UNIVERSITY OF CALIFORNIA,
IRVINE

Population and community consequences of ecological differences between the sexes

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

William Kevin Petry

Dissertation Committee:
Professor Kailen A. Mooney, Chair
Professor Diane R. Campbell
Professor Steven A. Frank
Professor Stephen G. Weller

2016

DEDICATION

To

Judy, for answering her own questions and inspiring mine,

and to

My parents, for nourishing my love of the natural world…and forgiving the times I brought the outdoors in.

"A la realidad le gustan las simetrías y los leves anacronismos."
Jorge Luís Borges, El Sur

"Otros refieren de otro modo la historia. En el mundo no puede haber dos cosas iguales."
Jorge Luís Borges, Parábola del Palacio

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I deeply grateful to my advisor, Kailen Mooney, whose unwavering support, enthusiasm, and mentorship made my time as a graduate student rewarding in innumerable ways. He has been fiercely committed to my growth as a scientist since our earliest interactions, showing a rare blend of generosity, creativity, and spirit of collaboration. It has been a distinct privilege to learn from such a gifted scientific teacher and role model. My science going forward will continue to echo the lessons Kailen has taught me – and it will be better for it.

I thank my committee members – Diane Campbell, Steve Frank, and Steve Weller – for their invaluable feedback at all stages of this dissertation. Their critiques helped to cultivate in me new ways of thinking about biology, and I deeply appreciate their intellectual contributions to this dissertation. Tom Miller served as an adopted committee member, taking me on as a student of population modeling. The trajectory of my dissertation was profoundly altered over the course of our interaction. Much of this dissertation simply wouldn't have been possible without his clarity of thinking, creativity, and patience.

I am indebted to my family and friends for their love and support during my graduate studies. I am particularly grateful to Emily Abbott. She has been a bastion of support and understanding when I needed it most and brightened my life through our shared adventures (and occasional misadventures). I thank my parents for nourishing my love of biology in all the forms that embodied from childhood through today. I am thankful for those who took me under their wing my first year at UC Irvine, especially Jenn Weber, Jason Brown, Jenny Talbot, Karla Feitl, and Stephanie Kivlin.

The Rocky Mountain Biological Laboratory became my home away from home over these past few years, and I am grateful to the RMBL community for facilitating my research and personal development. I have never encountered such an intellectually lively community. My conversations, hikes, and soaks with Mary Price, Nickolas Waser, Amy Iler, Paul CaraDonna, and Ross Brennan were particularly formative. I have benefitted enormously from the infectious spirit of the RMBL, particularly in the way that it facilitated my interaction with Judy Soule. Judy's dissertation served as an inspiration and sounding board for my understanding of the natural history and ecology of *Valeriana edulis*. Collaborating with her has been one of the most rewarding experiences of my professional life.

Many research assistants, citizen scientist volunteers, friends, and family members contributed to the collection of the data presented in this dissertation: Ana Chicas-Mosier, Jordan Croy, Peter Darnell, Scott David, Mark Dorf, Guadalupe Flores, Janet Haw, Lauren Morse, Chelsea Oeffler, Andrew Petry, Carolyn Petry, Catherine Prenot, Ariel Riggan, R. Alex Smith, Sage Smith, Louis-Jean Teste, and Su'ad Yoon. Two of my dissertation chapters have previously appeared in print. I recognize the contributions of my co-authors on these papers. Judith D. Soule, Amy M. Iler, David W. Inouye, Ana Chicas-Mosier, Tom E.X. Miller, and Kailen A. Mooney collaborated with me on Chapter 1. Kayla I. Perry, Aleshia Fremgen, Sarah K. Rudeen, Mitchell Lopez, John Dryburgh, and Kailen A. Mooney collaborated with me on Chapter 3.

I thank the Ecological Society of America and its publisher John Wiley and Sons for permission to include Chapter Three of my dissertation, which was originally published in *Ecology*. I am grateful for the financial support of my research by a National Science Foundation Graduate Research Fellowship, a NSF Doctoral Dissertation Improvement Grant (DEB-1407318), NSF DEB-1457029, the University of California Irvine Ayala School of Biological Sciences, and the Rocky Mountain Biological Laboratory.

CURRICULUM VITAE

William Kevin Petry

Education

2016 Ph.D. in Ecology and Evolutionary Biology, University of California, Irvine
Dissertation: Ecological consequences of intraspecific variation between the sexes
Advisor: Kailen A. Mooney

2010 B.S. in Biology, Truman State University
Honors thesis: A quantitative comparison of two sample methods for collecting *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) in Missouri
Advisors: Stephanie A. Foré and Laura J. Fielden

Research Appointments

2007 Missouri Department of Conservation, Northeast Region, Resource Science Division
2005 Missouri Department of Conservation, St. Louis Region, Fisheries Division

Publications (*undergraduate co-author; †authors contributed equally)

6. Moreira, X.†, **W.K. Petry**†, J. Hernández-Cumplido, S. Morelon, & B. Benrey. (In press) Plant defence responses to volatile alert signals are population-specific. Oikos
5. Moreira, X., K.A. Mooney, S. Rasmann, **W.K. Petry**, A. Carrillo-Gavilán, R. Zas, & L. Sampedro. (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters 17:537-546.
4. **Petry, W.K.**, K.I. Perry*, A. Fremgen*, S.K. Rudeen*, M. Lopez*, J. Dryburgh*, & K.A. Mooney (2013) Mechanisms underlying plant sexual dimorphism in multi-trophic arthropod communities. Ecology 94: 2055-2065.
3. Mooney, K.A., A. Fremgen*, & **W.K. Petry** (2012) Plant sex and induced responses independently influence herbivore performance, natural enemies and aphid-tending ants. Arthropod-Plant Interactions 6: 553-560.
2. **Petry, W.K.**, K.I. Perry*, & K.A. Mooney (2012) Influence of macronutrient imbalance on native ant interactions with aphids, aphid enemies, and host plant flowers in the field. Ecological Entomology 37: 175-183.
1. **Petry, W.K.**, S.A. Foré, L.J. Fielden, & H-J. Kim (2010) A quantitative comparison of two sample methods for collecting *Amblyomma americanum* and *Dermacentor variabilis* (Acari: Ixodidae) in Missouri. Experimental and Applied Acarology 52: 427-438.

Selected Fellowships and Research Awards

National Science Foundation

2015-2017 "Demographic consequences of sexually dimorphic responses to ongoing and experimental climate change" (PI: K.A. Mooney, Senior personnel: W.K. Petry and T.E.X. Miller)
2010-2014 Graduate Research Fellowship (2010-2014)
2014 Doctoral Dissertation Improvement Grant (2014)

Rocky Mountain Biological Laboratory

2015 Langenheim Fellowship
2012 Snyder Fellowship
2011 Kingsdale Fellowship

Selected Teaching & Mentoring Achievements (* = undergraduate; † = graduate; ‡ = public)

†Climate Action Program Data Science Workshop, co-organizer and co-instructor (2016)
‡PlantShift Citizen Science Program, creator and organizer (2013-2015)
†Quantitative Methods in Ecology & Evolutionary Biology, teaching assistant and guest lecturer (2014)
*R Statistical Workshop – UC Irvine Center for Environmental Biology internship program, organizer and instructor (2014)
*R Statistical Workshop – RMBL undergraduate education program, organizer and instructor (2014)

Selected Academic Citizenship and Service

Rocky Mountain Biological Laboratory Research Committee, member (2015-present)
Assistant Professor Search Committee, graduate student representative (2011-2012; resulted in the hiring of Sergio Rasmann)

ABSTRACT OF THE DISSERTATION

Ecological consequences of intraspecific variation between the sexes

By

William Kevin Petry

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2016

Professor Kailen A. Mooney, Chair

Males and females commonly differ in ecologically important traits. These traits mediate the acquisition and allocation of resources, altering individual interactions with the abiotic and biotic environment. Environmental change may therefore affect the performance of one sex more than the other, biasing the sex ratio. Despite this ecological divergence, the sexes remain co-dependent for reproduction. Low frequencies of one sex limit the mating opportunities for the more common sex, potentially reducing population growth rate. Currently very little is known about how demographic differences between the sexes scale up to produce widely documented patterns of skewed sex ratios. Moreover, the consequences of sexual variation for the dynamics of populations and communities are poorly understood. This dissertation aims to bridge these gaps by mechanistically decomposing the origins of biased sex ratios, tracing the consequences of biased sex ratios for population dynamics, and the effects of this form of intraspecific variation on the structure of associated communities. Population sex ratios in the long-lived dioecious plant *Valeriana edulis* (Caprifoliaceae) are shown to respond to climatic variation across the species elevation range and to recent anthropogenic climate change because the sexes differ in a key physiological trait, water use efficiency. Biased sex ratios arise because of sex

differences in multiple demographic processes including differential mortality and altered schedules of reproduction. Seed production is strongly limited when males are rare. However, population growth is not strongly affected by this mating failure, as this species' longevity buffers against perturbations in reproductive rates. Finally, the sex-specific patterns of association in a multi-trophic arthropod community are disentangled through a series of field experiments that show that arthropod preferences for female plants are driven by direct plant-arthropod interactions. Here even higher trophic levels respond more strongly to trait variation between plant sexes rather than to numerical or qualitative changes in their prey and mutualist partners. Overall, this work explores a poorly studied but widespread axis of intraspecific variation, showing that ecological difference between the sexes are powerful drivers of ecological pattern and process.

INTRODUCTION

The fundamental difference between the sexes is that males produce smaller gametes than females (Scudo 1967, Parker et al. 1972, Williams 1975, Charnov 1982, West 2009). This gametic asymmetry (i.e., anisogamy) has cascading effects across the physiology, morphology, and life history of the sexes. At the finest grain, females bear higher costs than males in the production of offspring. Accommodating this additional burden – or from the male perspective, avoiding it – restructures patterns of allocation to the core life history processes of growth, survival, and reproduction (Williams 1975, Stearns 1992, Delph 1999). Although elaborate mechanisms may equalize reproductive costs between the sexes through sexual selection, these too add allocation constraints asymmetrically between the sexes (Hoglund and Sheldon 1998). In some organisms, allocation dissimilarities manifest only in physiological differences whereas in other species morphological differences between the sexes are so exaggerated that early systematists classified males and females of the same species into entirely different genera (e.g., *Catonephele* butterflies; Bates 1865).

Sexual variation in physical and life history traits provoke different responses to biotic and abiotic stressors. Male animals can be more susceptible to parasites (Zuk and McKean 1996) and predation (Acharya 1995, Quinn and Kinnison 1999, Lodé et al. 2004), perform poorly in low quality environments (Trivers and Willard 1973, Schindler et al. 2015), and experience higher mortality rates (Comfort 1979). In contrast, male plants are generally less susceptible to pathogens (Kaltz and Shykoff 2001), herbivores (Cornelissen and Stiling 2005, Mooney et al. 2012), and abiotic stressors such as drought (Hultine et al. 2007, Xu et al. 2008a, 2008b) than females. The upshot is that differences between the sexes can alter their relative performance, leading to slower growth, higher mortality, compromised reproductive bouts, and greater

vulnerability to attack from natural enemies in the sex that is more strongly affected by the environment.

Population sex ratios can become unbalanced as a natural consequence of sustained performance differences between the sexes. Here a key distinction in types of sex ratios is important. Changes in the frequencies of the sexes arising from differential rates of growth, survival, and reproduction affect the sex ratio of individuals that go on to reproduce; this is the operational sex ratio (OSR). Fisher's classic argument for the evolutionary mechanisms maintaining balanced sex ratios only applies to the primary sex ratio – the frequency at which males and female offspring are produced – and makes no demands on the frequencies of the sexes after parental investment ends (Fisher 1930, Charnov 1982, West 2009). Sex differences in vital rates are only one proximate mechanism by which skewed OSR may arise (Shelton 2010a, 2010b, Jenouvrier et al. 2010). Sex-specific attack by natural enemies (Miller et al. 2007, Harrison et al. 2010), sex differences in phenology (Calabrese and Fagan 2004, Calabrese et al. 2008), sex ratio distorting symbionts and cellular components (Hurst 1993, Engelstädter and Hurst 2009, Himler et al. 2011), and sex-biased dispersal (Ranta et al. 1999, Veran and Beissinger 2009, Miller et al. 2011, Miller and Inouye 2013) have all been demonstrated to alter OSR. Natural populations vary in OSR from equal frequencies of the sexes to extreme bias towards one sex (Hardy 2002). Across species of dioecious plants, for example, the full range of sex ratios can be found. A recent survey of the literature (Field et al. 2013a) revealed that nearly half of species were reported to have balanced sex ratios, about 30% had male-biased sex ratios (up to 94% of individuals were male), and approximately 20% had female-biased sex ratios (up to 100% of individuals were females, persisting exclusively through clonal reproduction).

The sexes remain co-dependent for sexual reproduction, thus a limitation of one sex will limit the production of offspring. Several function forms for the relationship between OSR and offspring production – the mating function – have been proposed (Miller 2007). Different functional forms may more suitably model the biology of mating in a given species based on the species mating system (Rankin and Kokko 2007, Shelton 2008, 2010a). Despite strong differences in the projection of population dynamics under different mating function scenarios, the mating function has only been empirically estimated once (Miller and Inouye 2011).

Sex complicates life histories by inducing variation among individuals in demographic rates and nonlinear feedbacks between population sex ratios and the population growth. However, the ecological mechanisms driving biased OSR and its consequences are poorly known. Demographic approaches place the differences between the sexes within the backdrop of the full life cycle, enabling a decomposition of the origins of biased OSR and a contextualized understanding of their consequences. Recent advancements in population modeling allow arbitrarily complex life histories – including differences between the sexes – to be efficiently constructed and analyzed using a broad range of tools previously developed for matrix population models (Easterling et al. 2000, Caswell 2001, Ellner and Rees 2006, Merow et al. 2014).

Recent anthropogenic climate change underlines the importance of understanding how biological systems will respond to environmental change (Parmesan 2006); however, our understanding of the mechanistic links between individual-scale responses to change and their population- and species-level consequences is limited. Existing approaches to ecological phenomena have traditionally employed a "mean field" approach by that assumes all individuals of a species are ecologically identical, or at least that their ecological dynamics are well-captured

by the species average (Violle et al. 2012). Recent calls to account for intraspecific variation highlight the dynamical consequences of relaxing the assumption that all individuals are ecologically identical (Bolnick et al. 2003, 2011, Violle et al. 2012, Dall et al. 2012, de Roos and Persson 2013). The lingering challenge is to identify meaningful axes of ecological variation that efficiently predict the direction and degree to which individual differ. Ecological variation between the sexes represents a tractable but relatively unexplored form of intraspecific variation. Moreover, shedding light on sex-specific responses to the environment may improve ecological forecasts of the impact of climate change on natural systems.

Dissertation overview

My dissertation examines the impact of ecological variation between the sexes on population and community dynamics. In Chapter 1 I pair observational data across space and time to demonstrate that males and females of a long-lived plant species respond differently to the abiotic environment. Specifically, I show that physiological differences between the sexes lead to biased sex ratios that in turn are sufficient to affect reproduction. In Chapter 2 I use demographic models to explore the life history differences between the sexes across a climatic gradient, the mechanisms by which climate biases sex ratios, and the consequences of biased sex ratios for population dynamics. I demonstrate that climate has complex, sex-specific effects on demographic rates and biases population sex ratios, but that the loss of reproductive output because of unbalanced sex ratios has little effect on population growth. Finally, in Chapter 3 I document strong differences between the sexes in the structure of plant-associated arthropod communities, then I use a series of manipulative experiments to determine the species interaction mechanisms that give rise to these patterns. I show that direct arthropod-plant interactions

explain the differences in arthropod community structure between plant sexes with little effect of arthropod-arthropod interactions. In summary, my dissertation illustrates that intraspecific variation between the sexes is a potent driver of ecological patterns and dynamics across scales.

CHAPTER 1

Sex-specific responses to climate change alter population sex ratio and performance

ABSTRACT

Males and females are ecologically distinct in many species. Sex-specific responses to climate change may occur as a result, but such responses are virtually unstudied. Here we document the causes and consequences of a plant's sex-specific responses to climate change over the past 4 decades and across an elevation gradient. Water availability increases with elevation, driving males to low frequency because of sex differences in physiology and life history. Recent aridification has shifted sex ratios upslope at 175 m/decade, exceeding documented paces of species range shifts by an order of magnitude. The resulting increase in male frequency reduces the pollen limitation of female fitness and alters plant-dependent arthropod communities. Accordingly, climate change is driving previously unrecognized changes in sex ratios that govern fundamental evolutionary and ecological dynamics.

MAIN TEXT

Differences between the sexes in morphology and physiology often result in sex-specific responses to the environment (Lande 1980, Burk 1982, Zuk and McKean 1996, Geber et al. 1999). Climate change may affect the sexes differently, potentially creating an imbalance in the frequency of males and females (Bierzychudek and Eckhart 1988, Field et al. 2013b) and altering patterns of fertility and population demography (Le Galliard et al. 2005, Shelton 2008, Calabrese et al. 2008). Climate-skewed sex ratios – if sufficiently strong – could drive population decline (Le Galliard et al. 2005) and compromise the ability of species ranges to track shifting climate

envelopes (Miller and Inouye 2013). Although the potential for sex-specific responses to climate change is strong, no studies have focused directly on this problem.

Here we show that complementary axes of climatic variation over space (ca. 2000m elevation) and time (33 years of climate change) have parallel, sex-specific effects on a long-lived plant and dramatically skew population sex ratios. We studied *Valeriana edulis* (Caprifoliaceae), a dioecious herb with fixed, genetically-based sex expression (Meurman 1925, Soule 1981) and a wide elevation range from arid low-elevation scrublands to mesic alpine tundra. We paired historic and contemporary surveys of operational sex ratios (OSR: the proportion of flowering individuals that are male) with individual-based demographic and physiological data to assess: (i) sex ratio change, (ii) the sex-specific mechanisms underlying this change, (iii) how biased sex ratios influence individual fitness, and (iv) the extended consequences of biased sex ratios for populations and species interactions.

Climate varies considerably across the elevation range of *V. edulis* (Fig. S1.1A-D; Table S1.2). Contemporary climate data for our study area in the Rocky Mountains of Colorado (Fig. S1.2; Table S1.1) show that increasing elevation is accompanied by a decrease in mean growing season (June-August) temperature (−0.59°C per 100m), an increase in growing season precipitation (1.5mm per 100m), a delay in the date of snowmelt (4.1 days later per 100m) and a trend for increasing growing season soil moisture (1.09% per 100m). Collectively these clinal changes produce a gradient of decreasing aridity with increasing elevation.

We surveyed population sex ratios across this elevation gradient to test whether elevational variation in climate was accompanied by parallel variation in *V. edulis* population OSR. Surveys of 32 *V. edulis* populations across the species range in 2011 showed that males became rare with increasing elevation (Fig. 1.1A; $P = 0.003$, $R^2 = 0.26$), falling from a frequency

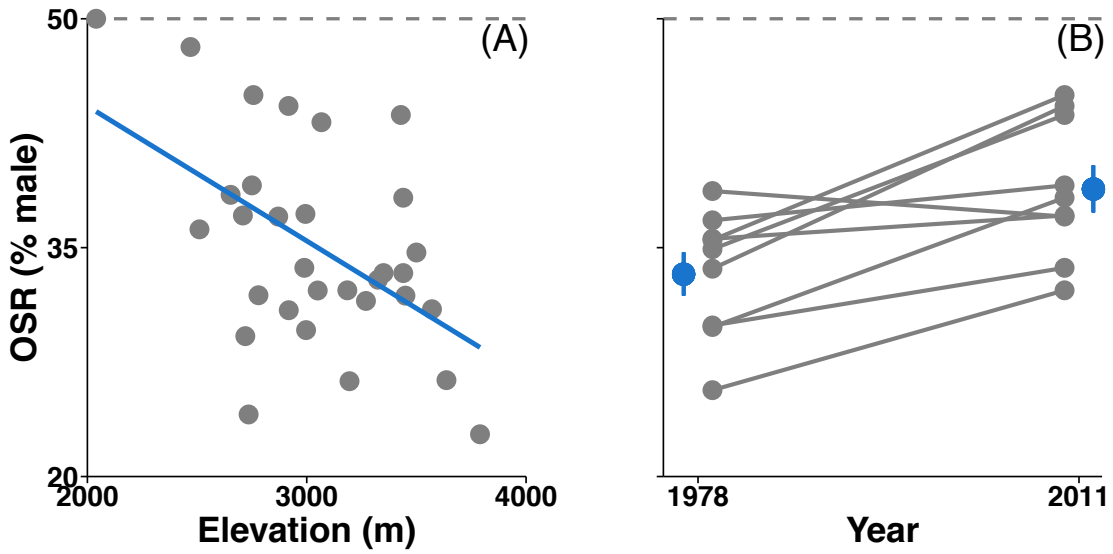


Figure 1.1. Operational sex ratio (OSR; % flowering plants that are male) of *Valeriana edulis* co-varies with spatial and temporal climatic variation. **(A)** OSR becomes significantly more female-biased with increasing elevation across the species elevation range in contemporary surveys (2011). **(B)** Males have significantly increased in frequency with climate change between 1978 and 2011 by an average of 5.5% (blue points ± 1 SE) representing 9 resurveyed populations (linked grey time points). A mean of 294 plants were sexed in each population; populations with < 100 flowering plants were censused completely.

of 50.0% at the lowest elevation population to 22.7% at the highest population for an average change of −0.88% per 100m of elevation. These findings suggest that spatial climatic variation has strong effects on *V. edulis* OSR and that similar shifts may occur in response to climate change over time.

Recent climate change has warmed and dried our study area, driving climatic isoclines up in elevation and providing a temporal axis of climate variation paralleling that occurring over the elevation gradient (Fig. S1.1E-H; Table S1.2). Data collected over the past four decades (1978–2014) show that regional mean temperature during the growing season has increased by 0.21°C/decade; growing season precipitation has decreased by 1.91 mm/decade; snowmelt date has marginally advanced by 2.9 d/decade; and soil moisture during the growing season has decreased by 1.5%/decade (Tables S1.1, S1.2). This change over time is equivalent to an upslope shift in the isoclines for mean growing season temperature, growing season precipitation, advancement of snowmelt, and soil moisture at velocities of 36 ± 8, 133 ± 26, 72 ± 40, and 195 ± 523 m/decade, respectively. Regional climatic projections suggest that climate will continue to change (Maurer et al. 2007, Stewart 2009). Nevertheless, the changes observed over the past four decades have been sufficient to alter the ecology of many flowering plant species in our study region (CaraDonna et al. 2014). In the case of *V. edulis*, we found that the onset of flowering has advanced by 3.1 d/decade ($P = 0.062$, $R^2 = 0.091$; Fig. S1.3) likely due to an advancing date of snowmelt and a strong association of snowmelt with flowering phenology in this species ($P < 0.0001$, $R^2 = 0.47$; see Supplemental Methods for analyses of peak and last flowering).

Recent climate change has in turn significantly shifted *V. edulis* OSR in a manner consistent with the upslope shift in climate. Surveys of OSR from nine populations in both 1978 and 2011 showed that males have become more frequent across the species' elevation range at a

rate of 1.28%/decade (paired t-test P = 0.047; Fig. 1.1B). By comparing this temporal shift with the independent, parallel pattern of OSR variation over space, we estimate that OSR isoclines are moving upslope at a rate of 175 m/decade [mean ± lower, upper SE: 87, 316 m/decade], mirroring shifts in precipitation and soil moisture. The parallel change in OSR over two independent climatic gradients – elevation and time – implicates climate as the driver of OSR variation, but does not reveal the processes by which this occurs.

To explore the mechanisms underlying sex-specific responses to climate change, we first quantified life history differences between the sexes in four populations spanning 1167m of elevation (2470–3637m) and varying 22% in OSR (48% to 26% male). We used sex- and size-structured rates of annual growth and mortality collected from 1978-1980 to calculate male and female life expectancy upon reaching reproductive maturity in each population. This metric integrates sex differences in demographic performance across the life span and reflects the average duration during which a plant contributes to OSR. Sex differences in reproductive life expectancy were concordant with population OSR, such that female-biased OSRs were associated longer reproductive life spans than males (Fig. S1.4). These findings are consistent with the hypothesis that the sex-specific effects of climate on life history drive population variation in OSR.

We next sought to determine the physiological basis for the sex-specific effects of climate by focusing on water, a key resource. A seasonal decline in water availability between snowmelt and the mid-summer monsoon characterizes the growing season of *V. edulis* (Fig. S1.5); a plant's water use efficiency (WUE: carbon assimilation per unit of water transpiration) will therefore mediate its ability to acquire energy within the short growing season. We hypothesized that sex differences in WUE – a trait known to differ between the sexes in many

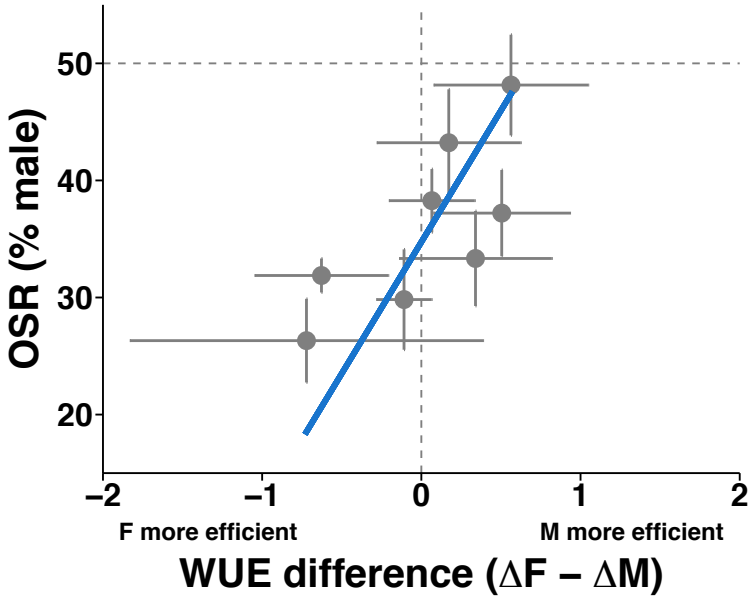


Figure 1.2. Sex differences in water use efficiency (WUE = carbon assimilated per unit water transpired) predict OSR in 8 populations spanning 2470–3637m. Water use efficiency is inferred from ^{13}C fractionation (Δ), and positive differences between the sexes (ΔF−ΔM) indicate WUE of females is lower than that of males and vice versa.

other plant taxa (e.g. Dawson and Bliss 1989) – underlie sex-differences in plant performance and drive patterns in OSR. We measured the integrated WUE of each sex as indicated by leaf carbon isotope ratios collected from eight populations that vary in OSR (Farquhar et al. 1989). Sex differences in WUE strongly predicted population OSR (Fig. 1.2; $t = 2.06$, $df = 6$, $P = 0.043$); females had higher WUE than males in strongly female-biased populations (low OSR), but males had higher WUE than females in populations with a higher proportion of males (higher OSR).

