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Hybrid apomicts trapped in the ecological niches of their sexual ancestors

Martin Mau[a,1], John T. Lovell[b], José M. Corral[c], Christiane Kiefer[d], Marcus A. Koch[e], Olawale M. Aliyu[a,f], and Timothy F. Sharbel[a]

[a]Apomixis Research Group, Leibniz Institute of Plant Genetics and Crop Plant Research, 06466 Gatersleben, Germany; [b]Department of Integrative Biology, The University of Texas at Austin, Austin, TX 78712; [c]Department of Bioanalytics, Coburg University of Applied Sciences and Arts, 96450 Coburg, Germany; [d]Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; [e]Department of Biodiversity and Plant Systematics, Centre for Organismal Studies Heidelberg, 69120 Heidelberg, Germany; and [f]Department of Crop Production, College of Agriculture, Kwara State University, PMB 1530 Ilorin, Kwara State, Nigeria

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Asexual reproduction is expected to reduce the adaptive potential to novel or changing environmental conditions, restricting or altering the ecological niche of asexual lineages. Asexual lineages of plants and animals are typically polyploid, an attribute that may influence their genetic variation, plasticity, adaptive potential, and niche breadth. The genus *Boechera* (Brassicaceae) represents an ideal model to test the relative ecological and biogeographic impacts of reproductive mode and ploidy because it is composed of diploid sexual and both diploid and polyploid asexual (i.e., apomictic) lineages. Here, we demonstrate a strong association between a transcriptionally conserved allele and apomictic seed formation. We then use this allele as a proxy apomixis marker in 1,649 accessions to demonstrate that apomixis is likely to be a common feature across the *Boechera* phylogeny. Phylogeographic analyses of these data demonstrate (*i*) species-specific niche differentiation in sexuals, (*ii*) extensive niche conservation between differing reproductive modes of the same species, (*iii*) ploidy-specific niche differentiation within and among species, and (*iv*) occasional niche drift between apomicts and their sexual ancestors. We conclude that ploidy is a substantially stronger and more common driver of niche divergence within and across *Boechera* species although variation in both traits may not necessarily lead to niche evolution on the species scale.

Boechera | *UPGRADE2* | *APOLLO* | geographic parthenogenesis | niche conservation

Sexual reproduction offers several evolutionary advantages over asexuality, including accelerated adaptation to variation in environments (1), competitors (2), and parasites (3). As such, evolutionary transitions from sexual to asexual reproduction or outcrossing to selfing may have a strong impact on an organism's ecological distribution and adaptive potential (4, 5). Because reproductive-mode divergence can occur on short temporal scales (6), comparisons between closely related taxa that differ in reproductive mode offer unique opportunities to study the adaptive significance of sexuality at micro- (i.e., population) (7) and macroevolutionary (i.e., species) (8) levels.

Apomixis, the asexual formation of seeds via meiotically unreduced gametes, is rare among angiosperm genera (~1.1%) (9). It is nonetheless an evolutionarily important trait capable of fixing the entire genome as one linkage group across generations, conferring potential fitness advantages associated with the now-fixed genotype, such as yield, in ecological (10) and agricultural settings (11). Apomicts seem to have evolved from sexual ancestors independently in several distantly related taxa (12) and can experience advantages, such as reduced or no allocation to male function (in hermaphroditic taxa) (13) and reproductive assurance (sensu ref. 14), which together enhance their colonizing abilities (15). These advantages may be tempered by disadvantages imparted by the absence of recombination, such as increased deleterious mutation accumulation (16) and poor responses to selection imposed by changing environments (17).

Comparisons between asexuals and their sexual ancestors shed light upon the processes contributing to the evolution and maintenance of apomixis. Both novel mutations (i.e., gain-of-function mutation; sensu ref. 18), and/or hybridization (19) have been proposed to induce apomixis although recurrent hybridization may obscure origin and age estimations of natural apomictic lineages [e.g., *Boechera* (20) and *Taraxacum* (21); but see ref. 22]. One explanation for the success of apomicts in mixed reproductive systems follows from the fact that many of them display strong evidence for niche differentiation from their sexual progenitors, a pattern termed "geographic parthenogenesis" (GP) (23, 24). The ubiquity of GP has led to the hypothesis that niche differentiation, rather than niche conservation, governs the ecology of apomictic lineages (ref. 25; but see ref. 26). GP could be explained by (*i*) an escape from competition between sexuals and apomicts occupying similar niches (27–29), (*ii*) selection for asexual genotypes with wider ecological tolerance compared with sexuals ("general purpose" genotype model) (30), and (*iii*) niche partitioning between sexual parents and their hybrid apomictic progeny, the latter of which have a fixed subset of genetic variation from the sexuals ("frozen-niche variation" model) (31). Despite substantial evidence for GP, the factors responsible for this pattern are poorly understood. For example, because GP is commonly observed in diploid sexual–polyploid asexual complexes (25, 32), it is speculated that ploidy could be the primary source of GP rather than reproductive mode (14, 25, 33).

Significance

Ecological-niche differentiation in diploid sexual–polyploid asexual complexes has been observed within and among many taxa, yet the relative contributions of reproductive system and ploidy are not fully understood. Here, we assess niche characteristics of sexual diploid, apomictic (asexual) diploid, and triploid *Boechera* (Brassicaceae) lineages. We find strong evidence for widespread hybridization and, to a lesser degree, ploidy variation as factors that together overcome the adaptive disadvantages of apomictic (i.e., asexual) reproduction. When controlling for ploidy, we find only modest evidence for putatively asexually driven ecological-niche divergence among reproductive systems, a finding that contradicts the well-supported patterns of geographic parthenogenesis.

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[1]To whom correspondence should be addressed. Email: mau@ipk-gatersleben.de.

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The North-American genus *Boechera* (Brassicaceae) is an ideal system to study the ecological and evolutionary dynamics of reproductive-mode divergence. The genus is monoecious, and, in addition to broad ecological ranges and high intra- and interspecies diversity (34), *Boechera* possesses three reproductive-mode classes: diploid sexuals versus triploid and diploid pseudogamous apomicts (35). A number of lines of evidence demonstrate that the switch from sex to apomixis has occurred multiple times during the evolution of *Boechera* (36–38). As such, *Boechera* offers replicated events of sexual-apomictic transitions within a ploidy level and enables comparisons between reproductive modes without the confounding effects of variable ploidy.

Here, we document the associations between reproductive-mode variation and the extensive ecological and physiological diversity of *Boechera* to test two hypotheses: (*i*) Apomixis is a recent evolutionary development arising only in a related subset of haplotypes, and (*ii*) niche evolution is an intrinsic factor of reproductive-mode divergence (i.e., geographic parthenogenesis) and not a covariate of ploidy variation. To assess these hypotheses, we examine the phylogeographic distribution of *APOLLO* (apomixis-linked locus) (39) and *UPGRADE2* (unreduced pollen grain development) (40), two alleles whose expression is highly correlated with apomeiotic egg and pollen formation in *Boechera*, respectively, to infer the ecological niches of 1,649 single samples from different populations per species of diploid sexual and apomictic *Boechera*. Our data provide phylogeographic evidence for multiple origins of apomictic cytotypes in *Boechera* and support a frozen-niche variation model for diploid apomixis niche evolution. Importantly, we provide statistical evidence that ploidy variation, both within and among species, is a stronger driver of niche evolution than reproductive mode.

