

# Ecological differentiation facilitates fine-scale coexistence of sexual and asexual *Boechera*

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**PREMISE OF THE STUDY:** Ecological differentiation (ED) between sexual and asexual organisms may permit the maintenance of reproductive polymorphism. Several studies of sexual/asexual ED in plants have shown that the geographic ranges of asexuals extend beyond those of sexuals, often in areas of higher latitude or elevation. But very little is known about ED at fine scales, wherein coexistence of sexuals and asexuals may be permitted by differential niche occupation.

**METHODS:** We used 149 populations of sexual and apomictic lineages in the genus *Boechera* (rock cress) collected across a portion of this mustard's vast range. We characterized reproductive mode, ploidy, and species identity or hybrid parentage of each individual, and then used a multipronged statistical approach to (1) identify ED between sexuals and asexuals; (2) investigate the impacts of two confounding factors, polyploidy and hybridization, on ED; and (3) determine the environmental variables underlying ED.

**KEY RESULTS:** We found that sexuals and asexuals are significantly ecologically differentiated across the landscape, despite fine-scale interdigitation of these two reproductive forms. Asexual reproduction was strongly associated with greater disturbance, reduced slope, and greater environmental variability. Although ploidy had little effect on the patterns observed, hybridization has a unique impact on the relationships between asexual reproduction and specific environmental variables.

**CONCLUSIONS:** Ecological differentiation along the axes of disturbance, slope, and climatic variability, as well as the effects of heterozygosity, may contribute to the maintenance of sexuality and asexuality across the landscape, ultimately impacting the establishment and spread of asexual lineages.

**KEY WORDS** apomixis; asexual reproduction; *Boechera*; Brassicaceae; ecological differentiation; environmental variability; geographic parthenogenesis; hybridization.

Classic evolutionary theory predicts that, in species with polymorphic reproductive systems, sexual and asexual reproduction cannot coexist stably (Maynard Smith, 1971; Marshall and Brown, 1981). Upon initiation of asexual reproduction, holding all else constant, the twofold cost of sex enables asexual organisms to produce twice as many offspring as sexuals, leading ultimately to extinction of the latter (Maynard Smith, 1978). However, empirical research has revealed numerous systems in which sexuals and asexuals coexist, including animals (Vrijenhoek and Lerman, 1982; Case, 1990; Halkett et al., 2005; Lehto and Haag, 2010; Neiman et al., 2011), fungi (Saleh et al., 2012; Castel et al., 2014), and plants (Meirmans et al., 2003; Krahulcová et al., 2009). Additionally, multiple theoretical models predict conditions that permit persistence of sexuals in the face

of asexual reproductive assurance, which enables rapid asexual population expansion following invasion of a sexual population. These conditions include population structure, which increases the time necessary for asexual fixation across subdivided populations (Hartfield et al., 2012); and the Hill-Robertson effect, whereby associations between deleterious loci result in the increase of modifiers associated with recombination (Peck, 1993; Hartfield et al., 2010).

Coexistence may also be maintained via ecological differentiation (ED) between reproductive strategies, where sexual and asexual organisms occupy different niches (Case and Taper, 1986; Rispe and Pierre, 1998; Burt, 2000; Doncaster et al., 2000; Agrawal, 2009). In animal systems, sexual/asexual reproductive variation is associated with environmental factors such as temperature (Dedryver

et al., 2001), xeric conditions (Kearney, 2003), disturbance (Ben-Ami and Heller, 2007), and temporal dynamics (Halkett et al., 2005). For example, sexually produced eggs may enable winter survival in aphids and in *Daphnia* Müller (Rispe and Pierre, 1998). To understand which specific variables affect coexistence, however, these environments must be dissected further. In *Daphnia*, multiple winter environmental characteristics such as photoperiod, drought, and low food availability may influence the transition to sexual reproduction (see, e.g., Stross and Hill, 1965). Additionally, multiple plant-feeding insects specialize on different hosts (a form of environmental differentiation), supporting the coexistence of sexuals and asexuals. For example, sexual individuals of the aphid *Rhopalosiphum padi* L. use  $C_3$  Poaceae as summer hosts, transitioning to *Prunus padus* L. in the autumn, whereas asexual individuals use mostly  $C_4$  Poaceae year-round (Gilabert et al., 2014). Cumulatively, ED appears to play a key role in the maintenance of multiple forms of reproductive polymorphism in nature, and may permit the establishment of asexual lineages or populations by preventing competition with other lineages.

In plants, there are fewer studies of sexual/asexual ED, and they have focused almost entirely on large-scale geographic patterns. At this scale, several key environmental characteristics have been shown to differentiate sexuals from apomicts (asexuals that reproduce via clonal seed). Apomictic *Ranunculus kuepferi* Greuter & Burdet commonly occurs in previously glaciated sites in the Alps, whereas sexual individuals are found only at lower latitudes, in areas that were unglaciated (Cosendai and Hörandl, 2010). Similarly, sexual *Townsendia hookeri* Beaman is restricted to sites beyond the boundaries of the Cordilleran and Laurentide ice sheets, whereas apomictic populations are found in both previously glaciated and unglaciated areas (Thompson and Whitton, 2006). Apomictic taxa have also invaded distant new habitats, which suggests an association between asexuality and disturbance or novel habitats (Amsellem et al., 2001; Chapman et al., 2004). These studies suggest that at broad scales, asexuality is promoted by glaciation or environmental characteristics associated with glaciation and novel habitats. But to our knowledge, only one previous study has examined ED at a fine scale. Verduijn et al. (2004) focused on contrasting microenvironments between sexual and asexual members of a single population of dandelions (*Taraxacum officinale* F.H. Wigg), finding that the two forms occurred in different microhabitats.

The lack of focus on sexual/asexual dynamics at a fine geographic scale has hindered our understanding of the ecology of asexual formation and spread, a fundamental gap in the ecology of sex. Additionally, none of these studies has been able to identify the specific factors driving sexual/asexual ED, due to the confounding effects of polyploidy as well as fundamental differences in reproductive assurance; in each of the previously mentioned plant systems (*Townsendia*, *Ranunculus*, and dandelion), asexuals are polyploid and by definition do not require mates, whereas sexuals are diploid and outcrossing and by definition require mates. Thus, our understanding of ecological factors driving sexual/asexual coexistence has been limited by a focus on broad geographic scales, and on taxa in which confounding factors may influence the observed patterns.

Apomixis is tightly linked not only with polyploidy but with hybridization. Apomictic lineages are often derived from hybridization events (Asker and Jerling, 1992). It is thus possible that sexual/asexual ED is not due to reproductive mode itself, but instead is driven by correlated factors. Numerous examples exist in

the literature of ED between polyploids and diploids (Kirchheimer et al., 2016; Sonnleitner et al., 2016) and between hybrids and their progenitors (Rieseberg et al., 2003). To our knowledge, no studies have examined ED among all combinations of asexuality and these associated traits. How might correlated factors influence our expectations of ED? Hybrid plants combine parental traits in novel ways, displaying traits that are unique to one parent, intermediate between parents, a combination of assorted characteristics of each parent, or entirely novel (Rieseberg and Ellstrand, 1993; Yakimowski and Rieseberg, 2014). Considering ecological niche as a trait, we may expect hybrid/progenitor ED to follow any of these predictions. Further, the increased heterozygosity associated with hybridization, which provides more genetic variability to hybrids, may permit expansion into new habitats, similar to theory proposed for polyploids (Stebbins, 1985).

Assuming that hybridization between sexual lineages results in the formation of apomicts, asexual and sexual lineages will briefly coexist immediately following the creation of new asexuals. The establishment of exclusively asexual populations requires that asexuals either outcompete sexuals or disperse outside of the site of origin, which may occur via differences in reproductive assurance, or dispersal or establishment potential. Indeed, the latter has been shown in *Crataegus*, where asexual fruits are larger than those produced by sexual trees, driving asexual range expansion (Coughlan et al., 2014). In mixed sexual/asexual populations where sexuals reproduce via outcrossing, asexual pollination of sexual ovules is predicted to result either in the spread of asexual alleles into formerly sexual genotypes or in the production of unfit hybrids, leading to asexual expansion and the loss of sexual lineages (Whitton et al., 2017). However, this is unlikely to be a factor in taxonomic groups with a high rate of self-fertilization. Alternatively, environmental associations with reproductive mode may permit asexual spread and establishment in niches that are unsuitable to sexuals, as seen in invasive *Rubus* (Amsellem et al., 2001) and hawkweed (*Hieracium lepidulum* (Stenstr.) Omang; Chapman et al., 2004). Although any of these processes may occur individually or in combination with one another, which environmental variables are associated with the spread of asexual lineages beyond their site of origin remains unknown.