Variation in OSR may feed back to affect population growth by altering pollen availability and seed set rates (Caswell and Weeks 1986, Knight et al. 2005). To investigate such dynamics in *V. edulis*, we measured the response of female seed set to an index of pollen availability that distance-weights male flower abundance within the range of pollen movement (Supplemental Methods). The majority (*ca*. 90%) of pollen was received from males within 10m of focal females (Fig. S1.6). Female seed set in turn increased with pollen availability in this mating neighborhood (Fig. 1.3), rising from 39.3% to 99% of flowers producing seed across the observed range of neighborhood pollen availability. We simulated the effect of population OSR on seed set across the range of observed OSRs (Fig. 1.1A; Supplemental Methods), and we found that the observed spatial variation in OSR was sufficient to significantly alter female fitness. The low frequency of males at high elevation (22.8%) reduced median pollen availability by 55% compared to low-elevation populations with balanced OSR, corresponding to a reduction from 95% seed set at low elevation to 76% at high elevation (19% overall across the *V. edulis* elevation range; Fig. 1.3).

A mechanistic understanding of OSR dynamics in this system enables projections of future state of *V. edulis* populations. Assuming the rate of increase in male frequency continues

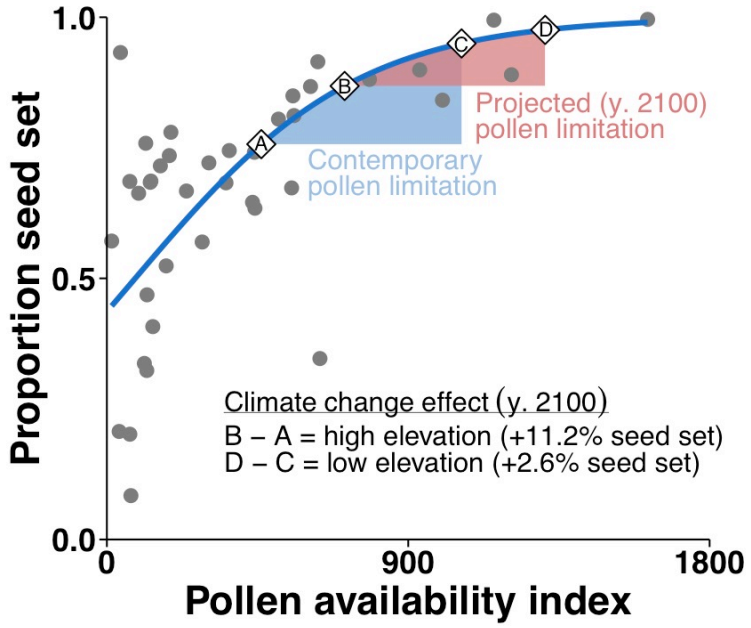


Figure 1.3. Pollen availability limits female reproductive success (points and line; controlling for competition with neighboring females for pollen). Estimated mean pollen availability for the range of contemporary OSRs observed across the *V. edulis* elevation range and expected OSRs in the year 2100 are indicated by the shaded regions and labeled points (**A-D**). A linear model was fit to logit-transformed data; the back-transformed model fit is shown (see Supplemental Methods for full model description).

(1.28%/decade; Fig. 1.1B), we predict that pollen limitation of reproduction in high-elevation populations will be halved by the year 2100 (a median seed set increase of 11.2%; Fig. 1.3), facilitating the upslope range expansion of this species. In contrast, we predict that increases in male frequency at low elevation will have little effect on seed set success (+2.6%) because females in those populations are pollen-saturated under contemporary, balanced OSRs (Fig. 1.3). Instead, increasing male-bias in OSR at low elevation may threaten population viability by replacing females with males and thus reducing population-level seed production (Caswell and Weeks 1986). Moreover, female *V. edulis* support dramatically higher densities of arthropods than do males, including several specialist herbivores that depend exclusively on *V. edulis* (Mooney et al. 2012, Petry et al. 2013). Accordingly, a climate-driven decline in female frequency may induce changes extending to higher levels of ecological organization (Thompson et al. 2013).

Previously reported rates of species range shifts in montane plants show a mean upslope shift of 11.1 m/decade (Supplementary Methods), dramatically slower than the analogous 175 m/decade upslope pace of OSR change in *V. edulis* (Fig. 1.4). Whereas numerous studies have characterized the biological effects of climate change in terms of species' range shifts poleward (km/decade) or upslope (m/decade; Chen et al. 2011), few have investigated the parallel, but relatively cryptic shifts in sex ratios or other traits occurring within the boundaries of a species range (Etterson and Shaw 2001, Thompson et al. 2013). The pace of species range shifts frequently lags behind the pace of climate change (Supplementary Methods), and such range disequilibria are frequently attributed to dispersal limitation (Sexton et al. 2009). In contrast, shifts in traits within species ranges may track climate change more closely because they are based on differential performance of genetically-based types that often already exist in many

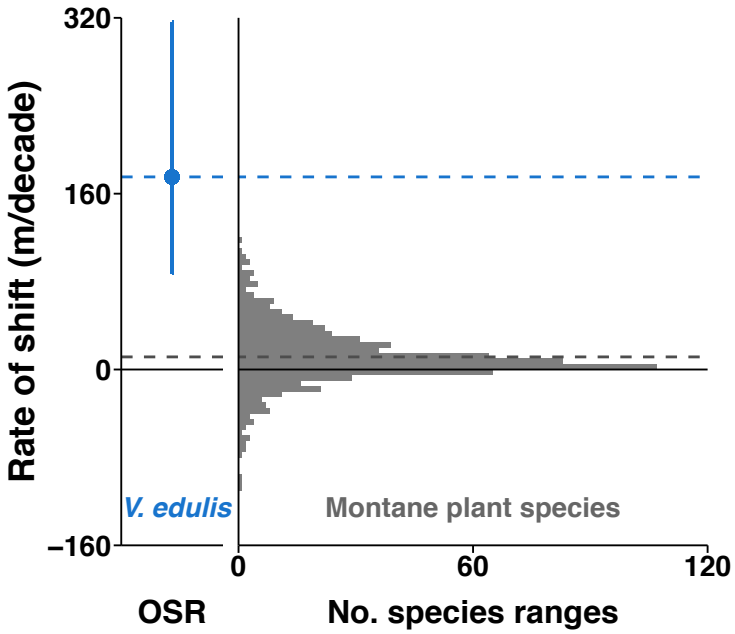


Figure 1.4. Elevation range shifts of montane plant species (677 observations encompassing 643 species reported in the literature) are on average an order of magnitude slower than the rate of *V. edulis* OSR change (blue point and dashed line). A histogram (left) shows the observed distribution of range shifts and the mean range shift (dashed grey line) is indicated.

populations across the range. Here we show that the pace of OSR change in *V. edulis* tracked climate change; it has kept pace with shifts in precipitation and soil moisture, and has exceeded those of temperature and snowmelt (Fig. S1.1).

We have demonstrated the occurrence of a previously unrecognized, but potentially widespread form of biological response to climate change. We show that sex-specificity of these responses can be exceptionally rapid with broad effects across multiple scales of ecological organization. Within populations, sex-specific responses to climate change can skew sex ratios – through sex differences in physiology and performance or otherwise (Morbey and Ydenberg 2001, Calabrese et al. 2008, Forrest 2014, Shaw and Kokko 2014) – and the resulting sex ratio biases may, by mediating reproduction, affect population growth rate, the risk of population extinction, and the rate of adaptation to changing climate by reducing effective population size (Rankin and Kokko 2007, Charlesworth 2009). In so doing, climate-driven changes in sex ratio may also control the tempo of species range shifts by mediating mate limitation at the leading or trailing range margins (Miller et al. 2011, Miller and Inouye 2013). Accordingly, a full understanding of biological responses to climate change requires a multi-scale approach that integrates the underlying, but cryptic, changes in intraspecific traits that give rise to higher order patterns.

SUPPLEMENTAL MATERIALS

Materials and Methods

Observed sex ratio assessment and analysis

This study was conducted in Gunnison County and Chaffee County, Colorado USA surrounding the Rocky Mountain Biological Laboratory (RMBL; Fig. S1.2). Observed population sex ratios of flowering individuals (hereafter OSR) were assessed in fifteen populations (2709-3440m elevation) between 1978 and 1980. Ten populations were relocated and nine were resurveyed in 2011 – the tenth population (Brush Creek Pasture elevation: 2735m) was excluded because the site was both intensely grazed by livestock and regularly hayed. We assessed OSR in these historic populations plus an additional 22 contemporary populations (2040-3790m elevation) between 2011 and 2014. At each population, we determined the sex of each flowering plant by visual inspection of the flowers until ca. 200 plants were counted along a paced transect or all flowering individuals were censused (range in sampled populations: 197-1284, range in censused populations: 27-172). Sex expression in *V. edulis* is genetically fixed and can only be determined morphologically while flowering. Individuals bearing flowers of both sexes are very rare (<1%), consistent with the rate of sexual inconstancy in other dioecious species (Charlesworth 2002). Insects are the primary pollinators, typically generalist flies and solitary bees, and *V. edulis* does not reproduce vegetatively. Mature plants flower in >90% of years, and plants may live more than 100 years (Soule 1981).

We analyzed changes in OSR over elevation and time after logit transformation because OSR is bound by 0 and 1 (i.e. all female and all male, respectively). We calculated the pace of upward OSR shift by estimating the contemporary elevations (Fig. 1.1A) that correspond to the mean OSRs in 1978 and 2011 (Fig. 1.1B), then dividing their difference by the intervening 33

years. We report the mean shift after propagating the errors associated with each parameter – the regression slope, the regression intercept, the mean historic OSR, the mean contemporary OSR – using the R package PROPAGATE (Spiess 2014). This package uses a parameter sampling approach and accounts for the covariance structure between parameters; each sample is drawn from a multivariate normal distribution with a covariance matrix constructed from parameter standard deviations. We based all inferences on 10^6 Monte Carlo simulations.

Phenological measurements

The flowering phenology of *V. edulis* has been monitored as part of a community-level flowering phenology survey ca. every 2 d during the flowering period from 1974-2014 in a series of 2 × 2m permanent plots at the RMBL (Inouye 2008). *Valeriana edulis* is present in 13 plots and has flowered in at least one of these plots every year. We recorded the number of stems with open flowers (as opposed to counting all open flowers) of *V. edulis* in each plot because individual plants contain numerous small flowers. There was no census in 1978 or 1990, leaving 39 years of data from 1974-2014.

We analyzed shifts in first, peak, and last flowering dates from the flowering distributions resulting from our surveys. We summed counts of flowering stems across plots in each year to determine the dates of first, peak, and last flowering. First flowering was the first day on which a flowering stem contained flowers in any of the plots, i.e. the very beginning of the across-plot cumulative flowering curve. Peak flowering was calculated as the day on which 50% of flowering stems were counted (following Iler et al. 2013). Last flowering was the last day on which open *V. edulis* flowers were observed. Total flower number was counted instead of the number of flowering stems in six of the 39 years; we removed these years from the analysis of

peak flowering date so that all data are consistent across years. The change in measurement unit does not affect the determination of first and last flowering dates.

We regressed each phenological response variable on year to assess shifts in phenology through time. To examine phenological responses to climate, we regressed each response variable on the timing of snowmelt. Timing of snowmelt was the day of first bare ground in a permanent plot at the RMBL (1975-2014; Table S1.1). We focused on timing of snowmelt as a climatic predictor of flowering phenology based on a previous analysis that used an earlier version of the phenology data (Iler et al. 2013). Based on previous studies that have found a threshold flowering response to snowmelt in high-altitude plant communities (Inouye 2008, Steltzer et al. 2009, Iler et al. 2013), we also fit a piecewise regression model relating snowmelt to all flowering responses using the R package SEGMENTED (Muggeo 2008). The piecewise model improved the fit for peak flowering date (ΔAIC < 2) but not first or last flowering date.

First, peak, and last dates of *V. edulis* flowering advanced by 0.60 ± 0.11, 0.79 ± 0.11, and 0.63 ± 0.16 days for every day that snowmelt advanced, respectively ($P < 0.0005$, $R^2 = 0.47$, $R^2 = 0.62$, $R^2 = 0.30$, respectively). Peak flowering dates did not advance over the earliest range of snowmelt dates on record in the piecewise statistical model ($R^2 = 0.69$). Regardless, the trend towards earlier snowmelt was mirrored by advancing trends in *V. edulis* flowering phenology (first flowering date: −3.1 d/decade, $P = 0.062$, $R^2 = 0.091$; peak: −3.5 d/decade, $P = 0.084$, $R^2 = 0.093$; last: −4.1 d/decade, $P = 0.050$, $R^2 = 0.10$).

Climate data

We assembled climate data across space and time from publicly available databases (Fan and van den Dool 2004, National Operational Hydrologic Remote Sensing Center 2004, PRISM Climate Group 2014) and records shared by colleagues at the RMBL (Tables S1.2, S1.4). Interpolated and fixed location data were cropped to a 1° × 1° study area centered on the RMBL and enclosing all study populations. Too few weather stations measuring temperature over time, precipitation over time, and soil moisture over space were available within this study area; stations within an expanded 2° × 2° study area were included for these variables. Datasets over time were truncated to years between 1978 and 2014. Elevation data for the study area were acquired from the 30m resolution NASA STRM dataset (Farr et al. 2007). Changes in climate variables over space and time were assessed with linear regression. Growing season means were determined as the arithmetic mean of monthly means from June-August, inclusive, corresponding to the period between leaf flush and above-ground senescence.

All climate variables were measured or interpolated using standard meteorological sensors with the exception of snowmelt data from personal sensors. Here snowmelt is inferred from HOBO Pendant Temperature/Light data loggers (Onset Computer Corporation, Bourne, Massachusetts) deployed at ground level following Kreuzer et al. (2003). Briefly, light reaching the subnivean zone is below the logger detection limit (1 lux between 150-1200 nm) and temperatures remain stable near 0°C. Light infiltration and diurnal fluctuations in temperature were used to mark the melting of snow.

The pace of climate change was calculated for each variable following a similar approach to the calculation of the pace of OSR change (see *Observed sex ratio assessment and analysis*). Briefly, for each climate variable we estimated the expected value at the endpoints of the regression over time (Fig. S1.1E-H). We then mapped these expected values onto the regressions

over space (Fig. S1.1A-D) to calculate the total elevation displacement over the focal time period (1978-2014). Finally, the pace was calculated by dividing the total displacement by the intervening number of decades (3.6). In parallel with our calculation of the pace of OSR shift, we propagated the errors associated with each regression parameter using 10^6 Monte Carlo simulations in the R package PROPAGATE (Spiess 2014).

Estimation and analysis of historic demographic rates

Sex- and size-specific annual rates of growth and survival were collected in four populations between 1978 and 1980 (Soule 1981). In each population, >600 individual plants were uniquely tagged. Adult individuals (79-88% of all plants) were binned into 4 size classes (3 in the case of one population, Brush Creek Pasture) by rosette diameter (Table S1.3). Plants with at least 10 rosette leaves or flowering were considered to be adults. The bounds of the adult size classes were chosen separately for each population using a method that minimizes sampling and distributional errors in the matrix model (Vandermeer 1978), thus the size classes are not identical among the populations. Sex was determined for all individuals by inspection of the flowers, and those not flowering in any of the 3 years (2-4% of adults) were excluded. Annual rates of growth were calculated as transitions from one size class to another, and shrinkage (i.e. negative growth) was allowed.

For each sex and population combination, annual growth and survival rates were used to populate square transition matrices (Caswell 2001), here restricted to adult stages after the onset of flowering (because pre-reproductive plants could not be sexed). Mean life expectancy for adults at size class 1 was calculated for each matrix following Cochran & Ellner (1992) as implemented by the R package IPMPACK v2.1 (Metcalf et al. 2012). This quantity measures the

remaining life expectancy of plants once they begin flowering and thus reflects the average duration during which a plant contributes to OSR. Relative life expectancy for each population was calculated as the ratio of male to female life expectancy.

Stable isotope analyses

Fully-expanded leaf samples were collected during peak flowering in the summer of 2011, immediately freeze-dried, then stored at −80°C. Samples were prepared for C isotope ratio analysis following Pratt and Mooney (Pratt and Mooney 2013). Briefly, samples were ground using a Wig-L-Bug bead mill (International Crystal Laboratories, Garfield, NJ) and ca. 1.3 mg of powdered leaf homogenate was packed into 5 × 9 mm tin capsules. Mass spectrometry was then performed at the University of California, Irvine Stable Isotope Ratio Mass Spectrometry Facility (Delta^{plus} XL, Thermo Finnigan, Asheville, NC). Carbon isotope discrimination, $\Delta^{13}C$, was calculated assuming that $\delta^{13}C_{air} = -8.0‰$ (Farquhar et al. 1989). Higher values of $\Delta^{13}C$ indicate lower WUE. For each population the mean $\Delta^{13}C$ and the standard error of the mean (SE) were calculated separately for each sex. Relative differences in $\Delta^{13}C$ between the sexes were calculated by subtracting mean male $\Delta^{13}C$ from the mean female $\Delta^{13}C$ and propagating the error. Because both the dependent and independent variables were estimated with error, a generalized Deming regression (R package DEMING v.1.0-1) that minimized the total sum of squares was used to account for error in both variables (Therneau 2014).

Effect of OSR on reproduction

Pollen movement distance was determined by dusting all dehiscent anthers of 12 male *V. edulis* with a fluorescent dye powder (a pollen analogue; Kearns and Inouye 1993) at Emerald Lake (3185m) in 2014, then collecting a subsample of flowers from the surrounding female plants along two perpendicular transects centered on the dyed male after 48 hours. All transects were at least 20m long, though females at distances up to 40m were sampled for a subset of 4 of the 12 dyed males. Dye particles were counted only if they were deposited on the stigma, and the distances of these stigmas to the dyed male were recorded. A Lomax (type-II Pareto) distribution was fit to the data to characterize the relationship between distance and dye, yielding parameters scale = 2.20 and shape = 1.24. This distribution predicts that 50% of dye – and by extension pollen – is deposited within 1.65m and 90% is deposited within 11.98m. To maximize replication in a limited space, we assumed a mating neighborhood radius of 10m.

Mating neighborhood (10m radius) composition was measured around 44 haphazardly chosen focal females at Emerald Lake (14 in 2014 and 30 in 2015 – five focal females in 2015 were later excluded because of heavy ungulate herbivory). A subset of open flowers on each focal female ($\bar{x}$ = 74, range: 21-329) was marked on the sepals with a black felt-tip marker, and these flowers were later measured for seed production (0 vs. 1 seed). The distance from the focal female to each plant within the neighborhood was measured to the nearest 5 cm, and all open flowers were counted on each. When the number of flowers was >100, an estimation technique was used. Briefly, all open flowers were counted in a subset of the inflorescence, then the number of these subsets needed to fill the entire inflorescence was estimated visually. Each observer estimated flowers, then exhaustively counted flowers on a calibration dataset of >20 individuals of each sex. Exploratory analyses revealed differences in estimation among observers, but no observer showed differences in estimations between the sexes. A linear

regression relating the estimated number of flowers to the true number of flowers was fit to each observer's calibration dataset. In all cases observer estimations were robust predictors of the true flower number ($0.81 < R^2 < 0.94$; 6 observers). Subsequently all observers used the estimation method and flower estimations were used to estimate the true number of flowers using the parameters of the observer's calibration regression.

Neighborhood composition was determined by measuring: (i) the distance of each neighbor to the focal female, (ii) the total number of plants regardless of sex (= density), and (iii) an index of pollen availability (PC). The PC index assumes that male plant pollen contribution to the focal female increases with the number of male flowers but declines as the distance to the focal female increases (Fig. S1.6). The PC index was calculated using a modified formula from Garcia-Camacho et al. (2009) that allows the decline in male pollen contribution to follow a Lomax distribution:

$$PC_i = \sum_{j=1}^{n} f_j p(x) d_{ij} \qquad \text{(Eq. 1.1)}$$

where n is the number of males within 10m of the focal female, f_j is the number of open flowers on male j, and $p(x)d_{ij}$ is the probability that pollen is transferred the distance d between focal female i and neighborhood male j given by the Lomax distribution fit to the fluorescent dye dataset.

The proportion of marked flowers on the focal female that set seed was modeled as dependent on neighborhood PC index and female floral density (a surrogate for competition with neighboring females for pollen) after a logit transformation to linearize the response variable (Table S1.5). Exponential spatial autocorrelation within each year was detected with an effective range of 14m, and this spatial non-independence between neighboring replicates was accounted

for in the model using generalized least squares (GLS) regression as implemented in the R package NLME (Pinheiro and Bates 2000, Pinheiro et al. 2015).

Simulation of OSR effect on pollen limitation

Detailed mating-scale data from the Emerald Lake population were used to estimate the effect of population-level OSR on mating-scale pollen limitation. Within an arbitrary 200m^2 of the population, all plants were mapped with a sub-meter accuracy GPS receiver (Trimble GeoXT 6000). The spatial arrangement of plants was modeled as a Thomas cluster point process with an intensity $\lambda = 0.890$, $\kappa = 0.123$, scale = 1.303 using the R package SPATSTAT (Baddeley and Turner 2005). For the most extreme OSRs observed across the elevation gradient (Fig. 1.1A), the fitted Thomas point process was simulated 100 times on a 50 × 50m region. Points were randomly assigned a sex based on the OSR and assigned a flower number drawn from the sex-specific distributions of flower number observed at Emerald Lake assuming that flowering frequency is 90%.

To ensure that population-level OSR is indicative of the mean pollination neighborhood composition, the positions of all flowering plants in similar plots were mapped at 3 other populations (collectively spanning 872m elevation and 21% OSR). The spatial arrangement of these plants was treated as a multivariate point pattern consisting of male and female individuals, and the spatial distribution of the sexes was assessed using K functions (la Cruz et al. 2008) that test whether one sex is more likely to be surrounded by individuals of the same (positive K_1-K_{12}) or opposite sex (negative K_1-K_{12}). These functions test for aggregation or segregation at multiple spatial scales simultaneously. Under the null hypothesis of random intermixing of the sexes, both K function operations equal zero. Permutations (n = 1,000) that randomly assign a

sex label to each point were used to construct 95% confidence intervals around the null hypothesis. Significant differences from random intermixing of the sexes occur when the test statistic exceeds the bounds of this interval. This analysis was carried out using the R package ECESPA (la Cruz 2008). Although many plant species with separate sexes show spatial segregation of the sexes (Bierzychudek and Eckhart 1988), there was no evidence of spatial aggregation or overdispersion of the sexes relative to one another in any population, demonstrating that population-level OSR reflects the central tendency of the distribution of the sexes at finer scales (Fig. S1.7).

The effect of distance on pollen availability index (PC) was estimated for each female following the methods described above (see *Effect of OSR on reproduction*). Simulated females less than 10m from the simulated region boundary were excluded to avoid edge effects. The distribution of the pollen availability index (PC) was right skewed, so the median was used to measure central tendency.

Pace of species range shifts

We compiled published reports of species range shifts from large comparative surveys of montane plant distributions over time (Parolo and Rossi 2008, Holzinger et al. 2008, Lenoir et al. 2008, Kelly and Goulden 2008, Bergamini et al. 2009, Crimmins et al. 2011, Telwala et al. 2013, Morueta-Holme et al. 2015). The total elevation displacement (i.e. the change in mean, cover-weighted, or maximum elevation) of each species range was standardized by the time between measurements (3–21 decades). We chose to fit a logistic distribution to the data ($\mu = 10.53$, $s = 14.25$; Fig. S1.8) because the data showed higher kurtosis than the Normal distribution; the logistic fit was better than the Normal fit ($\Delta AIC = 75.5$). Moreover, this is a conservative choice

because the higher kurtosis of the logistic distribution endows it with heavier tails than the Normal distribution.

Previously reported rates of upslope range shift in montane plants ranged from −106.4 to 116.2 m/decade with a mean velocity of 11.5 ± 1.1 m/decade (Fig. 1.4). Species range shifts of greater or equal magnitude to the pace of *V. edulis* OSR change (175 m/decade) are expected to be exceedingly rare (9.8×10^{-6}; Fig. S1.8). Moreover, species range shifts tend to lag behind the pace of climate change (Parolo and Rossi 2008, Kelly and Goulden 2008, Bergamini et al. 2009, Chen et al. 2011, Crimmins et al. 2011, Morueta-Holme et al. 2015).