Results

A Molecular Marker Predicts Apomixis in *Boechera*. We used the flow cytometric seed screen (FCSS) (41) to functionally infer reproductive mode in 275 *Boechera* accessions from 22 species (Dataset S1). Each plant was additionally genotyped for the presence or absence of apomixis-specific alleles (hereafter "allele class") of two genes associated with apomeiotic pollen (*UPGRADE2*) (40) and egg cell formation (*APOLLO*) (39) (Fig. S1). FCSS revealed that apomixis-specific alleles were nearly fixed among plants determined to be apomictic (*UPGRADE2*, 96.06%; *APOLLO*, 98.39%, respectively). Sexuals were virtually free from the apomictic *APOLLO* allele (frequency, 2.27%) whereas 34.48% of sexuals had the apomictic *UPGRADE2* allele. The tighter association of the apomictic *APOLLO* allele with apomixis (logistic regression model; predictor variable = *APOLLO*, covariates = FCSS and taxon data, $n = 256$, $e^B = 2835.48$, $\chi^2 = 306.49$, $P < 0.0001$) (Dataset S1), relative to the apomictic *UPGRADE2* allele (logistic regression model; predictor variable = *UPGRADE2*, covariates = FCSS and taxon data, $n = 272$, $e^B = 60.48$, $\chi^2 = 139.59$, $P < 0.0001$) (Dataset S1), led us to use the *APOLLO* polymorphism as a proxy marker for apomixis.

Broad Phylogenetic Distribution of Apomixis. We genotyped the *APOLLO* and *UPGRADE2* allele classes in 1,374 additional herbaria accessions, representing 84 of the 111 accepted *Boechera* species and nine species of four closely related genera (Dataset S1 and Table S1). A subset of 1,010 accessions were previously genotyped for several chloroplast markers (20). The chloroplast DNA (cpDNA) haplotypes were used to determine the phylogenetic distribution of *APOLLO* and *UPGRADE2* allele classes on a genus-wide scale ($n = 1,649$) because true species-specific cpDNA-haplotype lineages are rare (i.e., in total, seven maternal phylogenetic lineages) due to haplotype sharing among species (20).

On a genus-wide scale, apomixis, as defined by the presence of the apomictic *APOLLO* allele, was found in all cpDNA-haplotype lineages and in 49.31% of all *Boechera* cpDNA haplotypes (Dataset S1). Apomixis frequencies did not vary between the major cpDNA-haplotype lineages (two-tailed Fisher's exact tests between cpDNA-haplotype lineages I, II, and III; VI and VII were excluded because $n \leq 5$ accessions; $P \geq 0.071$) (Dataset S1), except for lineages IV and V, where apomixis frequencies were strongly reduced compared with 49.31% average apomixis frequency (two-tailed Fisher's exact test; lineage IV, 1 of 13 accessions, $P = 0.0033$; lineage V, 2 of 21 accessions, $P = 0.0002$) (Dataset S1).

Of the 31 *Boechera* species characterized by more than 10 accessions, 29 species contained both apomictic and sexual individuals (Table S1). There was wide variation in the frequency of apomictic individuals across species (range, 0–100%; median, 42.55%) (Fig. 1*A*). For example, *Boechera retrofracta* and *Boechera divaricarpa*, both large groups with wide geographic distributions, were characterized by both reproductive modes (61.96%, and 77.45% apomixis, respectively) (Fig. 1*A* and Table S1). By contrast, *Boechera stricta*, with the widest distribution of any species (42), was predominantly sexual with a few apomicts ($n = 214$, 83.71% sexual) (Fig. 1*A* and Table S1). As seen previously (43), our data also show that hybrids, such as *B. stricta* × *B. retrofracta* and *B. stricta* × *Boechera spatifolia*, have the highest frequencies of apomixis ($n = 11$, 93.33% and $n = 15$, 100%, respectively) (Fig. 1*A* and Table S1).

***UPGRADE2* and *APOLLO* Are Linked and Geographically Dispersed.** The apomixis-specific alleles of *UPGRADE2* and *APOLLO* were detected in 41.73% and 46.15% of the tested accessions, respectively (Dataset S1). For the purpose of this paper, we use the term "linkage" to describe cooccurrence or coabsence of the apomictic *UPGRADE2* and *APOLLO* alleles in single individuals, as determined through PCR. In that light, 77.08% of all accessions ($n = 1,584$) demonstrated linkage of both allele classes (i.e., both apo-alleles cooccurred or were coabsent in an individual). The number of accessions demonstrating linkage between both allele classes varied from 39.39% to 100% among species (Fig. 1*B* and Dataset S1).

Individuals carrying the oldest cpDNA haplotypes AA, AB, and AC, which are represented by suprahaplotype S8 (~0.7–2 million y) (44) (Fig. 2), had either none, both, or one of the apomixis-related *APOLLO* and *UPGRADE2* alleles. There was no evidence for overrepresentation of either allele class in ancient or recent cpDNA-haplotype carriers (*APOLLO*, $r^2 = 0.499$; *UPGRADE2*, $r^2 = 0.281$) (Table S2). We also found that all cpDNA haplotypes associated with the apomixis-specific alleles of one or both loci are interconnected in the phylogenetic network (Fig. 2).

The apomictic alleles of *APOLLO* and *UPGRADE2* were each observed only in a single accession of *Boechera* sister genera (*APOLLO, Cusickiella quadricostata*; *UPGRADE2, Polyctenium fremontii*) (Dataset S1 and Table S1). In contrast to the apomictic *APOLLO* allele, the apomictic *UPGRADE2* allele was not detected in any of the genera in neighboring clades (Table S1 and GenBank nucleotide collection search, www.ncbi.nlm.nih.gov/genbank/, release 205.0). However, the two apomictic alleles were never linked in outgroups of the *Boechera* phylogeny.

Sexual and Apomictic *Boechera* Do Not Differ in Genus-Wide Geographic Range. We used a constrained correspondence analysis (CCA) to compare the geographic distribution of both allele classes of the proxy apomixis marker *APOLLO* within and across species. Statistical differences among groups were determined by 10,000 permutations. First, tests of geographic divergence conducted by partitioning ecological-niche differentiation among accessions showed no significant differences between sexual and apomictic *Boechera* on a genus-wide scale ($n = 1,595$, $P_{spat} = 0.549$) (Fig. 3 and Table 1). Both allele classes of *APOLLO* spanned nearly the entire geographic distribution of the total sample, which has a

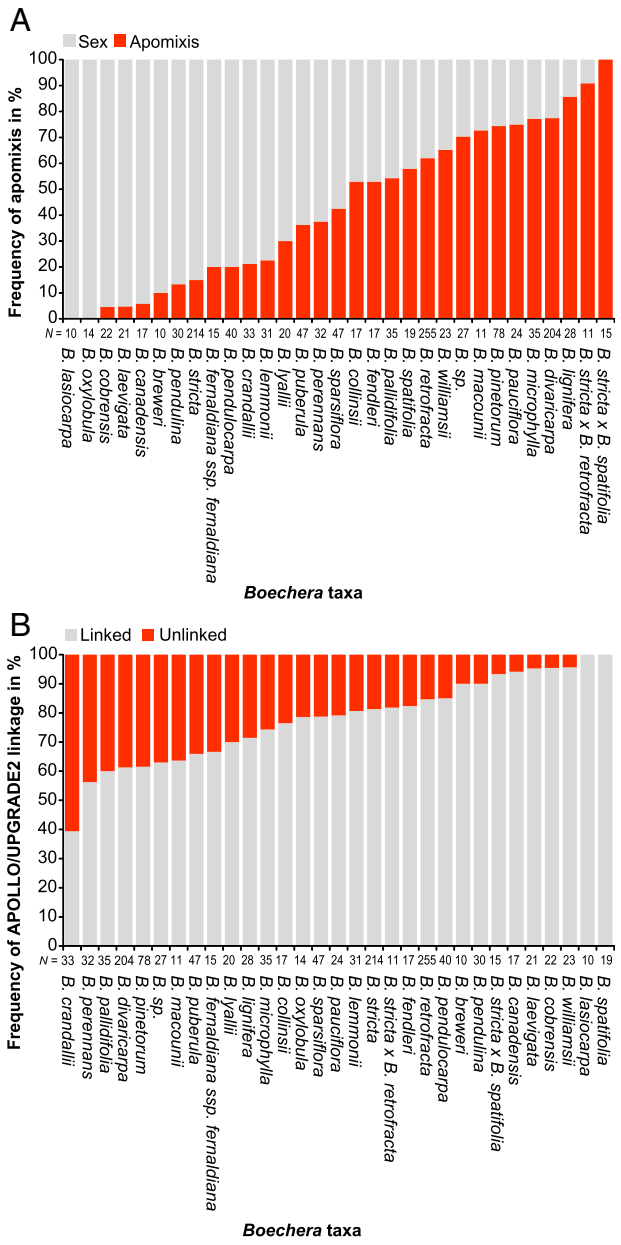

Fig. 1. Frequency of (*A*) apomixis inferred from the presence of the female-apomeiosis marker allele of *APOLLO* and (*B*) linkage of male- and female-apomeiosis marker alleles across *Boechera* species. Here, the term "linkage" refers to the cooccurrence or coabsence of both apomictic alleles from the *UPGRADE2* and *APOLLO* genes per individual accession.

latitude range from 31°34'N to 72°46'N and a longitude range from −50°28'W to −151°22'W. Thereby, sexuals ranged from 31°34'N, −91°12'W to 72°46'N, −56°10'W, and apomicts ranged from 32°2'N, −115°54'W to 69°40'N, −50°28'W (Fig. 3, Fig. S2, and Table S3).