The genus *Boechera* Á. Löve & D. Löve (“rock cress,” formerly *Arabis* L.; Brassicaceae) offers an ideal system for studying the impacts of asexual reproduction free from confounding factors. Consisting of  $\geq 70$  sexual species, *Boechera* has long been considered taxonomically difficult because of shifts to apomixis, the presence of multiple ploidal levels, and frequent hybridization among taxa (Al-Shehbaz and Windham, 2010). The range of the genus includes much of North America, from northern Mexico to the Canadian territories, and east to Greenland (Al-Shehbaz and Windham, 2010). The genus occupies a wide range of ecological conditions (Rushworth et al., 2011), allowing study of ecological and evolutionary mechanisms across diverse native habitats. Although some major clades within western *Boechera* have been identified, phylogenetic relationships among these clades remain largely unresolved (Alexander et al., 2013), which is consistent with recent rapid divergence. Most of the sexual diploid species that have been genetically characterized are largely self-fertilizing (Roy, 1995; Schranz et al., 2005; Beck et al., 2012; Li et al., 2017). Nonetheless, some low level of outcrossing must occur, as interspecific hybridization events have resulted in hundreds of unique hybrid combinations (Li et al., 2017). Throughout the *Boechera* literature, these

hybridization events are strongly associated with apomixis (Schranz et al., 2005; Beck et al., 2012; Li et al., 2017). These two traits have historically prevented reliable identification of most *Boecheera* specimens; polyploidy is likewise prevalent in the genus, with triploidy the most common higher ploidy level (Al-Shehbaz and Windham, 2010).

*Boecheera* is the only genus of flowering plants in which diploid apomicts are prevalent, providing a rare opportunity to disentangle asexuality from the effects of polyploidy (Beck et al., 2012). Additionally, the prevalence of self-fertilization in sexually reproducing *Boecheera* species ensures that both sexual and asexual *Boecheera* can reproduce without mates, removing the disparity in reproductive assurance often hypothesized to result in ED. Self-fertilization reduces heterozygosity by half with each generation, resulting in highly homozygous sexual genotypes, whereas the strong association between hybridization and apomixis in *Boecheera* results in the apomicts having fixed highly heterozygous genotypes. The presence of non-hybrid asexual *Boecheera* resulting from within-species outcrossing events further permits disentangling of the impacts of heterozygosity versus hybridization per se on sexual/asexual ED.

Our focal species for the present study is the highly selfing *Boecheera retrofracta* Graham (Á. Löve & D. Löve), the widest-ranging species in the genus, which occurs in all Canadian provinces and territories west from Quebec; in the United States, the genus is common from Utah to California and north to Alaska. Across its range, *B. retrofracta* occupies mostly sagebrush and open forest habitats 300–3300 m in elevation, and includes sexual diploids, apomictic diploids, and apomictic triploids, while hybridizing with many other *Boecheera* species (Al-Shehbaz and Windham, 2010).

We studied >100 populations of sexual and asexual *Boecheera* to characterize sexual/asexual ED, asking if ED facilitates the coexistence of these two forms of reproduction across populations. We first used a recently published multilocus genotyping database to identify the species or hybrid parentage of each individual with high accuracy. We found that *B. retrofracta* hybridizes and outcrosses extensively, forming numerous interspecific and intraspecific (i.e., hybrid and non-hybrid) apomictic lineages. We examined sexual/asexual ED among all individuals in the study area, and additionally controlled for polyploidy to address its unique impact on observed patterns. We next focused on sexual *B. retrofracta*, sexual members of the closely related *B. stricta* (Graham) Al-Shehbaz, their asexual hybrid, and asexual non-hybrid *B. retrofracta*, to disentangle the effects of hybridization on patterns of sexual/asexual ED. Lastly, we compared ecological characteristics of purely asexual vs. mixed sexual/asexual populations to pinpoint variables that may facilitate asexual spread across the landscape. This comprehensive approach allowed us to determine the environmental characteristics that shape sexual/asexual coexistence across populations of *Boecheera*, free from the confounding effects of polyploidy, hybridization, and reproductive assurance, a question that has thus far gone unaddressed in plants.

## MATERIALS AND METHODS

### Sampling and growth procedures

Seeds from 149 *Boecheera* sites across central Idaho and western Montana were collected between 2005 and 2009 (Appendix S1; see

the Supplemental Data with this article) and used for all subsequent analyses. Populations were defined as groups of individuals separated by  $\geq 1$  km, and collections ranged in size from one to 32 maternal seed families per population (mean = 6.85). Our focus was on *B. retrofracta*, which has been segregated from *B. holboellii* (Hornem.) Á. Löve & D. Löve, formerly known as *Arabis holboellii* Hornem. (Windham and Al-Shehbaz, 2006). We targeted individuals with morphological traits attributed to *B. retrofracta* and potential hybrids involving *B. retrofracta* and *B. stricta*, which is morphologically and genetically distinct from our focal species (Alexander et al., 2013). Seeds were grown on wet filter paper in Petri dishes until seed leaves emerged, then transferred to a soil mixture of Fafard 4P Mix and Sunshine MVP (Sun Gro Horticulture, Agawam, Massachusetts, USA) and grown in the Duke University Research Greenhouses. One individual was chosen at random to represent each seed family.

### DNA extraction and PCR

Multilocus genotypes for each individual were generated via DNA extraction and PCR. Leaf tissue was collected from a total of 1020 greenhouse-grown individuals. DNA extractions were performed on either fresh or silica-dried samples, by one of two methods: a KoAC protocol (Lee and Mitchell-Olds, 2011), or a CTAB protocol (Doyle and Dickson, 1987) modified as described in Beck et al. (2012). Thirteen multiplexed fluorescent-labeled microsatellite markers (Clauss et al., 2002; Dobeš et al., 2004b; Song et al., 2006) were amplified using the protocols of Beck et al. (2012); one individual did not amplify well and was removed from further analyses. Loci were selected to maximize allelic diversity in multiple species across the genus, which permitted locus amplification in interspecific hybrids (Appendix S2). Reactions were run on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) and analyzed using Genemarker (SoftGenetics, State College, Pennsylvania, USA).

### Species identification

*Boecheera* species and hybrids were identified via comparison of allelic profiles to the *Boecheera* Microsatellite Website (BMW), a 15-locus microsatellite database of >4000 herbarium specimens representing ~80 sexual diploid species and 400 unique hybrid parental combinations, considered asexual taxa (Li et al., 2017). BMW calculates an allelic similarity score for each multilocus genotype, corrected for missing data, and offers putative matches based on this score. Trials of known samples using BMW resulted in 96% correct identification to species (Li et al., 2017). Following BMW analysis, identifications were confirmed with morphological traits (Appendix S3). We used principal component analysis (PCA) of genetic data converted to Lynch distance (briefly, a measure of dissimilarity calculated as twice the number of bands two genotypes have in common, divided by the total number of bands in the two genotypes; Lynch, 1990) in the R package “Polysat” (Clark and Jasieniuk, 2011), to visually assess genetic intermediacy of putative crosses between *B. stricta* and *B. retrofracta* and within *B. retrofracta*.

### Apomict and polyploid identification

Several methods were used to determine ploidy level and reproductive mode in our collections. Ploidy level was first estimated using

the maximum allele number across loci in an individual, as in Beck et al. (2012). For 132 genotypes, one or two leaves from mature plants were also analyzed for ploidy level with a Partec PAS flow cytometer (Partec, Munster, Germany) using an internal standard of *Brassica rapa* L. as described in Schranz et al. (2005). Hybridization among species of different genome sizes occasionally led to flow-cytometry-based ploidy estimates that were inconsistent with inferences from maximum allele number. To confirm the accuracy of maximum allele number ploidy determinations, chromosome squashes were performed on 16 individuals (four putative triploid apomicts and 12 putative diploid apomicts; Appendix S4). In all cases, chromosome squashes confirmed the accuracy of maximum allele number inference over inferences based on flow cytometry (Appendix S5).