Supplemental Figures

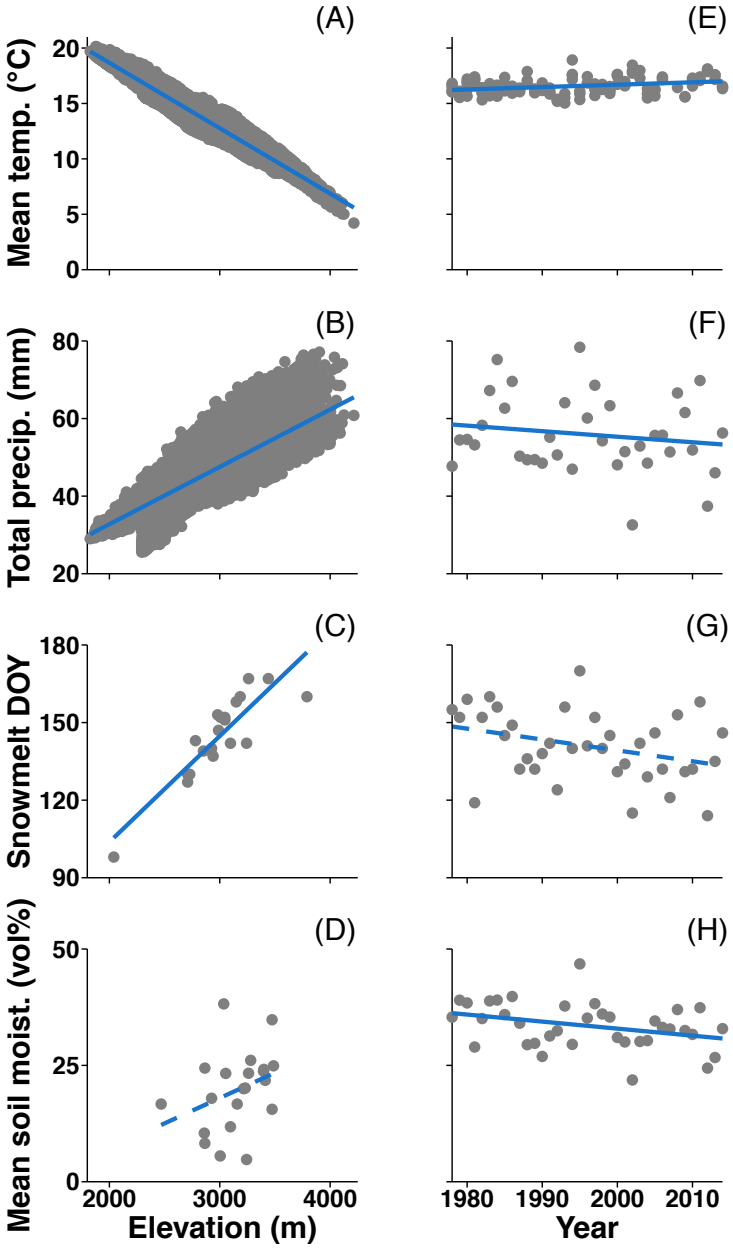


Figure S1.1. Climate changes over space and time in central Colorado, USA (see methodology in SI). Mean temperature during the growing season (June-August; 1981-2010 normals) declines with elevation **(A)** and has increased over time **(E)**. Growing season total precipitation increases with elevation **(B)**, but remains constant over time **(F)**. The date of snowmelt delays with elevation **(C)**, and has advanced over time **(G)**. Mean soil moisture during the growing season increases with elevation **(D)**, but decreases over time **(H)**. Solid and dashed lines indicate slopes

that differ from zero ($P < 0.05$) and trends ($P < 0.15$), respectively. See Table S1.1 for data sources and Table S1.2 for full summary statistics.

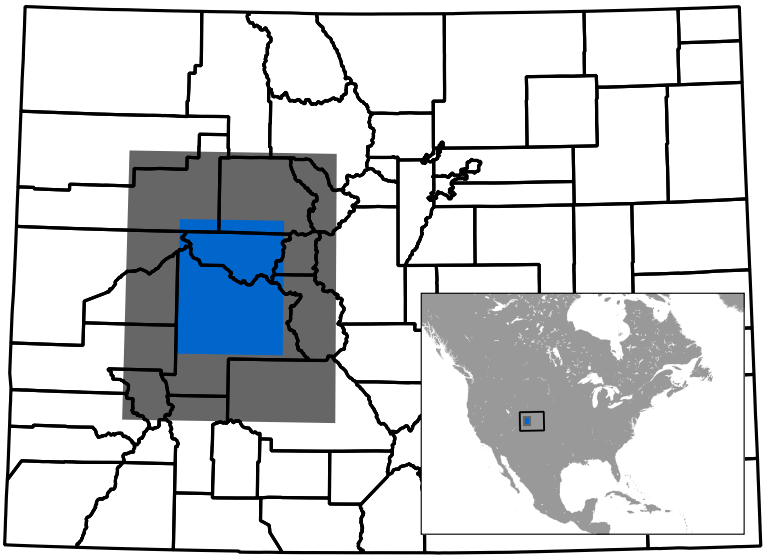


Figure S1.2. Map of study area centered on the Elk and West Elk Mountains of Colorado, USA (inset). Colored shaded regions show the study area (1° in blue and expanded 2° region in gray) in Colorado with county outlines in black.

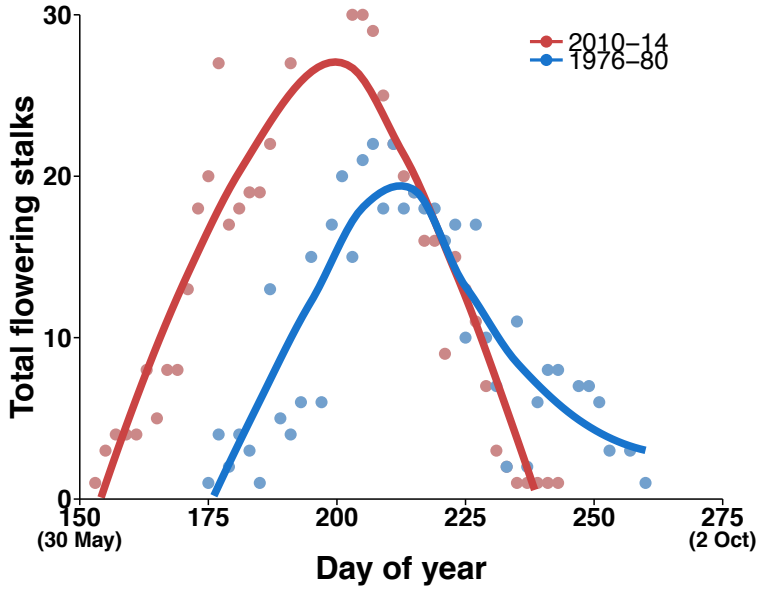


Figure S1.3. The flowering phenology of *Valeriana edulis* has advanced between 1976 and 2014. Flowering stem counts are summed across all plots in each 5-year window to generate the flowering curve.

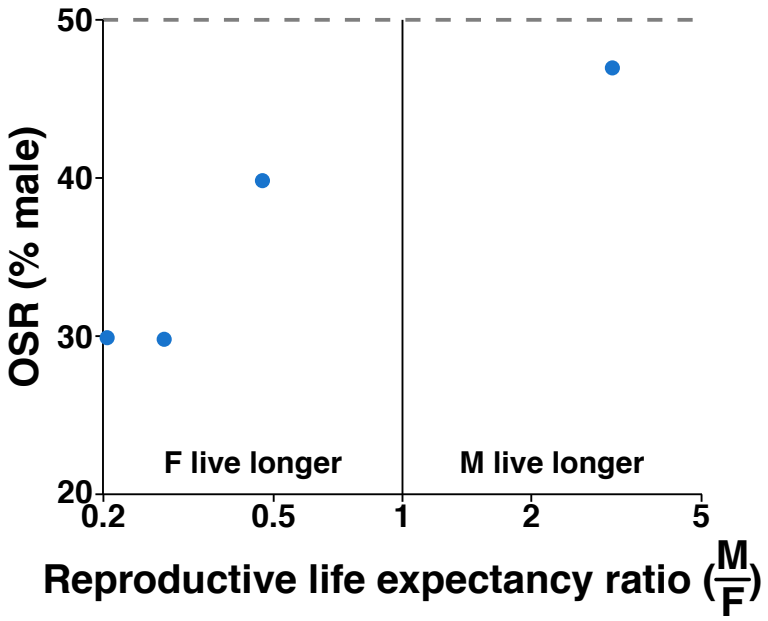


Figure S1.4. Relative life expectancy among the sexes (male/female) at the onset of reproductive maturity corresponds qualitatively to OSR (note $\log_{10}$ scale of the x-axis that scales deviations in each direction symmetrically about one). Demographic rates and OSR data were collected between 1978-1980 (see Supplemental Methods and Table S1.3).

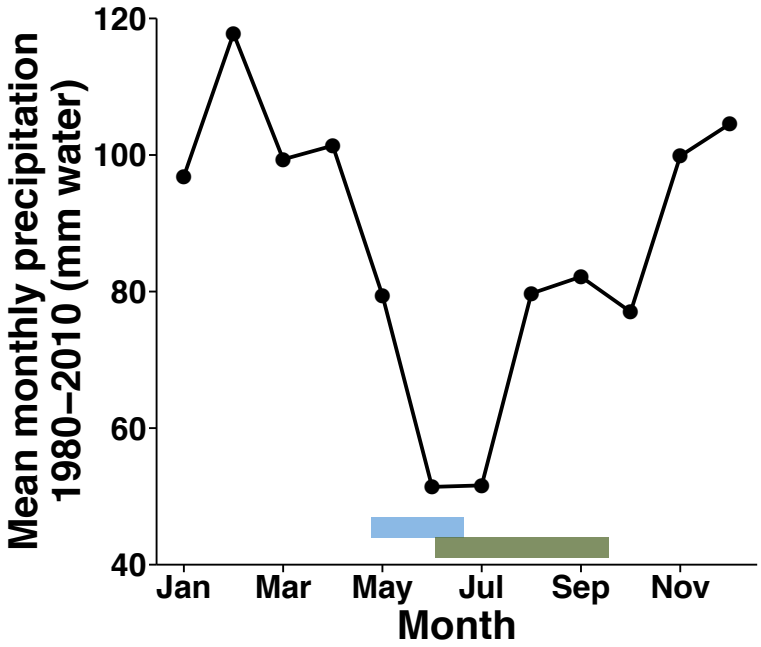


Figure S1.5. The growing season of *V. edulis* – beginning immediately following snowmelt (1980-2011 range in blue) and ending shortly after the termination of flowering (1980-2011 range in green) occurs during a mid-year dry period before the arrival of the late summer monsoon rains. Precipitation data are normals from 1980-2011 from PRISM Climate Group, snowmelt data are from billy barr (see Table S1.1 for full data source information).

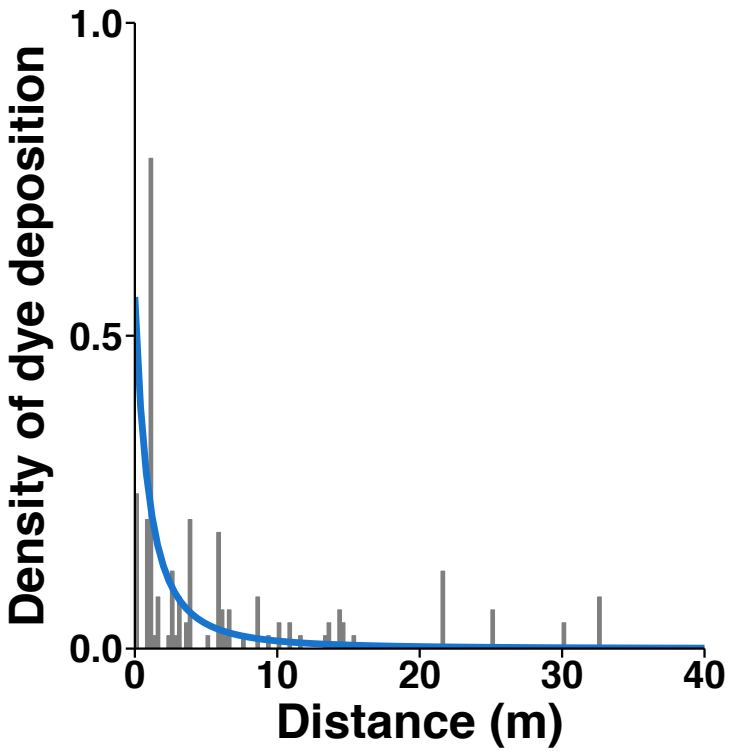


Figure S1.6. Lomax distribution fit to observed fluorescent dye (pollen analogue) movement by insect pollinators from *V. edulis* males to neighboring females. Pollen analogue deposition decreased sharply with distance, with >95% deposited within 11 m.

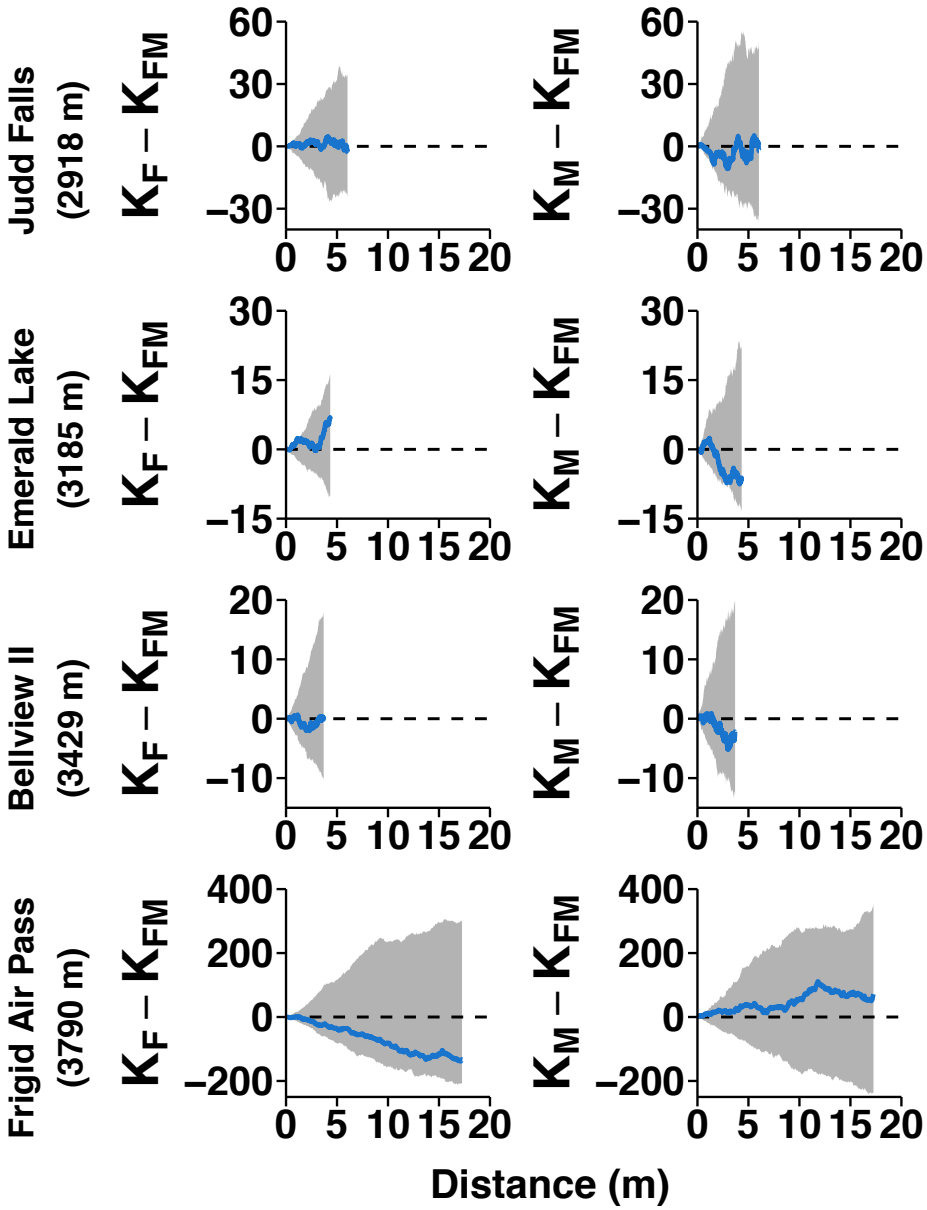


Figure S1.7. Male and female individuals in four populations of *V. edulis* are randomly intermixed in space, showing no evidence of sexual aggregation or segregation across a range of spatial scales (**A–H**). The left column shows tests for females, and the right column shows tests for males. The solid blue line shows the value of the test statistic ($K_X - K_{FM}$, where x is either M for males or F for females); positive values indicate aggregation, and negative values indicate overdispersion. Significant departures from the null hypothesis (0; dashed line) occur when the black line exits the 95% confidence interval around 0 based on 1,000 random permutations (grey

shaded region). Differences in the maximum spatial scale of inference are limited by data and spatial constraints of the population.

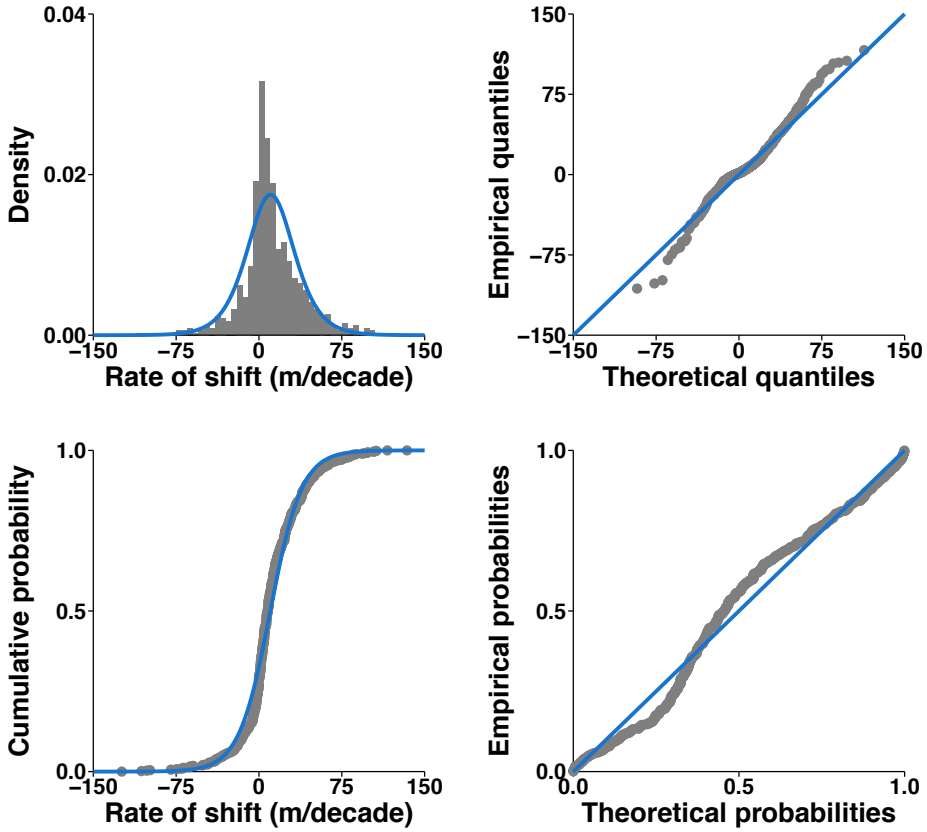


Figure S1.8. Diagnostic plots for the logistic distribution fit to species elevation range shifts (Fig. 1.4). Probability density function, quantile-quantile plot, cumulative density function, and probability-probability plot indicate a good fit to the data.

Supplemental Tables

Table S1.1. Data sources for climate analyses over space and time. The growing season for *V. edulis* is defined as June (rosette growth initiated) through August (seed set and rosette senescence). Comparisons over time refer to 1978-2014.

Variable	Gradient	Type	Dataset(s)	URL
Growing season mean temperature (°C)	Space	Interpolation	PRISM 1981-2010 Normals (30-arcsecond)	[1]
	Time‡	Aggregated point sources	NOAA GHCN-M v3	[2]
Growing season precipitation (mm)	Space	Interpolation	PRISM 1981-2010 Normals (30-arcsecond)	[1]
	Time‡	Aggregated point sources	NOAA GHCN-M v2 SNOTEL	[2] [3]
Snowmelt date (days since 1 Jan.)	Space†	Aggregated point sources	SNOTEL, author data loggers, and personal communications (b. barr, D. W. Inouye, J. D. Thompson)	[3,4]
	Time	Single point source	Personal communication (b. barr)	[4]
Soil moisture (vol. %)	Space†‡	Aggregated point sources	SNOTEL and RMBL weather stations	[3,5]
	Time	Model	NOAA CPC Soil Moisture v2 (0.5 degree)	[6]

†Data from 2014 only.
‡Study area extended to a 2°×2° around the RMBL because of low station density.
URLs: [1] http://www.prism.oregonstate.edu; [2] http://www.ncdc.noaa.gov/ghcnm/; [3] http://www.wcc.nrcs.usda.gov/snow/; [4] http://www.gothicwx.org; [5] http://www.wrcc.dri.edu/rmbl/; [6] http://www.esrl.noaa.gov/psd/

Table S1.2. Summary of linear regressions on climate variable changes over space (elevation in study area) and time (1978-2014). The pace of climate change is the rate of elevational shift in climate isoclines over time (see Supplemental Methods), and standard errors are included in parentheses when appropriate. Data sources for each analysis are listed in Table S1.1.

	Space (100 m^{-1})			Time (decade^{-1})			Pace of climate change (m/decade)
Climate variable	**Slope**	**R^2**	***P***	**Slope**	**R^2**	***P***	
Growing season mean temperature (°C)	−0.590 (±0.001)	0.94	**<0.001**	0.215 (±0.059)	0.09*	**<0.001**	36 (±8)
Growing season precipitation (mm)	1.478 (±0.009)	0.67	**<0.001**	−1.963 (±0.489)	0.02*	**<0.001**	133 (±26)
Snowmelt date (days since 1 January)	4.098 (±0.516)	0.77	**<0.001**	−2.928 (±1.849)	0.06	*0.12*	72 (±40)
Growing season mean soil moisture (vol%)	1.088 (±0.708)	0.11	*0.141*	−1.511 (±0.715)	0.11	**0.041**	195 (±523)

*Partial R^2 for time when controlling for weather station identity.

Table S1.3. Size class bounds for the four historic populations for which demographic data are available (1978-1980). Plant size was measured as the rosette diameter.

Population	Elevation (m)	Adult size 1 (cm)	Adult size 2 (cm)	Adult size 3 (cm)	Adult size 4 (cm)
Brush Creek Pasture	2735	1 – 4	5 – 7	8 – 21	———
Brush Creek Range	2780	1 – 4	5 – 8	9 – 14	15 – 28
North Rustler's Gulch	2989	1 – 6	7 – 10	11 – 22	23 – 38
Bellview I	3440	1 – 6	7 – 9	10 – 14	15 – 36

Table S1.4. Inventory of climate stations used to gather point measurements. Authoritative metadata for each station are available from the data host listed in Table S1.1. Abbreviations: GHCN = Global Historic Climate Network, SNOTEL = Snow Telemetry, USCRN = United States Climate Reference Network, RMBL = Rocky Mountain Biological Laboratory, T = Temperature, P = Precipitation, Sn = Snowmelt date, So = Soil moisture.

Network(s)	Station name	Station ID	T	P	Sn	So
GHCN	Collbran	42572476007 (v.2) 42500051741 (v.3)	■	■		
GHCN	Dillon 1E	42572469001 (v.2) 42500052281 (v.3)	■	■		
GHCN	Gunnison 3SW	42572476002 (v.2) 42500053662 (v.3)	■	■		
GHCN	Montrose #2	42572476001 (v.2) 42500055722 (v.3)	■	■		
GHCN	Saguache	42572462008 (v.2) 42500057337 (v.3)	■	■		
GHCN	Crested Butte	42572476004 (v.3)	■			
GHCN	Eagle	42574421001 (v.3)	■			
GHCN	Telluride	42574521006 (v.2)		■		
SNOTEL	Burro Mountain	378		■	■	■
SNOTEL	Middle Fork Camp	1014		■	■	*
SNOTEL	Summit Ranch	802		■	■	■
SNOTEL	Copper Mountain	415		■	■	■
SNOTEL	Fremont Pass	485		■	■	■
SNOTEL	Hoosier Pass	531		■	■	■
SNOTEL	Buckskin Joe	938		■	■	■
SNOTEL	Rough and Tumble	939		■	■	■
SNOTEL	Brumley	369		■	■	■
SNOTEL	Park Cone	680		■	■	■
SNOTEL	Upper Taylor	1141		■	■	■
SNOTEL	Butte	380		■	■	■
SNOTEL	Schofield Pass	737		■	■	■
SNOTEL	Park Reservoir	682		■	■	■
SNOTEL	Porphyry Creek	701		■	■	■
SNOTEL	Cochetopa Pass	1059		■	■	■
SNOTEL	Slumgullion	762		■	■	■
RMBL	Almont	——				■
RMBL	Judd Falls	——				■
RMBL	Kettle Ponds	——				■
RMBL	Mexican Cut	——				■
RMBL	Snodgrass	——				■

* Station excluded because of apparent failure of soil moisture sensors at the 20-25 cm depth during the focal time period (i.e. consistently registering 0 despite positive values at other measured soil depths).

Table S1.5. Parameter estimates from generalized least squares regression testing for an effect of a pollen availability index on female seed production after logit transformation and controlling for competition with neighboring female flowers for pollen (see Supplemental Methods). Parameters were fit by restricted maximum likelihood (REML) with an exponential spatial correlation structure (range = 15.3m), and all estimates are presented after back-transformation. Both lower and upper standard error estimates (LSE, USE) are reported when they are not approximately symmetric.

Model term	Estimate ± SE	t	P
Intercept	0.722 ± (0.080, 0.068)	2.570	0.0144
Pollen availability index	$0.500 \pm 2.19 \times 10^{-4}$	3.446	0.0015
Female flower density	$-0.501 \pm 3.87 \times 10^{-6}$	–3.325	0.0020

CHAPTER 2

Climate-skewed sex ratios: Demographic causes and consequences

ABSTRACT

Climate change and other forms of environmental variation are well known to affect population performance, but the mechanisms by which individual responses scale up to produce such effects remain largely unstudied. Exaggerated life history differences are common between the sexes, potentially shaping their individual responses to the environment and affecting population dynamics when sex-specific responses drive one sex to low frequency. Here, we measured life history differences between the sexes in a long-lived dioecious plant across its 2000m elevational range. Population sex ratios respond to changing temperature and aridity, yielding strongly female-biased populations at high elevation populations and rapid increases in male frequency in response to recent climate change. We used demographic modeling to determine the mechanisms by which climate affects population sex ratio across the elevation gradient, and to evaluate the consequences of climate-skewed sex ratios for population dynamics. The sexes differed in their rates of growth, survival, and flowering, and these life history differences interacted with elevation. Although many mechanisms contributed to the decline in male frequency with increasing elevation, this pattern was primarily driven by (i) increased male mortality, (ii) slowed production of new cohorts of balanced-sex seedlings, and (iii) reduced male flowering frequency. The addition of sex ratio-mediated mate limitation to our population projections reduced the rate of population growth by an average of 1.4%, far less than the observed variation among populations. In summary, climate variation – and presumably including climate change – can alter the composition of populations through multiple, complementary demographic pathways. Although the effects of biased sex ratios on population

dynamics observed here were relatively small in the context of the full life cycle, the ubiquity of ecological differences between the sexes and their reproductive co-dependence suggest that this dynamic may be important in other systems.

