Identifying the fitness consequences of sex in complex natural environments

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In the natural world, sex prevails, despite its costs. Although much effort has been dedicated to identifying the intrinsic costs of sex (e.g., the cost of males), few studies have identified the ecological fitness consequences of sex. Furthermore, correlated biological traits that differ between sexuals and asexuals may alter these costs, or even render the typical costs of sex irrelevant. We conducted a large-scale, multisite, reciprocal transplant using multiple sexual and asexual genotypes of a native North American wildflower to show that sexual genotypes have reduced lifetime fitness, despite lower herbivory. We separated the effects of sex from those of hybridity, finding that overwinter survival is elevated in asexuals regardless of hybridity, but herbivores target hybrid asexuals more than nonhybrid asexual or sexual genotypes. Survival is lowest in homozygous sexual lineages, implicating inbreeding depression as a cost of sex. Our results show that the consequences of sex are shaped not just by sex itself, but by complex natural environments, correlated traits, and the identity and availability of mates.

KEY WORDS: Apomixis, asexuality, *Boechera*, evolution of sex, herbivory, hybridization, reciprocal transplant, viability selection.

Impact Summary

The evolution of sex is a fundamental mystery in evolutionary biology. Why do organisms reproduce sexually, when asexuality allows organisms to avoid so much—the production of males, the risks of breaking up well-adapted genetic networks, the dangerous search for mates, and the maintenance of costly secondary sexual characteristics? Research has long predicted that the persistence of both sexual and asexual reproduction is due to a balance between the many costs and benefits of each. Yet few studies have compared the fitness of sexual and asexual organisms, and fewer still have been conducted in the natural environment in which they have evolved. Furthermore, although hybridization and whole genome duplication often co-occur with asexuality in many species, no studies to our knowledge have disentangled the effects of these complicating traits from those of sex itself. We conducted a multisite reciprocal transplant experiment of wild-collected sexual and asexual plants belonging to the mustard genus *Boechera*, which is highly self-fertilizing when sexual. Importantly, asexual *Boechera* are formed by outcrossing events either between or within species. We found that asexual genotypes had higher overall fitness at all sites and in both experimental years, and that this fitness increase was driven by higher overwinter survival of asexuals. Survival in heterozygous sexual lineages was similar to that of asexual lineages, suggesting that inbreeding depression may underlie the lower fitness of sexual genotypes in this plant. We further found that asexual lineages experienced greater insect herbivory than sexual lineages, and that herbivory was highest on hybrid (between-species) asexuals. Our study shows that the evolution of sex is driven by a combination of environmental factors and the availability and

identity of mates, and suggests a key role for correlated traits in this process so critical to all of evolution.

Despite over a century of research, sex remains a fundamental mystery in evolutionary biology. Although recombination removes deleterious alleles and unites beneficial ones (Hill and Robertson 1966; Keightley and Otto 2006), enabling organisms to adapt (Felsenstein 1974) and evolve with antagonists (Jaenike 1978), sex has many costs. These typically include the "twofold cost," wherein sexual organisms produce half as many offspring as asexuals due to their investment in male offspring (Maynard Smith 1978), as well as the disruption of co-adapted beneficial loci via recombination (Lehtonen et al. 2012). Although theory has identified many costs and benefits of sex, these are likely mediated by the biotic and abiotic selective environment, as well as the patterns of mating among sexuals, and the biological processes through which asexuality arises.

The genetic processes maintaining sex have been widely studied, but the ecological context in which sex occurs—the selective agents and patterns of selection occurring in the natural environment—has received far less attention. This is a crucial gap in our knowledge, as the processes governing sexual/asexual dynamics occur in complex natural environments. Much of our understanding of the ecology of sex comes from decades of powerful experimental work on the freshwater snail, which has established sterilizing trematode parasites as the primary selective agent maintaining sex via frequency-dependent selection. This system offers a key example of Red Queen dynamics, wherein sexual reproduction generates novel lineages that avoid infection. Water depth moderates parasite frequency, leading to a striking ecological pattern: sex predominates in shallow water, where secondary hosts occur, whereas asexuality is widespread in deeper water (Lively 1987; Jokela and Lively 1995; Lively and Dybdahl 2000; Vergara et al. 2014). This research shows that the abiotic and biotic environments operate in concert to shape sexual/asexual dynamics in the natural world. Critically, both ecology and its interactions with a system's biological features provide the context necessary to pinpoint the real-life costs and benefits of sex (Neiman et al. 2018).

Similarly, very few studies have accounted for the widespread observation that sex and asex are often differently associated with fundamental biological and life history traits, despite the critical role these traits play in shaping the costs of sex (Meirmans et al. 2012). For example, the cost of males will be minimized in plants with hermaphroditic, self-compatible flowers (Charlesworth 1980; Mogie 1996); as such, frequent inbreeding will further minimize this cost. Additional costs of asexuality may be rooted in the origin and composition of asexual lineages. Although polyploidy (whole genome duplication) and hybridity

(origins via hybridization) are overrepresented in asexual plants and vertebrates as compared to sexuals (e.g., Vrijenhoek 1978; Asker and Jerling 1992; Lutes et al. 2010), the impact of these key traits is rarely considered. Further costs of sex may be related to mate choice (inbreeding, outcrossing, and hybridization, or outcrossing between widely divergent taxa) and mate availability for sexual organisms, which have profound phenotypic and genetic consequences (Meirmans et al. 2012). Hybridization alters numerous fitness-related traits, which can increase or decrease fitness (Yakimowski and Rieseberg 2014). Importantly, a transition to asexuality enables persistence of hybrid genotypes, regardless of the fitness effects of hybridization (Asker and Jerling 1992). The genetic impacts of mate choice will similarly influence the evolution of sex. For example, sex via inbreeding can expose a recessive genetic load (Charlesworth and Willis 2009), whereas asexual reproduction may maintain heterozygosity. Such life history traits likely contribute substantially to sexual/asexual dynamics, but are rarely discussed in studies of the evolution of sex.

The mustard genus *Boechera* offers a rare opportunity to study the ecological costs of sex while accounting for multiple sex-associated biological traits. Apomixis (asexual reproduction via seed) is widespread in the genus (Böcher 1951; Schranz et al. 2005; Aliyu et al. 2010). These asexuals may be polyploid or diploid, enabling separate assessment of the effects of polyploidy and asexuality (Kantama et al. 2007; Beck et al. 2012; Mau et al. 2015). Furthermore, lineages that reproduce asexually are derived from outcrossing events, which may be either interspecific or intraspecific (Li et al. 2017; Rushworth et al. 2018), allowing separable examination of hybridity and asexuality. Finally, similar to other asexual systems (e.g., Lutes et al. 2010; Fradin et al. 2017), asexuals are highly heterozygous, whereas sexual *Boechera* are highly self-fertilizing and thus highly homozygous (Roy 1995; Song et al. 2006; Li et al. 2017). This particular trait is unusual in sexual/asexual plant systems, as nearly all known apomictic plants are derived from outcrossers (Asker and Jerling 1992). Yet inbreeding is of broad relevance to all sexual/asexual systems, as all sexual organisms undergo shifts in population size and variable biparental inbreeding. In other words, the consequences of sex will always depend in large part on the identity of available sexual partners. Contrasting asexual and inbred sexual lineages offers sharp insight into the importance of fixing heterozygous genotypes in the origins, maintenance, and spread of apomixis. For this reason, the well-characterized mating system of *Boechera* offers an ideal system for studying the evolution of sex.

Using a reciprocal transplant experiment in intact native habitat, we identified the fitness components underlying the ecological costs of sex in *Boechera*. We examined female lifetime fitness and insect herbivory of sexual and asexual plants,

finding strong overwinter viability selection against sexual lineages, which is lessened in heterozygous sexuals. This survival difference resulted in increased lifetime fitness for asexual lineages, despite elevated insect herbivory on hybrid asexuals. Importantly, the negative impacts of herbivory appeared in later life stages, reducing the probability of survival to a second year, consistent with a cost of hybridization. Numerous lines of evidence suggest that the cost of sex in this system is due at least in part to inbreeding depression in highly homozygous sexual lineages.