Because nearly all sexual species in *Boechera* are highly self-fertilizing, whereas apomixis is associated with outcrossing events, heterozygosity is often used to diagnose reproductive mode in the genus with high accuracy (Roy and Rieseberg, 1989; Sharbel and Mitchell-Olds, 2001; Schranz et al., 2006; Kantama et al., 2007; Beck et al., 2012; Li et al., 2017). To validate the application of a heterozygosity cutoff as in Li et al. (2017), we screened 7–10 offspring for each of 17 putatively apomictic lines (as determined by the heterozygosity cutoff), and verified that offspring were genetically identical to the parent (results not shown). Reproductive mode was thus inferred from observed heterozygosity in the remainder of our data set: individuals heterozygous at  $\geq 50\%$  of loci were considered apomictic, and those heterozygous at  $< 50\%$  of loci were inferred to reproduce via sex. This threshold is consistent with the bimodal distribution of heterozygosity across the genus (Li et al., 2017).

### Environmental variables

Environmental variables were harvested from the WorldClim database (Hijmans et al., 2005) using the R package “raster” (Hijmans, 2013). BIO1–BIO11 are temperature variables; BIO12–BIO19 estimate precipitation. The scale of these data is 1 km<sup>2</sup>, which may not entirely capture the conditions experienced by sessile organisms in environments with substantial topographic relief (Kirchheimer et al., 2016). Latitude, longitude, and three topographical variables (elevation, within-population slope, and distance to nearest road [a proxy for disturbance]) were extracted using Google Earth. Prior to harvesting environmental data, we merged populations that occurred within 4 km of each other and at similar elevations, and removed populations with fewer than four individuals, reducing our data set from 149 populations ( $N = 1020$ ) to 96 populations ( $N = 955$ ). Before statistical analyses, all variables except latitude and longitude were standardized to mean = 0 and SD = 1 to enable comparison on a common scale. Environmental variables are highly correlated with each other, which was accounted for either through PCA or through removal of highly correlated variables (see below).

### Focal comparisons

To elucidate the impacts of asexuality, polyploidy, and hybridization on ED, we subdivided the data set into four focal comparisons, which we denote “FC1” through “FC4.”

To identify variables broadly underlying sexual/asexual ED, FC1 contrasted all apomictic individuals (regardless of ploidy) vs. all sexuals (955 individuals, 96 populations). FC2 isolated the impacts

of polyploidy on the pattern observed through two different methods: first, discriminant analysis of principal components (DAPC) and multivariate analysis of covariance (MANCOVA) compared sexual diploids, asexual diploids, and asexual triploids; second, for simplicity, we dropped the triploids and included only diploid sexuals and asexuals in our generalized linear model (GLM; 812 individuals, 73 populations). To identify the effects of hybridization, FC3 (592 individuals, 67 populations) contrasted non-hybrid *B. retrofracta* apomicts (“asexual Br”), hybrid *B. retrofracta* × *B. stricta* apomicts (“Br × Bs”), and the two sexual parental species (*B. retrofracta* [“Br”] and *B. stricta* [“Bs”]). Only 10 polyploids were identified in the FC3 data set, and these were not removed from statistical analyses. Finally, to identify variables that may underlie the initial establishment of asexual populations, FC4 examined ED between purely asexual vs. mixed sexual/asexual populations (750 individuals, 75 populations: 51 mixed sexual/asexual, 24 purely asexual).

### Statistical analyses

Summary statistics were calculated for the entire data set, and a series of statistical analyses were subsequently implemented for each FC. Observed and expected heterozygosity and  $G_{IS}$  (Nei, 1987), an analogue of the inbreeding coefficient  $F_{IS}$ , were calculated for each population in GenoDive v2.0b27 (Meirmans and Van Tienderen, 2004; Appendix S1).  $G_{IS}$  was calculated only for sexual accessions in populations containing more than one sexual individual.

For each FC, the following statistical process was performed in R version 3.3.1 (R Core Team, 2016). We first conducted PCA to identify correlations among all standardized environmental and topographical variables, using the “DAPC” function in the R package “adeget” (Jombart, 2008). DAPC uses a combination of PCA and discriminant function analysis (DFA) to create synthetic variables that describe the greatest axes of differentiation between previously defined groups. These groups differed for each FC as follows: FC1, sexual and asexual individuals; FC2, sexual diploids, asexual diploids, and asexual triploids; FC3, each of the four focal taxa (asexual Br, asexual Br × Bs, sexual Br, and sexual Bs); and FC4, mixed sexual/asexual populations vs. pure asexual populations. To verify statistical significance of ED between groups, we used MANCOVA to compare PCA values for principal components (PCs) 1–5 as in Duncan and Rausher (2013), with population as a covariate. Predictor variables were as follows: for FC1, sex/asex; for FC2, diploid sexual vs. diploid asexual vs. triploid asexual; for FC3, Br vs. Bs vs. Br × Bs vs. asexual Br; and for FC4, mixed sexual/asexual vs. pure asexual. Univariate analyses of individual PCs were also conducted (not shown).

To determine the role of specific environmental variables contributing to ED, two approaches were used. First, loadings of each environmental variable to discriminant functions were identified in the DFA portion of DAPC. To investigate the possibility of additional important variables, we next implemented GLMs using the “glm” command in the R package “lme4” (Bates et al., 2014) with population-level data. In FC1–FC3, sex vs. asex was the response variable; for FC4, the response was pure asexual vs. mixed sexual/asexual. Note that, for simplicity, these response variables differ from the categories used in other statistical analyses. We selected uncorrelated climate variables as predictors, identified using PCA loadings, to emphasize the greatest amount of variation in climate when possible.  $P$  values were corrected for multiple comparisons

when necessary, using a sequential Bonferroni correction (Holm, 1979).

By definition, correlations vary among the FCs because the data sets and correlation structure differ. This necessitated the selection of relevant environmental variables for each GLM that minimized correlation. For each of the FCs, the following variables had correlation coefficients  $<0.7$  and were retained: FC1 and FC2—annual mean temperature (BIO1), annual temperature range (BIO7), total annual precipitation (BIO12), precipitation seasonality (BIO15), slope, elevation, and disturbance; FC3—annual mean temperature (BIO1), isothermality (BIO3), temperature seasonality (BIO4), precipitation seasonality (BIO15), slope, elevation, and disturbance; and FC4—isoothermality (BIO3), annual temperature range (BIO7), total annual precipitation (BIO12), precipitation seasonality (BIO15), slope, and disturbance. Isothermality represents the range of annual temperatures in a given location on a given day; high values of isothermality correspond to a more temperate environment, whereas low values correspond to locations where seasonal variation is high.