To determine the ecological niche of each *APOLLO* allele class, we assessed the values of 19 bioclimatic variables (www.worldclim.org/) and elevation for each accession. Random forest classification was used to select variables based on their importance for each model (Table 1). On the genus-wide scale, there was no signature of ecological-niche differentiation between sexuals and apomicts ($PP_{eco} = 0.159$) (Figs. 3 and 4 *A* and *C* and Table 1), with both reproductive modes typically found in temperate conifer forests and desert/xeric shrublands (82.96% and 84.87% respectively) (Fig. 3, Dataset S1, and Table S4). Sexuals and apomicts were found in similar mean annual temperatures (apomicts, lower quartile = 2 °C, upper quartile = 6 °C; sexuals, lower quartile = 2 °C, upper quartile = 7 °C), annual precipitation (apomicts, 356–603 mm, sexuals, 336–643 mm), and elevation (apomicts, 1,402–2,520 m; sexuals, 1,126–2,469 m) (Fig. 3 and Table S3). No geographic differentiation was found between ploidy classes across the entire sample ($P_{spat} = 0.687$) (Table 1). However, diploid genotypes had climatic distributions that were different from those of triploids ($PP_{eco} = 0.014$) (Table 1).

Comparisons between reproductive mode and ploidy class independently revealed genus-wide evidence of ecological-niche differentiation between sexual and apomictic genotypes, independent of ploidy ($PP_{eco} = 0.013$) (Table 1). Within apomicts, diploids and triploids displayed different ecological distributions ($PP_{eco} = 0.037$) (Fig. 4 *B* and *D* and Table 1). Combined, these results point to a weak, but significant, pattern of GP for both ploidy and reproductive mode across *Boechera*.

Ploidy and Reproduction Independently Influence Niche Partitioning in *Boechera*. Lack of niche differentiation at the genus level could be an artifact of among-species apomixis-independent divergence (Fig. 3). Alternatively, niche conservation may reflect extended periods of sympatry between sexuals and apomicts, as observed for other agamic complexes (e.g., *Taraxacum officinale*) (45). We thus tested the effect of both reproductive mode and ploidy separately on within-species niche variation. Across the 84 *Boechera* species, we had sufficient replication within each of the three ploidy classes and two reproductive modes to conduct within-species tests for 7 and 18 species, respectively.

There was no evidence for significant geographic divergence for 6 of 7 species at the ploidy level, and for 15 of 18 species at the reproductive-mode level (Table 1). This pattern was bolstered by a paired Student's *t* test demonstrating that the geographic range areas of sexuals and apomicts within species were similar ($r^2 = 0.83$, $P < 0.0001$; paired *t* test, df = 18, $P = 0.342$) (Table S3). We did detect geographic divergence between apomicts and sexuals in three species (*B. crandallii*, $P_{spat} = 0.0002$; *B. retrofracta*, $P_{spat} = 0.0001$; and *B. stricta*, $P_{spat} = 0.0003$) (Fig. 3, Table 1, and Fig. S3). Differences between ploidy levels were observed only in *B. retrofracta* ($P_{spat} = 0.0001$) (Table 1).

A combined CCA using niche models for each ploidy class or each reproductive-mode class per species in addition to spatial distribution as a covariate revealed significant local niche differentiation between sexuals and apomicts in 2 of 18 species (e.g., *B. retrofracta*) (Fig. 5 *A* and *C* and Table 1). A within-species test of the independent effects of allele and ploidy classes in 57 diploid sexual and 27 diploid apomictic *B. retrofracta* accessions confirmed niche differentiation between reproductive modes in diploids ($PP_{eco} = 0.0002$) (Fig. 5 *A* and *C* and Table 1). Additionally, comparisons between apomictic accessions also revealed significant niche differentiation between ploidy levels ($PP_{eco} = 0.0049$) (Fig. 5 *B* and *D*, Fig. S4, and Table 1). On the species level (i.e., for *B. retrofracta*), apomictic diploids had a wider ecological-niche distribution compared with apomictic polyploids whereas, at the genus-wide scale, the trend was opposite (Fig. S4). This observation points to varying directions of niche differentiation among species. Overall, local niche differentiation with ploidy as cofactor (4 of 7 species) (Table 1) occurred significantly more frequently than with reproductive-mode divergence (2 of 18 species, Fisher's exact test, $P = 0.032$) (Table 1).

Discussion

***APOLLO* and *UPGRADE2* Are Linked and Conserved in Apomicts.** We used quantitative analyses of the penetrance of apomictic seed formation and a large-scale screening of apomictic seed formation in a variety of *Boechera* taxa (Dataset S1) to demonstrate that presence of the female apomeiosis-linked allele of the

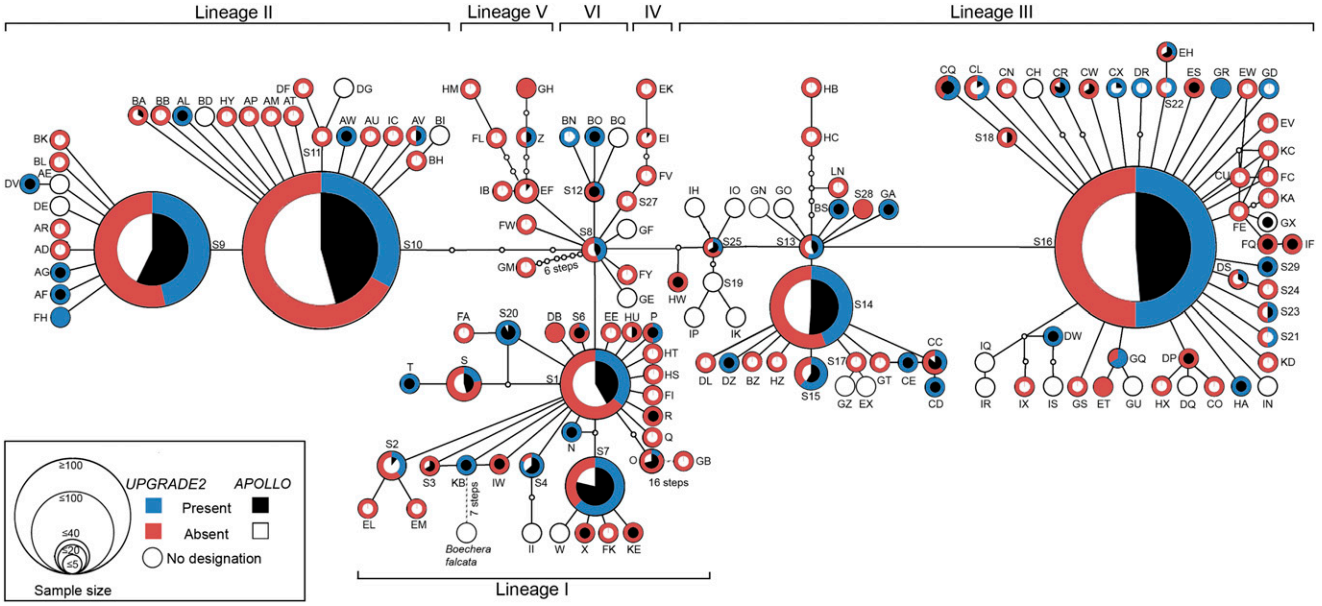

Fig. 2. Genus-wide phylogenetic distribution of apomixis in *Boechera*. The phylogenetic distributions of apomictic *APOLLO* and *UPGRADE2* alleles reflect the range of chloroplast haplotype diversity of sexual *Boechera* accessions. Haplotype node sectors indicate the frequency of accessions carrying the male and the female apomeiosis alleles versus those lacking one or both alleles.