INTRODUCTION

A mechanistic understanding of how species respond to climate – including climate change – is a fundamental goal of ecology. Most approaches to this challenge have described the effect of climate on individual-level processes or, alternatively, on population- and species-level patterns (e.g., Parmesan 2006, Chen et al. 2011, Elmendorf et al. 2012, CaraDonna et al. 2014). Translating individual-level responses to large-scale patterns would provide a predictive framework for addressing the impact of climate changes, but the population processes that aggregate individual-level responses are rarely studied under multiple climatic conditions (Compagnoni and Adler 2014, Merow et al. 2014, Salguero-Gómez et al. 2015, 2016). Comparative demographic approaches provide this mechanistic bridge by placing the responses of heterogeneous individuals to their environment in the context of the full life cycle, enabling projections of their effects on large-scale patterns (Birch 1953, Caswell 1989, 2001, Merow et al. 2014).

Males and females often show strong niche and life history differences, and thus they are likely to respond differently to environmental variation and global change (Shine 1989, Geber et al. 1999, Chapter 2). At the individual-level, climatic stressors often provoke different physiological responses between the sexes. For example, female plants are often less tolerant of drought than are males (Dawson and Geber 1999, Xu et al. 2008a, 2008b), and ectothermic animals may have sex-specific thermal optima (Lailvaux 2007). Sufficiently strong differences

between the sexes may bias sex ratios as suggested by correlations between environmental variables and sex ratios among microsites within populations (Waser 1984, Bierzychudek and Eckhart 1988, Ruckstuhl and Neuhaus 2006) and across broad climatic gradients (Grant and Mitton 1979, Alatalo and Molau 1995, Gauquelin et al. 2002, Pickering and Hill 2002, Pickering et al. 2003, Wearmouth and Sims 2008, Myers 2011). However, the few demographic decompositions of sex ratio variation have considered few populations (3) occurring in climatically-similar locales with small ranges of OSR (Veran and Beissinger 2009, Shelton 2010b). Most animal species and many ecologically dominant plants have separate sexes (Ghiselin 1969, Renner 2014), yet the consequences of sex-specific responses to the environment and global change is largely unknown.

Unbalanced sex ratios make it difficult for individuals of the common sex to find mates (Caswell and Weeks 1986). The number of offspring produced thus depends not only on the number of reproductive individuals, but also on the frequency of each sex – the operational sex ratio (OSR). Theory and a small number of case studies show that reproductive failure when OSR is biased can have strong negative impacts on population growth and slow the expansion of species ranges (Caswell and Weeks 1986, Calabrese et al. 2008, Miller et al. 2011). The effect of biased OSR on population growth depends on both the sensitivity of individual reproductive output to changes in OSR (termed the "mating function" or "marriage squeeze;" Caswell and Weeks 1986, Miller and Inouye 2011) and the sensitivity of population growth rate to changes in individual reproductive output. Although OSR has been shown to affect reproductive rates in many taxa (Shelton 2008, e.g. García-Camacho et al. 2009, Buckel et al. 2012, Chapter 2), less is known about the consequences of frequency-dependent reproduction for population dynamics (Miller and Inouye 2011).

Our previous work has shown that the OSR of a long-lived dioecious plant, *Valeriana edulis*, responds to climate over a steep elevation gradient and as a result of recent anthropogenic climate change (Chapter 2). At the individual-level, the sexes differ in water use efficiency: males expend less water than females while assimilating carbon under hot, arid climates, but females become more efficient than males as the climate cools and becomes more mesic. At the population level, these physiological differences between the sexes correlate with OSR where male frequency decreases to *ca.* 20% at cool, mesic high elevations. The rarity of males in these populations limits the amount of pollen available to females, reducing the average seed production by 19%. How climate affects the demographic performance of each sex to produce biased sex ratios and their consequences for population dynamics are unknown, and building the mechanistic links between individual-level process and population-level pattern would provide a powerful tool for predicting responses to future climate change.

Here we use a demographic approach to determine the mechanisms by which climate biases OSR and to measure the population-level consequences of OSR-mediated mate limitation. We constructed a sex- and size-structured demographic model and parameterized it with data from seven populations varying in both OSR and climate across the species elevation range. Compared model projections among these populations enabled us to address the following questions:

(1) Through which mechanisms (i.e., demographic vital rates) does climate differentially affect the life histories of the sexes and thus and equilibrium sex ratio (OSR_{eq})?

(2) What is the overall effect of climate on population growth rate (λ)?

(3) What proportion of the overall effect of climate on λ is driven by climate-skewed OSR?

By addressing these questions, our study provides a mechanistic understanding for the effects of climate on population dynamics and illuminates the effect of mate limitation that arises from sex-specific responses to the environment.

MATERIALS & METHODS

Study system

Valeriana edulis Nutt. ex Torr. & A. Gray (Caprifoliaceae) is a long-lived dioecious herb native to the montane west of North America. Individual plants consist of a basal rosette arising from a large taproot. Upon reaching reproductive size, *V. edulis* produces one-to-many flowering stems in most years, each stem bearing several dozen to several thousand flowers. Sex expression remains constant throughout life with the rare exception of males that produce a small proportion of viable female flowers ("male inconstancy;" Ehlers and Bataillon 2007). Small solitary bees and flies pollinate *V. edulis*. Most pollen movement is restricted to short distances such that females rarely receive pollen from males greater than 10m away, and there is no spatial segregation of the sexes (Chapter 2). The proportion of female flowers that set seed within this pollination neighborhood is significantly affected by the availability of males. Reproduction only occurs through seed.

In western Colorado, USA – our study region – *V. edulis* grows in discrete populations across a *ca.* 2000m elevation gradient over which climate becomes increasingly arid with decreasing elevation (Chapter 2). Population operational sex ratio is balanced (OSR = 0.500) at the low-elevation end of the species range but becomes increasingly biased towards females at high elevation (OSR = 0.228). Female-biased OSR at the upper species range limit induces pollen limitation, reducing population-level seed production by 19%. Recent climate change in

our study region, increasing temperatures and reducing plant available water, has rapidly increased male frequency over time. This parallel response of OSR to aridity suggests that spatial climate variation is a good surrogate for climate change.

Model structure

We built a sex- and size-structured integral projection model (IPM) to explicitly account for vital rate differences among individuals and the frequency-dependent availability of mates. We parameterized the model with field data to estimate the geometric rate of population growth (λ) and equilibrium OSR (hereafter OSR_{eq} to differentiate model projections from observed variation in OSR) across seven populations spanning the elevation range of *V. edulis*. The model predicts the change in the abundances and size distributions of females, *F(y)*, and males, *M(y)*, for each year, *t*. By tracking the abundance of each sex across the size distribution, we were able to project the number of flowering individuals of each sex and calculate the population OSR. With two sexes, four classes of transition rates are possible, two within a sex (F→F and M→M) and two between the sexes (F→M and M→F). We calculated an IPM kernel that described all possible transition rates for each class. We then grouped the kernels into a 2×2 "megamatrix" structure that allows all transitions across the whole population to be considered at once as:

$$\begin{pmatrix} F(y)_{t+1} \\ M(y)_{t+1} \end{pmatrix} = \begin{pmatrix} \int_{\Omega}[p^F(y,x) + (1-\mathfrak{r})f^F(y,x)]\,dx & 0 \\ \int_{\Omega}[\mathfrak{r} f^F(y,x)]\,dx & \int_{\Omega}[p^M(y,x)]\,dx \end{pmatrix} \begin{pmatrix} F(y)_t \\ M(y)_t \end{pmatrix} \quad \text{(Eq. 2.1)}$$

Sex-specific superscripts allow the sexes to have different demographic rates and Ω sets the bounds on plant size. Size-dependent growth and survival are represented in Eq. 2.1 by:

$$p(y,x) = s(x)g(y,x) \quad \text{(Eq. 2.2)}$$

Here *s(x)* is the probability that an individual of size *x* survives the one year period, and *g(y,x)* is the probability of growth from size *x* to size *y* over the interval. Variance associated with the

growth function distributes probability density around the mean predicted size. Reproduction, *f(y,x)*, represents the probability of an *x*-sized maternal plant producing *y*-sized seedlings. This reproduction term decomposes into:

$$f(y,x) = f_n(x)p_E d(y) \quad \text{(Eq. 2.3a)}$$

Here $f_n(x)$ is the number of seeds a maternal plant of size *x* produces, p_E is the probability of seedling establishment, and *d(y)* describes the distribution of seedling sizes. We assigned reproduction to females because they provide all the resources to developing seeds. However, females depend on pollen from males to produce seed. When males are rare (i.e., OSR is low), pollen limitation allows only a portion of potential seeds to be realized. To account for this, we added a mating function, $\mathfrak{B}_{OSR}$, to Eq. 2.3a that describes the functional relationship between OSR and the proportion of potential seeds that are produced. Thus the reproduction function, *f(y,x)*, becomes:

$$f(y,x) = \mathfrak{B}_{OSR} f_n(x)p_E d(y) \quad \text{(Eq. 2.3b)}$$

Each maternal plant produces offspring of both sexes, sons with probability, $\mathfrak{r}$, and daughters with probability, $1 - \mathfrak{r}$. This is the primary sex ratio (PSR), and the total seed production is allocated accordingly by multiplication.

Together the four IPM kernels that compose the megamatrix describe all possible transitions in a single year. The kernels along the main diagonal represent transitions within each sex, and the kernels in the off-diagonal represent transitions between the sexes. Males do not produce seeds, thus the reproduction term (Eq. 2.3b) is absent from the kernels in the second column of the megamatrix. Similarly, the model does not allow individual plants to change sex, and the corresponding kernels are replaced with zero with the exception of a reproduction-only kernel that allows females to produce male seedlings. We discretized the megamatrix of IPM

kernels into a single approximating matrix from which we calculated asymptotic dynamics (Easterling et al. 2000, Ellner and Rees 2006).

Model parameterization and tests for sex differences

We parameterized the IPM model with field data collected from 2013-2015 at seven sites across the *V. edulis* elevation range that span 1750m elevation and male frequencies of 22.8-50.0%. At each population, we tagged ca. 250 individual plants stratified by sex and across the size distribution. We measured plant size as the perimeter around the base of the rosette where the leaves emerge through the soil surface, recorded to the nearest 0.5cm. We assessed seed production by counting all seeds in subset of the inflorescence, and then we visually estimating the number of similarly sized subsets across all flowering stems. This method reliably predicted seed production ($R^2 > 0.8$ for all observers, data not shown). The length of our survey was insufficient to parameterize a stochastic model (Fieberg and Ellner 2001); we pooled all data points at each population into a single one-year census interval.

We estimated vital rates – growth, survival, and reproduction – as a function of plant sex (female or male) and the natural logarithm of plant size (range: 0.5-167.0 cm) using generalized linear models (GLMs) with weak Cauchy priors (implemented in the R package ARM v.1.8-6; Gelman et al. 2008). For each vital rate function at each population, we allowed plant sex to affect the slope and intercept of the function (Table 2.1 provides the biological interpretation of each vital rate function parameter). We could only determine plant sex during flowering, and plant sex was not observed for the 24% of tagged individuals that did not flower during any of our census years. Ignoring such missing data biases regression parameter estimates and inflates parameter uncertainty (Rubin 1976, Nakagawa and Freckleton 2011). We used multiple

Table 2.1. Description of model parameters and their biological meaning.

Demographic function	Function parameters	Fit separately by Sex	Fit separately by Population	Description and biological interpretation
Recruitment	Establishment probability		●	Probability that a seed successfully overwinters, germinates in the spring, and survives to the summer census. Estimated from seed addition plots.
	Seedling size mean			Mean perimeter of seedlings.
	Seedling size variance			Variance of the perimeter of seedlings.
Growth	Intercept*	●	●	Annual growth rate of plants with perimeter = 1cm. Describes the growth rate of small plants.
	Slope	●	●	Change in the annual growth rate of plants as plant size increases. A growth function slope <1 ensures that the largest plants shrink, preventing them from growing to infinite size.
	Slope standard error	●	●	Variation in the annual growth rate of plants that is not explained by plant size or sex. Accounts for occasional shrinkage of small plants and positive growth of large plants.
Survival	Intercept*	●	●	Log-odds that a plant with perimeter = 1cm will survive to the next year. Describes the survival rate of small plants.
	Slope	●	●	Change in the annual odds of survival as plant size increases. More positive survival function slopes indicate a smaller size threshold at which most plants assimilate enough resources to survive until the next year.
Flowering	Intercept*	●	●	Log-odds that a plant with perimeter = 1cm will flower in the current year. Describes the flowering rate of small plants.
	Slope	●	●	Change in the annual odds of flowering as plant size increases. More positive survival function slopes indicate a smaller size threshold at which most plants assimilate enough resources to survive until the next year.
Seed production	Intercept* (count component)		●	Number of seeds produced by a female plant with perimeter = 1cm* per reproductive bout. Describes the seed output of small reproductive females.
	Slope (count component)		●	Exponential rate of change in the number of seeds produced per reproductive bout as female plant size increases. A positive slope for the count component of the seed production function indicates that larger females produce more seed than smaller individuals
	Intercept* (zero-inflating component)		●	Log-odds that a female plant with perimeter = 1cm will produce at least one seed in the current year. Describes the rate of reproductive failure for small females.
	Slope (zero-inflating component)		●	Change in the annual odds of reproductive failure as female plant size increases. More positive survival function slopes indicate a smaller size threshold at which most plants begin reproducing.

Note: * The intercept is not at perimeter = 0cm because size was natural-logarithm transformed prior to fitting each vital rate function.

imputation to correct for this bias and uncertainty, allowing robust estimates of each vital rate. The probability that a plant's sex was not observed depended on plant size because small individuals have low probabilities of flowering (Fig. S2.1). The missing data were thus "missing at random" (sensu Rubin 1976). We generated 100 replicate imputations of plant sex (F or M) for these missing cases using the MICE package in R (v. 2-25; van Buuren and Groothuis-Oudshoorn 2011, R Core Team 2015). We then fit each vital rate GLM to each imputed dataset and averaged the resulting coefficients.

For growth, $g(y,x)$, we regressed natural log-transformed size in year $t+1$ on size in year t using linear regression with normally-distributed residuals. Survival from year t to $t+1$, s(x), was modeled using a binomial GLM. We estimated the reproduction function, $f_n(x)$, by fitting natural log-transformed size in year t to seed production, also in year t. We used a zero-inflated negative binomial GLM with $log(size_t)$ as the predictor for both the count and zero-inflating model components because non-flowering individuals produced no seeds and caused model overdispersion (Zeileis et al. 2008, Jackson 2015). We calculated recruitment rates, p_E, from seed addition plots established each year in each population by dispersing a total of 250 seeds from 25 maternal plants in a 0.5×0.5m plot. Recruitment was low, thus estimated the distribution of seedling sizes from data pooled across all populations ($\bar{x}$ = 1.03cm, s^2 = 0.02cm). We assumed that primary sex ratio was balanced ($\mathfrak{r}$ = 0.5) and recruitment rates were independent of sex because we were unable to determine the sex of seeds or seedlings. We have previously estimated the mating function, $\mathfrak{B}_{OSR}$, at Emerald Lake (Chapter 2); we assumed that this relationship held at all populations.

Each IPM kernel in the megamatrix was discretized into a 200×200 matrix for analysis. We used 90% of the smallest observed seedling size and 110% of the maximum observed plant

size observed at each population as integration limits. These size buffers prevented "eviction" from the model by limiting the artificial inflation of mortality rates when individuals grow or shrink outside of the integration limits (Williams et al. 2012). Because the reproduction function, *f(y,x)*, depends on OSR in year *t*, the model is non-linear and its asymptotic dynamics can only be determined through numerical simulation. We recalculated *f(y,x)*, rebuilt the IPM kernels in the megamatrix, and re-discretized the model at each of 500 time steps to obtain stable estimates of λ and OSR_{eq}.

Determining the demographic origin of biased OSR

We compared parameterizations of the IPM model across the elevation gradient using a life table response experiment (LTRE; Caswell 2001). This retrospective approach uses vital rate sensitivities to quantify the relative contributions of each demographic rate to the observed differences in demographic statistics. Although most commonly used to identify the drivers of differences in λ between populations, LTRE analyses are equally applicable to other demographic statistics such as OSR_{eq} (Caswell 2001). We partitioned the change in OSR_{eq} over elevation as the sum of contributions from each vital rate as:

$$\frac{d\mathrm{OSR}_{eq}}{dx} = \sum_i \frac{\partial \mathrm{OSR}_{eq}}{\partial \alpha_i} \frac{\partial \alpha_i}{\partial x} \qquad \text{(Eq. 2.4)}$$

Here each vital rate contribution to the overall change in OSR_{eq} is calculated as the sensitivity of OSR_{eq} to perturbations of the vital rate (α_i) multiplied by the change in the vital rate with elevation (x). These terms are analogous to a slope and displacement of the independent variable in a regression equation, respectively. This decomposition reveals which vital rates of each sex change with elevation, but also shows which of these vital rate changes are sufficiently strong to affect OSR_{eq}.

Effect of climate and biased OSR on population dynamics

We subjected our model to perturbation analyses to determine the sensitivity of population growth rate, λ, to reproduction (Caswell 2001). The magnitude of this sensitivity paired with our previous estimate of the effect of OSR on seed production (Chapter 2) provides an upper bound to strength of OSR influence on λ. We produced a variant of the IPM model by setting seed production per female to a constant value regardless of OSR (i.e. a female-dominant mating function Caswell and Weeks 1986, Miller and Inouye 2011). Comparing the projections of λ from each population under this alternate model with projections from the main IPM reveals the extent to which λ is suppressed by OSR-driven pollen limitation. We then used a second LTRE analysis where λ was the demographic statistic responding to variation in climate PC axis score to calculate the total effect of climate on λ.

RESULTS

Climate had widespread effects on the vital rates of both sexes, affecting some rates equally between the sexes whereas others produced strong differences between the sexes (Fig. S2.2). Because our questions center on the mechanisms that drive biases in OSR, we focus on these vital rate changes and their consequences below. We provide a full treatment of climate and sex effects on vital rates in the Supplemental Materials.

Demographic mechanisms of the change in operational sex ratio over elevation

Demographic differences among populations and between the sexes (Fig. S2.2) resulted in 36% variation in equilibrium operational sex ratio (OSR_{eq}: 23.7-59.9% male), with OSR_{eq}

declining with increasing elevation (Fig. 2.1). This pattern mirrored the decrease in male frequency with increasing elevation that we have observed in the focal populations and across 24 other natural populations (Chapter 2). However, most populations were not in equilibrium as indicated by higher male frequencies in OSR_{eq} than we observed in these populations.

Our LTRE analysis partitioned the total change in OSR_{eq} with elevation into contributions from each vital rate (Fig. 2.2). Negative values indicated that the change in the vital rate with increasing elevation served to decrease equilibrium male frequency, whereas positive contributions worked to increase equilibrium male frequency. Most contributions from both sexes were negative, overwhelming the few mechanisms that opposed the decrease in OSR_{eq} with increasing elevation. Vital rate parameters differed in the magnitude of their contributions, and the sexes differed in which parameter(s) most strongly influenced the overall pattern in OSR_{eq}. The three strongest contributions were from the male survival function, the female seed production function, and the male flowering function. First, small males at high elevation were subject to increasing mortality with increasing elevation (Fig. S2.2), thereby driving down OSR_{eq} (Fig. S2.3). Second, the parameters of the seed production function each contributed to female-biased OSR_{eq} at high elevation. This effect was primarily driven by the lowest elevation site where small plants were less likely to suffer reproductive failure and produced similar amounts of seed as larger plants (Fig. S2.2). Together these changes pushed reproduction to smaller sizes, increasing the frequency that new cohorts of individuals (with balanced sex ratios) were produced. Finally, males at high elevation delayed flowering until they had achieved larger sizes as indicated by the intercept parameter (Figs. S2.2, S2.3). Although males were present at high elevation, OSR_{eq} only counts flowering individuals.

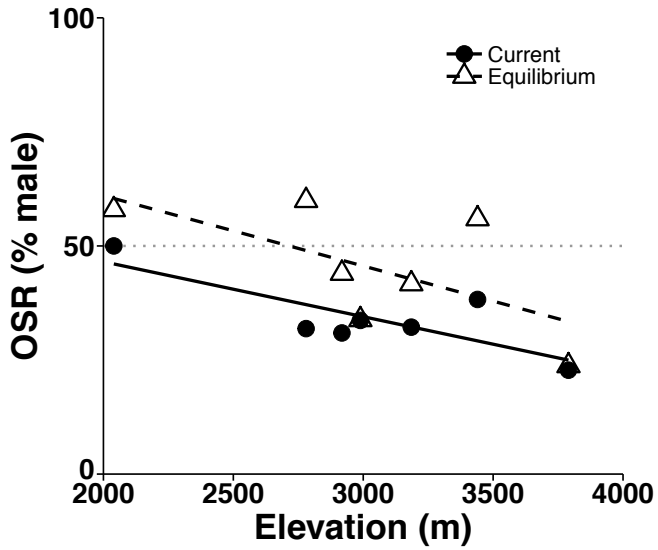


Figure 2.1. Current and projected equilibrium operational sex ratios (OSR) show corresponding declines across the elevation gradient. Most populations are not in equilibrium, and male frequencies are projected to increase over time. Both patterns are consistent with our previous report of OSR responses to climate variation over space and climate change over time (Chapter 2).

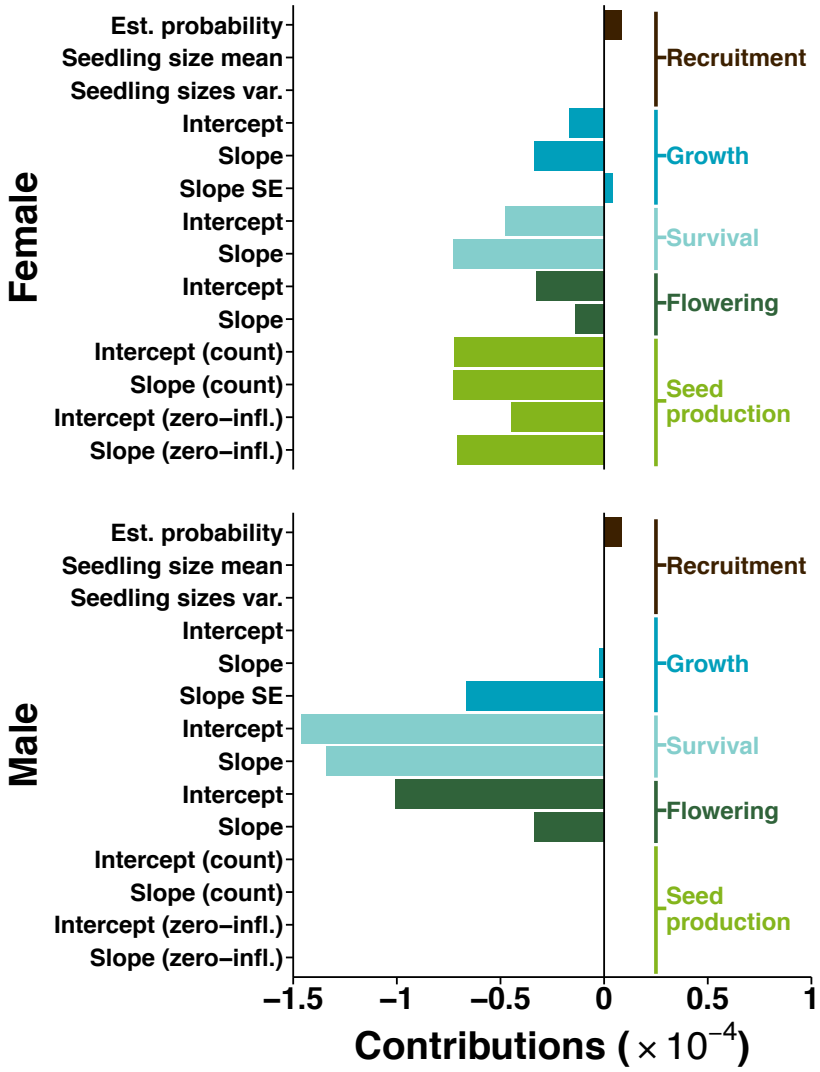


Figure 2.2. Vital rate changes with elevation and their effect on equilibrium operational sex ratio (OSR_{eq}) explain the negative slope between OSR_{eq} and elevation. A LTRE analysis revealed that the contributions (x-axis) of each vital rate parameter (y-axis) to the slope between OSR_{eq} and elevation differed by sex and life history process (indicated by color and label). Parameters with zero contribution either did not vary clinally (e.g., seedling size mean and variance), varied clinally but had negligible effect on OSR_{eq} (e.g., male growth intercept), or were fixed at zero to reflect the life history of *V. edulis* (e.g. males do not produce seed). Figure abbreviations: est. =

establishment, var. = variance, SE = standard error, zero-infl. = zero-inflation. See Table 2.1 for a biological interpretation of each parameter.

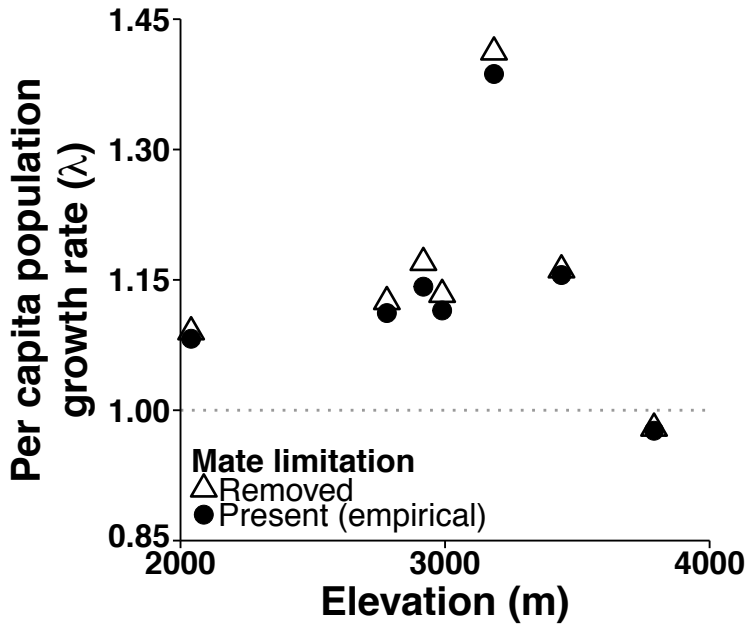


Figure 2.3. Effect of skewed OSR on population growth rate ($\lambda > 1$ indicates positive population growth). Mate limitation due to biased OSR was either removed from the model (open triangles) or present in the model based on empirical values (filled circles).