## RESULTS

### Multiple sexual and apomictic taxa in a small geographic area

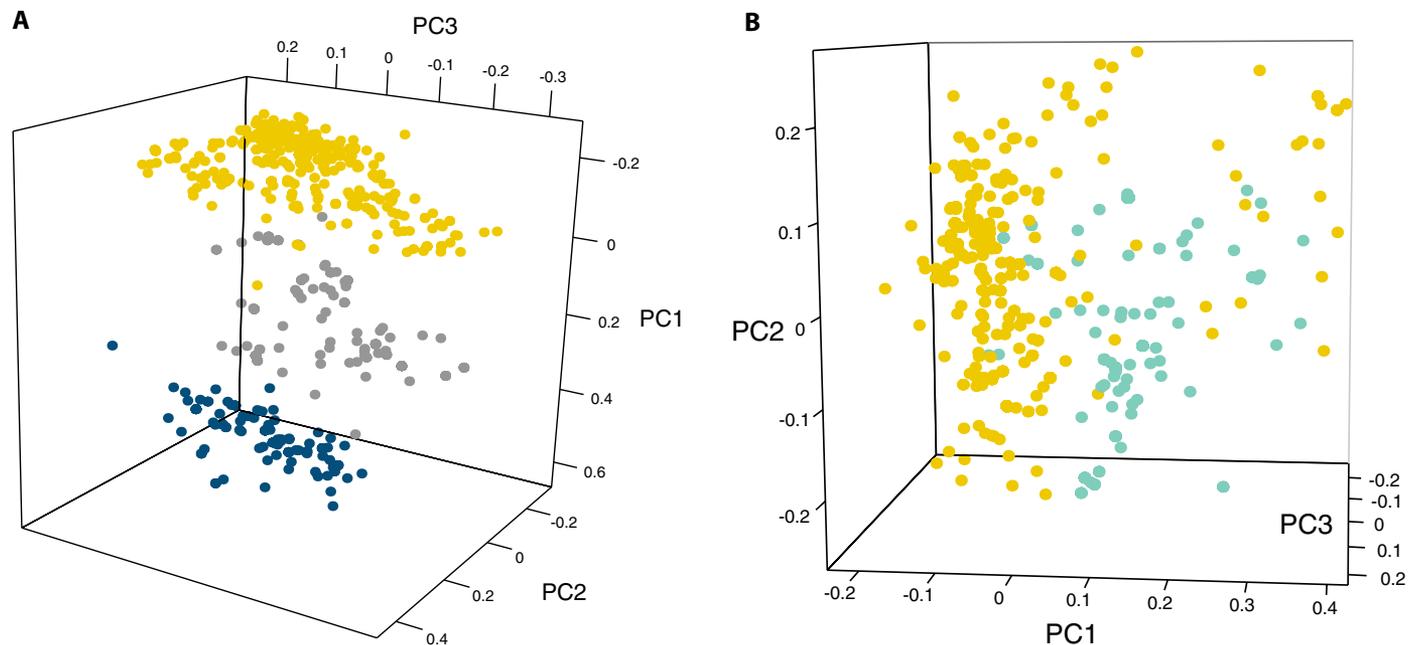
Of 1020 individuals, 983 could be confidently identified to species or hybrid combination; those that could not were excluded from subsequent analyses (Appendix S1). Four sexual species were present in the data set (464 *B. retrofracta* individuals, 112 *B. stricta*, 22 *B. pendulocarpa* [A. Nelson] Windham & Al-Shehbaz, and six *B. williamsii* [Rollins] Dorn, its first report

outside of Wyoming). We identified 16 well-supported hybrid species combinations representing various configurations of *B. retrofracta*, *B. stricta*, *B. pendulocarpa*, *B. williamsii*, *B. sparsiflora* (Nutt.) Dorn, *B. fecunda* (Rollins) Dorn, and *B. exilis* (A. Nelson) Dorn. (Appendix S6). In addition, a small number of hybrids were tentatively identified as involving *B. puberula* (Nutt.) Dorn, *B. microphylla* (Nutt.) Dorn, *B. subpinnatifida* (S. Watson) Al-Shehbaz, or *B. paupercula* (Greene) Windham & Al-Shehbaz (Appendix S1).

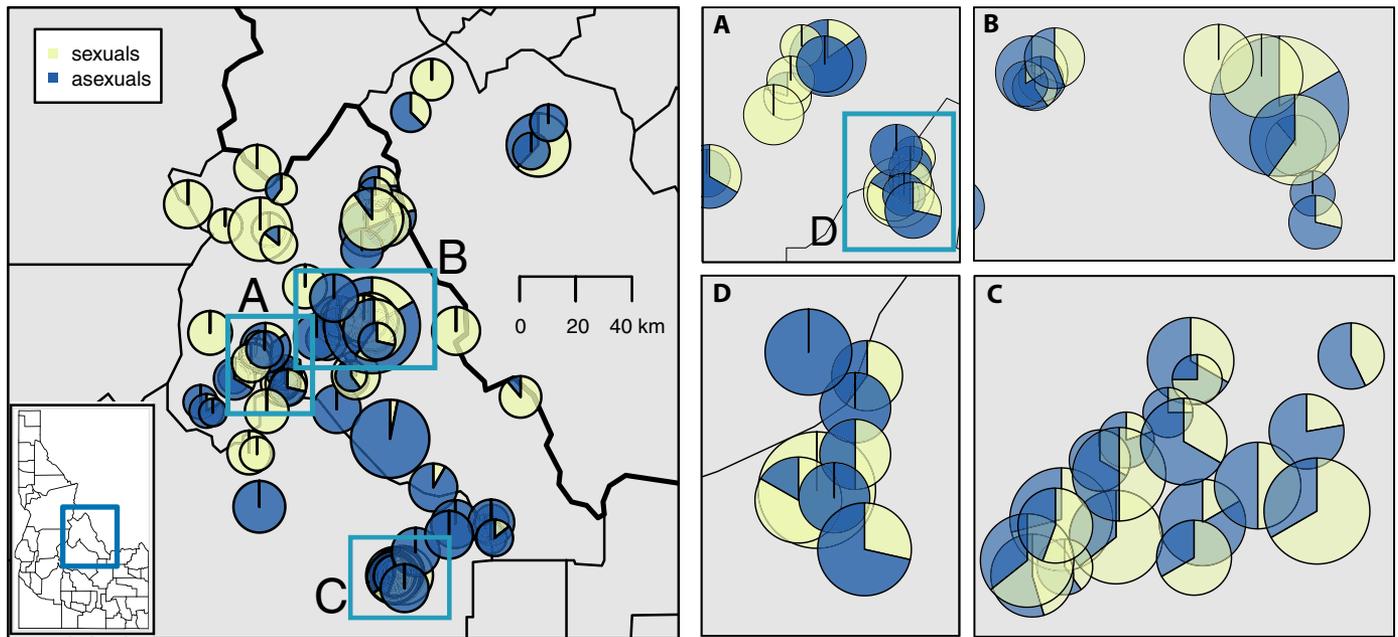
Average observed homozygosity of a sexual *B. retrofracta* individual was 0.94 (range: 0.58–1.0), and average  $G_{IS}$  for sexual *B. retrofracta* populations was 0.665, confirming that *B. retrofracta* is highly self-fertilizing like most sexual *Boecheera* species (Beck et al., 2012). The morphology of *B. retrofracta* is also consistent with self-fertilization, in that its flowers are small and white, typical of the selfing syndrome (Al-Shehbaz and Windham, 2010). Highly heterozygous, within-species apomicts were present in *B. retrofracta*, *B. pendulocarpa*, and *B. stricta*. Examination of these individuals determined that they were morphologically consistent with their species designation. PCA visualizations of genetic data for 114 putative *B. retrofracta*  $\times$  *B. stricta* hybrids and heterozygous asexual *B. retrofracta* accessions were likewise consistent with their hypothesized origins, indicated by genetic intermediacy between the putative parents (Fig. 1).

### No evidence for geographic structure of reproductive mode

Over half (575/1020; 56%) of the individuals sampled for this study reproduced asexually. Consistent with earlier studies, we found that apomictic individuals were most often diploid or triploid, with 300 diploid and 275 triploid asexuals sampled. Two putative tetraploids



**FIGURE 1.** Principal component analysis (PCA) of microsatellite data depicting genetic intermediacy of asexual *Boecheera* between putative parental taxa. (A) PCA of *B. stricta* (dark blue), *B. retrofracta* (gold), and *B. retrofracta*  $\times$  *B. stricta* hybrids (gray), which are both morphologically and genetically intermediate between the two parental species. The first three axes of variation explain 66.7% of the variation. (B) PCA of homozygous *B. retrofracta* (gold) and heterozygous asexual *B. retrofracta* (aqua). Heterozygous *B. retrofracta* are genetically intermediate between clusters of homozygous *B. retrofracta*, consistent with outcrossing between divergent lineages of *B. retrofracta* resulting in asexuality. The first three axes of variation explain 53.2% of the variation.



**FIGURE 2.** Map of 96 *Boechera* populations analyzed statistically in this study, located in central Idaho and western Montana. Pie charts, scaled for population size, represent individual populations, with sexuals shown in yellow and asexuals in blue; geographic scale is indicated (main map). (A–D) show individual populations in areas of high population density. Inset D is within the area of inset A.

**TABLE 1.** Summary of reproduction and polyploidy in populations included in this study, with percentage of total populations in parentheses.

Reproduction	Individuals	Populations	Diploid populations	Triploid populations	Mixed ploidy populations
Sexual	444	36 (24.1)	36 (48.6)	0 (0)	0 (0)
Apomictic	575	58 (38.9)	21 (28.4)	21 (36.2)	16 (29.6)
Mixed	–	55 (36.9)	17 (23.0)	0 (0)	38 (70.4)

were removed from further analyses. Asexuals were widespread across the study area, occurring in 113 of 149 populations, and co-occurring with sexual reproduction in 55 populations (36.9% of all populations; Fig. 2). By comparison, 38.9% (58/149) of populations were entirely asexual, while 24.1% (36/149) consisted of only sexual individuals (Table 1). A *G*-test showed that these ratios deviated from those expected by chance ( $G = 6.04$ ,  $df = 2$ ,  $P = 0.049$ ), with pure asexual and mixed sexual/asexual populations overrepresented.