APOLLO gene (39) can be used as a proxy marker for apomixis. A parallel analysis of the same samples for the presence of the male-apomeiosis allele *UPGRADE2* (40) demonstrated a weaker positive correlation with apomictic seed production (Dataset S1). These results imply segregation between the *UPGRADE2* and *APOLLO* alleles and are consistent with the fact that not all apomictic genotypes produce unreduced pollen (40, 46). For example, *APOLLO* and *UPGRADE2* are unlinked in some accessions of *Boechera microphylla* (31.82%) (Fig. 1*B*), which could explain the absence of unreduced pollen in some apomictic accessions (47).

We used the apomeiosis-linked *APOLLO* allele to screen for the potential for apomictic seed production in a large number of herbarium accessions (n = 1,373; taken from ref. 20) for which no seed material existed. These results demonstrated that (*i*) some taxa that were previously classified as purely sexual [e.g., *B. stricta* (48) and *B. crandallii* (49)] are likely to contain apomicts (Fig. 1*A* and Table S1) and (*ii*) taxa formerly considered as purely apomictic [e.g., *B. divaricarpa* (37, 48)] are likely to be characterized by both sexual and apomictic members (Fig. 1*A* and Table S1). Our demonstration that the majority of tested taxa (93.55% of the 31 *Boechera* species with $n \geq 10$ accessions) (Table S1) contain both sexual and apomictic members is consistent with recent taxonomic reassessments of *Boechera*, whereby morphological differences between sexual and hybrid apomictic members of a species are considered as significant characters for taxon subdivision (see the Flora of North America (Vol. 7) website, floranorthamerica.org/). Nonetheless, our data imply that morphological divergence has not yet been accompanied by niche differentiation (as measured here) between apomicts and sexuals in the majority of tested species (88.89%) (Table 1). Considering the already established complex influences of adaptation, hybridization, and polyploidy on the morphological evolution of *Boechera*, for example with previously observed variability in relative levels of meiotically reduced and unreduced pollen even among obligate apomicts (40), it is not surprising that our ability to resolve ploidy and/or reproduction-associated effects relied upon species-level rather than genus-level comparisons.

The high frequency of phylogenetically and geographically distant taxa in which the apomixis-specific alleles of *APOLLO* or *UPGRADE2* were linked in a subset of their individuals (96.43%) (Dataset S1), in conjunction with their conserved polymorphisms and complex DNA sequences (39, 40) and the fact that each allele was discovered in an independent experiment (39, 40), implies that each allele is part of, or is tightly linked to, the genetic networks leading to apomeiotic egg and pollen formation.

The cooccurrence of apomixis-specific alleles of both the *APOLLO* and *UPGRADE2* genes across the majority of *Boechera* taxa (Table S1) is indicative of either their independent origins, followed by complementation through hybridization, or common ancestry with regard to their origin. Our data more strongly support the former by the fact that the 5′ UTR polymorphism that defines the apomictic *APOLLO* allele predates the origin of the genus *Boechera* (39). In contrast to the apomictic *APOLLO* allele, the apomictic *UPGRADE2* allele was only found in two single accessions among 2 of 14 species tested, belonging to a more broadly defined genus *Boechera* (e.g., *Boechera laevigata*) (50) or closely related genera (i.e., *Cusickiella*) while not being detected in distant plant taxa (e.g., *Arabidopsis* and *Brassica*) (Dataset S1, Table S1, and GenBank nucleotide collection, www.ncbi.nlm.nih.gov/genbank/, release 205.0).

Similar Haplotype Diversity in Sexuals and Apomicts Mirrors Reticulate Spread of Apomixis Alleles. Hybridization can be considered as a potential inducer of apomixis (19). Intra- and interspecific gene flow from apomicts to sexuals via apomictic pollen is possible (48) and likely facilitated the horizontal transfer of apomixis across *Boechera* (43, 51). Nevertheless, in *Boechera*, hybridization and apomixis are closely (47) but not exclusively (43) associated. Thus, if hybridization per se is not the induction mechanism of apomixis in *Boechera*, our data together imply that (*i*) *APOLLO* and *UPGRADE2* arose independently of one another in different *Boechera* species/populations and (*ii*) these apomixis alleles were brought together via hybridization between plants carrying *APOLLO* and/or *UPGRADE2*, which facilitated the transfer of both alleles into different sexual genetic backgrounds (i.e., species), leading to the reticulate phylogeographic pattern shown here (Figs. 2–4, Table 1, Fig. S3, and Tables S3 and S4).