Effect of climate on population growth rate and contribution of climate-biased OSR

Although populations varied 1.4–fold in equilibrium per capita population growth rate (λ: 0.977-1.387), there was no overall cline in population growth rate across the elevation range of *V. edulis*. We altered the observed mating function to eliminate pollen limitation brought about by skewed OSR, allowing females to produce convert 100% of their flowers into seed. Comparing model projections of λ with and without pollen limitation of female fertility revealed that climate-skewed OSR reduced λ in all populations by an average of 0.014 (range: –0.028 to –0.003; Fig. 2.3).

DISCUSSION

Integrating across sex-specific responses to climatic variation revealed the mechanisms by which climate skews OSR and the population-level consequences of mate limitation. Our results provide evidence that population sex ratios are lagging behind the pace of climate change. Model projections based on contemporary vital rates suggest that males will continue to increase in frequency over time as they have over the past several decades (Chapter 2). The variation in demographic vital rates that we measured across the elevation range of *V. edulis* showed that climate decreases male frequency through multiple life history mechanisms. The three most important mechanisms underlying this pattern reduced male survival, slowed the rate that new cohorts of individuals with balanced sex ratios recruited into the population, and increased the size threshold for male flowering as elevation increased. Although these mechanisms increased mate limitation, the effect of climate-skewed OSR on population growth was weak and likely would have been stronger in a short-lived species where recruitment has a larger impact on population size. Overall, our results disentangle the complex mechanisms by which climate

impacts population structure and dynamics, showing the value of a demographic approach that integrates across the organism's life cycle.

Although the response of *V. edulis* OSR to climate change has been exceptionally rapid over the past several decades (Chapter 2), the current vital rate differences between the sexes should lead to greater frequencies of males than we observed in most populations (Fig. 2.1). This lag suggests that OSR changes are slower than the pace of climate change; if climate change were to stop, we would expect to see continued change in OSR until the population reached OSR_{eq}. A key assumption of analyzing demographic models at equilibrium is that the environment does not change (Caswell 2001). Indeed environmental changes are the primary reason that demographic models fail to predict future population changes in nature (Crone et al. 2013). However, parameterizing demographic models across an environmental gradient as we have done here allows for a space-for-time substitution that may yield more robust predictions of population responses to climate change (Elmendorf et al. 2015).

Through demographic analysis, we have revealed the mechanistic basis for the remarkable cline in sex ratio observed in this species. Multiple mechanisms contributed to increasing female frequency at high elevation with few, weak countervailing mechanisms favoring higher male frequency. The three dominant mechanisms affected a wide range of life history processes. First, reduced male survival at high elevation removed males from high elevation populations across the life cycle rather than at a single early life history stage (Clutton-Brock 1986, Eppley 2001, Veran and Beissinger 2009). Differential survival between the sexes has been reported in the few other demographic decompositions of OSR variation. A study of green-rumped parrotlets (*Forpus passerinus*) showed that male-biased sex ratios arose from low survival of female juveniles (Veran and Beissinger 2009). Similarly, a small survival advantage

across the life cycle of female seagrasses (*Phyllospadix scouleri* and *P. serrulatus*) produced strongly female-biased sex ratios (Shelton 2010a, 2010b). Mechanisms that bias OSR through survival operate on the timescale of generations, thus we predict that OSR changes by these mechanisms will integrate long-term trends in climate in *V. edulis*. The second strongest contributor to female-biased OSR at high elevation was the slower generation cycling caused by females delaying reproduction until they had achieved larger sizes. We know that this mechanism was mediated through seed production because simple changes in female flowering had much smaller contributions to OSR. Because *V. edulis* is not clonal, the production of new seeds is the only mechanism by which new individuals of both sexes are produced. Our model assumed that the sex ratio of seeds (i.e., the primary sex ratio) was balanced. Elongating the time interval between germination and first reproduction allowed other demographic differences between the sexes to more strongly bias OSR before new, balanced sex cohorts were produced. Life history theory predicts that high survival and high gains in fecundity should favor individuals that delay reproduction to later ages or larger sizes (Reznick et al. 1990, Kozłowski 1992, Stearns 1992, Wesselingh et al. 1997). We see evidence for both higher survival and higher seed production per reproductive bout at high elevation (Figs. S2.1, S2.2). The third strongest mechanism delayed male flowering to larger sizes at high elevation as well, possibly operating as a result of the same forces acting to delay reproduction when survival is high. Here the effect of male reproductive delay was more direct on OSR, operating by directly changing the number of individuals that flowered and thus were counted by our method of estimating OSR.

Despite significant pollen limitation caused by female-biased OSR, the impact of reduced seed production had little effect on population growth rate. Previous studies have suggested that

a strong effect of OSR on seed production is necessary for biased OSR to impact population dynamics (Caswell and Weeks 1986, Shelton 2010a). However, ours is only the second study to our knowledge – in the first in natural populations – to empirically measure the mating function and incorporate it into a demographic model (Miller and Inouye 2011). We suspect that *V. edulis* populations are insulated from most fluctuations in seed production because it is a long-lived iteroparous species, exemplifying a life history pattern that often shows low elasticities of λ to reproduction (Franco and Silvertown 2004). For these species OSR effects on survival are much more likely to disrupt population dynamics, for example in lizards where male harassment of females reduced female survival and caused population extinction (Le Galliard et al. 2005). Our model did not account for frequency-dependent mortality that could, for example, operate through a competitive advantage of one sex over the other (Oddie 2000, García-Ramos et al. 2007). At a broader level, comparative elasticity analyses may serve as a useful guide to predict the effects of under-studied population processes. Although the effects of biased sex ratios on population dynamics were small in this system, their effects on other species may be more pronounced. The multitrophic arthropod communities associated with *V. edulis* differ strongly between the sexes where females host higher abundances of a specialist aphid herbivore, of aphid-tending ants, and of aphid predators (Petry et al. 2013). To these arthropods, the sexes represent different qualities of habitat. Although males can support these species, the decline in higher-quality females with climate changes may destabilize arthropod populations, especially for the specialist herbivore. Overall, our results highlight the importance of placing demographic processes in the context of the full life cycle and their potential impact of other species to evaluate their ecological consequences.

In conclusion, our study illustrates a diverse set of mechanisms by which climate produces dramatic skews in population sex ratios. Although these sex-specific responses to the environment did not meaningfully alter population growth, shorter-lived species may be more susceptible to population decline when sex ratios are biased. Our work showcases the value of integrating demographic processes across the life cycle to determine the origin and consequences of population change.

SUPPLEMENTAL MATERIALS

Effects of plant sex and climate on individual-level demographic rates

We found variation in vital rates both between the sexes and among populations of *V. edulis* (Fig. S2.1). Below we describe these patterns and their biological significance (Table 2.1; Fig. S2.2).

Establishment of recruits

Because seedling sex was not known, we did not test for variation between the sexes at this life stage. Seedling recruitment was low in all populations, ranging from less than 1% to just over 3%, with a weak trend for increasing recruitment rates with increasing elevation. Seedling size and seedling size variance did not vary among populations because we pooled estimates of these parameters across all populations.

Growth function

Growth functions differed both among populations and between the sexes (Fig. S2.1A). As described in "Methods: Model parameterization and tests for sex differences", the growth function (a linear regression) included three parameters, the intercept, the slope, and the standard error of the slope. There was no clear trend across the elevation gradient or between the sexes for growth of small individuals, as indicated by the intercept of the growth function. Similarly, the change in growth rate with size showed no clear pattern in response to climate or sex, as indicated by the slope of the growth function. However, the variability in growth rate, reflected in the standard error of the growth slope, increased at higher elevation for males but stayed

constant for females. At high elevation, males were thus more prone to dramatic changes in size between years (i.e., growth or shrinkage) than were similarly sized females.

Survival function

Survival functions differed both among populations and between the sexes (Fig. S2.1B). As described above, the survival function (a logistic regression) included two parameters, the intercept and the slope. Small females were less likely to survive than small males at low elevation, but the reverse was true at high elevation. In contrast, survival of larger plants did not differ by sex, but was higher than small plants and increased with elevation. As a consequence, survival increased with size, but did so more strongly at low elevations where small plant survival was lowest. Increasing slopes of the survival function with increasing elevation for both sexes reflected this change.

Flowering function

Flowering functions differed both among populations and between the sexes (Fig. S2.1C). Two parameters – the intercept and the slope – controlled the shape of this function, which we fit with a logistic regression. Small females were more likely to flower than similarly sized males at low elevation, but the reverse was true at high elevation; this was indicated by decreasing and increasing intercepts with increasing elevation for female and males, respectively. The probability of flowering increased with increasing plant size. However, this change was more pronounced for females at high elevation and males at low elevation than for females at low elevation and males at high elevation, as indicated by decreasing survival function slopes with increasing elevation for females and the opposite relationship for males.

Opposing patterns for both parameters between the sexes resulted in a switch in the relative flowering frequency between the sexes across their size distribution where the direction of the switch varied with elevation. At low elevation, females began flowering at smaller sizes than males, but large males flowered more frequently than large females. At high elevations, this pattern was reversed: males were more likely to flower at small sizes than were females, but large females flowered more frequently than large males.

Seed production function

Seed production functions were highly variable among populations (Fig. S2.1D). Our parameterization of this function consisted of four parameters divided into two components that we describe separately below. Seed production was limited to females in the model, thus there was no effect of plant sex on these parameters.

The count component of the function described the number of seeds that a female produced in a reproductive bout and depended on the current size of the female. Small females at low elevation were able to produce more seed per reproductive bout than similarly sized plants at high elevation, indicated by a decreasing intercept with increasing elevation. Seed production by larger females at higher elevations quickly exceeded that of plants at low elevation as indicated by an increase in the slope with increasing elevation.

The zero-inflating component of the function described the probability that females would fail to produce seed either because they did not flower or no flowers were able to set seed (e.g. due to desiccation, herbivory, or pathogen attack). Small plants at high elevation were more likely to avoid reproductive failure compared to similarly size plants at low elevation, as

indicated by an increase in the intercept. Reproductive failure rates across the elevation gradient converged with increasing plant size, as indicated by higher slopes at low elevation.

The opposing patterns between the two function components resulted in no clear clines in individual seed production.

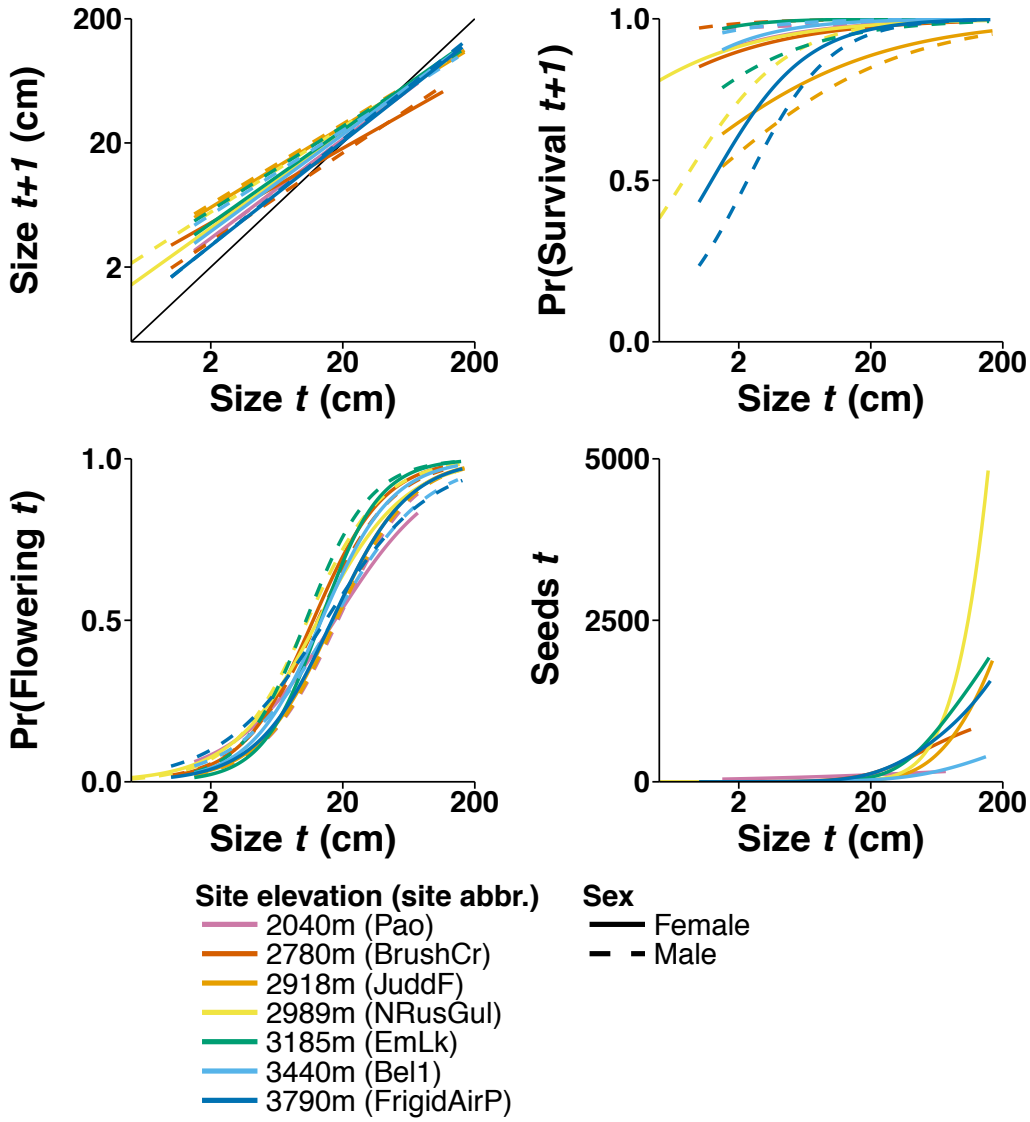


Figure S2.1. Sex- and size-specific **(A)** growth rate, **(B)** survival probability, **(C)** flowering probability, and **(D)** seed production for seven populations of *Valeriana edulis* spanning *ca.* 1800m elevation. Colors denote separate populations with warmer hues at low elevation and cooler hues at high elevation, whereas solid and dashed lines indicate females and males, respectively. Size at times *t* and *t+1* are on a logarithmic scale.

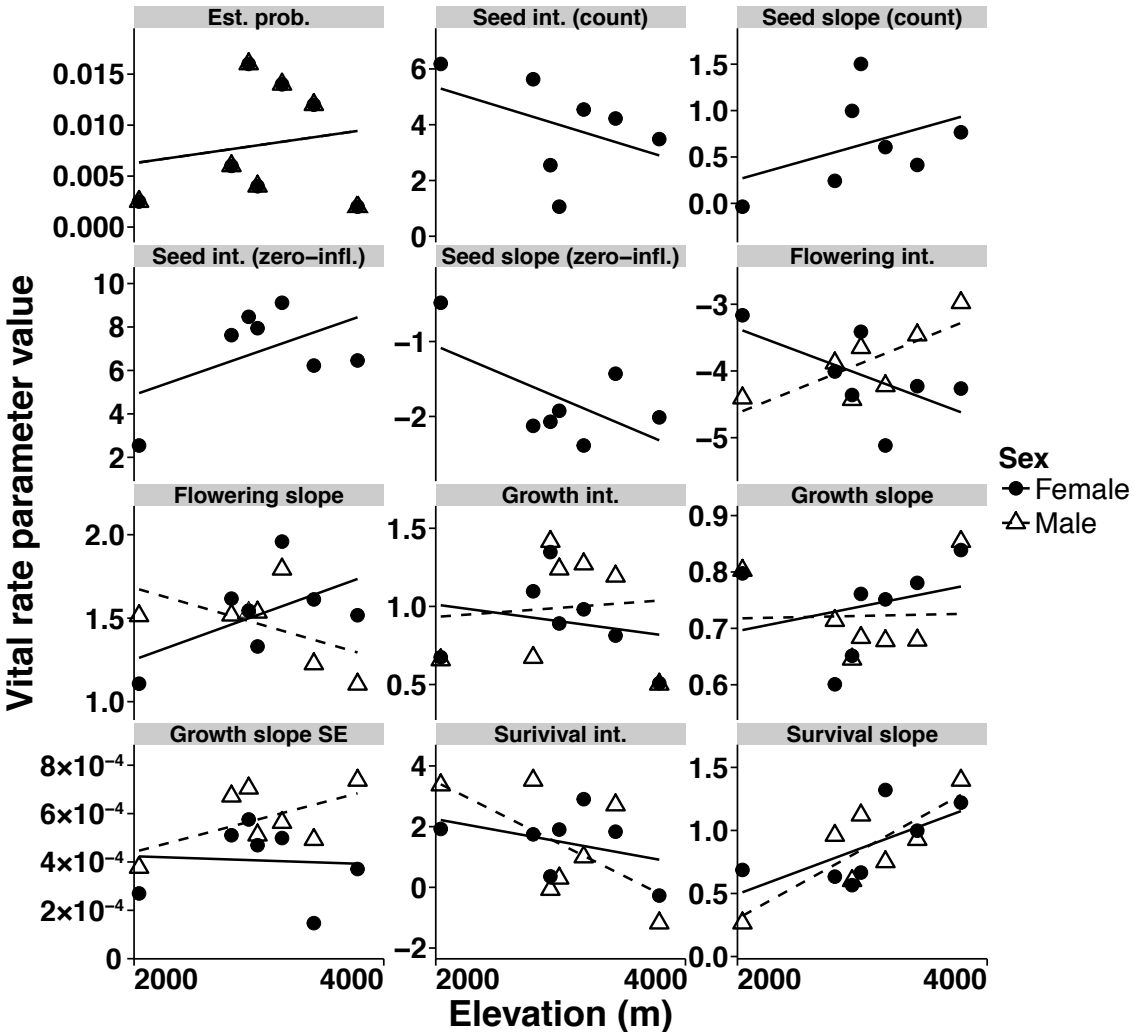


Figure S2.2. Vital rate parameter changes for both sexes across the elevation gradient. Each point represents a population where females are shown as solid circles (solid lines) and males are shown as open triangles (dashed lines). The slope of each fit corresponds to the second term in Eq. 2.4. See Fig. 2.2 for abbreviations and Table 2.1 for biological interpretation of each vital rate parameter.

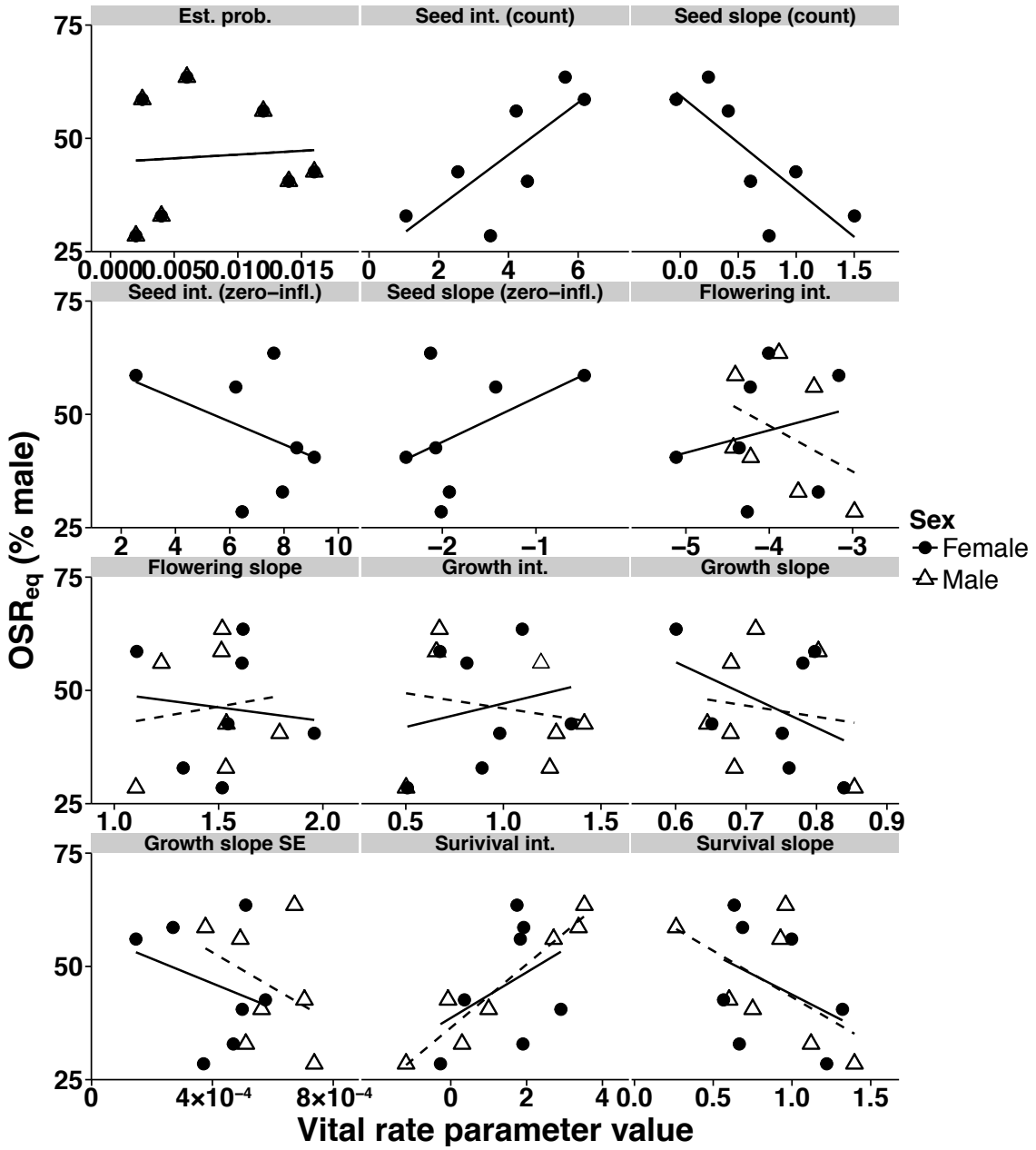


Figure S2.3. Sensitivity of OSR_{eq} to vital rates. Each point represents a population where females are shown as solid circles (solid lines) and males are shown as open triangles (dashed lines). The slope of each fit corresponds to the first term in Eq. 2.4. See Fig. 2.2 for abbreviations and Table 2.1 for biological interpretation of each vital rate parameter.

CHAPTER 3

Mechanisms underlying plant sexual dimorphism in multi-trophic arthropod communities

ABSTRACT

A growing body of research documents the importance of plant genetic effects on arthropod community structure. However, the mechanisms underlying these effects are often unclear. Additionally, plant genetic effects have largely been quantified in common gardens, thus inflating the estimates of their importance by minimizing levels of natural variation. Using *Valeriana edulis,* a dioecious plant with genetically based sex determination, we conducted surveys and experiments on wild-grown individuals to document field patterns of arthropod association between the sexes and the mechanisms underlying these plant genetic effects. Three years of surveys revealed strong and consistent sex-biased arthropod association in wild-grown plants: female plants supported 4-fold, 1.5-fold, and 4-fold higher densities of aphids, aphid predators, and aphid-tending ants, respectively, compared to males. There was mixed evidence that the female bias for aphids was due to higher plant quality, while we found no difference between plant sexes in aphid preference or the top-down effects of predators and tending ants. Female bias for ants was due to both the greater attractiveness of female plants (direct effect mediated by floral nectar) and an independent, weaker effect of higher aphid abundance on females (density-mediated indirect effect). Conversely, the female bias for predators was driven solely by the greater attractiveness of female plants. We did not find interaction modification, i.e., ant-aphid and predator-aphid interactions were equivalent between plant sexes. Plant sex explained 0.24%, 2.28%, and 4.42% of the variance in aphids, predators and ants, respectively, values comparable to but slightly weaker than those previously reported from common-garden

studies. In contrast to the prediction of diminished plant genetic effects with increasing trophic level, we show how weak indirect effects on predators and parasitoids (via herbivores) can be complemented by strong direct effects via common plant traits (floral resources). In summary, we document direct and indirect effects of genetically based sex on a multi-trophic arthropod community that were expressed in wild-grown plants across multiple years.

INTRODUCTION

There is rapidly accumulating evidence that genetic variation in one species can have far-reaching effects on the structure and dynamics of communities and ecosystems (Whitham et al. 2003, 2006, Johnson and Stinchcombe 2007). Particularly well studied are the consequences of genetic variation within plant species for the structure of associated arthropod communities (Wimp and Whitham 2001, Johnson and Agrawal 2005, Whitham et al. 2006). This body of literature shows that plant genotype identity can explain a large proportion of the variation in metrics of multi-trophic arthropod community structure, including total arthropod abundance, abundance of herbivores and predators, species richness, and the stability of community composition over time (Wimp and Whitham 2001, Johnson and Agrawal 2005, Keith et al. 2010). While making notable contributions, the studies investigating such effects have also suffered from two shortcomings. First, they are based upon experimental common gardens (with the exception of three systems: *Populus* spp. (Wimp et al. 2004, Bangert et al. 2006b, 2006a, Wimp et al. 2007, Meneses et al. 2012), a tropical tree (Zytynska et al. 2011), and a bromeliad (Zytynska et al. 2012)) that by design minimize natural variation and thus over-estimate the contribution of plant genetics to variance in arthropod community structure (Tack et al. 2012). And second, few have explored the mechanisms by which genetic variation in plant traits

propagates through arthropod food webs to produce these community-level patterns (but see Wimp and Whitham 2001, Johnson 2008, Mooney and Agrawal 2008).

Plant genetic variation may affect arthropod abundance directly and indirectly. Direct effects result from differences in traits among genotypes that affect plant-arthropod pairwise interactions. Indirect effects are downstream effects of plant traits and may be further subdivided into density-mediated indirect effects (interaction chains, sensu Wootton 1994) and trait-mediated indirect effects (interaction modification, sensu Wootton 1994, Peacor and Werner 1997). In the first case, plant traits that directly affect the density of one arthropod species in turn propagate to indirectly affect additional arthropod species. Here the parameter(s) of the functional response (linear or nonlinear) of one arthropod to another arthropod remain constant among plant genotypes. In the second case, plant traits modify the functional response parameters. Distinguishing among these non-mutually exclusive effects of plant genotype provides the mechanistic framework needed to predict the ecological and evolutionary consequences of plant genetic variation for multi-trophic arthropod communities (Strauss et al. 2005, Mooney and Singer 2012). Furthermore, this mechanistic approach allows for comparisons of the relative strengths of these community-organizing interactions and for a test of the hypothesis that plant genetic effects diminish with increasing trophic level due to the primacy of indirect effects at these levels (Johnson and Agrawal 2005, Mooney and Agrawal 2008).