Methods

STUDY SYSTEM

Boechera retrofracta (Brassicaceae) is a short-lived perennial wildflower native to western North America. Multiple *Boechera* species are highly self-fertilizing (Hamilton and Mitchell-Olds 1994; Roy 1995; Song et al. 2006), and *B. retrofracta*'s high microsatellite homozygosity (mean homozygosity = 0.94; Rushworth et al. 2018) and small flowers that self-pollinate before anthesis (C. Rushworth, pers. obs.) suggest frequent self-fertilization. Nonetheless, *B. retrofracta* forms hybrids with at least 12 species (Windham and Al-Shehbaz 2006). Asexuality is tightly associated with outcrossing and hybridization in *Boechera*; the vast majority of wild-collected asexual lineages appear to originate through interspecific or intraspecific outcrossing (Schranz et al. 2005; Kantama et al. 2007; Beck et al. 2012; Rushworth et al. 2018). Apomixis in this system is pseudogamous, requiring endosperm (but not embryo) fertilization (Böcher 1951). Because sexuals are highly selfing and asexuals must also produce a small amount of viable pollen, several of the traditional costs of sex are minimized in this system, allowing investigation of broader ecological and biological factors maintaining sex.

SAMPLING AND GROWTH METHODS

We used 53 wild-collected diploid genotypes (24 sexual and 29 asexual) in a multisite, reciprocal transplant experiment (see Table S1). Species (or parental species, in the case of asexuals) were determined based on microsatellites and morphology (Rushworth et al. 2018) using the *Boechera* Microsatellite Website (Li et al. 2017). Heterozygosity, estimated from microsatellites, was calculated for each maternal lineage. Twenty-one sexual genotypes were entirely homozygous, and three were not (heterozygosity ranging from 0.08 to 0.42); heterozygosity of all but one asexual genotypes was >0.5 (Figure S1). Eighteen asexual genotypes were "hybrid asexuals," resulting from interspecific hybridization events, whereas 11 are "nonhybrid asexuals," resulting from within-species outcrossing. One sexual genotype was not *B. retrofracta*, and was not analyzed; all asexual genotypes but one ("A5") involved *B. retrofracta* as a parental species.

Other parental species represented in hybrids were *Boechera sparsiflora*, *Boechera exilis*, *Boechera pendulocarpa*, *Boechera stricta*, and *Boechera puberula*. To ensure that experimental selection pressures approximated those of each genotype's native environment, we selected lines based on proximity or similarity of collection and garden environments. All but two genotypes were collected within 10 km of an experimental garden.

To minimize maternal environmental effects, genotypes were grown in controlled conditions for one generation before the experiment. Seeds were germinated on wet filter paper in petri dishes and transplanted as seedlings onto a blend of Fafard 4P Mix and MetroMix 200 (Sun Gro Horticulture, Agawam, MA, USA) in Ray Leach Cone-tainers (Steuwe and Sons, Tangent, OR, USA) in the Duke University greenhouses, and shipped as rosettes for transplanting at 6 weeks of age. Transplantation into replicated, randomized blocks occurred in fall for data collection the following season, allowing synchronization of flowering time with the native vegetation.

TRANSPLANT EXPERIMENT

Subsets of the 53 genotypes were planted in two cohorts of replicated, randomized blocks in the fall of 2012 and 2013. Data were collected for two growth seasons for each planting year, totaling 3 years of data collection. Planting year 1 consisted of 2000 individuals representing 19 asexual and 22 sexual genotypes, planted in five gardens with 400 plants/site (ALD, CAP, CUM, MIL, and SIL; Table S1). In year two, 2080 individuals representing 18 asexual and 21 sexual lineages were transplanted at the same sites (520 plants/site; Figure S2). However, the SIL site was excluded from year 2 because its dense vegetation and high humidity are atypical *B. retrofracta* habitat. Overwinter survival was scored the following spring. Plants that survived were measured for width, height, flower number, and fruit number. Due to low reproduction, MIL in year 1 ($N = 34$ reproducing plants) and CUM in year 2 ($N = 2$) were excluded from analyses. Leaf damage was quantified visually by trained observers, as in Prasad et al. (2012). The CUM site was excluded in herbivory analyses, as no herbivory measurements were made there. Prior to fruit dehiscence, one to four fruits were collected from two to three replicates of each genotype within each garden. Seeds were counted from each fruit and averaged across replicates to estimate average seed set per genotype per garden. Fecundity was estimated as average seed set per fruit multiplied by individual fruit number. This average was rounded to the nearest integer to allow us to model seed set as a negative binomially distributed random variable.

POLLEN VIABILITY

To evaluate how sexual system impacted pollen viability, we grew 25 sexual and 26 asexual genotypes in a growth chamber at the Duke University Phytotron. We collected mature developing buds

(stage 12; Smyth et al. 1990) from one to three individuals per genotype ($N = 112$ individual plants), and stained pollen to differentiate viable from inviable grains (Peterson et al. 2010), averaging pollen viabilities across replicates. For each individual, 100 pollen grains were randomly counted from a microscopy slide visualized on a (Leica Camera AG, Wetzlar, Germany) MZ7.5 Stereozoom dissecting microscope and imaged using a (Canon Inc., Tokyo, Japan) EOS Rebel T3i digital camera.

STATISTICAL ANALYSES

Our primary aim was to evaluate the association between Reproductive System (*RS*; a factor that takes the value of sexual or asexual) and group (*group*, a factor that can take the value of sexual, hybrid asexual, or nonhybrid asexual) with fitness components over time and space. Because sexuals and asexuals differ intrinsically in heterozygosity, any observed fitness differences may stem from inbreeding or outbreeding depression. We conducted additional analyses to address this possibility.

All analyses were performed using R version 3.5.2 (R Core Team 2018). Outliers were visually identified using an adjusted boxplot analysis suitable for far-right-skewed data (Hubert and Vandervieren 2008), with robustbase (Maechler et al. 2019). To analyze fitness data, we used generalized linear mixed models (GLMMs) in glmmTMB (Brooks et al. 2017). Model diagnostics were conducted using DHARMa (Hartig 2019) and marginal means were estimated using the package ggeffects (Lüdecke 2018). Lifetime fitness was estimated using a zero-inflated negative binomial distribution with the canonical link functions, where structural zeros represent plants that did not survive the winter and the conditional portion of the model represents fecundity of survivors. Separate fecundity and survival models were also run, using the negative binomial and binomial error distributions, respectively. The model used was as follows:

$$y \sim RS + garden + RS \times garden + year + RS \times year$$

where *y* indicates a fitness component, lifetime fitness, or herbivory. Two random effects were included in all models: block nested in site and genotype nested in *RS*. The same structure was used for the zero inflation and conditional portions of the zero-inflated negative binomial model for lifetime fitness. In all estimable models, a random effect of genotype nested in reproductive system, crossed with garden, accounted for essentially no variance, and was therefore removed from analyses. A scaled covariate for width at planting was included in survival models, whereas a scaled covariate of plant height was added to all herbivory models to account for a link between plant size and insect attraction.

We next tested for fitness differences among hybrid asexual genotypes, nonhybrid asexual genotypes, and sexual genotypes,

by running the same models as above, replacing the *RS* term with the *group* term. To assess the effects of heterozygosity on survival, we used fixed-effects-only models to analyze survival of heterozygous and homozygous sexual lineages, and complementary analyses (heterozygous sexuals vs. asexuals; homozygous sexuals vs. asexuals) were also conducted. Significance testing for fitness analyses was conducted via likelihood ratio tests (Bolker et al. 2009) with Holm corrections (Holm 1979). Although the variance in fitness likely plays an important role in the long- and short-term maintenance of sex (Agrawal 2006), data structured with the mean-variance relationship modeled by GLMMs prevent the independent estimation of mean and variance. We therefore do not discuss the variance in fitness.