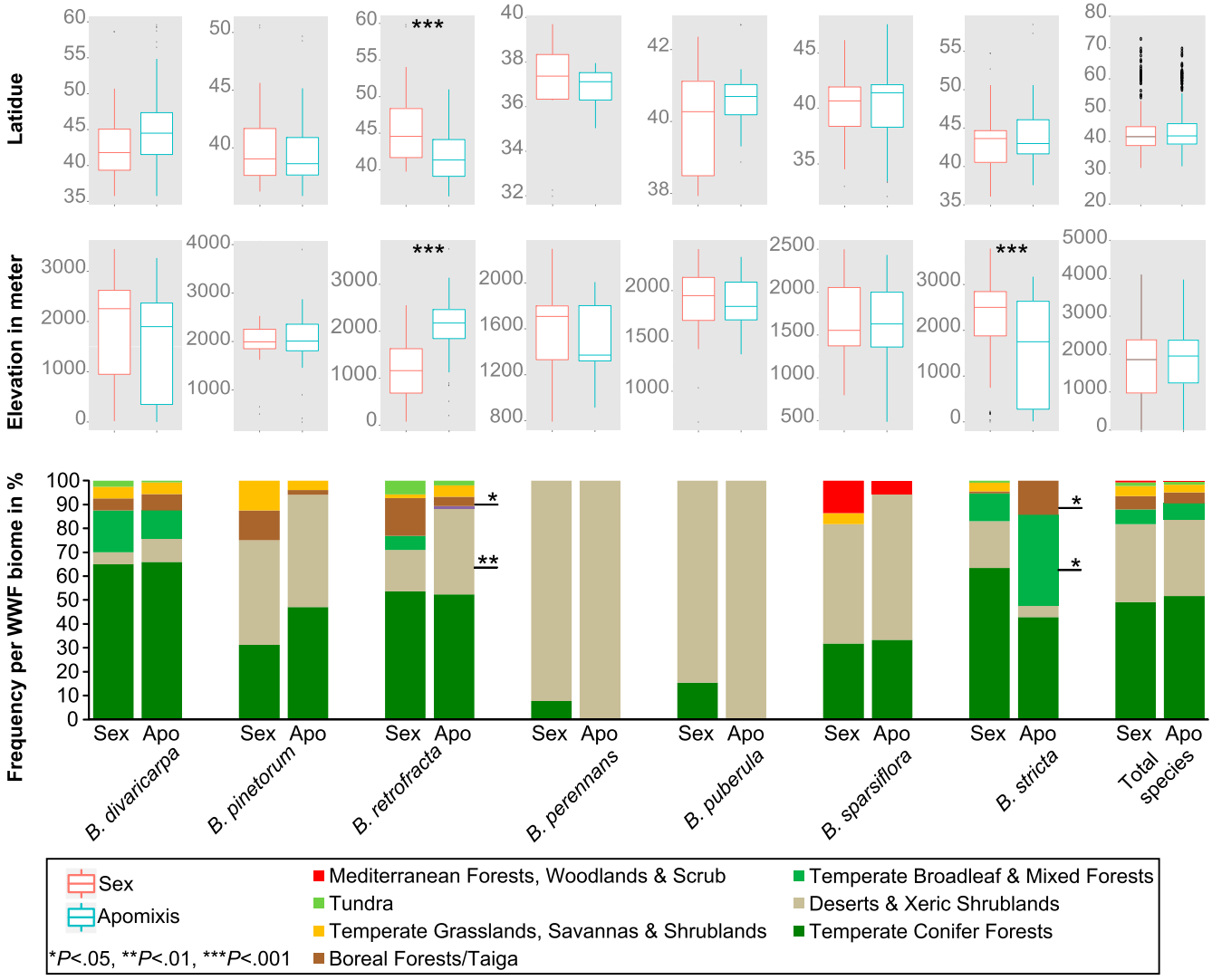

Fig. 3. Species-specific variation of niche occupation and niche partitioning between sexual and apomictic *Boechera* in a subset of species where statistical comparisons could be made. In some species, apomixis is constrained to a subset of climates (e.g., *B. retrofracta*) whereas, in others, apomixis is found across the entire ecological niche (e.g., *B. divaricarpa*). Asterisks denote significant differences between distribution of sexual and apomictic *Boechera* based on two-tailed Fisher's exact tests ($\alpha = 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$).

Widespread hybridization (44, 48) reflects similar apomixis frequencies across the major cpDNA-haplotype lineages (lineage I = 54.26%, lineage II = 47.74%, and lineage III = 50.64% apomixis) (Figs. 1*B* and 2 and Tables S2 and S4), corroborates previous findings of high genotypic diversity in other agamic complexes (e.g., *T. officinale*) (52), and is furthermore supported by a computational study (53). It remains unclear whether the observed phylogenetic and geographic cooccurrence of apomixis-specific alleles is due to multiple independent introgressions of both alleles, or a single introgression event involving both alleles followed by dispersion of the linked alleles via hybridization throughout the genus.

Niche Conservation Between Reproductive Modes, Isolation-By-Ploidy, and Occasional Niche Drift Between Apomicts and Their Sexual Ancestors. Seen from a genus-wide level, apomicts and sexuals share similar habitats and climatic limits (Figs. 3 and 4 *A* and *C* and Table 1), an observation that could be explained by the spread of apomixis into different sexual backgrounds in an "infectious" fashion (sensu ref. 54). A dynamic equilibrium between generation and neutral loss of asexual lineages (55) could thus have led to the broad niche conservation and lack of GP in *Boechera*, in contrast to other agamic complexes (e.g., *Ranunculus auricomus* complex) (56).

Despite the decreased statistical power of species-level analyses due to our inability to infer and test multiple populations per species from the herbarium dataset, these analyses were still able to resolve divergent patterns. Species-specific niche occupation (Fig. 3, Fig. S3, and Table S3) reflects the divergence and adaptation processes that characterize the evolutionary success of *Boechera* (34). However, there is also significant local adaptation within *Boechera* species [e.g., *B. stricta* (57) and *B. spatifolia* (58)] that seems to be associated with reproductive mode, which covaried with niche occupation in 2 of 18 species with $n \geq 5$ accessions per reproductive mode (Fig. 5 *A* and *C* and Table 1). Importantly, these niche differences were present despite identical ploidy levels (see *B. retrofracta* and *Boechera williamsii*) (Table 1).

Niche conservation between reproductive modes in the majority of the tested species (88.89%) (Table 1) could have a number of explanations. First, an ancestral and independently derived apomictic lineage may have evolved to occupy a similar niche as a particular sexual species (i.e., evolutionary convergence). This

Table 1. Widespread niche conservation between reproductive modes, and isolation-by-ploidy niche differentiation within and among species of *Boechera*

Subset	Sex	Apo	Variables selected*	F_{spat}[†]	P_{spat}[†]	F_{eco}[‡]	P_{eco}[‡]	PP_{eco}[‡]	α[§]
Subset "reproduction"									
All accessions[¶]	869	726	Bio10, elevation	0.32	0.5498	0.59	0.1615	0.1593	0.0500
All 2×[¶]	269	125	Bio2, bio6, bio8, bio9, bio11, bio12, bio19, elevation	2.86	0.0789	2.94	0.0145	**0.0129**	0.0250
All 3×[¶]	22	101	Bio14, bio16	0.02	0.8647	2.00	0.2398	0.2405	0.0250
Boechera collinsii	8	9	Bio3, elevation	1.94	0.1613	0.51	0.3015	0.3086	0.0026
Boechera crandallii	26	7	Bio3, bio16	17.74	**0.0002**	6.34	0.0177	0.0200	0.0026
Boechera divaricarpa[¶]	46	158	Bio4, bio14, bio18	0.39	0.5236	1.79	0.1579	0.1554	0.0026
Boechera fendleri	8	8	Bio3, bio9	0.27	0.5884	2.45	0.1330	0.1324	0.0026
Boechera lemmonii	23	7	Bio2, bio5, bio7, elevation	2.04	0.1618	3.50	0.0080	0.0093	0.0026
Boechera lyallii	14	5	Bio8, bio18	0.67	0.4204	0.05	0.8187	0.8164	0.0026
Boechera microphylla	8	27	Bio6, bio11	1.08	0.3161	5.82	0.0108	0.0105	0.0026
Boechera pallidifolia[¶]	16	19	Bio5, bio8	0.10	0.7508	1.94	0.1662	0.1647	0.0026
Boechera pauciflora	6	18	Bio4, bio9, bio10	1.32	0.2665	1.42	0.2516	0.2503	0.0026
Boechera pendulocarpa	32	8	Bio16, elevation	8.57	0.0074	2.92	0.0799	0.0719	0.0026
Boechera perennans[¶]	20	11	Bio3, bio18	0.11	0.7428	0.53	0.5492	0.5456	0.0026
Boechera pinetorum[¶]	20	57	Bio3, bio14, bio17	0.38	0.5571	1.27	0.2539	0.2582	0.0026
Boechera puberula[¶]	30	17	Bio5, bio9	0.49	0.4937	1.82	0.1619	0.1627	0.0026
Boechera retrofracta (all)[¶]	97	158	Bio2, bio3, elevation	34.22	**0.0001**	24.35	**0.0001**	**0.0001**	0.0026
Boechera retrofracta (2×)[¶]	30	27	Bio2, bio3, elevation	7.25	0.0074	14.30	**0.0001**	**0.0002**	0.0026
Boechera sparsiflora[¶]	25	20	Bio5, bio8, bio12, bio18, bio19	0.67	0.4087	0.68	0.5215	0.5189	0.0026
Boechera spatifolia	8	11	Bio15, bio19	1.97	0.1453	8.14	0.0131	0.0113	0.0026
Boechera stricta[¶]	182	32	Bio16, bio18	14.05	**0.0003**	0.16	0.8313	0.8291	0.0026
Boechera williamsii	8	15	Bio2, elevation	5.18	0.0199	14.67	**0.0010**	**0.0008**	0.0026
Subset "ploidy"	2x	3x							
All[¶]	414	123	Bio3, bio4, bio5, bio11, bio15, bio18	0.15	0.6877	0.30	0.5885	**0.0144**	0.0500
Sex[¶]	269	22	Bio1, bio11, bio14	0.49	0.4668	6.97	0.0052	**0.0177**	0.0250
Apo[¶]	125	101	Bio4, bio18	0.08	0.7725	0.06	0.8132	0.0379	0.0250
Boechera collinsii	3	3	Bio4, bio6	7.84	0.0974	31.10	0.0129	0.0108	0.0063
Boechera divaricarpa	3	35	Bio4, bio9, bio19	3.10	0.0748	2.87	0.0968	**0.0063**	0.0063
Boechera lignifera	4	3	Bio7, bio8	0.07	0.8595	12.18	0.0285	0.0293	0.0063
Boechera pallidifolia	22	3	Bio2, bio17	0.43	0.4872	11.58	**0.0008**	**0.0009**	0.0063
Boechera retrofracta	57	35	Bio3, elevation	16.82	**0.0001**	17.85	**0.0001**	**0.0001**	0.0063
Boechera retrofracta (apo)[¶]	27	35	Bio3, elevation	5.71	0.0217	5.13	0.0043	**0.0049**	0.0063
Boechera spatifolia	16	3	Bio4, bio18	1.21	0.3187	0.63	0.3567	0.3462	0.0063
Boechera stricta × *spatifolia* (apo)	10	5	Bio4, bio5	0.62	0.6798	51.04	**0.0001**	**0.0001**	0.0063