One form of genetic variation in plants with important ecological and evolutionary consequences is that of plant sex in dioecious species (Ågren et al. 1999, Cornelissen and Stiling 2005). Dioecy is a sexual system that occurs in 37 of 51 plant orders and 10% of all angiosperm species (Geber et al. 1999, Cornelissen and Stiling 2005). Because plant sex is by in large genetically determined (Ming et al. 2011) and easily identified in the field, dioecy (and other

polymorphic sexual systems) allows for rapid *in situ* genotyping of wild-growing plants with respect to the sex determination loci. Despite the relatively few genes that typically underlie sex determination (Ming et al. 2011), plant sex is associated with a substantial degree of ecological dimorphism. Female plants typically invest more in reproduction than males (Lloyd and Webb 1977, Delph 1999), grow more slowly, have higher levels of defenses against herbivores, support lower herbivore densities, and receive less herbivore damage (Ågren et al. 1999, Cornelissen and Stiling 2005). In contrast to these well-documented effects of plant sex on herbivores, far less is known about effects on higher trophic levels (but see Ashman and King 2005, Mooney et al. 2012).

This study investigates the mechanisms by which genetically based plant sex influences the tripartite interactions among ants, ant-tended herbivores, and herbivore predators. These food web vignettes (sensu Polis and Strong 1996) are experimentally tractable, consisting of as few as four species (plant, herbivore, ant and predator), yet still encompass the ecological complexity of tri-trophic interactions, herbivory, predation, and mutualism. Surveys of naturally occurring male and female *Valeriana edulis* over three consecutive years demonstrated strong and consistent plant genetic effects over arthropod community structure, with females supporting aphid, predator, and ant abundances that were 4-fold, 1.5-fold, and 4-fold greater than those of males (Fig. 3.1A). These surveys were accompanied by a series of manipulative studies to determine which of the direct effects and density- and trait-mediated indirect effects of plant sex (Fig. 3.1B) underlie these field patterns. Uniquely, this study identifies the mechanisms of plant genetic control over a multi-trophic arthropod community, and juxtaposes the strength of these effects against realistic levels of natural variation by studying wild-growing plants across multiple years.

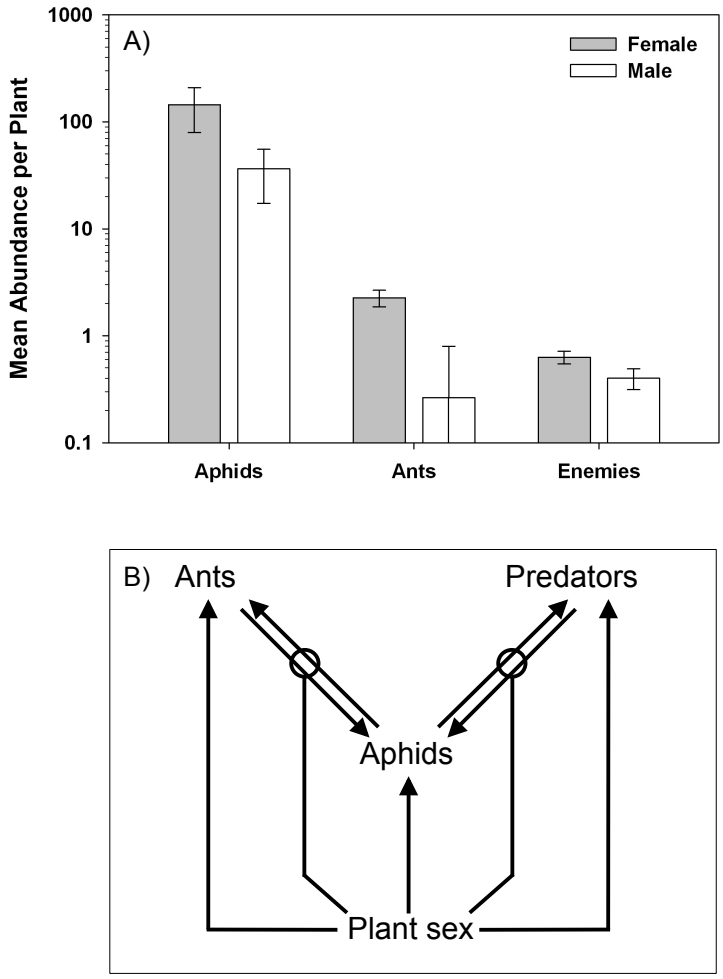


Figure 3.1. (A) Abundance (mean ± SE) of aphids, ants, and predatory arthropods on male and female *Valeriana edulis* from three years of surveys. There was significant plant sexual dimorphism in the abundance of aphids (P = 0.007) and ants (P = 0.001), and marginally significant sexual dimorphism in predators (P = 0.067). **(B)** The hypotheses for mechanisms underlying these patterns. Arrows indicate direct effects; lines with circles indicate interaction modification.

METHODS

STUDY SITES

This study was conducted in montane meadows (described by Langenheim 1962) in Gunnison County, Colorado, USA at 2918 m (38.967° N, 106.995° W) near the Judd Falls Trailhead parking area (hereafter JF) and at 2780 m elevation and 12 km distant from JF, referred to as Brush Creek (hereafter BC; 38.895° N, 106.890° W). Where experiments were replicated at both sites, the timing was staggered (JF initiated approximately 7 days later) such that both experiments began at a similar phase with respect to plant and arthropod phenology.

NATURAL HISTORY

The plant *Valeriana edulis* Nutt. ex Torr. and A. Gray subsp. *edulis*, is a dioecious perennial herb distributed throughout western North America (Meyer 1951). Plants grow for several years as a basal rosette of leaves before flowering via one to many inflorescences of small (3-5 mm diameter) white flowers (Soule 1981). Inflorescences bolt during the second half of June and consist of a stalk supporting one to several compound cymes. Like its dioecious congener *V. dioica* L. (Meurman 1925), sex determination in this species appears to be genetically controlled by an XY sex chromosome system given that monitoring of 776 plants for three consecutive years never found changes in individual sex expression (Soule 1981). After bolting following snowmelt, *V. edulis* is fed upon by the ant-tended aphid *Aphis valerianae* Cowen (Aphididae, Heteroptera), which then persists into early September. This aphid feeds on the upper portions of the main inflorescence stalk and within compound cymes on the stalks supporting individual cymes. Ants that frequently collect aphid honeydew and *V. edulis* floral nectar include *Tapinoma sessile* (Say), *Formica podzolica* Francoeur, *F. neogagates* group,

Camponotus modoc (Wheeler), and *Myrmica incompleta* Provancher. None of the ant species have been observed to prey on or move aphids among feeding sites (W. K. Petry personal observation, Petry et al. 2012, Mooney et al. 2012). The community of predators observed to feed on *A. valerianae* includes Araneae, Coccinellidae (Coleoptera), Syrphidae (Diptera), Miridae and Reduviidae (Heteroptera), Braconidae, Ichneumonidae and Vespidae (Hymenoptera), and Chrysopidae (Neuroptera).

SURVEYS OF PLANT TRAITS AND ARTHROPODS

In the summer of 2009, plant traits and arthropod abundance were measured on 97 randomly selected flowering plants (67 female and 30 male). In the summers of 2010 and 2011, similar surveys of 13 male and 16 female permanently marked plants were conducted, again measuring plant traits and arthropod abundance. Plant traits measured included several metrics of plant size and reproduction (fully described in Supplemental Materials: Appendix A). Though some plant traits varied among years, there was no difference between the sexes in plant traits except flower number and inflorescence volume (Supplemental Materials: Appendix A). Nectar was difficult to quantify due to its small quantity and high viscosity, but female nectar was frequently visible while male nectar was rarely so. Aphids, ants, and predators were surveyed in all three years through an exhaustive search of each plant. All individuals of taxa commonly reported to be predatory (see Natural history) were counted regardless of their observed activity. For each taxon actual predation events were observed on one or more occasions. Survey data for predators and ants were supplemented with observations from plants used in several experiments (see Mechanistic studies) to which aphids were added, but no other manipulations were imposed. Consequently, survey sample size for aphids, predators and ants was 166 (2009, n = 97; 2010, n

= 29; 2011, n = 40), 162 (2009, n = 30; 2010, n = 100; 2011, n = 32) and 156 (2009, n = 97; 2010, n = 29; 2011, n = 30) observations, respectively. Mean abundances for each plant were derived by standardizing the number of arthropods observed to the number of times that individual plant was surveyed to avoid pseudoreplication.

The abundance of each arthropod group was analyzed using ANOVA, with plant sex and observation year as independent factors. When residuals were not normally distributed or variances were heteroscedastic, resampling analyses (following Manly 2007) were conducted. Briefly, arthropod abundances were modeled as dependent upon plant sex and year using an ANOVA to generate observed F statistics for each model term. P values were estimated by comparing observed F statistics to null F distributions based on 10^3 permutations of the dependent variable and the result is P_{perm}. In addition, the presence/absence of each arthropod group was analyzed using logistic regression, with plant sex and observation year as independent factors. In these surveys and in all experiments, statistical analyses were conducted using R 2.15.0 (R Core Team 2012).

MECHANISTIC STUDIES

The plant surveys just described revealed a consistent female bias for aphid, predator, and ant abundance across three years (Results, Fig. 3.1A). Accordingly, manipulative experiments and additional analyses of survey data were conducted to elucidate the mechanisms underlying these patterns (Fig. 3.1B).

Plant effects on aphids

We used two methods to test for direct plant effects on aphids. First, to test for sexual dimorphism in plant quality to aphids, the growth rate of experimentally established aphid populations was compared between male and female plants in both 2009 and 2010 at JF. This study was based on 60 plants in 2009 (30 male, 30 female) and 100 plants in 2010 (49 male, 51 female). Aphid populations were established at mean initial densities of 8 ± 0.6 (mean ± SE) and 11 ± 0.2 wingless adults per plant (in 2009 and 2010, respectively) within mesh bags that excluded both predators and ants. Population growth was monitored for 18 days (beginning on 5 July) and 8-12 days (beginning 14 July) for 2009 and 2010, respectively. Population growth rate was calculated according to the following equation:

$$r = \frac{\ln(N_{t1}) - \ln(N_{t0})}{t_1 - t_0} \qquad \text{(Eq. 3.1)}$$

where N_{t0} and N_{t1} are population densities at time t_0 and t_1, respectively. For extinct populations at t_1, r was set to zero. Because analysis residuals for population growth rate did not follow normal or Poisson distributions, resampling analyses were used (as above for survey data).

Second, the relative preference of dispersing aphids for male and female plants was assessed to test whether differences in aphid abundance were due to host plant selection. In 2011, 18 male and 13 female aphid-free plants were selected at JF on 29 June, while 18 male and 24 female aphid-free plants were selected for observation at BC on 11 July. Each plant was then inspected at intervals of 3 to 7 days for the occurrence of newly arrived, individual, winged aphids. While this survey interval may have missed early predation events, it is likely adequate to detect effects of plant sex on aphid establishment and initial reproduction. When ~70% of monitored plants were colonized (after 34 and 14 days for JF and BC, respectively) the occurrence of colonization was modeled using a logistic regression with plant sex, site, and their interaction as independent factors.

Predator effects on aphids

To compare the effects of predators on aphids between male and female plants, pairs of similarly sized inflorescence stalks were selected at JF in 2009 on 15 male and 15 female plants (n = 30 paired inflorescences). Ants were excluded from all inflorescences using tape coated with a sticky paste barrier (Tanglefoot Company, Grand Rapids, Michigan, USA) around the base of the stem. None of the most abundant predators in this system – winged predators (e.g. adult coccinellids, wasps, adult hemipterans) or apterous predators deposited as eggs by winged adults (e.g. larval coccinellids and syrphids) – are affected by these barriers. Both inflorescences were enclosed in breathable mesh bags, and for half of the bags several vertical slits were cut into bags to allow predator access. Although this design may have allowed emigration of aphids in the treatment open to predators, the goal of this experiment was to test for sexual dimorphism in predator effects rather than to quantify the effect of predators absolutely. Moreover, substantial emigration is unlikely because (1) the production of winged dispersal morphs was rare and (2) this specialist species does not appear to disperse among hosts by walking, as we have never observed individuals ascending or descending the plant stems subtending inflorescences (W. K. Petry, personal observation). On 3 August, aphid populations were established at a mean initial density of 37 ± 2 aphids per plant and monitored for 10 days. Aphid population growth rate (Eq. 3.1) was then modeled as a dependent variable on predator exclusion, plant sex, and their interaction, with initial aphid population as a covariate. Here the main effects test the hypothesis that predators and plant sex influence aphid performance while their interaction tests the hypothesis that plant sex modifies the top-down effect of predators on aphid performance.

Ant effects on aphids

To compare the effects of ants on aphids between male and female plants, experiments were conducted at both JF and BC in 2011. Eight blocks of plants were selected per site with two male and two female plants each, and two experimental inflorescences per plant for a total of 128 experimental inflorescences. Within each block, one male and one female plant were assigned to receive a single unwinged aphid on each inflorescence, representing colony establishment. The other male and female plant received aphids at initial densities of 34 ± 2 aphids, simulating an established colony. Within each plant, ants were then excluded from one inflorescence, with the second inflorescence serving as a control. Aphid populations were monitored for 14 days (beginning on 26 July) and 15 days (beginning on 20 July) for JF and BC, respectively. Aphid population growth rate (Eq. 3.1) was modeled as dependent on plant sex, initial aphid density, site, and their two- and three-way interactions with block as a random effect. This model tests for effects of ant exclusion, aphid density, and plant sex on aphid population growth (all main effects) as well as sex modification of both ant effects on aphids (plant sex × ant exclusion) and aphid density effects (plant sex × aphid density) and the density-dependent effects of ants (plant sex × density × ant exclusion interaction).

Plant and aphid effects on predators

Further analysis of survey data were used to identify the pathways (direct, aphid density-mediated, or interaction modification) underlying the higher abundance of predators on female plants. Predator abundance (n = 162 replicate plants) was modeled as dependent upon plant sex, aphids, and their interaction while controlling for year using ANCOVA. This model

simultaneously tests for direct, aphid density-mediated and interaction modification effects of plant sex on predators. A significant effect of plant sex, while controlling for variation in aphid abundance, is indicative of a direct effect. Similarly, a significant effect of aphid abundance while controlling for plant sex, in combination with the greater abundance of aphids naturally occurring on female plants, is evidence for a density-mediated indirect effect. Finally, a significant plant sex × aphid abundance interaction indicates an interaction modification, as the slope of the relationship between aphid and predator abundance, representing the per capita effect of aphids on predators, would differ between the sexes.

Plant and aphid effects on ants

Further analysis of survey data were used to identify the pathways underlying the higher abundance of ants on female plants using an ANCOVA in a fashion parallel to that for predators (see Plant and aphid effects on predators). Ant abundance (n = 156 replicate plants) was modeled as dependent upon the main effects of plant sex (direct effect) and aphid abundance (aphid-mediated indirect effect) and their interaction (interaction modification effect) while controlling for year.

We also used a manipulative experiment to specifically test whether sexual dimorphism in the effect of floral nectar influenced ants and ant-aphid interactions. In 2011, 10 pairs of male and female plants were selected at both JF and BC, with two experimental inflorescences per plant (total n = 80 inflorescences). For each plant, one inflorescence was randomly selected to receive a nectary-blocking treatment (Rudgers 2004). Glue (Aleene's OK to Wash-It fabric glue; Duncan Enterprises, Fresno, California, USA) was applied to each flower on nectary exclusion plants, while a similar amount of glue was applied to the stems immediately subtending those

flowers on control plants. Plants were then stocked with aphids at a mean initial density of 12 ± 1 aphids per plant. Counts of both ants and aphids were made across 19 days (beginning on 19 July) and 11 days (beginning on 10 July) at JF and BC, respectively. Ants were counted separately with respect to whether they were tending aphids or foraging on inflorescences.

This experiment tested three sets of hypotheses regarding the role of nectar in plant-aphid-ant interactions. First, ant abundance at floral nectaries (averaged across all repeated surveys) was modeled as dependent upon plant sex, exclusion of nectaries, and their interaction, with field site and block included as random effects and aphid abundance as a covariate. Second, the abundance of ants tending aphids (averaged across all repeated surveys) was analyzed with the same independent variables as the model used to analyze flower visitation. In these analyses of ant visitation to flowers or aphids, the main effect of plant sex tests for sexual dimorphism in ant abundance, the main effect of nectar exclusion tests whether floral resources influence ant abundance, while the nectar exclusion × plant sex interaction tests whether differences in the attractiveness of floral resources between sexes underlie any direct influence of plant sex on ant abundance.

A complementary analysis tested for aphid performance responses to plant sex and floral nectar exclusion (mediated through ant or predator recruitment to nectar). Aphid population growth rate was modeled as dependent upon plant sex, nectary blocking, and their interaction, with field site included as a random effect. In this analysis, the main effect of plant sex tests for sexual dimorphism in plant quality (thus complementing the test of direct effects of plant sex on aphids described in Plant effects on aphids), and the main effect of nectary blocking tests whether floral resources (indirectly) influence aphid performance. Because glue did not directly affect aphid performance (experiment described in Supplemental Materials: Appendix B), a

significant main effect of nectary blocking is likely mediated through higher trophic levels. Finally, the nectary blocking × plant sex interaction tests whether any difference in aphid performance between male and female plants is underlain by sexual dimorphism in the higher-trophic-level-mediated effects of floral nectar.

Partitioning variance in arthropod abundance

The magnitude of plant sex effects on arthropods can be assessed not only by comparing mean abundances between males and females as above, but also by quantifying the proportion of overall variance explained (R^2_{sex}). When these R^2 calculations are made with respect to variance among plant genotypes (e.g., clonal replicates, half- or whole-sib families), such values are similar to broad-sense heritabilities (H^2) for arthropod abundance as an extended plant phenotype (Shuster et al. 2006). In the present case, R^2_{sex} assesses the subset of H^2 that can be ascribed to plant sex determination genes.

The approach for estimating R^2_{sex} for each arthropod group's abundance (survey data) was first to use a sequential sum of squares (type I) ANOVA model to estimate the sum of squares for plant sex (SS_{sex}) and year (SS_{year}) terms as well as the model's total sum of squares (SS_{total}). Here the total proportion of variance in arthropod abundance explained by plant sex (direct + indirect effects) was calculated as SS_{sex} divided by SS_{total} with interannual variation in abundance removed ($SS_{total} - SS_{year}$). Because plant sex affected aphid presence (vs. absence) but not abundance when aphids were present (analysis not shown), we used aphid presence in partitioning analyses. For aphids and predators, this value was assumed to be solely due to the direct effect of plant sex based upon our analyses of survey and experimental data (see Results). For ants, however, the total effect of plant sex on ant abundance could be partitioned into direct

and aphid-mediated indirect pathways by directly calculating the R^2_{sex} for the direct pathway and subtracting it from the total R^2_{sex} (i.e., from direct + indirect) to yield the indirect R^2_{sex}. Here the direct R^2_{sex} is calculated from a similar model and using the same sum-of-square terms as above, except that a term for aphids is fit before plant sex.

RESULTS

SURVEYS OF PLANT TRAITS AND ARTHROPODS

Preliminary analyses showed patterns of sexual dimorphism in arthropod associations to be consistent across years (i.e., no sex × year interaction, $P \geq 0.12$), and this interaction term was excluded from all models. There was strong sexual dimorphism in the abundance of aphids, ants, and predators (Fig. 3.1). Female plants hosted fourfold more aphids compared to males (Table 3.1). Aphids were also more likely to be present (vs. absent) on females than males, occurring on 43% and 24% of plants, respectively (Table 3.1). Aphid presence appeared to drive the significant difference in aphid abundance: on those plants with one or more aphids, there was no difference in aphid abundance ($F_{1, 59} = 2.50$, $P = 0.12$), though the trend was for a greater abundance (back-transformed mean ± asymmetric SE: lower, upper) on females (50.6 ± 11.0, 19.0) than males (22.8 ± 6.7, 15.0). There was a trend ($F_{1, 158} = 3.48$, $P = 0.067$) for female plants to host more predators compared to males (1.5×; Table 3.1). Predators were equally likely to be present (vs. absent) on female than male plants (46% of plants; Table 3.1). On those plants with one or more predators during any survey visit, females again hosted marginally more predators ($F_{1, 71} = 3.59$, $P = 0.06$), with means of 1.08 ± 0.11 and 0.81 ± 0.11 predators per survey visit, respectively. Female plants hosted fourfold more ants compared to males (Table 3.1). Ants were more likely to be present (vs. absent) on females than males, occurring on 51% and 16% of

Table 3.1. Analysis of variance tables of tests for arthropod community dimorphism between female and male *Valeriana edulis* plants.

Arthropod	Source	F	df	P
Aphid abundance	**Sex**	7.44	1, 162	**0.007**
	Year	30.83	2, 162	**<0.001**
Aphid presence/absence	**Sex**	4.94	1, 162	**0.028**
	Year	21.80	2, 162	**<0.001**
Ant abundance	**Sex**	9.13	1, 152	**0.001**
	Year	4.97	2, 152	**0.007**
Ant presence/absence	**Sex**	17.73	1, 152	**<0.001**
	Year	5.08	2, 152	**0.007**
Predator abundance	**Sex**	3.48	1, 158	**0.067**
	Year	14.02	2, 158	**<0.001**
Predator presence/absence	Sex	<0.01	1, 158	0.999
	Year	3.56	2, 158	**0.031**

Notes: Significant ($P < 0.05$) and marginally significant ($P < 0.10$) model terms and their p-values are shown in bold. No sex × year interactions were significant ($P > 0.12$).

plants, respectively (Table 3.1). On those plants with one or more ants during any survey visit, there was a trend for ant abundance to be higher on females than on males ($F_{1, 55} = 3.93$, $P = 0.05$), with means of 2.91 ± 0.23 and 1.37 ± 0.57 ants per survey visit, respectively.

MECHANISTIC STUDIES

Plant effect on aphids

When aphids were reared within mesh bags in the absence of ants and predators, population growth rate (*r*) was not significantly different between male and female plants (Fig. 3.2A; $F_{1, 156} = 0.12$, $P_{perm} = 0.72$) when controlling for a significant year effect ($F_{1, 156} = 9.96$, $P_{perm} = 0.001$). Similarly, dispersing aphids also did not show an overall preference for female or male plants (Fig. 3.2B; $F_{1, 69} = 0.77$, $P = 0.38$) and this pattern was consistent across both sites (i.e., no site effect, $F_{1, 69} = 0.266$, $P = 0.61$, or sex × site interaction, $F_{1, 69} = 0.005$, $P = 0.94$).

Predator effects on aphids

Comparing aphid population growth between predator exclusion and control treatments on male and female plants, predators had a negative effect on aphid performance ($F_{1, 55} = 6.22$, $P_{perm} = 0.014$) when controlling for initial aphid abundance ($F_{1, 55} = 10.75$, $P_{perm} = 0.001$), reducing population growth rate by 19% (predator exclusion, 0.074 ± 0.007; control, 0.060 ± 0.007). Although there was a trend toward greater predator abundance on female plants (see Results: Surveys of plant traits and arthropods), there was no evidence that plant sex mediated the effect of predators on aphid performance (i.e., no trait-mediated indirect effect indicated by an insignificant sex × predator treatment interaction; $F_{1, 55} = 0.42$, $P_{perm} = 0.53$).

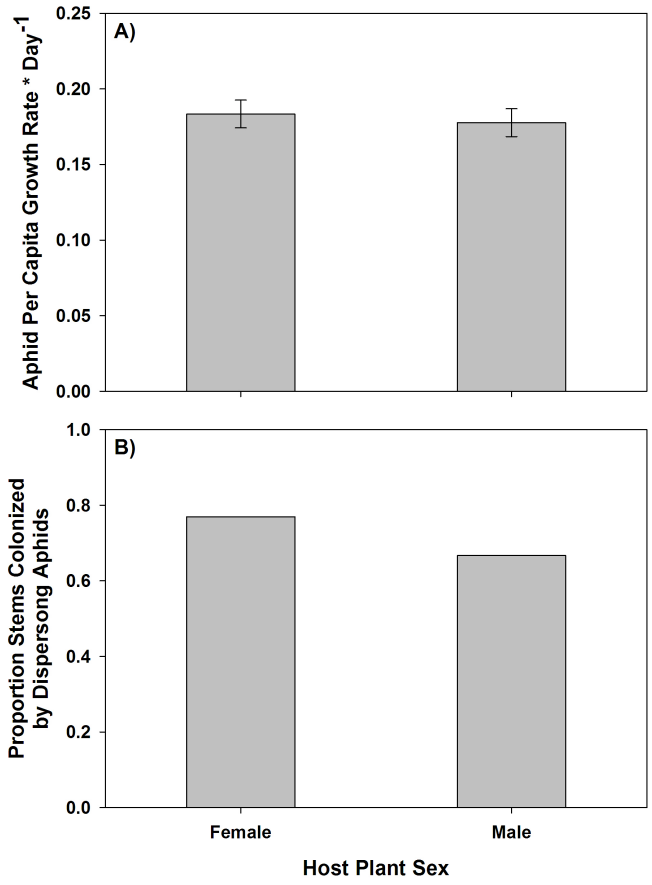


Figure 3.2. (A) Daily per capita daily growth rate (mean ± SE) of the aphid *Aphis valerianae* on female and male *Valeriana edulis*, controlling for year. Plant sex did not influence aphid growth rate (P_{perm} = 0.72; see *Methods: Surveys of plant traits and arthropods* for definition of P_{perm}). **(B)** The proportion of female and male plants colonized by winged aphids did not differ (P = 0.38) at both study sites, BC and JF.

Ant effects on aphids

The exclusion of ants (Fig. 3.3) increased aphid population growth rate by 46% ($F_{1, 119}$ = 20.83, $P_{perm} < 0.001$) and did so to an equal extent on male and female plants, i.e., no ant × plant sex interaction ($F_{1, 119} = 0.03$, $P_{perm} = 0.87$). Furthermore, aphid performance was 41% higher on females than males ($F_{1, 119} = 6.84$, $P_{perm} = 0.011$), contrasting the assessment of host plant quality to aphids within mesh bags (see Plant effects on aphids). Neither aphid density ($F_{1, 119} = 0.14$, $P_{perm} = 0.24$), study site ($F_{1, 119} = 0.98$, $P_{perm} = 0.32$), nor any two- and three-way interactions between the four main effects were significant ($P_{perm} > 0.60$).