### Sexuals and asexuals are ecologically differentiated

Despite substantial environmental overlap shown by PCA, sexuals and asexuals were ecologically differentiated (variable means summarized in Appendix S7 and PC means summarized in Appendix S8), and significantly so (MANCOVA for FC1,  $P < 2.20 \times 10^{-16}$ ; all MANCOVA results summarized in Table 2). Comparing diploid sexuals, diploid asexuals, and triploid asexuals (FC2), MANCOVA again showed that the groups were ecologically differentiated ( $P < 2.20 \times 10^{-16}$ ). Contrasts showed that sexuals differ from both diploid apomicts ( $P = 6.60 \times 10^{-16}$ ) and triploid apomicts ( $P = 6.60 \times 10^{-16}$ ), and that diploid and triploid apomicts differ from one another ( $P = 7.82 \times 10^{-8}$ ). Sexual Br, sexual Bs, their hybrids, and non-hybrid Br (FC3) also differ in ecological preference ( $P < 2.20 \times 10^{-16}$ ). Contrasts showed that the two sexual species differ significantly ( $P = 1.10 \times 10^{-15}$ ), and both apomictic groups differ from sexual Br (hybrid,

**TABLE 2.** Results from MANCOVA showing significant ecological differentiation for climatic variables among groups, including a covariate for population. (A) FC1: comparison of all sexuals with all asexuals. (B) FC2: full model comparing sexuals ("2xS"), diploid asexuals ("2xA"), and triploid asexuals ("3xA"), followed by contrasts. (C) FC3: full model comparing sexual *Boechera retrofracta* ("sexual Br"), sexual *B. stricta* ("sexual Bs"), non-hybrid asexual *B. retrofracta* ("asexual Br"), and hybrid asexual *B. stricta* × *B. retrofracta* ("Br × Bs"), followed by contrasts. (D) FC4: full model comparing pure asexual with mixed sexual/asexual populations.

Model	Wilks' $\lambda$	Approx. <i>F</i>	df (n, d)	<i>P</i>
A Full	0.85	32.87	5, 949	<2.20e-16
B Full	0.85	33.81	5, 949	<2.20e-16
2xS vs. 2xA	0.89	17.34	5, 696	6.60e-16
2xA vs. 3xA	0.92	8.60	5, 498	7.82e-08
2xS vs. 3xA	0.83	29.28	5, 698	6.60e-16
C Full	0.29	64.78	15, 1720.2	<2.20e-16
Sexual Br vs. sexual Bs	0.23	294.09	5, 431	1.10e-15
Sexual Br vs. asexual Br	0.77	26.05	5, 188	1.10e-15
Br × Bs vs. asexual Br	0.77	11.30	5, 188	1.12e-15
Br × Bs vs. sexual Bs	0.31	90.56	5, 202	1.10e-15
Br × Bs vs. sexual Br	0.77	27.30	5, 455	1.10e-15
D Full	0.88	19.79	5, 743	<2e-16

$P = 1.10 \times 10^{-15}$ ; non-hybrid,  $P = 1.10 \times 10^{-15}$ ) as well as from each other ( $P = 1.12 \times 10^{-9}$ ). Mixed sexual/asexual populations were also significantly ecologically differentiated from pure asexual populations

( $P < 2e-16$ ). PCs 1–5 explained >94% of the variation in each focal comparison (data not shown).

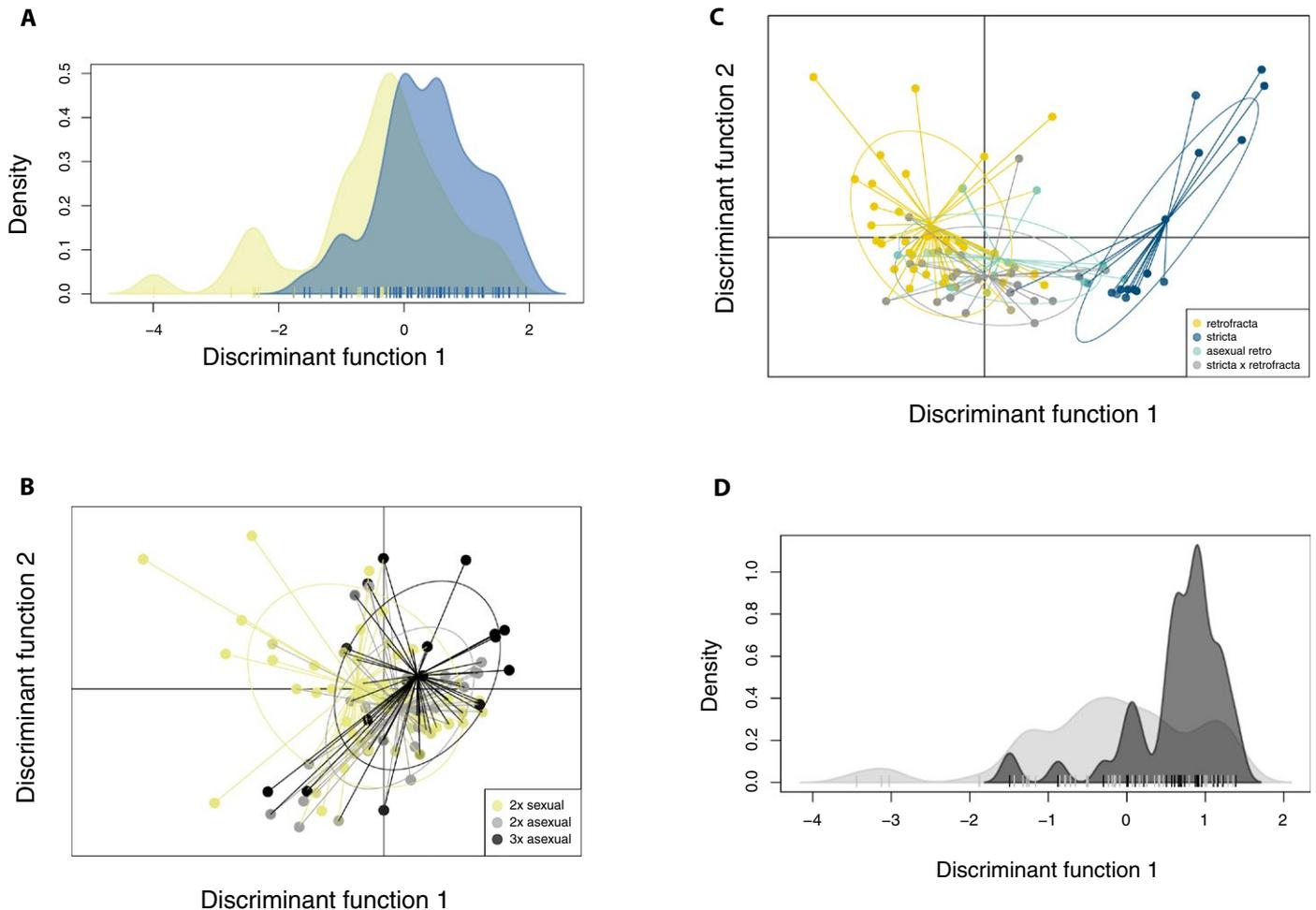
**Disturbance and slope differentiate asexuals and sexuals**

Visualization of DAPC discriminant functions shows broad overlap between sexual and asexual niches, with some ED apparent nonetheless (Fig. 3A). Overall, diploid asexuals show more environmental overlap with triploid asexuals than diploid sexuals do with either group (Fig. 3B).

Discriminant function loading values show that two variables—slope and disturbance—contributed heavily to axes differentiating sexuals from asexuals in all FCs, with asexuals occurring in flatter, more disturbed sites (Appendix S7 and Appendix S9). In montane environments, slope and distance to nearest road might be correlated, as roads are generally built in accessible locations; however, the correlation between these variables in the full data set was only 0.01. Compared to sexuals, asexuals on average occur in areas with

greater yearly drought stress (indicated by lower precipitation in both the wettest month and wettest quarter) and areas of greater temperature stress (indicated by lower minimum temperature in the coldest month; Appendix S7). Asexuals overall occur in locations slightly farther south than sexuals (asexual mean latitude  $44.71 \pm 0.02$  compared to the sexual mean latitude  $44.87 \pm 0.02$ , a difference of ~10 km; Appendix S7).