*Bio1, annual mean temperature; bio2, mean monthly temperature range; bio3, isothermality; bio4, temperature seasonality; bio5, maximum temperature of warmest month; bio6, minimum temperature of coldest month; bio7, temperature annual range; bio8, mean temperature of wettest quarter; bio9, mean temperature of driest quarter; bio10, mean temperature of warmest quarter; bio11, mean temperature of coldest quarter; bio12, annual precipitation; bio13, precipitation of wettest month; bio14, precipitation of driest month; bio15, precipitation seasonality; bio16, precipitation of wettest quarter; bio17, precipitation of driest quarter; bio18, precipitation of warmest quarter; bio19, precipitation of coldest quarter.

[†]Post hoc permutation test for CCA on geographic distances to detect differences in spatial patterns between each sample group. Significant differences are shown in bold.

[‡]Post hoc permutation test for CCA on ecological variables (bio1 to 19, elevation) without (P_{eco}) and with spatial covariate (partial P_{eco}). Significant values are shown in bold.

[§]Bonferroni corrected threshold for partial *P* values assuming $\alpha^* \approx \alpha/M$ with M = number of independent tests.

[¶]Maxent ecological-niche model available in Figs. 4 and 5 and Figs. S3 and S4.

scenario is unlikely considering (*i*) multiple lines of evidence for repeated separate transitions from sex to apomixis in *Boechera* (36–38), (*ii*) that cpDNA haplotypes are distributed across multiple habitats, (*iii*) that cpDNA haplotypes are partially shared by sexual and apomictic accessions (24.63%, $n = 203$) (Tables S3 and S4), and (*iv*) that sexuals and apomicts display a similar range of genetic diversity as a reflection of their phylogenetic relationships (i.e., cpDNA haplotypes per individual) (Table S4) (59). Therefore, a more parsimonious scenario is favored whereby introgression of apomixis factors into different sexual backgrounds is accompanied by the establishment of independent apomixis lineages. Considering this, the observed niche conservation between sexual and apomictic conspecifics could be explained by the apomictic lineages being too young to have diverged (i.e., recently induced) (60) or that niche differentiation is not possible due to genetic constraints: for example, a genetic bottleneck having stronger effects on sexuals versus apomicts due to inbreeding (61).

Stronger patterns of GP in other agamic complexes could reflect the fact that apomicts in most other plant species are polyploids (reviewed in ref. 56; but see ref. 62) whereas diploid apomixis is relatively frequent in *Boechera* (i.e., apomixis frequency in diploids, 31.33%, $n = 399$; and in polyploids, 81.40%, $n = 129$) (Dataset S1). Ploidy variation, rather than reproductive-mode divergence, seems to be the common driver of niche differentiation (Table 1) although we cannot yet infer which specific aspect of polyploidy (e.g., genetic composition, genome size, or deleterious allele masking, etc.) accounts for the observed differences in niche occupation between diploids and polyploids. Reproductive isolation-by-ploidy between mostly diploid

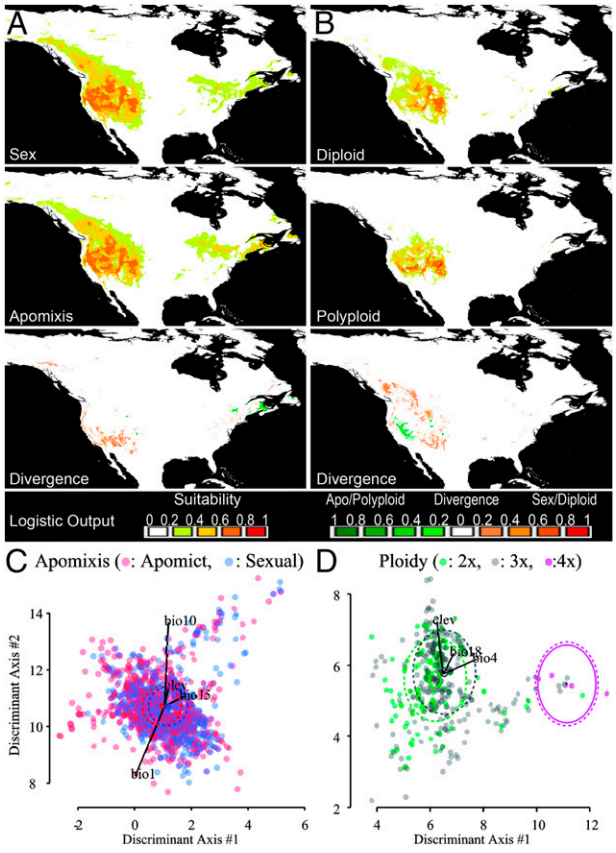

Fig. 4. Lack of niche differentiation on genus-wide level. Across all *Boechera* species, there is no evidence of an association between niche differentiation and reproductive-mode divergence (*A* and *C*), but significant evidence between diploids and triploids (*B* and *D*). Strong differentiation between tetraploidy and all other ploidy levels is observed although this result is based on only six observations. The *Upper* panels show Maxent predictive ecological models of *Boechera* accessions. Habitat suitability is represented using different colors from low (green) to high (red). Strength of distribution differences is displayed for the surplus of apomicts or polyploids (shades of green) and the surplus of sexuals or diploids (shades of red). The *Lower* panels depict constrained discriminant function score distributions, where dashed ellipses represent 50% normal intervals and solid black vectors represent the scaled direction and effect of the labeled explanatory variables.

sexuals and polyploid apomicts in other agamic complexes is enhanced by reduced fertility in their hybrid progeny (48), reduced levels of backcrossing (e.g., in the *R. auricomus* complex) (63), and enhanced colonizing abilities for disturbed areas and species-range edges due to altered ecological tolerances (60, 64), processes that would equally explain the more common pattern of ploidy-driven niche divergence within and across *Boechera* species. Our inability to identify geographic range size variation between reproductive modes (Table S3), or through divergence between geographic distance (i.e., latitude levels) and niche specificity (Table 1), underlines the importance of multiparametric analyses for tests of GP between reproductive groups.

Conclusions

Here, we present, to our knowledge, the first evidence of isolation-by-ploidy and reproductive mode as independent forces of species-specific niche differentiation in an agamic complex. Together with species-specific habitat variation (Fig. 3 and Table S3), isolation-by-ploidy has a more ubiquitous effect than reproductive mode on niche differentiation across *Boechera* (Table 1). Niche conservation between reproductive modes within species is the most distinctive pattern observed (Table 1), and therefore we reject our initial hypothesis of niche evolution as an intrinsic factor of reproductive-mode divergence.

Our data alternatively support an extended frozen niche variation model to explain habitat differences between sexuals and apomicts. This model implies that niche occupation by apomicts reflects a subset of that of their parental sexual taxa, whereby apomictic progeny adopt the adaptive peak of sexuals to their ecological niche (31).

This extended model hinges upon multiple origins of the apomictic phenotype from different sexual backgrounds in *Boechera*. Introgression of apomixis alleles from apomictic diploids into different diploid sexual genotypes (43, 48) may have facilitated the enormous genetic diversity characteristic of apomictic *Boechera* (Table S4) (see also ref. 43). Our data demonstrate that apomictic *Boechera* for the most part drift into new niches by virtue of ploidy variation although evidence for occasional ploidy-independent niche drift was also found (e.g., Fig. 5 *A* and *C*, Table 1, and Fig. S3).