Leaf damage was log-transformed and analyzed using a linear mixed model (LMM) in lme4 (Bates et al. 2015). Significance testing was conducted using *F* tests with Kenward-Roger approximation of denominator degrees of freedom (Kenward and Roger 1997) using pbkrtest (Halekoh and Højsgaard 2014).

To understand the relationship between herbivory and fitness, and how it differs among groups, two additional models were estimated with log herbivory and its interaction with group and garden as predictors. Each model examined a fitness component: fecundity and survival to a second year, both of which are conditional upon survival to year 1 and nonzero herbivory. We did not include higher order interaction terms, as our experimental questions were satisfied by the following simpler model:

$$y \sim group + garden + year + group \times garden + group \times year$$
$$+ herbivory + group \times herbivory + garden \times herbivory$$

Results

HIGHER ASEXUAL LIFETIME FITNESS DUE TO SURVIVAL ADVANTAGE

Higher asexual lifetime fitness

Lifetime fitness, the product of survival and reproduction, was higher in asexual than sexual genotypes ($\chi^2 = 11.90$, df = 2, $P = 7.82 \times 10^{-3}$). The effect of RS depended on year ($\chi^2 = 34.74$, df = 2, $P = 1.72 \times 10^{-7}$) and garden ($\chi^2 = 23.45$, df = 8, $P = 0.016$; Table S2). To disentangle the effects of hybridization and sex, we analyzed the same data with the group term. This model similarly showed an effect of group ($\chi^2 = 13.33$, df = 4, $P = 0.039$), and its interaction with both year ($\chi^2 = 36.87$, df = 4, $P = 1.53 \times 10^{-6}$) and garden ($\chi^2 = 66.70$, df = 16, $P = 3.00 \times 10^{-7}$; Tables 1 and S2). Estimated lifetime fitness for each group varied across year and site, but was elevated in both asexual groups, with consistently high hybrid asexual fitness (Figure S3). While our model design did not allow us to predict overall lifetime fitness for each group without accounting for interactions, raw

Table 1. Reproductive system (sexual vs. asexual) and hybridity predict lifetime fitness via spatially and temporally variable selection. Results for the main effect of group and two interactions (group and garden, and group and year) for zero-inflated negative binomial GLMMs of lifetime fitness. Left, the zero-inflation portion models structural zeros or plants that did not survive to reproduce. Center, the conditional portion models seed set in plants that survived (fecundity). Right, significance estimates for the overall model, incorporating both portions. Group indicates sexuals "sex" versus asexual hybrids "hyb" versus asexual nonhybrids, the reference category. Estimates come from conditional models, whereas test statistics (χ^2 deviance, degrees of freedom, and P-values) come from likelihood ratio tests for each overall effect. Significant P-values are in bold. Full results from each separate model are in Supplementary Information.

| Term Condition		df	Zero-inflation				Conditional				Overall model		
			Coef.	SE	χ^2	P-value	Coef.	SE	χ^2	P-value	df	χ^2	P-value
Group	sex	2	0.339	0.468	12.22	**0.01**	−0.329	0.254	1.12	0.57	4	13.33	**0.039**
	hyb		−0.528	0.487			−0.357	0.250					
Group × Garden	sex × CAP	8	0.129	0.342	42.82	**6.66×10^{-6}**	0.048	0.221	23.70	**0.01**	16	66.70	**3.00×10^{-7}**
	hyb × CAP		0.977	0.349			0.485	0.208					
	sex × CUM		−0.596	0.495			0.377	0.319					
	hyb × CUM		0.376	0.517			0.682	0.322					
	sex × MIL		−0.278	0.394			0.052	0.233					
	hyb × MIL		1.063	0.402			0.435	0.228					
	sex × SIL		−1.167	0.661			1.843	0.481					
	hyb × SIL		−1.746	0.663			1.895	0.463					
Group × Year	sex × 2	2	1.103	0.378	23.56	**5.36×10^{-5}**	0.594	0.194	13.32	**5.16×10^{-3}**	4	36.87	**1.53×10^{-6}**
	hyb × 2		−0.402	0.407			0.071	0.193					
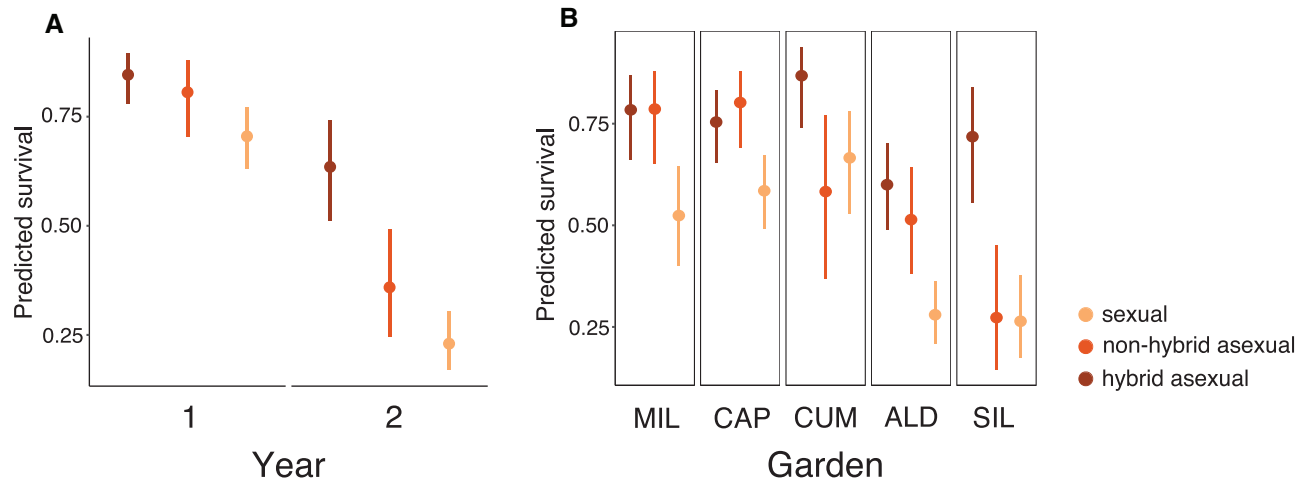
Figure 1. Asexual survival is higher than sexual. Estimated marginal means for survival show substantial temporal (A, group by year interaction) and spatial variation (B, group by garden interaction). (A) Survival is reduced in sexuals in both experimental years. (B) In all sites, hybrid asexuals have higher overwinter survival than sexuals; in three of five sites, nonhybrid asexual survival is similarly high. Bars show 95% confidence intervals. SIL was only included in year 1, but fitness was predicted at this site.

summaries showed higher overall fitness in both groups of asexuals, although years varied slightly (Year 1 means: sexual, 211.54; hybrid asexual, 289.40; nonhybrid asexual, 299.93; Year 2 means: sexual, 76.29; hybrid asexual, 181.57; nonhybrid asexual, 145.55; Figure S4). Estimated marginal means for all models are in Appendix S1.

Higher asexual survival

We next asked if fitness disparities were attributable to differences in survival or fecundity. The zero-inflation portion of a zero-inflated negative binomial GLMM addresses structural zeros, or the probability of not surviving to reproduce. Overwinter survival was significantly elevated in asexual genotypes compared to sexuals. In year 1, 81% of asexuals survived, whereas 64.3% of sexuals survived. In year 2, 56.5% of asexuals survived, compared with only 23.8% of sexuals.

Survival differed by reproductive system ($\chi^2 = 11.82$, df $= 1$, $P = 2.35 \times 10^{-3}$), and across year ($\chi^2 = 9.16$, df $= 1$, $P = 0.04$) and garden ($\chi^2 = 46.73$, df $= 4$, $P = 1.21 \times 10^{-8}$; Table S3A). In both years, asexuals had higher survival; the interactions between year and RS, and garden and RS, were also significant (year: $\chi^2 = 22.88$, df $= 1$, $P = 8.60 \times 10^{-6}$; garden: $\chi^2 = 16.23$, df $= 4$, $P = 0.016$; Table S3A). Similar results were seen in a separate model of survival (Table S4A). Plant size also increased plant survival ($\chi^2 = 54.20$, df $= 1$, $P = 7.24 \times 10^{-13}$).