To examine ED of apomicts and sexuals on a finer scale, we compared sexual Bs, sexual Br, apomictic Br, and apomictic Br × Bs hybrids (FC3). The two sexual species occupied non-overlapping regions of environmental space along discriminant function 1, while the two apomicts spanned the interspecies gap (Fig. 3C). Slope and disturbance were again heavily loaded in FC3, as was latitude (Appendix S9, section C). Both hybrid and non-hybrid apomicts occurred substantially closer to roads than sexual Bs or Br, indicating an association between asexuality and disturbance (Appendix S7, section C). However, both forms of asexuals occurred in locations with intermediate slopes and



**FIGURE 3.** Visualization of ecological differentiation in four different focal comparisons via discriminant analysis of principal components (DAPC). (A) Asexuals (blue) and sexuals (yellow) co-occur in multiple populations but are ecologically differentiated. (B) Diploid asexuals (gray) and triploid asexuals (black) overlap substantially in ecological space compared with diploid sexuals (yellow). (C) *Boechera retrofracta* (gold) is ecologically well-differentiated from *B. stricta* (blue), whereas asexual *B. retrofracta* (green) and asexual hybrid *B. retrofracta* × *B. stricta* (gray) occupy environments intermediate between the two species. (D) Purely asexual populations (black) are concentrated in a narrow range of ecological conditions, compared with mixed sexual/asexual populations (gray).

**TABLE 3.** Results of GLM (family = binomial) predicting apomixis/sex for interspecific hybrids, with significant *P* values in bold. (A) Triploids included (FC1, *N* = 96 populations) for an overall sexual vs. asexual comparison. (B) Analysis limited to diploid sexuals and diploid asexuals (FC2, *N* = 73 populations).

A.				
Variable	Estimate	SE	Z	P
BIO1	0.581	0.481	1.208	0.227
BIO7	-1.132	0.173	-6.527	<b>2.02e-10</b>
BIO12	-0.619	0.273	-2.266	0.053
BIO15	-0.778	0.196	-3.967	<b>2.18e-04</b>
Slope	0.698	0.099	7.067	<b>6.32e-12</b>
Elevation	-0.426	0.403	-1.055	0.291
Disturbance	0.275	0.084	3.293	<b>2.97e-03</b>
B.				
Variable	Estimate	SE	Z	P
BIO1	2.563	0.730	3.509	<b>8.98e-04</b>
BIO7	-1.065	0.241	-4.428	<b>1.90e-05</b>
BIO12	-0.187	0.439	-0.426	0.67
BIO15	-0.853	0.299	-2.850	<b>0.009</b>
Slope	0.595	0.122	4.893	<b>2.97e-06</b>
Elevation	0.960	0.565	1.698	0.053
Disturbance	0.274	0.112	2.446	<b>0.029</b>

between the two sexual species, as well as intermediate latitudes (Br, high slope and high latitude; Bs, low slope and low latitude; Appendix S7, section C).

Pure asexual populations were concentrated in a small range of environmental conditions compared with mixed sexual/asexual populations (Fig. 3D). Slope and disturbance were also heavily loaded in the comparison of pure asexual and mixed sexual/asexual populations, with pure asexual populations occurring in locations that are more disturbed (asexual,  $19.40 \pm 6.46$  m from nearest road vs. mixed,  $244.34 \pm 68.81$  m from nearest road) and flatter (asexual slope,  $0.20 \pm 0.03$  vs. mixed slope,  $0.23 \pm 0.02$ ).

#### Apomicts occur in areas of greater environmental variability

We next used GLMs to evaluate the impact of ploidy level and hybridization on environmental associations with reproductive mode. In the full data set, Moran's *I* showed no evidence of spatial autocorrelation, although this was not the case in FC3 (observed Index = 0.11, expected Index = -0.02, *P* = 0.01) or FC4 (observed Index = 0.33, expected Index = -0.01, *P* = 3.40e-14). Thus, some of the signal observed in these subsets may be underlain by geographic structure. In GLMs for FC1, FC2, and FC3, slope was the most significant predictor of reproductive mode, with apomicts occurring in locations of lower slope (flatter topography) than sexuals, and closer to roads, consistent with a positive relationship between apomixis and disturbance. GLMs also indicated a strong positive association between asexual reproduction and temperature and precipitation seasonality, with asexual percentage (hybrid vs. non-hybrid) affecting the patterns observed. Comparing mixed sexual/asexual and pure asexual populations, we found that increased temperature variation predicted pure asexuality.

FC1 included all individuals regardless of species or ploidy level. Of the seven variables incorporated, four had a significant relationship with reproductive mode after correcting for multiple

comparisons (BIO7, BIO12, BIO15, slope, disturbance; Table 3A). The prevalence of sex increased as a function of slope ( $0.70 \pm 0.10$ , *Z* = 7.07, *P* = 6.32e-12) and disturbance ( $0.28 \pm 0.08$ , *Z* = 3.29, *P* = 2.97e-03), and decreased with increased temperature range (BIO7,  $-1.13 \pm 0.17$ , *Z* = -6.53, *P* = 2.02e-10) and greater seasonality of precipitation (BIO15,  $-0.78 \pm 0.20$ , *Z* = -3.97, *P* = 2.18e-04). Cumulatively, these relationships suggest that apomixis in *Boechea* is predicted not only by slope and disturbance, but also by increased variability in temperature and precipitation.

In analyses controlling for ploidy (FC2), significance was retained for BIO7, BIO15, slope, and distance to nearest road (Table 3B). Controlling for ploidy resulted in one new significant effect—annual mean temperature (BIO1; *Z* = 3.51, *P* = 8.98e-04), with asexuals occurring at lower mean temperatures. All relationships between apomixis and environmental variables maintained directionality, with the exception of elevation, which was nonsignificant.

In FC3 (isolating the effects of interspecific hybridization from asexuality), reproductive mode was predicted by BIO1 (annual mean temperature, *Z* = 3.83, *P* = 3.78e-04), BIO3 (isothermality, *Z* = -2.47, *P* = 0.03), BIO4 (temperature seasonality, *Z* = -4.39, *P* = 1.13e-05), BIO15 (precipitation seasonality, *Z* = -4.36, *P* = 5.12e-05), slope (*Z* = 3.79, *P* = 3.02e-04), and disturbance (*Z* = 3.88, *P* = 4.24e-04; Table 4). Although both hybrid and non-hybrid asexuals occur in more disturbed locations than either sexual species, asexual means for slope and annual mean temperature were intermediate between those of the sexual species (Appendix S7, section C). Temperature and precipitation seasonality exhibited more complex relationships. Non-hybrid asexual Br occurs in more environmentally extreme locations (i.e., those with greater temperature and precipitation seasonality, as well as sites with lower values of isothermality) than either sexual species or hybrid asexuals. In contrast, mean precipitation seasonality for hybrids is intermediate between that of their parental species, while mean temperature seasonality for hybrids is lower than that of either parental species; isothermality values are highest for hybrids as well. Cumulatively, these relationships suggest that hybrids have reduced robustness to variation in temperature.

The FC4 GLM comparing pure asexual and mixed sexual/asexual populations indicated an association between temperature range and asexuality, with pure asexual populations occurring in sites associated with greater range in temperatures (estimate = 1.49, *Z* = 2.449, *P* = 0.01; Table 5). Slope and disturbance were not significant in this model, despite vastly different mean values (mixed sexual/asexual  $244.34 \pm 68.81$  m vs. pure asexual  $19.40 \pm 6.46$  m). This disparity may suggest that disturbance facilitates the establishment of pure asexual populations.

**TABLE 4.** Results of GLM (family = binomial) predicting apomixis/sex for FC3, with significant *P* values in bold (*N* = 67 populations).

Variable	Estimate	SE	Z	P
BIO1	3.269	0.853	3.834	<b>3.78e-04</b>
BIO3	-0.551	0.223	-2.468	<b>0.027</b>
BIO4	-1.132	0.258	-4.390	<b>1.13e-05</b>
BIO15	-0.591	0.136	-4.363	<b>5.12e-05</b>
Slope	0.508	0.134	3.790	<b>3.02e-04</b>
Elevation	1.737	0.731	2.376	0.053
Disturbance	0.786	0.203	3.876	<b>4.24e-04</b>

**TABLE 5.** Results of GLM (family = binomial) predicting pure asexual vs. mixed sexual/asexual populations, with significant *P* values in bold (FC4, *N* = 75 populations).