Plant and aphid effects on predators

We further analyzed survey data to assess the association between predators and aphids on male and female plants. Plant sex affected predators directly, but not through aphid density-mediated indirect effects or modification of aphid-predator interactions. Predator abundance was higher on female plants ($F_{1, 157} = 3.86$, $P_{perm} = 0.050$), and there was no evidence for an influence of aphid abundance ($F_{1, 157} = 0.476$, $P_{perm} = 0.49$), nor that the attractiveness of aphids to predators differed between plant sexes, i.e., there was no plant sex × aphid abundance interaction ($F_{1, 156} = 0.683$, $P_{perm} = 0.41$). Following this unusual lack of association between aphids and their predators, we performed a Fisher's exact test of association between plants hosting aphids and those hosting their predators but found no significant association (n = 162 observed plants; P = 0.68). An analysis (not shown) of predator abundance on plants without aphids (n = 29 observed plants) revealed a similar but nonsignificant (P = 0.35) pattern of higher abundance on females.

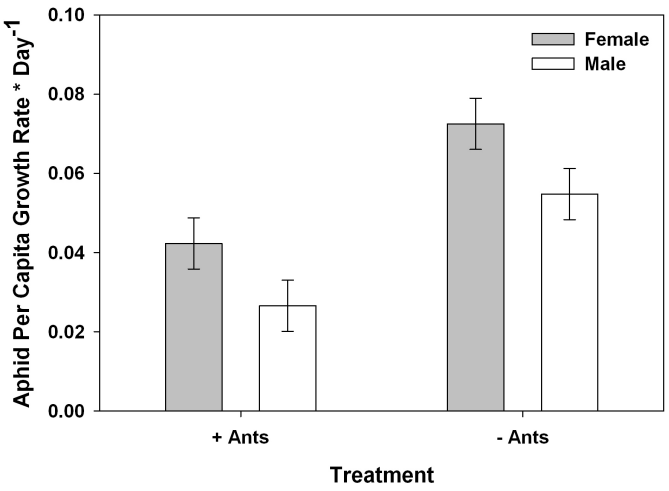


Figure 3.3. Daily per capita daily growth rate (mean ± SE) of aphids on female (dark bars) and male (light bars) plants, with ants allowed (+) or excluded (−) controlling for field site. Aphids performed better on females ($P_{perm} = 0.011$), and ants strongly reduced aphid performance ($P_{perm} < 0.001$).

Plant and aphid effects on ants

We further analyzed survey data to assess the association between ants and aphids on male and female plants. Plant sex affected ants directly and indirectly through aphids, but did not modify aphid effects on ants. Ant abundance was higher on female plants ($F_{1, 298} = 8.85$, $P = 0.003$) and was positively associated with aphid abundance ($F_{1, 298} = 6.39$, $P = 0.012$), supporting a direct and aphid-density-mediated attraction of ants to female plants. The attractiveness of aphids to ants did not differ between plant sexes, i.e. there was no plant sex × aphid abundance interaction ($F_{1, 297} = 0.05$, $P = 0.82$). Thus ants were attracted to female plants additively and independently by direct and aphid-mediated indirect pathways. An analysis (not shown) of those plants without aphids (n = 97 plants) revealed 3.3× higher ant abundance on females than males ($P = 0.011$).

We compared ant abundance between experimentally blocked and control floral nectaries on male and female plants with aphids. This manipulative experiment showed that floral nectar drives the direct effect of plant sex on ants and that nectar- and aphid-mediated ant recruitment operated independently. Although there was biased ant visitation toward female flowers across both nectary blocking and control treatments ($F_{1 ,67} = 4.91$, $P_{perm} = 0.016$), this effect of plant sex depended upon nectar blocking ($F_{1, 67} = 5.48$, $P_{perm} = 0.01$); there was strong female-bias in ant visitation to flowers when nectaries were open, but visitation was lower and no longer sexually dimorphic when nectaries were blocked (Fig. 3.4A). In addition, the abundance of aphids did not influence ant visitation to flowers ($F_{1, 67} = 0.193$, $P_{perm} = 0.66$). Despite this greater recruitment of ants to female nectar, the abundance of aphid-tending ants was influenced by aphid abundance alone ($F_{1, 67} = 19.04$, $P_{perm} < 0.001$), and not by plant sex ($F_{1, 67} = 0.70$, $P_{perm} = 0.41$), the blocking

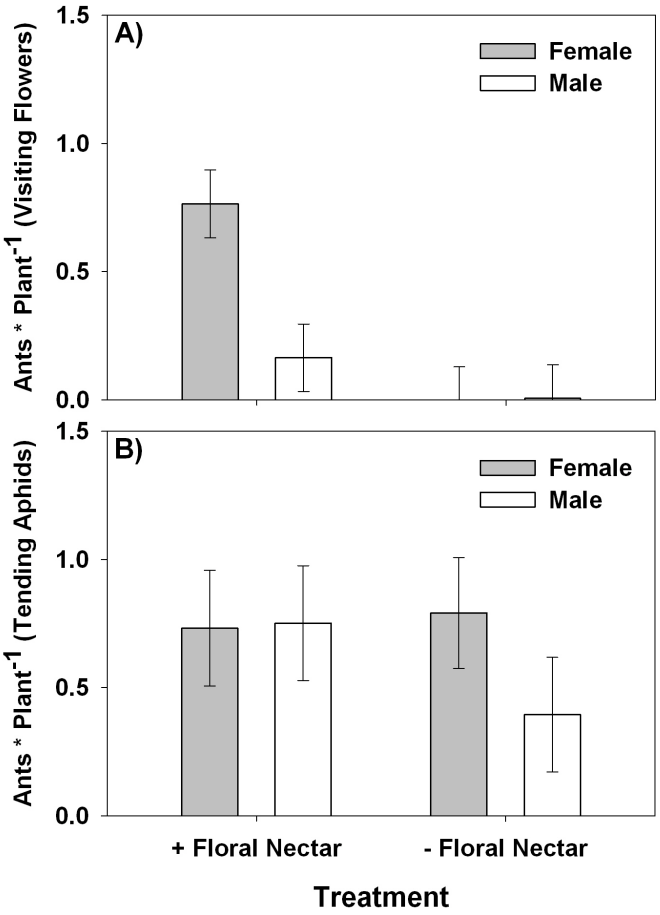


Figure 3.4. (A) Number (least-square mean ± SE) of ants visiting flowers on female (dark bars) and male (light bars) *Valeriana edulis* with floral nectaries open (+) or blocked with glue (−). Floral visitation was higher on females on control plants but was negligible on plants with blocked floral nectaries ($P_{perm} = 0.016$). **(B)** Number (least-square mean ± SE) of total ants tending aphids on female (dark bars) and male (light bars) plants with floral nectaries open (+) or blocked with glue (−). Ant tending of aphids was not affected by plant sex ($P_{perm} = 0.41$) or floral nectar availability ($P_{perm} = 0.49$).

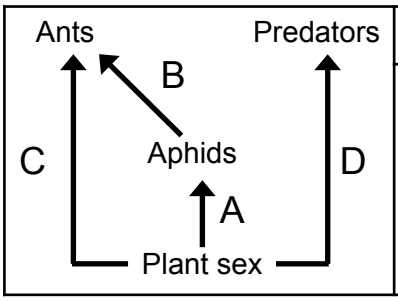
of floral nectar ($F_{1, 67} = 0.04$, $P_{perm} = 0.84$), or their interaction ($F_{1, 67} = 0.93$, $P_{perm} = 0.34$; Fig. 3.4B).

Partitioning variance in arthropod abundance

Sequential fitting of plant sex and aphid model terms to both predator and ant abundance partitioned the variance in arthropod abundance among the significant mechanistic pathways (Fig. 3.5). Plant sex explained a relatively small amount of variance (R^2_{sex}) in aphid presence (0.24%) and slightly more for the abundances of predators (2.28%) and ants (4.42%). Only ants had statistical support for both direct and indirect effects of plant sex. Decomposing the total effect on ants into direct and indirect pathways showed direct recruitment to females was fourfold stronger than the aphid-mediated pathway (3.61% vs. 0.81%).

DISCUSSION

Plant surveys conducted over three consecutive years demonstrated strong and consistent evidence for plant genetic control over arthropod community structure, with female plants having 4-fold more aphids, 1.5-fold more predators, and 4-fold more ants as compared to male plants. Mechanistic studies demonstrated plant genetic effects directly on aphids, predators, and ants, as well as weaker indirect plant genetic effects on ants (but not predators) mediated by aphid abundance (Fig. 3.5). These direct and indirect effects on ants operated independently of each other and there was no evidence for interaction modification, i.e., aphid-ant and aphid-predator interactions were not influenced by the sex of the plant upon which they occurred. Plant sex explained somewhat less variance in aphid, predator, and ant abundance (0.24%, 2.28%, and 4.42% respectively) relative to past studies documenting plant-genetic effects on arthropod



Plant sex effects on:	R^2_{sex}	F vs. M
Aphids, direct (A)	0.24%	4-fold
Ants, direct (C)	3.60%	4-fold
Ants, indirect (AB)	0.81%	
Predators, direct (D)	2.28%	1.5-fold

Figure 3.5. The subset of potential mechanisms (Fig. 3.1B) determined to underlie sexual dimorphism in arthropod abundances **(left)** and comparisons of mechanism effect sizes **(right)**. Mechanism effect sizes (arrows and values labeled A–D) are quantified as both percentage of variance explained (R^2_{sex}) and difference in abundance between female and male plants (F vs. M).

communities (reviewed in Johnson and Agrawal 2005). This study is unusual among those investigating plant genetic effects on arthropod community structure in its documentation of mechanism across multiple trophic levels, and as only the fourth system demonstrating the strength of plant genetic effects for wild-grown plants under non-experimental conditions.

Despite a consistent female-bias in aphid abundance, our results were ambiguous with respect to the mechanism. There were two experiments in which aphid population growth rate was compared between male and female plants. When aphids were isolated from interactions with predators and ants in mesh bags (Fig. 3.2A) there was no detectable difference in aphid performance. In contrast, in a separate experiment where aphids were unbagged, their performance was stronger on females (Fig. 3.3), although such effects did not depend on the presence of ants or predators. While our experiments provide some positive evidence for higher quality of female plants, consistent with the female-bias observed in surveys (Fig. 3.1), there was no corresponding female bias in aphid host plant selection. In addition, plant sex did not mediate the top-down effects of predators and ants on aphids; we did not detect differences in the rates of association between either tending ants or predators and aphids (density-mediated indirect effects), nor in the strength of their effects on aphid performance (interaction modification). So while there is some evidence superior plant quality underlies greater aphid abundance on females, further studies are required to definitively resolve this.

Plant genetic effects on ants were driven by two mechanisms. First, plant sex directly influenced ants, with female plants being more attractive than male plants due to differences in nectar rewards. Past studies have similarly shown dimorphism in ant recruitment to male and female flowers, although these studies are evenly divided with respect to which is the more attractive sex (Ashman and King 2005, Mooney et al. 2012). Second, the higher abundance of

aphids on female plants resulted in an indirect genetic effect on ants, a dynamic that has previously been observed in four other plant-aphid systems (Wimp and Whitham 2001, Johnson 2008, Mooney and Agrawal 2008, Abdala-Roberts et al. 2012). Although these two plant genetic effects on ants operated in parallel, each contributing to greater ant abundance on female plants, they operated independently in that ant recruitment to aphids did not vary between male and female plants, even though there were more ants associated with female flowers. The additive recruitment of ants to these co-occurring resources runs counter to the prediction that plant resources should mediate ant interactions with herbivores (Becerra and Venable 1989, Ness et al. 2009). A better understanding of dual resource effects on arthropods (i.e., synergistic attraction or antagonism) may come from comparing the nutritive composition of honeydew and floral nectar (e.g., Blüthgen et al. 2004).

We observed higher abundances of predators on female plants that was not driven by sexual dimorphism in aphid abundance, and the effects of predators on aphids was equal between plant sexes. That is, unlike ant abundance that was driven by both direct and indirect effects of plant sex, only direct effects influenced predator abundance. Higher predator abundance on females is consistent with a direct effect of sexual dimorphism in floral rewards (Pacini and Nepi 2007). Because predator effects on aphids were equivalent between male and female plants, we speculate that sexual dimorphism in floral attraction of predators did not mediate predator attack of aphids.

While both ants and predators were more abundant on female plants (Fig. 3.1A), the strength of the direct plant genetic effect was greater for ants. This finding is consistent with past studies that show plant genetic effects on ant but not predator abundance (Johnson 2008, Mooney and Agrawal 2008, Abdala-Roberts et al. 2012). The emerging generality of this pattern

may be due to the social foraging of ants vs. the generally solitary foraging of predators. This provides a pathway to greater recruitment to resources that are underlain by genetically variable plant traits. For these higher trophic levels more broadly, the strength of direct effects of plant sex exceeded indirect (herbivore-density-mediated) effects. This suggests that, although plant genetic effects on higher trophic levels may be diluted through intervening trophic links (e.g., herbivores), plant genetic variation in traits directly attractive to these arthropods may outweigh weak herbivore density-mediated effects. Specifically, consideration of floral nectar may be particularly important for understanding the strength of direct plant genetic effects. Floral nectar is attractive to both predators and ants (Wäckers 2005), and nectar traits can differ among genotypes (Eckhart 1999, Mitchell 2004). Furthermore, there is growing evidence that plant genetic effects on higher trophic levels are more apparent when floral rewards are available (this study; Crutsinger et al. 2008) vs. when they are absent (Johnson 2008, Mooney and Agrawal 2008, Abdala-Roberts et al. 2012).

Plant genetic effects on arthropod communities can be quantified both in terms of difference between genotype means (Fig. 3.1A), and in terms of the percentage of variance explained by genotype (community level heritability sensu Goodnight and Craig 1996, Shuster et al. 2006). Johnson and Agrawal (2005) reviewed the percentage of variance in arthropod groups attributable to plant genotype from plant collections across multiple spatial scales. In the 26 plant-arthropod interactions measured at a comparable spatial scale to this study, they showed that plant genotype explains an average of 8.5% of the variation in arthropod abundance. Consequently, the variance in aphid, predator, and ant abundance due to plant genotype (sex) in this study (0.24%, 2.28%, and 4.42% respectively) was comparable, although somewhat lower than that generally observed. By using wild-grown plants *in situ*, this study measured plant

genetic effects against levels of environmental variation that are greater, and more realistic, than that occurring in the experimental common gardens used in past studies (see reviews in Johnson and Agrawal 2005, Tack et al. 2012). Thus our study adds to a small literature showing the importance of plant genetic effects on arthropod communities in natural settings previously known only from two tree systems, *Populus* spp. (Wimp et al. 2004, Bangert et al. 2006b, 2006a, Wimp et al. 2007, Meneses et al. 2012) and *Brosimum alicastrum* (Zytynska et al. 2011), and the bromeliad *Aechmea bracteata* (Zytynska et al. 2012). It is perhaps not surprising to find less variance explained by a small proportion of the genome, the sex-determining genes (Ming et al. 2011), as compared to studies using clones, half or full siblings that measure the influences of whole plant genomes. Our study methodology thus offers a conservative estimate of extended consequences of plant genetics on arthropod community structure.

In summary, our study adds a mechanistic perspective to the growing literature on plant genetic control over arthropod community structure. Genetically based sex affected arthropod abundances though both direct and density-mediated indirect effects, but not through interaction modification, suggesting that the latter interaction type may not always play a role in structuring arthropod communities. Our findings also challenge the perspective that plant genetic effects diminish with increasing trophic level (Johnson and Agrawal 2005, Mooney and Agrawal 2008), observing that strong direct effects overshadowed this prediction. Thus genetic variation in traits both widely known to directly influence higher trophic levels (e.g., extrafloral nectaries, domatia, and volatiles) and those less studied traits like floral traits (this study) and plant morphology (Marquis and Whelan 1996) deserve attention. Furthermore, we measured these effects in wild-grown plants, finding patterns that were consistent across multiple years and comparable in strength to those previously found in more controlled, common garden experiments. This study

thus documents the mechanisms underlying plant genetic control of arthropod community structure in a natural setting.

SUPPLEMENTAL MATERIALS

Appendix A – Statistical methods and detailed results of tests for sexual dimorphism in plant traits.

Plant traits were compared between sexes using ANOVA, with the plant trait modeled as dependent upon plant sex and, where applicable, year of the observation and the sex-by-year interaction. When sex-by-year interaction terms were not significant ($P > 0.15$), they were removed. In the case of flower number, non-normal model residuals were corrected using resampling techniques and ANOVA following Manly (Manly 2007). Briefly, the observed test statistic (F) is compared to a null distribution of the test statistic calculated from 10^3 random arrangements of the dependent variable with respect to the independent variables.

Though some plant traits varied among years, there was no difference between the sexes in the height of tallest bolting inflorescence, mean height of bolting stems, number of stems, length of longest leaf, width of longest leaf, number of leaves, or basal diameter of the rosette. Only flower number and inflorescence volume differed significantly, with females producing more flowers despite males having larger cymes (Table S3.1).

Appendix B – Statistical methods and detailed results of tests for direct effects of nectary gluing on aphids.

A separate experiment tested for artifactual effects of gluing on aphid performance (i.e. direct toxicity effects, or effects mediated through changes in plant quality). Twenty male and twenty female plants were stocked with 25-75 aphids and bagged to exclude predators and ants. Glue was applied to floral nectaries of half of the plants, and the control plants (unlike in the experiment described in the main text) remained free of glue. The experiment was initiated in

two groups, one on 29 July 2011 and the other on 10 August 2011. After 10 days, aphid population growth rate was calculated (see main text). Aphid population growth rate was modeled as dependent upon glue treatment, plant sex, and the interaction between sex and glue treatment controlling for the experimental start date. Here the main effect of glue treatment tests for artifactual effects of gluing, and the main effect of plant sex offers another test for differences in host plant quality to aphids.

There was no main effect of gluing ($F_{1, 35} = 0.04$, $P = 0.83$), suggesting that glue did not affect aphid performance. Likewise, there was no main effect of plant sex ($F_{1, 35} = 2.12$, $P = 0.16$) or interaction between glue treatment and plant sex ($F_{1, 35} = 0.22$, $P = 0.63$) on aphid performance when controlling for a significant difference in aphid performance between start dates ($F_{1, 35} = 10.42$, $P = 0.003$).

SUPPLEMENTAL TABLES

Table S3.1. Analysis of variance tables of tests for trait dimorphism between female and male *Valeriana edulis* plants and least square mean ± SE trait values.

Trait	Year(s)	Source	F	df	p-value	Term	LS Mean ± SE
Tallest stem (cm)	2009, 2010	Sex	1.25	1, 117	0.265	Female	76.3 ± 3.4
						Male	72.9 ± 4.8
		Year	25.91	1, 117	**<0.001**	2009	71.7 ± 3.1
						2010	89.8 ± 6.3
Mean stem (cm)	2009, 2010	Sex	1.1	1, 117	0.296	Female	70.8 ± 3.2
						Male	67.8 ± 4.6
		Year	5.15	1, 117	0.025	2009	68.3 ± 2.9
						2010	76.0 ± 6.0
Stem number	2009, 2010	Sex	0.08	1, 227	0.772	Female	3.2 ± 0.4
						Male	3.1 ± 0.5
		Year	1.27	1, 227	0.26	2009	3.0 ± 0.5
						2010	3.3 ± 0.4
Width longest leaf (cm)	2010	Sex	0.92	1, 107	0.34	Female	5.6 ± 0.3
						Male	5.4 ± 0.4
Length longest leaf (cm)	2010	Sex	2.42	1, 107	0.123	Female	29.4 ± 1.3
						Male	27.8 ± 1.5
Leaf number	2010	Sex	2.05	1, 108	0.155	Female	137.0 ± 21.1
						Male	115.8 ± 19.4
Rosette diameter (cm)	2010	Sex	1.93	1, 108	0.167	Female	44.8 ± 3.3
						Male	41.3 ± 3.7
Mean cyme volume (cm^3)	2009	**Sex**	16.19	1, 95	**<0.001**	Female	5.1 ± 0.7
						Male	8.4 ± 1.9
Flower number*	2011	**Sex**	3.95	1, 39	**0.049**	Female	106.8 ± 17.6
						Male	56.8 ± 18.0

*p-value based on 10^3 random permutations (see text)

REFERENCES

Abdala-Roberts, L., A. A. Agrawal, and K. A. Mooney. 2012. Ant–aphid interactions on *Asclepias syriaca* are mediated by plant genotype and caterpillar damage. Oikos 121:1905–1913.

Acharya, L. 1995. Sex-biased predation on moths by insectivorous bats. Animal Behaviour 49:1461–1468.

Alatalo, J. M., and U. Molau. 1995. Effect of altitude on the sex ratio in populations of *Silene acaulis* (Caryophyllaceae). Nordic Journal of Botany 15:251–256.

Ashman, T.-L., and E. A. King. 2005. Are flower-visiting ants mutualists or antagonists? A study in a gynodioecious wild strawberry. American Journal of Botany 92:891–895.

Ågren, J., K. Danell, and T. Elmqvist. 1999. Sexual dimorphism and biotic interactions. Pages 217–246 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer, Berlin.

Baddeley, A., and R. Turner. 2005. spatstat: An R package for analyzing spatial point patterns. Journal of Statistical Software 12:1–42.

Bangert, R. K., G. J. Allan, R. J. Turek, G. M. Wimp, N. Meneses, G. D. Martinsen, P. Keim, and T. G. Whitham. 2006a. From genes to geography: a genetic similarity rule for arthropod community structure at multiple geographic scales. Molecular Ecology 15:4215–4228.

Bangert, R. K., R. J. Turek, B. J. Rehill, G. M. Wimp, J. A. Schweitzer, G. J. Allan, J. K. Bailey, G. D. Martinsen, P. Keim, R. L. Lindroth, and T. G. Whitham. 2006b. A genetic similarity rule determines arthropod community structure. Molecular Ecology 15:1379–1391.

Bates, H. W. 1865. Notes upon the variation of sexes in *Argynnis diana*. Proceedings of the Entomological Society of Philadelphia 4:204–207.

Becerra, J. X. I., and D. L. Venable. 1989. Extrafloral nectaries - a defense against ant-homoptera mutualisms. Oikos 55:276–280.

Bergamini, A., S. Ungricht, and H. Hofmann. 2009. An elevational shift of cryophilous bryophytes in the last century – an effect of climate warming? Diversity and Distributions 15:871–879.

Bierzychudek, P., and V. M. Eckhart. 1988. Spatial segregation of the sexes of dioecious plants. The American Naturalist 132:34–43.

Birch, L. C. 1953. Experimental background to the study of the distribution and abundance of insects: I. The influence of temperature, moisture and food on the innate capacity for increase of three grain beetles. Ecology 34:698–711.

Blüthgen, N., G. Gottsberger, and K. Fiedler. 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Austral Ecology 29:418–429.

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26:183–192.

Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: Incidence and implications of individual specialization. The American Naturalist 161:1–28.

Buckel, C. A., C. A. Blanchette, R. R. Warner, and S. D. Gaines. 2012. Where a male is hard to find: Consequences of male rarity in the surfgrass *Phyllospadix torreyi*. Marine Ecology Progress Series 449:121–132.

Burk, T. 1982. Evolutionary significance of predation on sexually signaling males. The Florida

Entomologist 65:90–104.

Calabrese, J. M., and W. F. Fagan. 2004. Lost in time, lonely, and single: Reproductive asynchrony and the allee effect. The American Naturalist 164:25–37.

Calabrese, J. M., L. Ries, S. F. Matter, D. M. Debinski, J. N. Auckland, J. Roland, and W. F. Fagan. 2008. Reproductive asynchrony in natural butterfly populations and its consequences for female matelessness. Journal of Animal Ecology 77:746–756.

CaraDonna, P. J., A. M. Iler, and D. W. Inouye. 2014. Shifts in flowering phenology reshape a subalpine plant community. Proceedings of the National Academy of Sciences 111:4916–4921.

Caswell, H. 1989. Analysis of life table response experiments I. Decomposition of effects on population growth rate. Ecological Modelling 46:221–237.

Caswell, H. 2001. Matrix population models. Sinauer Associates Inc, Sunderland, Massachusetts.

Caswell, H., and D. E. Weeks. 1986. Two-sex models: Chaos, extinction, and other dynamic consequences of sex. The American Naturalist 128:707–735.

Charlesworth, B. 2009. Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10:195–205.

Charlesworth, D. 2002. Plant sex determination and sex chromosomes. Heredity 88:94–101.

Charnov, E. L. 1982. The theory of sex allocation. Monographs in Population Biology. Princeton University Press, Princeton, N.J.

Chen, I. C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science 333:1024–1026.

Clutton-Brock, T. H. 1986. Sex ratio variation in birds. Ibis 128:317–329.

Cochran, M. E., and S. Ellner. 1992. Simple methods for calculating age-based life history parameters for stage-structured populations. Ecological Monographs 62:345–364.

Comfort, A. 1979. The biology of senescence. Third edition. Elsevier, New York.

Compagnoni, A., and P. B. Adler. 2014. Warming, soil moisture, and loss of snow increase Bromus tectorum's population growth rate. Elementa: Science of the Anthropocene 2:000020.

Cornelissen, T., and P. Stiling. 2005. Sex biased herbivory: a meta analysis of the effects of gender on plant herbivore interactions. Oikos 111:488–500.

Crimmins, S. M., S. Z. Dobrowski, J. A. Greenberg, J. T. Abatzoglou, and A. R. Mynsberge. 2011. Changes in climatic water balance drive downhill shifts in plant species' optimum elevations. Science 331:324–327.

Crone, E. E., M. M. Ellis, W. F. Morris, A. Stanley, T. Bell, P. Bierzychudek, J. Ehrlén, T. N. Kaye, T. M. Knight, P. Lesica, G. Oostermeijer, P. F. Quintana-Ascencio, T. Ticktin, T. Valverde, J. L. Williams, D. F. Doak, R. Ganesan, K. McEachern, A. S. Thorpe, and E. S. Menges. 2013. Ability of matrix models to explain the past and predict the future of plant populations. Conservation Biology 27:968–978.

Crutsinger, G. M., W. N. Reynolds, A. T. Classen, and N. J. Sanders. 2008. Disparate effects of plant genotypic diversity on foliage and litter arthropod communities. Oecologia 158:65–75.

Dall, S. R. X., A. M. Bell, D. I. Bolnick, and F. L. W. Ratnieks. 2012. An evolutionary ecology of individual differences. Ecology Letters 15:1189–1198.