Overwinter survival differed by group ($\chi^2 = 12.22$, df $= 2$, $P = 0.01$; Tables 1 and S3B), with both types of asexuals surviving better than sexuals in both cohorts (Figure 1A). The effects of group differed temporally (group × year interaction: $\chi^2 = 23.56$, df $= 2$, $P = 5.36 \times 10^{-5}$) and spatially (group × site interaction: $\chi^2 = 42.82$, df $= 8$, $P = 6.66 \times 10^{-6}$; Figure 1B; Tables 1

and S3B). A separate model of survival showed similar results (Table S4B). Hybrid asexuals were larger than other groups at planting ($\chi^2 = 53.79$, df $= 1$, $P = 7.24 \times 10^{-13}$; Table S4B). Across gardens, hybrid asexuals had higher predicted survival than sexuals, whereas nonhybrid asexual survival was more variable (Figure 1B). In three of five sites, survival was roughly equivalent in both asexual groups. In two sites, survival was similarly low for sexuals and nonhybrid asexuals.

Heterozygosity also impacted survival. Survival of heterozygous sexuals exceeded that of homozygous sexuals ($\chi^2 = 7.07$, df $= 1$, $P = 0.024$; Figure S5, Table S5), but did not result in increased total fitness for heterozygous sexuals (Table S6). Asexual and heterozygous sexual survival did not differ ($\chi^2 = 0.47$, df $= 1$, $P = 1$). Together, these results are consistent with inbreeding depression manifesting as reduced overwinter survival in homozygous sexual lineages.

No differences in fecundity

We next examined the conditional portion of the zero-inflated negative binomial GLMM, which analyzed fecundity (seed set conditional on survival). In total, 995 individuals set seed (597 asexuals and 398 sexuals); removing nine outliers left a dataset of 986 individuals. Seed set did not differ intrinsically between sexuals and asexuals, but the influence of RS varied by year ($\chi^2 = 11.86$, df $= 1$, $P = 1.72 \times 10^{-3}$; Table S3A). The conditional group model produced similar results: although the main effect of group was not significant, nonhybrid asexual fecundity was lowest in both years (Figure S6A). The influence of group varied by year ($\chi^2 = 13.32$, df $= 2$, $P = 5.13 \times 10^{-3}$; Tables 1 and S3B) and by garden ($\chi^2 = 23.70$, df $= 8$, $P = 0.01$; Tables 1 and S3B; Figure S6B). There were substantial environmental effects
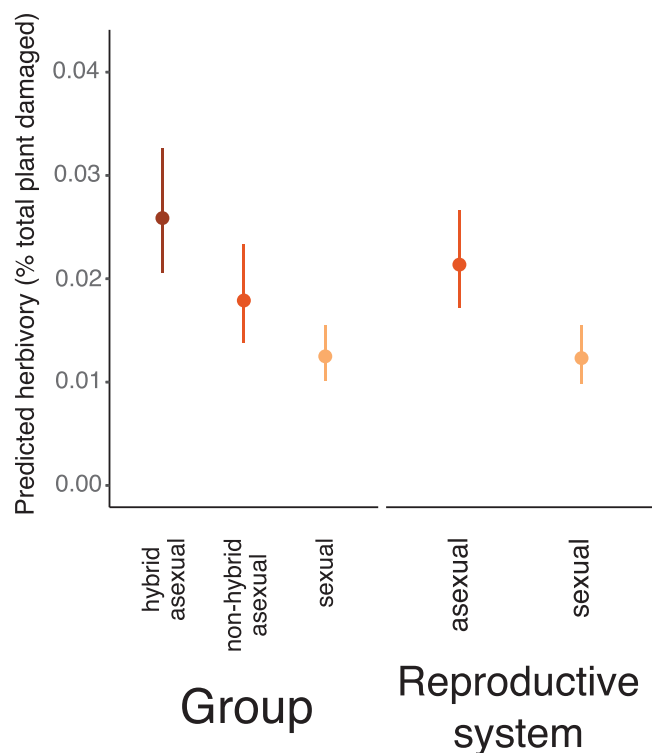
Figure 2. Herbivory is elevated in hybrid asexuals. Estimated marginal means for herbivory from LMMs show the main effects of group (sexual vs. nonhybrid asexual vs. hybrid asexual, left) and reproductive system (sexual vs. asexual, right). Bars show 95% confidence intervals.

on seed set (Table S3). Similar results were found in the separate fecundity models (Table S7). Pollen viability assays revealed no difference between sexuals and asexuals (pooled t-test: $t = 0.599$ on 49 df, $P = 0.55$), suggesting that any differences in seed set may be due to either maternal investment or pollen quantity. Heterozygosity had no impact on sexual fecundity ($\chi^2 = 1.24$, df $= 1$, $P = 0.26$; Table S8).

HERBIVORES TARGETED HYBRID ASEXUALS

A total of 33% of the experiment (1379 of 4080 plants) experienced leaf herbivory. The removal of 29 high-herbivory outliers resulted in a dataset of 1350 individuals (809 asexuals and 541 sexuals). Asexual herbivory was higher than sexual, with an average of 5.45% $\pm$ 0.002% leaf area damaged per asexual individual (estimated marginal mean: 2.14% $\pm$ 0.048%; 95% CI: 1.72–2.66%). By comparison, mean sexual leaf damage across years was 3.16% $\pm$ 0.002% (estimated marginal mean: 1.23% $\pm$ 0.05%; 95% CI: 0.98–1.55%), a significant difference ($F = 22.55$, df $= 1$, 48.29, $P = 9.33 \times 10^{-5}$; Table 2A; Figure 2). Herbivory varied by garden ($F = 6.40$, df $= 3$, 62.99, $P = 2.99 \times 10^{-3}$; Table 2A; Figure S7), consistent with spatial variation in herbivore communities. Herbivory was highest in hybrid asexu-

als (Figure 2). Group had a significant effect on herbivory ($F = 18.08$, df $= 2$, 42.39, $P = 1.26 \times 10^{-5}$; Table 2B), with both hybrid and nonhybrid asexuals experiencing higher leaf herbivory than sexuals (Figure 2). Plant height was significant in both models (Table 2).

HERBIVORY NEGATIVELY IMPACTED SURVIVAL TO SUBSEQUENT YEARS

What are the effects of herbivory on fitness, and do these effects differ between sexuals and asexuals? We next predicted two separate fitness components, fecundity and survival to a second year, using models incorporating a main effect of log-transformed herbivory, and its interaction with both group and garden.

Over 90% of experimental fitness occurred in the first year of life. In both years, more asexuals than sexuals survived to a second year. In experimental year 1, 8.2% of plants survived for a second year ($N = 163$: 108 asexuals and 55 sexuals). Similarly, 5.9% of plants in year 2 survived for a second year ($N = 123$: 104 asexuals and 19 sexuals). Roughly 1% of plants reproduced in their second year (Year 1: 1.4%, $N = 28$: 24 asexuals and four sexuals; Year 2: 1.3%, $N = 26$: 25 asexuals and one sexual). Nevertheless, survival to subsequent years could substantially increase lifetime fitness.

In temperate montane environments, herbivory and seed set co-occur during the short growing season, and may thus influence one another. We found no effect of herbivory on fecundity ($\chi^2 = 0.28$, df $= 1$, $P = 0.598$; Table S9), but herbivory decreased survival to a second year ($\chi^2 = 21.26$, df $= 1$, $P = 8.02 \times 10^{-6}$; Table S10). Group also affected subsequent survival ($\chi^2 = 32.88$, df $= 2$, $P = 5.79 \times 10^{-7}$; Table S10), with more hybrid asexuals than nonhybrids or sexuals surviving (Year 1: 82 hybrid asexuals, 17 nonhybrid asexuals, and 41 sexuals; Year 2: 75 hybrid asexuals, 28 nonhybrid asexuals, and 17 sexuals). In all groups, herbivory and probability of survival were negatively correlated (Figure 3). Although the interaction between group and herbivory was nonsignificant, the negative relationship was strongest in hybrid asexuals (Figure 3), consistent with herbivory limiting hybrid asexual fitness.