Variable	Estimate	SE	Z	<i>P</i>
BIO3	-0.147	0.429	-0.342	0.732
BIO7	1.493	0.610	2.449	<b>0.014</b>
BIO12	2.188	0.921	2.449	0.053
BIO15	1.265	0.653	1.939	0.530
Slope	-0.437	0.343	-1.273	0.203
Disturbance	-2.595	2.246	-1.155	0.248

## DISCUSSION

Across our study area, ecological differentiation between sexuals and asexuals is widespread in *Boechera*. This pattern is characterized by differences in disturbance, slope, and environmental variability, with asexual individuals tending to occur in flatter, more disturbed, and more variable environments than their sexual counterparts. Although these relationships are mostly unaffected by polyploidy, hybridization does have an impact, with hybrid asexuals present in environments that are intermediate between parental habitat, novel from parental habitat, or similar to the habitat of one parent or the other. Variability in temperature also differs between pure asexual and mixed sexual/asexual populations, suggesting that differences in temperature might permit the initial establishment and spread of asexual *Boechera*. Collectively, these results offer one of very few examples of ED maintaining coexistence of sexual and asexual plants across the landscape. Furthermore, this is the only study, to our knowledge, disentangling the confounding effects of polyploidy and hybridization on sexual/asexual ED.

### Rampant apomixis and hybridization across the landscape

With over half of our accessions identified as apomicts, and 379 (65%) of those representing interspecific hybrids comprising 16 unique species combinations, this study offers further evidence for the widespread nature of apomixis and hybridization in *Boechera*. The pervasiveness of these phenomena is consistent with the findings of Beck et al. (2012) and Li et al. (2017), which together documented hundreds of novel asexual hybrid combinations in *Boechera*. These observations provide strong support for the hypothesis that apomixis has arisen repeatedly in the genus (Sharbel and Mitchell-Olds, 2001; Dobeš et al., 2004a; Kiefer and Koch, 2012; Aliyu et al., 2013). Given this evidence for multiple origins of asexuality in *Boechera*, it may be unsurprising that we found no coarse geographic patterns in the distribution of sexuals and asexuals. Instead, sexuals and asexuals co-occur within roughly one-third of the populations surveyed. Co-occurrence could be due in part to a single older origin of an asexual lineage followed by mutation accumulation and dispersal. Support for this process comes from one recent study that examined multiple lineages from a single pair of parental species and reported close genetic relationships among daughter lineages, consistent with one origin followed by subsequent mutation (Lovell et al., 2017).

Alternatively, asexual lineages may be the result of migration events, and unrelated to the sexual genotypes with which they co-occur. Our results mostly support the latter process. For example, in the present study, hybrids involving *B. sparsiflora* are distributed across 23 populations, but sexual representatives of this species are

not present in the data set at all. Although sampling error may have introduced this pattern (rare sexual *B. sparsiflora* individuals might occur in the study area, but just were not sampled), this is unlikely to be the full explanation, as the northern range limit of *B. sparsiflora* is far south of our study area. This pattern of apomicts ranging beyond their sexual progenitors suggests two potential scenarios: first, that apomictic plants may have more effective dispersal or colonization abilities in comparison with sexuals; or second, that apomictic hybrids originated concurrently with range contractions of sexual congeners. Sexual populations of *Townsendia hookeri*, the Easter daisy, occur beyond both the northern and southern limits of apomictic populations, and patterns of diversity suggest that glacial activity produced the large latitudinal gap between sexual populations (Thompson and Whitton, 2006). Montane glaciers were present in the Rockies of central Idaho during the Pleistocene (Porter et al., 1983; Young et al., 2011), and these similarly may have fragmented distributions of widespread sexual species. *Boechera* hybrid apomicts may have formed in glacial refugia, where sexual species came into secondary contact (Dobeš et al., 2004a). As glaciers retreated, asexuals could either have dispersed into newly available habitat more quickly than their sexual progenitors or dispersed at the same rate but outcompeted sexual lineages. Further analyses are needed to determine the processes that have led to the presence of apomictic hybrids in locales distant from their sexual parental species.

### Environmental variation, slope, and disturbance underlie sexual/asexual ecological differentiation

In all four focal comparisons, our results show that asexual reproduction occurs in areas associated with disturbance, low slopes, and substantial variation in temperature and/or seasonal precipitation (Tables 3–5 and Appendix S9). Asexuality in both animals and plants has long been associated with “marginal” or “extreme” ecology (Glesener and Tilman, 1978; Hörandl, 2006). Glesener and Tilman (1978) hypothesized that asexuality is associated with environmental uncertainty (higher elevation or latitude, extreme drought conditions, greater disturbance) after reviewing data available for asexual animal populations and their sexual progenitors. Indeed, asexual plants are routinely found at higher elevations or latitudes than sexual plants (Bierzychudek, 1985; Hörandl, 2006). In high-elevation habitats, organisms may be subjected to lower overall temperatures, large daily temperature differentials, and substantial variation in monthly precipitation. In the Frank Church–River of No Return Wilderness, where many of our populations are located, low temperatures may reach  $-34^{\circ}\text{C}$  or lower, and high temperatures  $>37^{\circ}\text{C}$  have been recorded; the average temperature differential on a given summer day is  $22^{\circ}\text{C}$ . Average annual precipitation is similarly highly variable, with total rain and melted snow along the Salmon River estimated to be 7–10 inches, and 50–60 inches in western areas of the Wilderness (Finklin, 1988). In an area in which climatic variation is substantial during the course of a single day, the association of asexual *Boechera* with high variability is consistent with a link between asexuality and unforgiving environments.

Disturbance is likewise considered an element of environmental uncertainty and has historically been associated with asexuality (Glesener and Tilman, 1978). In our study area, comprising the mountainous and heavily forested Lemhi and Custer counties in Idaho and Beaverhead County in Montana, anthropogenic disturbance is the exception. At one site in this region, the vegetative

community has been shown to be intact over the last several thousand years (Mehring et al., 1977). In this area, roads are a prime example of recent anthropogenic disturbance of pristine habitat, as the presence of roadways is rare across the majority of *Boechera* populations.

In marginal habitats, asexuals may escape biotic pressures such as competitors, pathogens, or herbivores, offering another mechanism for ED (Glesener and Tilman, 1978). Few empirical examples of this phenomenon exist, but decades of research show a strong association between sex and pathogen infection in the New Zealand mudsnail, with sexuals occupying high infection risk sites (Jokela and Lively, 1995; Lively and Jokela, 2002; King et al., 2009) and asexuals occurring in low-risk areas (Gibson et al., 2016), showing evidence for niche differentiation between sexuals and asexuals that is mediated by biotic interactions. Likewise, Verhoeven and Biere (2013) found that soil microbes occurring in northern apomictic dandelion populations were less pathogenic than those from southern sexual populations; asexual populations also experienced reduced weevil infestation. Thus, biotic interactions may substantially influence sexual/asexual niche differentiation and alter the geographic distribution of each reproductive mode.

Slope is significant in three of our focal comparison GLMs (Tables 3 and 4) and heavily weighted in all discriminant functions (Appendix S9), although Br × Bs hybrids in FC3 are found at slopes intermediate between their parental species. Several factors could potentially explain the importance of slope to ED. Soil density or composition could differ between locations that vary in drainage capability, or asexuals may require more water than sexuals during the growing season (water availability will be higher in flat locations). Consistent with this hypothesis is evidence that facultatively asexual *Boechera* produce more sexual ovules in stressful environments (Mateo de Arias, 2013).

Compared to mixed sexual/asexual populations, purely asexual populations are found in more disturbed and less sloped environments, and in those with greater range in temperature, although only temperature range is significant in our GLM. This suggests that, beyond mere association with asexual reproduction, these factors may facilitate the spread of asexual genotypes beyond their site of origination, consistent with the link between asexuality and invasion (see, e.g., Amsellem et al., 2001; Chapman et al., 2004). Although a recent study found that asexuals and sexuals do not differ in their proclivity for invasion (Dellinger et al., 2016), numerous counterexamples exist. In one recent example of invasive asexuality, the marbled crayfish (a polyploid hybrid asexual) has spread to multiple freshwater systems around the world and expanded its range 100-fold during a 10-year invasion in Madagascar (Gutkunst et al., 2018). Doubtless, reproductive assurance plays an important role in the invasiveness of asexual animals, and likewise, for apomictic plants that are descended from outcrossing sexuals. However, it is possible that other factors may allow asexual invasion, including epigenetic variation, permissive or beneficial environmental conditions, and increased heterozygosity due to polyploidy or hybridization.