Therefore, the evolutionary success of apomictic *Boechera* seems to have been driven by a number of processes. First, we hypothesize that the ongoing hybridization-driven spread of apomixis alleles in an infectious manner into different sexual genetic

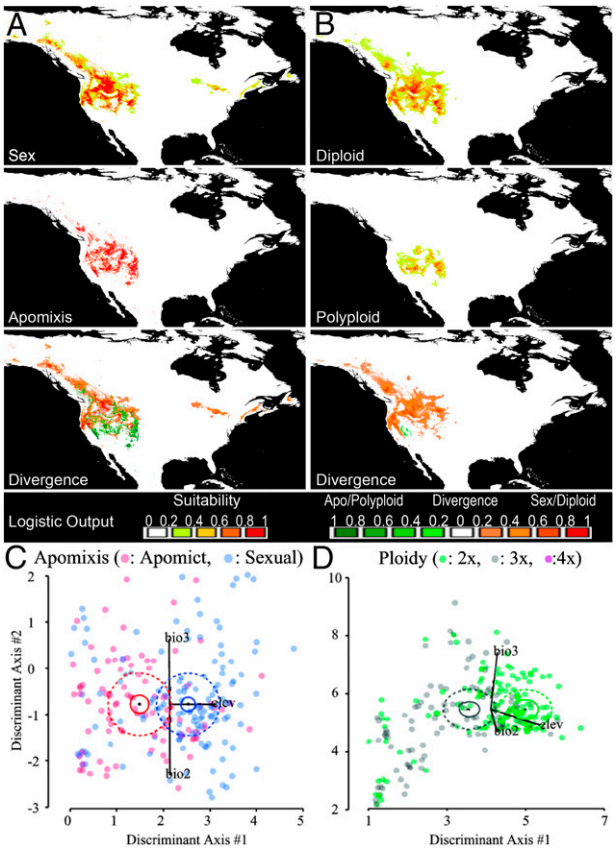

Fig. 5. Additive effects of ploidy and reproductive mode for niche partitioning in *B. retrofracta*. Within *B. retrofracta*, significant differentiation among reproductive mode (*A* and *C*) and, to a much stronger degree, ploidy (*B* and *D*) is observed. The *Upper* panels show Maxent predictive ecological models of different accessions. Habitat suitability is represented by different colors from low (green) to high (red). Strength of distribution differences is displayed for the surplus of apomicts or polyploids (shades of green) and the surplus of sexuals or diploids (shades of red). The *Lower* panels depict constrained discriminant function score distributions, where dashed ellipses represent 50% normal intervals and solid black vectors represent the scaled direction and effect of the labeled explanatory variables.

backgrounds has led to the establishment of apomixis in different niches. In a second step, we believe that recurrent polyploidy mediated by the production of meiotically unreduced gametes has, through a yet-unknown mechanism, enabled polyploid apomicts to diverge into novel niches. In addition, ploidy-independent niche differentiation arising after reproductive-mode transition further complicates the signature of natural selection in wild populations. In this regard, analyses of comprehensive data matrices have enabled us to disentangle at least some determinants of niche differentiation in a mixed reproductive mode and have led us to question whether geographic parthenogenesis in plants is an exception rather than the rule.

Materials and Methods

Detailed methods can be found in *SI Materials and Methods*. Briefly, 1,649 accessions of *Boechera* and closely related genera were screened for apomixis by a PCR-based detection method of apomixis-specific polymorphisms in two independent genetic factors (39, 40). Phylogenetic distribution of apomixis in *Boechera* was plotted onto a haplotype network using the TCS 1.21 software (65) with a connection limit of 95% and the *trnLF* dataset from ref. 20. Maxent ecological-niche variation models (66) for constraining variables such as reproductive modes and ploidy were plotted with DIVA GIS version 7.5 (www.diva-gis.org/) using ecological parameters from the WorldClim database (67). Geographic and ecological distances between ploidy levels or between reproductive modes were evaluated with a stepwise constrained correspondence analysis (CCA) (68) using the R programming environment version 3.1.1 (69) and the vegan package version 2.0-10 (70), followed by permutations to test for significance (70).

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Supporting Information

Mau et al. 10.1073/pnas.1423447112

SI Materials and Methods

Genetic Resources. Here, we analyzed 1,649 accessions (i.e., single samples from different populations per species) obtained from three pools of seed families: (*i*) 200 accessions of 11 *Boechera* taxa (Dataset S1), (*ii*) 75 accessions of 18 *Boechera* taxa (1), and (*iii*) 1,374 accessions of all available taxa, which covers 84 of the currently accepted 111 *Boechera* taxa. All seven major *Boechera* cpDNA-haplotype lineages (*Boechera* taxa of the three pools partially overlap) (Dataset S1) (2) were represented in the *Boechera* samples. In addition, nine taxa of neighboring genera of the tribe Boecheraea were included for all analyses (2, 3). We used a three-step approach to infer the reproductive mode of each genotype. First, accessions from seed pool *i* were grown in a common garden at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) plant growth facility, and DNA was extracted using the Agencourt Chloropure DNA extraction kit (Beckman Coulter). Reproductive mode was determined by the flow cytometric seed screen (1, 4) of 10–24 seeds from each plant (Dataset S1). Diploids producing >50% seeds with diploid [2C = (1Cmaternal) + (1Cpaternal)] embryos and triploid [3C = (2Cmaternal) + (1Cpaternal)] endosperm were defined as sexual whereas those producing >50% seeds with any deviation from this particular embryo–endosperm ratio were defined as apomictic (1, 5, 6), providing us with "sexual" and "apomictic" reproductive-mode classes. Second, using *Boechera* accessions from seed pools *i* and *ii*, we conducted a PCR-based analysis for the presence/absence of the candidate marker gene for either male (*UPGRADE2*) (7) or female apomeiosis (*APOLLO*) (8). Third, we used dried herbarium material from which seeds could not be collected (pool *iii*) to perform a PCR-based screen for the presence/absence of either *UPGRADE2* or *APOLLO*. Plant material representing 1,373 accessions for DNA analysis was obtained from herbarium accessions from Heidelberg University [Heidelberg Botanic Garden and Herbarium (HEID), Heidelberg; Marcus Koch, Department of Biodiversity and Plant Systematics; taxonomic information according to ref. 3; Dataset S1].

Processing and Analysis of DNA Sequences. PCR primers for a 645-bp fragment of the male-apomeiosis marker gene *UPGRADE2* ("PC1pol1-L", 5′-CTTTTCCGTTGACTTTCCGACAAAT-3′; and "PC1pol1-R", 5′-TCGATCAATCTCATTCGGGATCTAT-3′) (7) and of a 234-bp fragment spanning the apomixis-specific 5′ UTR polymorphism of the female-apomeiosis marker gene *APOLLO* ("Lara5-F", 5′-CCTCATCGTACCGTTGCTTCTCTC-3′; and "TSP1-R", 5′-GATAGCCCCAAACTCCAAAATCGC-3′) (8) were designed with Primer3 v0.4.0 (Fig. S1). PCR was performed in a volume of 10 μL, using 10 μM of each primer, 2.0 mM $MgCl_2$, and 0.5 U of BioTaq polymerase (Bioline). The housekeeping gene *ACTIN2* was used as external template control ("RTAct2T7-L", 5′-GTTCCACCACTGAGCACAATGTTACC-3′; and "RTAct2T7-R", 5′-AGTCTTGTTCCAGCCCTCTTTTG-TG-3′). The amplifications were run on a Mastercycler EP Gradient S (Eppendorf) under the following conditions: 5 min initial denaturation at 95 °C; 32 cycles of amplification with 30 s at 95 °C, 30 s at 60 °C, and 1 min at 72 °C; and 10 min of final elongation at 72 °C. PCR success was verified with agarose gel electrophoresis.