Discussion

Here, we show that both abiotic and biotic factors play key roles in the maintenance of sex in nature. Although viability selection favors both inter- and intraspecific asexuals, implicating shared causative traits such as asexuality or heterozygosity, biotic interactions are especially influenced by hybridization. Together, these results suggest that, although survival of adverse abiotic conditions is facilitated by asexuality or associated heterozygosity, hybridization plays a singular role in the interaction between asexuality and herbivory. The precise targets of selection are

Table 2. Herbivory varies by reproductive system, hybridity, and garden. (A) Model results for reproductive system ("RS," sexual vs. asexual) LMM and (B) group (sexual "sex" vs. asexual hybrid "hyb" vs. asexual nonhybrid, the reference category) LMM. Significant *P*-values are in bold. Estimates apply to the conditional models, whereas test statistics (*F*-statistics, degrees of freedom, and *P*-values) come from significance tests for each overall effect.

A.

Term	Condition	Estimate	SE	df	F	P-value
	Intercept	−1.633	0.071	–	–	–
RS	Sexual	−0.299	0.079	1, 48.29	22.55	**9.33 × 10⁻⁵**
Garden	CAP	−0.002	0.074	3, 62.99	6.40	**2.99 × 10⁻³**
	MIL	0.340	0.095			
	SIL	−0.002	0.103			
RS × Garden	Sexual × CAP	0.049	0.080	3, 1280.95	1.74	0.372
	Sexual × MIL	0.059	0.115			
	Sexual × SIL	0.234	0.105			
Year	2	−0.045	0.076	1, 67.59	0.38	1
RS × Year	Sexual × 2	−0.013	0.084	1, 1175.63	0.02	0.876
Plant height	–	0.045	0.016	1, 1318.25	7.52	**0.019**

B.

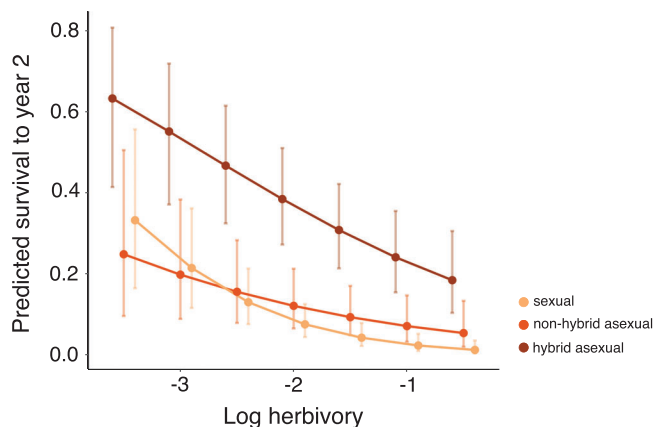| Term	Condition	Estimate	SE	df	F	P-value
	Intercept	−1.719	0.095	–	–	–
Group	Sex	−0.210	0.010	2, 42.39	18.08	**1.26 × 10⁻⁵**
	hyb	0.141	0.102			
Garden	CAP	−0.035	0.098	3, 62.98	6.37	**2.99 × 10⁻³**
	MIL	0.332	0.122			
	SIL	0.239	0.149			
Group × Garden	CAP × sex	0.083	0.102	6, 1273.93	1.99	0.065
	CAP × hybrid	0.060	0.102			
	MIL × sex	0.067	0.139			
	MIL × hybrid	0.016	0.132			
	SIL × sex	−0.005	0.150			
	SIL × hybrid	−0.324	0.152			
Year	2	−0.087	0.099	1, 66.98	0.30	1
Group × Year	Sex × 2	0.023	0.105	2, 1056.46	0.45	0.77
	Hybrid × 2	0.092	0.105			
Plant height	–	0.042	0.016	1, 1320.42	6.96	**0.019**

Figure 3. Plants with less herbivory are more likely to survive to the next year. Herbivory has a significant impact on survival to a second year, suggesting that the detrimental consequences of herbivory may not be observed in a single growing season. Although the correlation between year 2 survival and herbivory differs qualitatively by group (sexual vs. nonhybrid asexual vs. hybrid asexual), the interaction is not significant. Estimated marginal means are shown averaged across five different values of log herbivory. Points are jittered for ease of reading. Bars show 95% confidence intervals.

currently unknown, but our results suggest that the ecological fitness consequences of sex depend on ones' sexual partners, and may be mediated by interactions with biological antagonists.

THE COMPLEX ECOLOGY OF SEX

Despite extensive theoretical work and recurrent calls for field-based studies (Agrawal, 2006, 2009), few studies have examined the ecology of sex. In reviewing previous work, Becks and Agrawal (2010) found eight studies that compared fitness of sexual and asexual organisms, only one of which was conducted in the field. This study, focusing on vernal sweet grass planted out into the natural environment, found that sexual individuals had nearly a 1.5-fold fitness advantage over asexual individuals (Kelley et al. 1988). More recently, Hersh (2020) conducted a long-term reciprocal transplant of sexual and asexual *Townsendia*, finding that sexual fitness was comparable or higher outside their native range, whereas asexual fitness decreased when planted in the sexual portion of the range. Identifying ecologically relevant traits associated with sex, and the selective processes acting in natural environments, could elucidate the phenotypes and ultimately the molecular mechanisms underlying the cost of sex in wild populations. More studies of the evolution of sex in the wild are needed.

Although numerous selective agents are likely important to the evolution of sex (Neiman et al. 2018), we have little understanding of how these biotic and abiotic selection pressures maintain sexual reproduction in the wild. Previous work shows that

survival is elevated in sexual genotypes during abiotic environmental shifts (Stross and Hill 1965) or that sexual reproduction allows hosts to avoid biotic interactions via the generation of novel allelic combinations (Lively 1987). We found that strong viability selection early in life results in higher lifetime fitness for asexual lineages (Figure 1), but increased insect herbivory may provide an upper limit on asexual fitness (Figure 3). That different agents act at different life stages suggests that long-term studies are key to understanding how sexual/asexual dynamics play out in the natural environment.

Our reciprocal transplant used wild-collected sexual and asexual lineages to assess fitness in the natural environment. Other researchers have created sexual and asexual lineages de novo from the same parental genotypes, which permits control of parent of origin effects and disparities in lineage age (e.g., Kelley et al. 1988). Although fully characterizing the precise impacts of sex on fitness without any complicating parent of origin effects requires generating de novo sexual and asexual lineages from the same parents, our focus on field-collected samples ensures that the genotypes studied have ecological relevance—that is, that they have experienced natural selection in wild populations and survived, and thus provide an accurate understanding of processes playing out in the native environment. Comparison of de novo sexual and asexual flowering plant genotypes is an area where further research is needed.

The use of wild-collected genotypes requires careful consideration of correlated traits. Many of the best-characterized asexual systems are polyploid (e.g., Fox et al. 1996; Thompson and Whitton 2006), hybrid (e.g., Xu et al. 2015), or both (e.g., Lutes et al. 2010; Gutekunst et al. 2018). Yet neither of these biological features is often considered in studies of these systems or in broader discussions of the costs of sex. We explicitly considered traits correlated with reproductive mode in assessing patterns of selection on sexual reproduction, finding that viability is especially reduced in homozygous sexual lineages, consistent with inbreeding depression acting as a cost of sex. Hybrid asexuals were more prone to herbivory, suggesting that the costs of asexuality may be related to the correlated trait of hybridity. Clearly, selective processes that influence the evolution of sex are shaped by both the origin and composition of asexual lineages and patterns of mating in sexuals.

MATING SYSTEM INFLUENCES THE EVOLUTION AND ECOLOGY OF SEX

The fundamental role of sex in all organisms is to reshuffle variation across the genome through recombination and segregation. But the effects of these processes are tempered by the efficacy of such shuffling—in other words, recombination is less effective at creating new allelic combinations in organisms or genomic regions that are highly homozygous (Nordborg 2000). When sexual

partners resemble one another due to population structure, limited population genomic variation, and/or inbreeding, the traditional costs and benefits of sex can change dramatically (De Visser and Elena 2007). For example, one of the key benefits of sexual reproduction outlined in the literature is its ability to unite beneficial mutations arising in different lineages, which high rates of self-fertilization prevent. Practically speaking, the costs and benefits of sex are always a function of mate availability and identity, and inbreeding should thus be considered in the evolution of sex.