#### Disentangling the influence of confounding factors on sexual/asexual ED

In flowering plants, apomixis nearly always co-occurs with hybridization and polyploidy (Asker and Jerling, 1992). Each of these features will uniquely influence morphological traits that, in turn, affect reproductive isolation and gene flow, with consequences for

niche occupation and range limits. For example, most asexuals enjoy reproductive assurance, unlike their sexual progenitors, which is a likely cause of range expansion in many taxa (Hörandl, 2006). Another example is that of recently derived polyploids, which are instantly reproductively isolated from their progenitors because of meiotic failure in interploidy hybrids (Coyne and Orr, 2004). Without novel available niches, neopolyploids may be driven to extinction; those polyploids that survive are likely to be ecologically differentiated from their homoploid relatives.

We found that ED is significant both with and without controlling for polyploidy (Table 2). These results suggest that polyploidy has a minimal impact on ED itself, a conclusion that is further bolstered by the maintenance of significance and directionality in the specific environmental variables associated with sexual/asexual ED, with a minor impact on the relationship between elevation and asexuality. The relatively slight effect of polyploidy in our study system is in contrast to results in other taxa (Bierzychudek, 1985; Lo et al., 2013; Schinkel et al., 2016; Sonnleitner et al., 2016). This also conflicts with previous work in *Boechera*, which detected little niche differentiation between sexual and asexual accessions across the full geographic range, and which attributed ecological differences to polyploidy (Mau et al., 2015). This disparity in results could arise from a number of factors. First, unlike Mau et al. (2015), our study takes advantage of large sample sizes focused on a small portion of the geographic and taxonomic range of *Boechera*, which may provide greater statistical power to detect ecological divergence between sexual and apomictic genotypes. Second, the taxonomy of *Boechera* is complex, and hybrid parentage and identity of the numerous apomictic *Boechera* taxa have been nearly impossible to establish on the basis of morphology alone (Windham and Al-Shehbaz, 2006). Our fine-scale investigation of ED was possible only because of a recently published method for precise *Boechera* identification (Li et al., 2017). Third, Mau et al. (2015) highlighted *B. retrofracta* as one of the few ecologically differentiated *Boechera* species; patterns observed in our *B. retrofracta*-heavy data set may thus differ from taxonomically broader data sets. Lastly, it is possible that scale affects the patterns observed—fine-scale ED may not operate in the same way across the full geographic range of the genus, due to evolutionary or stochastic processes that play out over vast time scales. This may reflect the often-observed disparity between microevolutionary and macroevolutionary patterns in evolutionary biology as a whole (see, e.g., Rabosky and Matute, 2013).

Previous studies have implicated hybridization as an important driver of ED (Kearney, 2005; Coughlan et al., 2014), and hybridization appears to be an important driver of sexual/asexual ED in *Boechera* as well. Although hybridization can have detrimental effects on fitness, the introduction of new genetic material may increase evolutionary amplitude (Arnold and Martin, 2010; Yakimowski and Rieseberg, 2014). Shifts in habitat preference may occur in hybrids (Rieseberg et al., 2003), which can permit invasion of new habitats (Schierenbeck and Ellstrand, 2009; Hovick and Whitney, 2014). In a review of hybrid plant morphology, Rieseberg and Ellstrand (1993) found that most plant traits were either intermediate between the parents (45%) or indistinguishable from one parent or the other (45%). Alternatively, hybrid traits may be more extreme than either parent, which has been shown to scale with the genetic distance between progenitor species (Stelkens and Seehausen, 2009). Even if transgressive segregation does not occur, interspecific hybridization will result in novel trait combinations with potentially profound evolutionary and ecological implications (Seehausen, 2004).

Our focused comparison of two sexual species, their asexual hybrid, and asexual non-hybrids reveals complex patterns of ED. Considering environmental preference a trait of an organism, we find that hybrid *Boechera* meet each of the patterns predicted in the hybridization literature—hybrids occur in environments that are intermediate between their parents (slope and precipitation seasonality), similar to one parent or the other (precipitation in wet-test quarter and mean diurnal range), or more extreme than either parent (disturbance and isothermality; Appendix S7, section C). Cumulatively, our results suggest that hybridization has a unique impact on ED that differs from the effects of asexuality.

Why do the effects of hybridization differ between the full data set and FC3? Several explanations are possible. First, particular taxa and their niche preferences may simply produce different patterns. If FC3 included a different set of species, or consisted of a greater proportion of the ~80 sexual *Boechera* species and their hybrids, our conclusions would likely have differed. This issue underscores the importance of taxon identification in any study of asexual organisms; a given result will vary depending on the taxonomic understanding of the study group. Second, ED may depend on hybrid age; older hybrids could have established ecological niches that differ from those of their parental species, whereas recently derived hybrids may simply have lacked time to do so (Mau et al., 2015). The age of hybridization events could well differ between our full and reduced data sets; hybridization event(s) giving rise to the Br × Bs apomicts may have been recent, whereas FC1 may contain older hybrids, leading to a different pattern. Evidence for both recent (Koch et al., 2003; Dobeš et al., 2004b; Kiefer et al., 2009) and older (Dobeš et al., 2004a) Pleistocene hybridization events suggests a range of ages for apomictic lineages in *Boechera*. Further work is needed to accurately age the formation of hybrid lineages across the genus, and to assess the relative frequency of recent vs. older hybridization events and their respective impacts on ED.

In our comparison of non-hybrid and hybrid asexuals with their parental species, our results show that it is non-hybrid asexuals that occur in the most variable environments, rather than hybrids. Reproductive assurance does not differ between sexual and asexual rock cresses, and other associated traits are more likely to cause this observation. Chief among candidate traits is heterozygosity, which is low in selfing organisms and high in those produced via hybridization or outcrossing. In most studies of sexual/asexual ED, the effects of heterozygosity cannot be disentangled from those of hybridization or polyploidy. Indeed, although the masking of deleterious alleles accumulated via Muller's ratchet could facilitate rapid adaptation or increase tolerance of stressful environments, this process may be caused by polyploidy (Chao et al., 2013), by hybridization (reviewed in Abbott et al., 2013), or simply by disparities in heterozygosity (Gutekunst et al., 2018). Increased heterozygosity via outcrossing is sufficient to mask deleterious alleles, as evidenced by the vast increase in yield and improved drought resistance of hybrid maize (Crow, 1998). The results presented here are consistent with enhanced tolerance of environmental variability conferred by heterosis, although further studies are required to confirm the consequences of heterozygosity on fitness in rock cress.

## CONCLUSIONS

This study has addressed a critical gap in our understanding of the ecology of sex: What environmental variables underlie ecological

differentiation between sexual and asexual plants? Although addressed in studies spanning the entire range of several plant species, only one study, to our knowledge, has asked this question at a densely sampled focal geographic scale, and no previous work has been able to isolate the effects of asexuality from those of polyploidy, hybridization, and reproductive assurance. The intermediate scale of this work provides robust statistical power to detect patterns across a large number of populations, without the confounding influence of large-scale climatic variation or substantial differences in biogeographic history. Our extensive sampling of the mustard genus *Boechera* showed that sexuals and asexuals are ecologically differentiated across a swath of *Boechera*'s native range. Asexuality is associated with increased disturbance and environmental variability, as well as flatter topography; hybridization affects these associations. It is possible that these environmental conditions promote the establishment of asexual populations, although further study is needed. This work has important implications for the study of sexual/asexual coexistence in plants, and the genetically and empirically tractable system *Boechera* offers a unique opportunity for further investigation.

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## AUTHOR CONTRIBUTIONS

C.A.R. and T.M.-O. designed the research. C.A.R. and R.A.K. collected the data. C.A.R. and M.D.W. analyzed the data. C.A.R. and T.M.-O. interpreted the data. C.A.R., M.D.W., and T.M.-O. wrote the manuscript.

## DATA ACCESSIBILITY

Original data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.1jn5887> (Rushworth et al., 2018).

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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