Dawson, T. E., and L. C. Bliss. 1989. Patterns of water use and the tissue water relations in the dioecious shrub, *Salix arctica*: The physiological basis for habitat partitioning between the sexes. Oecologia 79:332–343.

Dawson, T. E., and M. A. Geber. 1999. Dimorphism in physiology and morphology. Pages 175–216 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer, Berlin.

de Roos, A. M., and L. Persson. 2013. Population and community ecology of ontogenetic development. Princeton University Press.

Delph, L. F. 1999. Sexual dimorphism in life history. Pages 149–174 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer, Berlin.

Easterling, M. R., S. P. Ellner, and P. M. Dixon. 2000. Size-specific sensitivity: Applying a new structured population model. Ecology 81:694–708.

Eckhart, V. M. 1999. Sexual dimorphism in flowers and inflorescences. Pages 123–148 *in* M. A. Geber, T. E. Dawson, and L. F. Delph, editors. Gender and sexual dimorphism in flowering plants. Springer.

Ehlers, B. K., and T. Bataillon. 2007. "Inconstant males" and the maintenance of labile sex expression in subdioecious plants. The New Phytologist 174:194–211.

Ellner, S. P., and M. Rees. 2006. Integral projection models for species with complex demography. The American Naturalist 167:410–428.

Elmendorf, S. C., G. H. R. Henry, R. D. Hollister, A. M. Fosaa, W. A. Gould, L. Hermanutz, A. Hofgaard, I. I. Jónsdóttir, J. C. Jorgenson, E. Lévesque, B. Magnusson, U. Molau, I. H. Myers-Smith, S. F. Oberbauer, C. Rixen, C. E. Tweedie, and M. Walker. 2015. Experiment, monitoring, and gradient methods used to infer climate change effects on plant communities yield consistent patterns. Proceedings of the National Academy of Sciences 112:448–452.

Elmendorf, S. C., G. H. R. Henry, R. D. Hollister, R. G. Björk, A. D. Bjorkman, T. V. Callaghan,

L. S. Collier, E. J. Cooper, J. H. C. Cornelissen, T. A. Day, A. M. Fosaa, W. A. Gould, J. Grétarsdóttir, J. Harte, L. Hermanutz, D. S. Hik, A. Hofgaard, F. Jarrad, I. S. Jónsdóttir, F. Keuper, K. Klanderud, J. A. Klein, S. Koh, G. Kudo, S. I. Lang, V. Loewen, J. L. May, J. Mercado, A. Michelsen, U. Molau, I. H. Myers-Smith, S. F. Oberbauer, S. Pieper, E. Post, C. Rixen, C. H. Robinson, N. M. Schmidt, G. R. Shaver, A. Stenström, A. Tolvanen, Ø. Totland, T. Troxler, C.-H. Wahren, P. J. Webber, J. M. Welker, and P. A. Wookey. 2012. Global assessment of experimental climate warming on tundra vegetation: Heterogeneity over space and time. Ecology Letters 15:164–175.

Engelstädter, J., and G. D. D. Hurst. 2009. The ecology and evolution of microbes that manipulate host reproduction. Annual Review of Ecology, Evolution, and Systematics 40:127–149.

Eppley, S. M. 2001. Gender-specific selection during early life history stages in the dioecious grass Distichlis spicata. Ecology 82:2022–2031.

Etterson, J. R., and R. G. Shaw. 2001. Constraint to adaptive evolution in response to global warming. Science 294:151–154.

Fan, Y., and H. van den Dool. 2004. Climate Prediction Center global monthly soil moisture data set at 0.5 resolution for 1948 to present. Journal of Geophysical Research 109:D10102.

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40:503–537.

Farr, T. G., P. A. Rosen, E. Caro, R. Crippen, R. Duren, S. Hensley, M. Kobrick, M. Paller, E. Rodriguez, L. Roth, D. Seal, S. Shaffer, J. Shimada, J. Umland, M. Werner, M. Oskin, D. Burbank, and D. Alsdorf. 2007. The shuttle radar topography mission. Reviews of

Geophysics 45:RG2004.

Fieberg, J., and S. P. Ellner. 2001. Stochastic matrix models for conservation and management: a comparative review of methods. Ecology Letters 4:244–266.

Field, D. L., M. Pickup, and S. C. Barrett. 2013a. Comparative analyses of sex-ratio variation in dioecious flowering plants. Evolution 67:661–672.

Field, D. L., M. Pickup, and S. C. Barrett. 2013b. Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations. Annals of Botany 111:917–923.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Forrest, J. R. K. 2014. Plant size, sexual selection, and the evolution of protandry in dioecious plants. The American Naturalist 184:338–351.

Franco, M., and J. Silvertown. 2004. A comparative demography of plants based upon elasticities of vital rates. Ecology 85:531–538.

García-Camacho, R., M. Méndez, and A. Escudero. 2009. Pollination context effects in the high-mountain dimorphic *Armeria caespitosa* (Plumbaginaceae): Neighborhood is something more than density. American Journal of Botany 96:1620–1626.

García-Ramos, G., C. Stieha, D. N. McLetchie, and P. H. Crowley. 2007. Persistence of the sexes in metapopulations under intense asymmetric competition. Journal of Ecology 95:937–950.

Gauquelin, T., A. Bertaudière Montès, W. Badri, and N. Montès. 2002. Sex ratio and sexual dimorphism in mountain dioecious thuriferous juniper (*Juniperus thurifera* L., Cupressaceae). Botanical Journal of the Linnean Society 138:237–244.

Geber, M. A., T. E. Dawson, and L. F. Delph. 1999. Gender and sexual dimorphism in flowering

plants. Springer, Berlin.

Gelman, A., A. Jakulin, M. G. Pittau, and Y.-S. Su. 2008. A weakly informative default prior distribution for logistic and other regression models. Annals of Applied Statistics 2:1360–1383.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. The Quarterly Review of Biology 44:189–208.

Goodnight, C. J., and D. M. Craig. 1996. Effect of coexistence on competitive outcome in *Tribolium castaneum* and *Tribolium confusum*. Evolution 50:1241–1250.

Grant, M. C., and J. B. Mitton. 1979. Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. Evolution 33:914–918.

Hardy, I. C. W., editor. 2002. Sex ratios. Cambridge University Press, Cambridge.

Harrison, A., M. Scantlebury, and W. I. Montgomery. 2010. Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. Oikos 119:1099–1104.

Himler, A. G., T. Adachi-Hagimori, J. E. Bergen, A. Kozuch, S. E. Kelly, B. E. Tabashnik, E. Chiel, V. E. Duckworth, T. J. Dennehy, E. Zchori-Fein, and M. S. Hunter. 2011. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. Science 332:254–256.

Hoglund, J., and B. C. Sheldon. 1998. The cost of reproduction and sexual selection. Oikos 83:478–483.

Holzinger, B., K. Huelber, M. Camenisch, and G. Grabherr. 2008. Changes in plant species richness over the last century in the eastern Swiss Alps: Elevational gradient, bedrock effects and migration rates. Plant Ecology 195:179–196.

Hultine, K. R., S. E. Bush, A. G. West, and J. R. Ehleringer. 2007. Population structure, physiology and ecohydrological impacts of dioecious riparian tree species of western North America. Oecologia 154:85–93.

Hurst, L. D. 1993. The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. Biological Reviews 68:121–194.

Iler, A. M., T. T. Høye, D. W. Inouye, and N. M. Schmidt. 2013. Nonlinear flowering responses to climate: are species approaching their limits of phenological change? Philosophical Transactions of the Royal Society B: Biological Sciences 368:20120489.

Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362.

Jackson, S. 2015. pscl: Classes and methods for R developed in the Political Science Computational Laboratory, Stanford University. CRAN.

Jenouvrier, S., H. Caswell, C. Barbraud, and H. Weimerskirch. 2010. Mating behavior, population growth, and the operational sex ratio: a periodic two-sex model approach. The American Naturalist 175:739–752.

Johnson, M. T. J. 2008. Bottom-up effects of plant genotype on aphids, ants, and predators. Ecology 89:145–154.

Johnson, M. T. J., and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). Ecology 86:874–885.

Johnson, M. T. J., and J. R. Stinchcombe. 2007. An emerging synthesis between community ecology and evolutionary biology. Trends in Ecology & Evolution 22:250–257.

Kaltz, O., and J. A. Shykoff. 2001. Male and female *Silene latifolia* plants differ in per-contact

risk of infection by a sexually transmitted disease. Journal of Ecology 89:99–109.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado.

Keith, A. R., J. K. Bailey, and T. G. Whitham. 2010. A genetic basis to community repeatability and stability. Ecology 91:3398–3406.

Kelly, A. E., and M. L. Goulden. 2008. Rapid shifts in plant distribution with recent climate change. Proceedings of the National Academy of Sciences 105:11823–11826.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, R. J. Mitchell, and T.-L. Ashman. 2005. Pollen limitation of plant reproduction: Pattern and process. Annual Review of Ecology, Evolution, and Systematics 36:467–497.

Kozłowski, J. 1992. Optimal allocation of resources to growth and reproduction: Implications for age and size at maturity. Trends in Ecology & Evolution 7:15–19.

Kreuzer, M., Jr, A. M. Ray, and R. S. Inouye. 2003. The use of data loggers to monitor environmental state changes: Snow melt and loss of surface water. Bulletin of the Ecological Society of America 84:27–29.

la Cruz, de, M. 2008. Metodos para analizar datos puntuales. Pages 76–127 *in* F. T. Maestre, A. Escudero, and A. Bonet, editors. Introduccion al Analisis Espacial de Datos en Ecologia y Ciencias Ambientales Metodos y Aplicaciones. Madrid.

la Cruz, de, M., R. L. Romao, A. Escudero, and F. T. Maestre. 2008. Where do seedlings go? A spatio-temporal analysis of seedling mortality in a semi-arid gypsophyte. Ecography 31:720–730.

Lailvaux, S. P. 2007. Interactive effects of sex and temperature on locomotion in reptiles.

Integrative and Comparative Biology 47:189–199.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.

Langenheim, J. H. 1962. Vegetation and environmental patterns in the Crested Butte area, Gunnison County, Colorado. Ecological Monographs 32:249–285.

Le Galliard, J.-F., P. S. Fitze, R. Ferrière, and J. Clobert. 2005. Sex ratio bias, male aggression, and population collapse in lizards. Proceedings of the National Academy of Sciences 102:18231–18236.

Lenoir, J., J. C. Gegout, P. A. Marquet, P. de Ruffray, and H. Brisse. 2008. A significant upward shift in plant species optimum elevation during the 20th century. Science 320:1768–1771.

Lloyd, D. G., and C. J. Webb. 1977. Secondary sex characters in plants. Botanical Review 43:177–216.

Lodé, T., M. J. Holveck, D. Lesbarrères, and A. Pagano. 2004. Sex–biased predation by polecats influences the mating system of frogs. Proceedings of the Royal Society B: Biological Sciences 271:S399–S401.

Manly, B. F. J. 2007. Randomization, bootstrap, and Monte Carlo methods in biology. Third edition. Chapman & Hall, London.

Marquis, R. J., and C. Whelan. 1996. Plant morphology and recruitment of the third trophic level: Subtle and little-recognized defenses? Oikos 75:330–334.

Maurer, E. P., L. Brekke, T. Pruitt, and P. B. Duffy. 2007. Fine-resolution climate projections enhance regional climate change impact studies. Eos, Transactions American Geophysical Union 88:504–504.

Meneses, N., J. K. Bailey, G. J. Allan, R. K. Bangert, M. A. Bowker, B. J. Rehill, G. M. Wimp,

R. L. Lindroth, and T. G. Whitham. 2012. Arthropod community similarity in clonal stands of aspen: a test of the genetic similarity rule. Écoscience 19:48–58.

Merow, C., A. M. Latimer, A. M. Wilson, S. M. McMahon, A. G. Rebelo, and J. A. Silander. 2014. On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data. Ecography 37:1167–1183.

Metcalf, C. J. E., S. M. McMahon, R. Salguero-Gómez, and E. Jongejans. 2012. IPMpack: an R package for integral projection models. Methods in Ecology and Evolution 4:195–200.

Meurman, O. 1925. The chromosome behaviour of some dioecious plants and their relatives with special reference to the sex chromosomes. Societas Scientiarum Fennica, Commentationes Biologicae II 2:1–104.

Meyer, F. G. 1951. *Valeriana* in North America and the West Indies (Valerianaceae). Annals of the Missouri Botanical Garden 38:377–503.

Miller, M. R., A. White, K. Wilson, and M. Boots. 2007. The population dynamical implications of male-biased parasitism in different mating systems. PLoS One 2:e624.

Miller, T. E. X. 2007. Demographic Models Reveal the Shape of Density Dependence for a Specialist Insect Herbivore on Variable Host Plants. Journal of Animal Ecology 76:722–729.

Miller, T. E. X., A. K. Shaw, B. D. Inouye, and M. G. Neubert. 2011. Sex-biased dispersal and the speed of two-sex invasions. The American Naturalist 177:549–561.

Miller, T. E. X., and B. D. Inouye. 2011. Confronting two-sex demographic models with data. Ecology 92:2141–2151.

Miller, T. E. X., and B. D. Inouye. 2013. Sex and stochasticity affect range expansion of experimental invasions. Ecology Letters 16:354–361.

Ming, R., A. Bendahmane, and S. S. Renner. 2011. Sex chromosomes in land plants. Annual Review of Plant Biology 62:485–514.

Mitchell, R. J. 2004. Heritability of nectar traits: Why do we know so little? Ecology 85:1527–1533.

Mooney, K. A., A. Fremgen, and W. K. Petry. 2012. Plant sex and induced responses independently influence herbivore performance, natural enemies and aphid-tending ants. Arthropod-Plant Interactions 6:553–560.

Mooney, K. A., and A. A. Agrawal. 2008. Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. The American Naturalist 171:E195–E205.

Mooney, K. A., and M. S. Singer. 2012. Plant variation in herbivore–enemy interactions in natural systems. Pages 107–130 *in* T. Ohgushi, O. J. Schmitz, and R. D. Holt, editors. Ecology and evolution of trait-mediated indirect interactions: Linking evolution, community, and ecosystem. Cambridge University Press, Cambridge.

Morbey, Y. E., and R. C. Ydenberg. 2001. Protandrous arrival timing to breeding areas: a review. Ecology Letters 4:663–673.

Morueta-Holme, N., K. Engemann, P. Sandoval-Acuña, J. D. Jonas, R. M. Segnitz, and J.-C. Svenning. 2015. Strong upslope shifts in Chimborazo's vegetation over two centuries since Humboldt. Proceedings of the National Academy of Sciences 112:12741–12745.

Muggeo, V. 2008. Segmented: an R package to fit regression models with broken-line relationships. R News 8:20–25.

Myers, J. P. 2011. A test of three hypotheses for latitudinal segregation of the sexes in wintering birds. Canadian Journal of Zoology 59:1527–1534.

Nakagawa, S., and R. P. Freckleton. 2011. Model averaging, missing data and multiple imputation: a case study for behavioural ecology. Behavioral Ecology and Sociobiology 65:103–116.

National Operational Hydrologic Remote Sensing Center. 2004. Snow Data Assimilation System (SNODAS). National Snow and Ice Data Center, Boulder, Colorado.

Ness, J. H., W. F. Morris, and J. L. Bronstein. 2009. For ant-protected plants, the best defense is a hungry offense. Ecology 90:2823–2831.

Oddie, K. R. 2000. Size matters: Competition between male and female great tit offspring. Journal of Animal Ecology 69:903–912.

Pacini, E., and M. Nepi. 2007. Nectar production and presentation. Pages 167–214 *in* S. W. Nicolson, M. Nepi, and E. Pacini, editors. Nectaries and nectar. Springer, Dordrecht, The Netherlands.

Parker, G. A., R. R. Baker, and V. G. F. Smith. 1972. Origin and evolution of gamete dimorphism and male-female phenomenon. Journal of Theoretical Biology 36:529–553.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Parolo, G., and G. Rossi. 2008. Upward migration of vascular plants following a climate warming trend in the Alps. Basic and Applied Ecology 9:100–107.

Peacor, S. D., and E. E. Werner. 1997. Trait-mediated indirect interactions in a simple aquatic food web. Ecology 78:1146–1156.

Petry, W. K., K. I. Perry, and K. A. Mooney. 2012. Influence of macronutrient imbalance on native ant foraging and interspecific interactions in the field. Ecological Entomology 37:175–183.

Petry, W. K., K. I. Perry, S. K. Rudeen, M. Lopez, J. Dryburgh, and K. A. Mooney. 2013. Mechanisms underlying plant sexual dimorphism in multi-trophic arthropod communities. Ecology 94:2055–2065.

Pickering, C. M., and W. Hill. 2002. Reproductive ecology and the effect of altitude on sex ratios in the dioecious herb *Aciphylla simplicifolia* (Apiaceae). Australian Journal of Botany 50:289–300.

Pickering, C. M., M. Jordan, and W. Hill. 2003. Sexual dimorphism and sex ratios of two Australian dioecious species of alpine pineapple grass, *Astelia alpina* var. *novaehollandiae* and *Astelia psychrocharis* (Asteliaceae). Nordic Journal of Botany 23:225–236.

Pinheiro, J., and D. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer Science & Business Media, New York.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, R Core Team. 2015. nlme: Linear and nonlinear mixed effects models. CRAN.

Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. The American Naturalist 147:813–846.

Pratt, J. D., and K. A. Mooney. 2013. Clinal adaptation and adaptive plasticity in *Artemisia californica*: Implications for the response of a foundation species to predicted climate change. Global Change Biology 19:2454–2466.

PRISM Climate Group. 2014. PRISM. Oregon State University.

Quinn, T. P., and M. T. Kinnison. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia 121:273–282.

R Core Team. 2012. R: A language environment for statistical computing. R Foundation for Statistical Computing.

R Core Team. 2015. R: A language environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rankin, D. J., and H. Kokko. 2007. Do males matter? The role of males in population dynamics. Oikos 116:335–348.

Ranta, E., V. Kaitala, and J. Lindström. 1999. Sex in space: Population dynamic consequences. Proceedings: Biological Sciences 266:1155–1160.

Renner, S. S. 2014. The relative and absolute frequencies of angiosperm sexual systems: Dioecy, monoecy, gynodioecy, and an updated online database. American Journal of Botany 101:1588–1596.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural-population. Nature 346:357–359.

Rubin, D. B. 1976. Inference and missing data. Biometrika 63:581–592.

Ruckstuhl, K., and P. Neuhaus. 2006. Sexual segregation in vertebrates. Cambridge University Press.

Rudgers, J. A. 2004. Enemies of herbivores can shape plant traits: Selection in a facultative ant-plant mutualism. Ecology 85:192–205.

Salguero-Gómez, R., O. R. Jones, C. R. Archer, C. Bein, H. Buhr, C. Farack, F. Gottschalk, A. Hartmann, A. Henning, G. Hoppe, G. Römer, T. Ruoff, V. Sommer, J. Wille, J. Voigt, S. Zeh, D. Vieregg, Y. M. Buckley, J. Che-Castaldo, D. Hodgson, A. Scheuerlein, H. Caswell, and J. W. Vaupel. 2016. COMADRE: a global data base of animal demography. Journal of Animal Ecology 85:371–384.

Salguero-Gómez, R., O. R. Jones, C. R. Archer, Y. M. Buckley, J. Che-Castaldo, H. Caswell, D. Hodgson, A. Scheuerlein, D. A. Conde, E. Brinks, H. de Buhr, C. Farack, F. Gottschalk, A.

Hartmann, A. Henning, G. Hoppe, G. Römer, J. Runge, T. Ruoff, J. Wille, S. Zeh, R. Davison, D. Vieregg, A. Baudisch, R. Altwegg, F. Colchero, M. Dong, H. de Kroon, J.-D. Lebreton, C. J. E. Metcalf, M. M. Neel, I. M. Parker, T. Takada, T. Valverde, L. A. Vélez-Espino, G. M. Wardle, M. Franco, and J. W. Vaupel. 2015. The COMPADRE Plant Matrix Database: an open online repository for plant demography. Journal of Ecology 103:202–218.

Schindler, S., J.-M. Gaillard, A. Grüning, P. Neuhaus, L. W. Traill, S. Tuljapurkar, and T. Coulson. 2015. Sex-specific demography and generalization of the Trivers-Willard theory. Nature 526:249–252.

Scudo, F. M. 1967. The adaptive value of sexual dimorphism: I, Anisogamy. Evolution 21:285–291.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics 40:415–436.

Shaw, A. K., and H. Kokko. 2014. Mate finding, Allee effects and selection for sex-biased dispersal. Journal of Animal Ecology 83:1256–1267.

Shelton, A. O. 2008. Skewed sex ratios, pollen limitation, and reproductive failure in the dioecious seagrass *Phyllospadix*. Ecology 89:3020–3029.

Shelton, A. O. 2010a. The ecological and evolutionary drivers of female-biased sex ratios: Two-sex models of perennial seagrasses. The American Naturalist 175:302–315.

Shelton, A. O. 2010b. The origin of female-biased sex ratios in intertidal seagrasses (*Phyllospadix* spp.). Ecology 91:1380–1390.

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. The Quarterly Review of Biology 64:419–461.

Shuster, S. M., E. V. Lonsdorf, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2006.

Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. Evolution 60:991–1003.

Soule, J. D. 1981. Ecological consequences of dioecism in plants: a case study of sex differences, sex ratios and population dynamics of *Valeriana edulis* Nutt. Michigan State University, East Lansing.

Spiess, A.-N. 2014. propagate: Propagation of uncertainty. CRAN.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press.

Steltzer, H., C. Landry, T. H. Painter, J. Anderson, and E. Ayres. 2009. Biological consequences of earlier snowmelt from desert dust deposition in alpine landscapes. Proceedings of the National Academy of Sciences 106:11629–11634.

Stewart, I. T. 2009. Changes in snowpack and snowmelt runoff for key mountain regions. Hydrological Processes 23:78–94.

Strauss, S. Y., H. Sahli, and J. K. Conner. 2005. Toward a more trait-centered approach to diffuse (co)evolution. New Phytologist 165:81–89.

Tack, A. J. M., M. T. J. Johnson, and T. Roslin. 2012. Sizing up community genetics: it's a matter of scale. Oikos 121:481–488.

Telwala, Y., B. W. Brook, K. Manish, and M. K. Pandit. 2013. Climate-induced elevational range shifts and increase in plant species richness in a Himalayan biodiversity epicentre. PLoS One 8:e57103.

Therneau, T. 2014. deming: Deming, Thiel-Sen and Passing-Bablock regression. CRAN.

Thompson, J., A. Charpentier, G. Bouguet, F. Charmasson, S. Roset, B. Buatois, P. Vernet, and P. H. Gouyon. 2013. Evolution of a genetic polymorphism with climate change in a Mediterranean landscape. Proceedings of the National Academy of Sciences 110:2893–

2897.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90–92.

van Buuren, S., and K. Groothuis-Oudshoorn. 2011. mice: Multivariate imputation by chained equations in R. Journal of Statistical Software 45:1–67.

Vandermeer, J. 1978. Choosing category size in a stage projection matrix. Oecologia 32:79–84.

Veran, S., and S. R. Beissinger. 2009. Demographic origins of skewed operational and adult sex ratios: Perturbation analyses of two-sex models. Ecology Letters 12:129–143.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: Intraspecific variability in community ecology. Trends in Ecology & Evolution 27:244–252.

Waser, N. M. 1984. Sex ratio variation in populations of a dioecious desert perennial, *Simmondsia chinensis*. Oikos 42:343–348.

Wäckers, F. L. 2005. Suitability of (extra-) floral nectar, pollen, and honeydew as insect food sources. Pages 17–74 *in* F. L. Wäckers, P. C. J. van Rijn, and J. Bruin, editors. Plant-provided food for carnivorous insects a protective mutualism and its applications. Cambridge, UK.

Wearmouth, V. J., and D. W. Sims. 2008. Sexual segregation in marine fish, reptiles, birds and mammals. Advances in Marine Biology 54:107–170.

Wesselingh, R. A., P. G. L. Klinkhamer, T. J. DeJong, and L. A. Boorman. 1997. Threshold size for flowering in different habitats: Effects of size-dependent growth and survival. Ecology 78:2118–2132.

West, S. A. 2009. Sex allocation. (S. A. Levin and H. S. Horn, Eds.) Monographs in Population

Biology. Princeton University Press, Princeton.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. LeRoy, E. V. Lonsdorf, G. J. Allan, S. P. DiFazio, and B. M. Potts. 2006. A framework for community and ecosystem genetics: From genes to ecosystems. Nature Reviews Genetics 7:510–523.

Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster, G. M. Wimp, D. G. Fischer, J. K. Bailey, and R. L. Lindroth. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. Ecology 84:559–573.

Williams, G. C. 1975. Sex and evolution. Princeton University Press.

Williams, J. L., T. E. X. Miller, and S. P. Ellner. 2012. Avoiding unintentional eviction from integral projection models. Ecology 93:2008–2014.

Wimp, G. M., and T. G. Whitham. 2001. Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. Ecology 82:440–452.

Wimp, G. M., S. Wooley, R. K. Bangert, W. P. Young, G. D. Martinsen, P. Keim, B. J. Rehill, R. L. Lindroth, and T. G. Whitham. 2007. Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. Molecular Ecology 16:5057–5069.

Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Keim, and T. G. Whitham. 2004. Conserving plant genetic diversity for dependent animal communities. Ecology Letters 7:776–780.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443–466.

Xu, X., F. Yang, X. Xiao, S. Zhang, H. Korpelainen, and C. Li. 2008a. Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. Plant, Cell and Environment 31:850–860.

Xu, X., G. Peng, C. Wu, H. Korpelainen, and C. Li. 2008b. Drought inhibits photosynthetic capacity more in females than in males of *Populus cathayana*. Tree Physiology 28:1751–1759.

Zeileis, A., C. Kleiber, and S. Jackman. 2008. Regression models for count data in R. Journal of Statistical Software 27:1–25.

Zuk, M., and K. A. McKean. 1996. Sex differences in parasite infections: Patterns and processes. International Journal for Parasitology 26:1009–1024.

Zytynska, S. E., M. F. Fay, D. Penney, and R. F. Preziosi. 2011. Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. Philosophical Transactions of the Royal Society B: Biological Sciences 366:1329–1336.

Zytynska, S. E., M. S. Khudr, E. Harris, and R. F. Preziosi. 2012. Genetic effects of tank-forming bromeliads on the associated invertebrate community in a tropical forest ecosystem. Oecologia 170:467–475.