Despite the widespread existence of asexual reproduction in flowering plants, little is known about the costs of sex in angiosperms. Researchers studying the evolution of sex in natural populations have largely focused on animal systems, where the existence of separate sexes provides clear predictions about the relevant costs and benefits (Lehtonen et al. 2012). Although hermaphroditic asexuals are still predicted to have a 1.5-fold fitness advantage, sex via self-fertilization muddles these predictions (Charlesworth 1980; but see Orive 2020), as do additional biological traits common in plants (Mogie 1996). For example, both selfing and pseudogamous apomictic *Boechera* must produce sufficient pollen for self-fertilization, reducing the applicability of any cost of males. Additionally, the strong link between hybridization and asexuality in numerous vertebrate and plant taxa (Asker and Jerling 1992; Kearney 2005) suggests that recurrent independent hybridization events generate novel asexuals. In *Boechera*, both sexual and asexual lineages have limited variation, but high diversity among asexual lineages may be generated by multiple independent origins of asexuality. Such cases require consideration of additional costs and benefits of sex absent from most models.

Theory predicts three main benefits of asexuality: first, asexual lineages enjoy reproductive assurance (the ability to reproduce even when mates are lacking), whereas sexual lineages do not, resulting in rapid population expansion in any given environment; second, asexuality avoids the "twofold cost of sex"; third, asexuality fixes beneficial heterozygous and epistatic allelic combinations indefinitely (Maynard Smith 1978). To our knowledge, *Boechera* is the only apomictic plant system in which sexual lineages are self-compatible (Asker a Jerling 1992). This unusual trait places *Boechera* in a unique position in which to explore the advantage of heterozygosity and heterosis in asexual fitness, an understudied characteristic of asexual lineages more broadly. Furthermore, the sharp contrast of inbred sexual genotypes with asexual lineages originating through outcrossing enables study of inbreeding depression as a cost of sexual reproduction. Although inbreeding depression is attenuated in highly self-fertilizing populations by purging (the loss of deleterious alleles following exposure to selection), moderate-effect deleterious mutations may still persist and reduce fitness (Arunkumar et al. 2015; Roessler et al. 2019).

We hypothesize that inbreeding depression manifests as the primary fitness cost of sexual genotypes in sexual *Boechera*. Our experiment showed that these lineages experienced fitness costs and benefits that follow predictions for inbreeding depression. Sexuals had reduced overwinter survival (Figure 1), particularly in homozygous lineages (Figure S4), that varied both spatially and temporally (Figure 1; Tables 1 and S4), consistent with inbreeding depression (Armbruster and Reed 2005; Cheptou and Donohue 2010). Our experiment contained few heterozygous sexual genotypes, and was thus underpowered to detect a strong relationship between heterozygosity and fitness. Nonetheless, our results suggest that the main cost associated with sex in *Boechera* may be due to deleterious load, and that outcrossing may be sufficient to increase survival in this system. This pattern, known as a heterozygosity-fitness correlation or HFC, is widespread in wild systems (Szulkin et al. 2010). Despite reduced survival in homozygous sexual lineages, we saw no evidence that survival differed between asexuals and heterozygous sexuals (Table S5), consistent with the masking of genome-wide recessive load underlying increased survival.

Although inbreeding likely poses a main cost of sex in this system, other factors may also be at play. First, asexual lineages likely arise frequently and those found in nature have survived selection, potentially resulting in an overrepresentation of high-fitness asexual genotypes in the field. Indeed, the majority of de novo *Boechera* hybrids have lower fitness than their selfed progenitors (Rushworth and Mitchell-Olds 2020), suggesting that fitness variation may be more common in the wild than our experiment suggests. Additionally, the recurrent origins of asexual lineages (Sharbel and Mitchell-Olds 2001) suggest that asexuals comprise a range of ages. With deleterious mutations accumulating over the lifespan of a given asexual lineage, older lineages may have reduced fitness due to mutation accumulation (Gabriel et al. 1993). Last, reproductive isolation that varies across populations may also result in fitness that depends on interactions between parental genotypes. Each of these scenarios is plausible and provides avenues for further research.

Studies of sexual and asexual fitness often assess fitness variance in addition to mean, because variance in fitness may present a long-term benefit that favors sexual reproduction (Agrawal 2006). Theory predicts that allele shuffling via sex will reduce or increase genetic variance, depending on the direction of association between alleles (Agrawal 2006). Intuitively, it may seem that increased variance is desirable. But depending on relative fitness of genotypes, increasing variance may be beneficial over long time scales, but detrimental over the short term (Agrawal 2006). Because of statistical limitations, we do not discuss fitness variance in the present study, although raw data show higher variance in hybrid asexual than sexual plants (Figure S4). Additionally, existing theory assumes that sexual reproduction occurs via

outcrossing, and thus sex provides a mechanism of creating new allelic combinations. This relationship is reversed in *Boechera*, where sex via self-fertilization reduces genetic variance, whereas the formation of asexual lineages via hybridization and outcrossing creates novel combinations of alleles. Furthermore, this variance may be equally fixed in wild asexual lineages and sexual lineages that self-fertilize. Future theoretical work is needed to incorporate self-fertilization into our predictions of genetic and fitness variance under sexual and asexual reproduction.

Self-fertilization has clear fitness benefits as well. Sex and recombination enable adaptation to novel environments (Felsenstein 1974), and inbreeding may speed local adaptation. In situations of ongoing environmental change, fixation of beneficial recessive alleles through self-fertilization results in rapid adaptation (Glémin and Ronfort 2012). Self-fertilization will also reduce the potential for reshuffling of beneficial allelic combinations, as high homozygosity limits opportunities for functional disruption (Hartfield et al. 2017). Our results showed that sexual lineages experienced less insect herbivory than asexuals (Figure 2). Local adaptation underlies variation in chemical defense both among populations of *Boechera stricta* (Prasad et al. 2012) and between species (Windsor et al. 2005). We thus hypothesize that interactions between divergent alleles in outcrossed asexuals disrupt chemical defenses, consistent with outbreeding depression.

HERBIVORY AS AN ECOLOGICAL COST OF ASEXUALITY

Classic literature posits multiple costs of asexuality, including the accumulation of deleterious mutations and the extinction of asexual clones with the highest mutation load (Müller 1964; Felsenstein 1974). Although asexual *Boechera* do show elevated levels of putatively deleterious mutations (Lovell et al. 2017), the heterozygous state of these mutations, the higher fitness of asexuals observed here, and the replenishment of asexual lineages by novel hybridization events all suggest that these intrinsic genetic costs have relatively little to do with the costs of asexuality in *Boechera*.

Rather, we found evidence for a short-term ecological cost associated with asexuality in this system—a 73% increase in asexual insect herbivory (Figure 2), consistent with predictions from theoretical literature (Stearns 1987). This pattern may be underlain by asexuality, heterozygosity, hybridization, or some combination of all three. In functionally asexual *Oenothera*, generalist herbivory is elevated across multiple species (Johnson et al. 2009), supporting a role for asexuality in the macroevolution of plant defense. Microevolutionary patterns suggest the same; viral infection is elevated in asexual and common sexual grass genotypes (Kelley 1994). Although elevated in both asexual groups, insect herbivory is highest in hybrid asexuals

(Figure 2). Ample research also shows that hybridization strongly alters plant defense and herbivore preference (Strauss 1994; Fritz 1999; Whitham et al. 1999), and hybrid taxa may produce greater or lesser quantities of chemical compounds, and/or novel ineffective compounds (Orians 2000). Disentangling the influence of asexuality and hybridization on defense offers a fruitful avenue for future research.

Although the costs of herbivory were insufficient to overcome the other benefits of asexuality in our 2-year study, these costs could manifest in other traits or at later life stages (Marquis 1992). We found that herbivory decreased survival to subsequent years (Figure 3), consistent with the negative correlation between fitness and herbivory documented in *Boechera stricta* (Prasad et al. 2012). Similar patterns have been identified in animals, suggesting that downstream consequences of parasitism or herbivory are broadly applicable. One recent study of Soay sheep showed that fecundity and parasite load collectively reduce overwinter survival (Leivesley et al. 2019). Additionally, we found that the relationship between second-year survival and herbivory is strongest in hybrid asexuals, suggesting that hybridization may limit the spread of asexuality via herbivory. However, more hybrids survive to a second year of life overall. Further work, including herbivore exposure experiments, is needed to understand the interplay between fitness and herbivory in this system.

The work presented here shows that sexual/asexual dynamics in wild plant populations are largely shaped by environmental complexity and correlated traits, including mating system. Asexual *Boechera* genotypes have consistently higher fitness and higher herbivory damage than self-fertilizing sexual lineages, and survival is lowest in homozygous sexual lineages. Our results highlight the importance of both the biotic and abiotic environments in the evolution of sex, and suggest that inbreeding and outbreeding depression underlie the ecological costs and benefits of sex in this system. Collectively, this work shows that the complex natural environment and the choice of a sexual partner work in tandem to shape patterns of reproductive variation in the wild.

DATA ARCHIVING

All original data and R scripts for statistical analyses are archived in Dryad (https://doi.org/10.5061/dryad.n5tb2rbt9).

LITERATURE CITED

Agrawal, A. F. 2006. Evolution of sex: why do organisms shuffle their genotypes? Curr. Biol. 16, R696–R704.

———. 2009. Why reproduction often takes two. Nature 462, 294–295.

Aliyu, O. M., M. E. Schranz, and T. F. Sharbel. 2010. Quantitative variation for apomictic reproduction in the genus *Boechera* (Brassicaceae). Am. J. Bot. 97, 1719–1731.

Armbruster, P. and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity 95, 235–242.

Arunkumar, R., R. W. Ness, S. I. Wright, and S. C. H. Barrett. 2015. The evolution of selfing is accompanied by reduced efficacy of selection and purging of deleterious mutations. Genetics 199, 817–829.

Asker, S. E. and L. Jerling. 1992. Apomixis in plants. CRC Press, Inc., Boca Raton, FL.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. lme4: linear mixed-effects models using Eigen and S4. J. Stat. Softw. 67, 1–48.

Beck, J. B., P. J. Alexander, L. Allphin, I. A. Al-Shehbaz, C. Rushworth, C. D. Bailey, et al. 2012. Does hybridization drive the transition to asexuality in diploid *Boechera*? Evolution 66, 985–995.

Becks, L. and A. F. Agrawal. 2010. The effect of sex on the mean and variance of fitness in facultatively sexual rotifers. J. Evol. Biol. 24, 656–664.

Böcher, T. W. 1951. Cytological and embryological studies in the amphi-apomictic *Arabis holboellii* complex. Kongel. Danske Vidensk. Selskab. Biol. Skr. 6, 1–59.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–35.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9, 378–400.

Charlesworth, B. 1980. The cost of sex in relation to mating system. J. Theor. Biol. 84, 655–671.

Charlesworth, D. and J. H. Willis. 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10, 783–796.

Cheptou, P. - O. and K. Donohue. 2010. Environment-dependent inbreeding depression: its ecological and evolutionary significance. New Phytol. 189, 395–407.

De Visser, J. A. G. M. and S. F. Elena. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. Nat. Rev. Genet. 8, 139–149.

Felsenstein, J. 1974. Evolutionary advantage of recombination. Genetics 78, 737–756.

Fox, J., M. Dybdahl, J. Jokela, and C. Lively. 1996. Genetic structure of co-existing sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50:1541–1548.

Fradin, H., K. Kiontke, C. Zegar, M. Gutwein, J. Lucas, M. Kovtun, et al. 2017. Genome architecture and evolution of a unichromosomal asexual nematode. Curr. Biol. 27, 2928–2939.e6.

Fritz, R. S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? Ecology 80, 382–391.

Gabriel, W., M. Lynch, and R. Burger. 1993. Muller's Ratchet and mutational meltdowns. Evolution 47, 1744–1757.

Glémin, S. and J. Ronfort. 2012. Adaptation and maladaptation in selfing and outcrossing species: new mutations versus standing variation. Evolution 67, 225–240.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, et al. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nat. Ecol. Evol. 2, 567–573.

Halekoh, U. and S. Højsgaard. 2014. A Kenward-Roger approximation and parametric boostrap methods for tests in linear mixed models - the R package pbkrtest. J. Stat. Softw. 59, 1–30.

Hamilton, M. and T. Mitchell-Olds. 1994. The mating system and relative performance of selfed and outcrossed progeny in *Arabis fecunda* (Brassicaceae). Am. J. Bot. 81, 1252–1256.

Hartfield, M., T. Bataillon, and S. Glémin. 2017. The evolutionary interplay between adaptation and self-fertilization. Trends Genet. 33, 420–431.

Hartig, F. 2019. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models.

Hersh, E. W. 2020. A clone together: exploring the causes and consequences of range divergence between sexual and asexual Easter daisies. Univ. of British Columbia, Vancouver, Canada.

Hill, W. G. and A. Robertson. 1966. Effect of linkage on limits to artificial selection. Genet. Res. 8, 269–294.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.

Hubert, M. and E. Vandervieren. 2008. An adjusted boxplot for skewed distributions. Comput. Stat. Data Anal. 52, 5186–5201.

Jaenike, J. 1978. A hypothesis to account for the maintenance of sex within populations. Evol. Theory 3, 191–194.

Johnson, M. T. J., S. D. Smith, and M. D. Rausher. 2009. Plant sex and the evolution of plant defenses against herbivores. Proc. Natl. Acad. Sci. USA 106, 18079–18084.

Jokela, J. and C. M. Lively. 1995. Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution 49, 1268–1271.

Kantama, L., T. F. Sharbel, M. E. Schranz, T. Mitchell-Olds, S. de Vries, and H. de Jong. 2007. Diploid apomicts of the *Boechera holboellii* complex display large-scale chromosome substitutions and aberrant chromosomes. Proc. Natl. Acad. Sci. USA 104, 14026–14031.

Kearney, M. 2005. Hybridization, glaciation and geographical parthenogenesis. Trends Ecol. Evol. 20, 495–502.

Keightley, P. D. and S. P. Otto. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. Nature 443, 89–92.

Kelley, S. E. 1994. Viral pathogens and the advantage of sex in the perennial grass *Anthoxanthum odoratum*. Philos. Trans. R. Soc. B Biol. Sci. 346, 295–302.

Kelley, S. E., J. Antonovics, and J. Schmitt. 1988. A test of the short-term advantage of sexual reproduction. Nature 331, 714–716.

Kenward, M. G. and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53, 983–997.

Lehtonen, J., M. D. Jennions, and H. Kokko. 2012. The many costs of sex. Trends Ecol. Evol. 27, 172–178.

Leivesley, J. A., L. F. Bussière, J. M. Pemberton, J. G. Pilkington, K. Wilson, and A. D. Hayward. 2019. Survival costs of reproduction are mediated by parasite infection in wild Soay sheep. Ecol. Lett. 22, 1203–1213.

Li, F. - W., C. A. Rushworth, J. B. Beck, and M. D. Windham. 2017. *Boechera* microsatellite website: an online portal for species identification and determination of hybrid parentage. Database 2016, 1–11.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328, 519–521.

Lively, C. M. and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405, 679–681.

Lovell, J. T., R. J. Williamson, S. I. Wright, J. K. McKay, and T. F. Sharbel. 2017. Mutation accumulation in an asexual relative of *Arabidopsis*. PLoS Genet. 13, e1006550-14.

Lüdecke, D. 2018. ggeffects: tidy data frames of marginal effects from regression models. J. Open Source Softw. 3, 772.

Lutes, A. A., W. B. Neaves, D. P. Baumann, W. Wiegraebe, and P. Baumann. 2010. Sister chromosome pairing maintains heterozygosity in parthenogenetic lizards. Nature 464, 283–286.

Maechler, M., P. Rousseeuw, C. Croux, V. Todorov, A. Ruckstuhl, M. Salibian-Barrera, et al. 2019. robustbase: basic robust statistics.

Marquis, R. J. 1992. Selective impact of herbivores. Pp. 301–325 *in* R. S. Fritz and E. L. Simms, eds. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. Univ. of Chicago Press, Chicago.

Mau, M., J. T. Lovell, J. M. Corral, C. Kiefer, M. A. Koch, O. M. Aliyu, et al. 2015. Hybrid apomicts trapped in the ecological niches of their sexual ancestors. Proc. Natl. Acad. Sci. USA 112, E2357–E2365.

Maynard Smith, J. 1978. The evolution of sex. Cambridge Univ. Press, Cambridge, U.K.

Meirmans, S., P. G. Meirmans, and L. R. Kirkendall. 2012. The costs of sex: facing real-world complexities. Q. Rev. Biol. 87, 19–40.

Mogie, M. 1996. Is there a cost of sex in hermaphrodites? Naturwissenschaften 83, 225–226.

Müller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. 1, 2–9.

Neiman, M., P. G. Meirmans, T. Schwander, and S. Meirmans. 2018. Sex in the wild: how and why field-based studies contribute to solving the problem of sex. Evolution 72, 1194–1203.

Nordborg, M. 2000. Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. Genetics 154, 923–9.

Orians, C. 2000. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. Am. J. Bot. 87, 1749–1756.

Orive, M. E. 2020. The evolution of sex. Pp. 273–295 *in* S. M. Scheiner and D. P. Mindell, eds. The theory of evolution. Univ. of Chicago Press, Chicago.

Peterson, R., J. P. Slovin, and C. Chen. 2010. A simplified method for differential staining of aborted and non-aborted pollen grains. Int. J. Plant Biol. 1, 1–5.

Prasad, K. V. S. K., B. - H. Song, C. Olson-Manning, J. T. Anderson, C. - R. Lee, M. E. Schranz, et al. 2012. A gain-of-function polymorphism controlling complex traits and fitness in nature. Science 337, 1081–1084.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Roessler, K., A. Muyle, C. M. Diez, G. R. J. Gaut, A. Bousios, M. C. Stitzer, et al. 2019. The genome-wide dynamics of purging during selfing in maize. Nat. Plants 5, 980–990.

Roy, B. A. 1995. The breeding systems of six species of *Arabis* (Brassicaceae). Am. J. Bot. 82, 869–877.

Rushworth, C. A. and T. Mitchell-Olds. 2020. The evolution of sex is tempered by costly hybridization in *Boechera* (rock cress). bioRxiv. https://doi.org/10.1101/2020.02.11.944256

Rushworth, C. A., M. D. Windham, R. A. Keith, and T. Mitchell-Olds. 2018. Ecological differentiation facilitates fine-scale coexistence of sexual and asexual *Boechera*. Am. J. Bot. 105, 1–14.

Schranz, M. E., C. Dobes, M. A. Koch, and T. Mitchell-Olds. 2005. Sexual reproduction, hybridization, apomixis, and polyploidization in the genus *Boechera* (Brassicaceae). Am. J. Bot. 92, 1797–1810.

Sharbel, T. F. and T. Mitchell-Olds. 2001. Recurrent polyploid origins and chloroplast phylogeography in the *Arabis holboellii* complex (Brassicaceae). Heredity 87, 59–68.

Smyth, D. R., J. L. Bowman, and E. M. Meyerowitz. 1990. Early flower development in Arabidopsis. Plant Cell 2, 755–67.

Song, B. H., M. J. Clauss, A. Pepper, and T. Mitchell-Olds. 2006. Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of *Arabidopsis*. Mol. Ecol. 15, 357–369.

Stearns, S. C. 1987. The evolution of sex and its consequences. Birkhäuser, Basel, Switzerland.

Strauss, S. Y. 1994. Levels of herbivory and parasitism in host hybrid zones. Trends Ecol. Evol. 9, 209–214.

Stross, R. G. and J. C. Hill. 1965. Diapause induction in *Daphnia* requires two stimuli. Science 150, 1462–1464.

Szulkin, M., N. Bierne, and P. David. 2010. Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64, 1202–17.

Thompson, S. L. and J. Whitton. 2006. Patterns of recurrent evolution and geographic parthenogenesis within apomictic polyploid Easter daises (*Townsendia hookeri*). Mol. Ecol. 15, 3389–3400.

Vergara, D., J. Jokela, and C. M. Lively. 2014. Infection dynamics in coexisting sexual and asexual host populations: support for the red queen hypothesis. Am. Nat. 184, S22–S30.

Vrijenhoek, R. C. 1978. Coexistence of clones in a heterogeneous environment. Science 199, 549–552.

Whitham, T. G., G. D. Martinsen, K. D. Floate, H. S. Dungey, B. M. Potts, and P. Keim. 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. Ecology 80, 416–428.

Windham, M. D. and I. A. Al-Shehbaz. 2006. New and noteworthy species of *Boechera* (Brassicaceae) I: sexual diploids. Harv. Pap. Bot. 11: 61–88.

Windsor, A. J., M. Reichelt, A. Figuth, A. Svatoš, J. Kroymann, D. J. Kliebenstein, et al. 2005. Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). Phytochemistry 66, 1321–1333.

Xu, S., K. Spitze, M. S. Ackerman, Z. Ye, L. Bright, N. Keith, et al. 2015. Hybridization and the origin of contagious asexuality in *Daphnia pulex*. Mol. Biol. Evol. 32:3215–3225.

Yakimowski, S. B. and L. H. Rieseberg. 2014. The role of homoploid hybridization in evolution: a century of studies synthesizing genetics and ecology. Am. J. Bot. 101, 1247–1258.

Associate Editor: S. Wright

Supporting Information

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

Figure S1. Histogram of heterozygosity for maternal genotypes.

Figure S2. Map of experimental study location and garden sites in central Idaho, USA.

Figure S3. Predicted lifetime fitness of asexuals is higher than that of sexuals.

Figure S4. Raw data show lifetime fitness of asexuals is higher than sexuals.

Figure S5. Heterozygous sexuals have higher over-winter survival than homozygous sexuals.

Figure S6. Temporally and spatially-variable fecundity selection.

Figure S7. Herbivory is spatially variable.

Table S1. Genotypes used in the study.

Table S2. Likelihood ratio tests for significance of terms in lifetime fitness models, calculated collectively across both the zero-inflation and conditional components.

Table S3. Results for model of lifetime fitness (A) by reproductive system, RS; (B) by group (sexual vs. hybrid asexual "hybrid" vs. non-hybrid asexual, the reference category).

Table S4. Results for model of survival (A) by reproductive system, RS; (B) by group (sexual vs. hybrid asexual "hybrid" vs. non-hybrid asexual, the reference category).

Table S5. Results for models comparing survival of heterozygous sexuals, homozygous sexuals, and asexuals (collectively, "Category").

Table S6. Results for models comparing lifetime fitness of heterozygous sexuals, homozygous sexuals, and asexuals (collectively, "Category").

Table S7. Results for model of fecundity by reproductive system, RS (A) and by group (B; sexual vs. hybrid asexual "hybrid" vs. non-hybrid asexual, the reference category).

Table S8. Results for models comparing fecundity of heterozygous sexuals, homozygous sexuals, and asexuals (collectively, "Category").

Table S9. Results from model of fecundity including log herbivory and its interaction with garden and group (sexual vs. hybrid asexual "hybrid" vs. non-hybrid asexual, the reference category).

Table S10. Results from model of survival to second year including log herbivory and its interaction with garden and group (sexual vs. hybrid asexual "hybrid" vs. non-hybrid asexual, the reference category).

Appendix S1. Tables of estimated marginal means from mixed models for fitness components, indicated by the sheet name.