Phylogenetic Distribution of *UPGRADE2* and *APOLLO*. (Supra) cpDNA-haplotype designations based on *trnL-F* sequence data (EU154066–EU154341; GenBank Nucleotide database, www.ncbi.nlm.nih.gov/nuccore) of 1,010 investigated accessions are available from ref. 2 (i.e., haplotypes collapsed into suprahaplotypes when sharing the same base order with exception for pseudogene-rich regions). Network reconstruction was conducted using the TCS 1.21 software with a connection limit of 95% (9) according to the parsimony analysis in ref. 2. Classification of accessions from lineages IV and V (Southeast United States) to either *Boechera* or to the closely related *Borodinia* is an ongoing debate (10, 11) and led to exclusion of 38 accessions from lineages IV and V from statistical analyses. Only taxa with a statistically valuable number of accessions ($n \geq 10$) were used for statistical analyses using SPSS v20 (LEAD Technologies).

Niche Variation Models. The nearly total association between *APOLLO* presence and the apomixis phenotype (see *Results*) and the hypothesized association with unreduced egg formation (8) led us to use the presence of *APOLLO* as a surrogate for labeling a herbarium sample as apomictic. Sample coordinates of 97% (n = 1,595) of the 1,649 successfully screened *Boechera* accessions were taken from refs. 2 and 12. We used DIVA GIS v7.5 (www.diva-gis.org/) to calculate the geographic range area for species with at least five accessions in each of the two reproductive classes. For the geographic range of each reproductive class per species, we created a minimum convex polygon, clipped these polygons to North America (i.e., excluding accessions in Greenland), removed oceanic coverage, and calculated the area of each polygon in square kilometers (13). We used minimum convex polygons to estimate species-specific reproductive-mode geographic range because this approach provides a way to consistently calculate range across taxa. Calculations of species-specific niche models for apomicts and sexuals were performed with Maxent version 3.3.3 (default settings, replicates = 15, random seed, training set = 80%, test set = 20%, regularization multiplier = 1; convergence threshold = 0.00001, maximum iterations = 5,000) (14). For reasons of model stability only species with at least 10 observations in each reproductive-mode class were considered for Maxent niche models using the 2.5 arcminute (~5 km^2) climate and elevation grids including all climatic layers (n = 19) from the WorldClim database (15). Maxent generated a threshold-independent, continuous output for climatic suitability range (0–1) of each sample subset based upon its biogeographic abundance. The model performance was then evaluated using the receiver operating characteristic (ROC) analysis (16) with the area under ROC curve (AUC) index (17). An AUC value of 0.5 indicates that the performance of the model meets randomness whereas values closer to 1.0 indicate better model performance. Map reconstructions were performed with DIVA GIS v7.5 (www.diva-gis.org/).

To statistically evaluate the true ecological distance between apomictic and sexual accessions under different constraining variables (ploidy and geographic distance) separately, species with at least five observations per reproductive-mode class and at least three observations for each ploidy class were used in a stepwise constrained correspondence analysis (CCA) (18) using the R programming environment version 3.1.1 (19) and the vegan package version 2.0–10 (20). To prevent over-fitting, bioclimatic variables with minor importance for each separate ecological-niche model were removed by a random-forest backward-elimination analysis of all 19 bioclimatic variables and elevation using the varSelRF package version 0.7-3 (21). Random forest generates multiple classification trees from bootstrap samples. Each time, a subset of the sample [i.e., out-of-bag (OOB) samples] is used to calculate an estimate of the classification error along the addition of trees to the forest. The se-

lected variables are those that yield the smallest OOB error rates using standard parameters (ntree = 5,000, mtryFactor = 1) (21). The selected bioclimatic variables with clear biological significance (i.e., smallest OOB error rate) were added sequentially (first to last) to the CCA, which was performed with and without geographic distance as a partial constraint. Permutation tests for CCA (number of permutations = 10,000; implemented as *anova* function in ref. 20) under a reduced model were applied to calculate the significance of relationships between (*i*) ecological niche and reproductive mode including ploidy variation, (*ii*) ecological niche and ploidy including reproductive-mode variation, (*iii*) ecological niche and reproductive mode independently of ploidy, (*iv*) ecological niche and ploidy independently of reproductive mode, and (*v*) spatial distribution and reproduction independently of the ecological niche. The probability of targeted type 1 error (α) with a *P* value threshold of α = 0.05 was conservatively adjusted using Bonferroni correction (critical threshold for *P* values = $\alpha^* \approx \alpha/M$, *M* = number of independent tests) (22).

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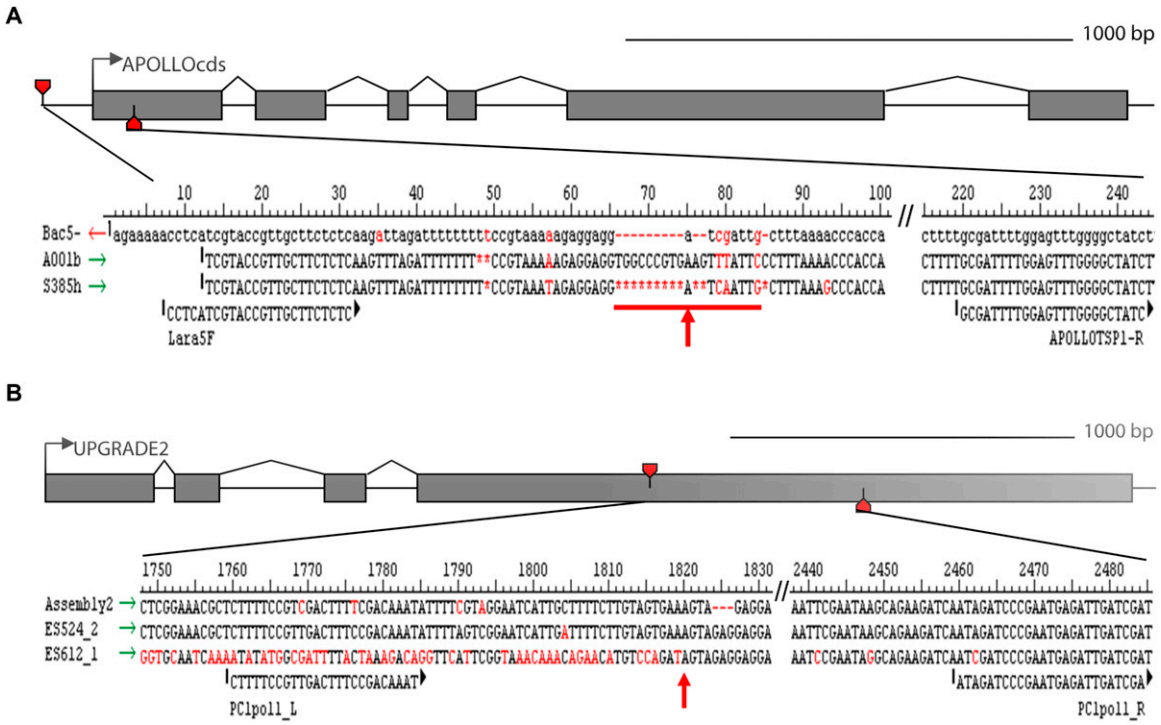

Fig. S1. Structure of apomixis marker genes *APOLLO* (*A*) and *UPGRADE2* (*B*). Red pins on sequence structure denote priming sites of primers used for PCR-based screen of apomixis-specific sequence polymorphism (red arrows). Bac5 and Assembly 2 denote different genomic BAC DNA sites from the same apomictic individual; A001b and ES524_2 denote the genomic DNA sequence of both factors, respectively, in apomictic accessions (i.e. apo allele); and S385h and ES612_1 denote the genomic DNA sequence of both factors, respectively, in sexual accessions (i.e. sex allele) (1, 2).

1. Mau M, et al. (2013) The conserved chimeric transcript UPGRADE2 is associated with unreduced pollen formation and is exclusively found in apomictic Boechera species. *Plant Physiol* 163(4):1640–1659.
2. Corral JM, et al. (2013) A conserved apomixis-specific polymorphism is correlated with exclusive exonuclease expression in premeiotic ovules of apomictic boechera species. *Plant Physiol* 163(4):1660–1672.

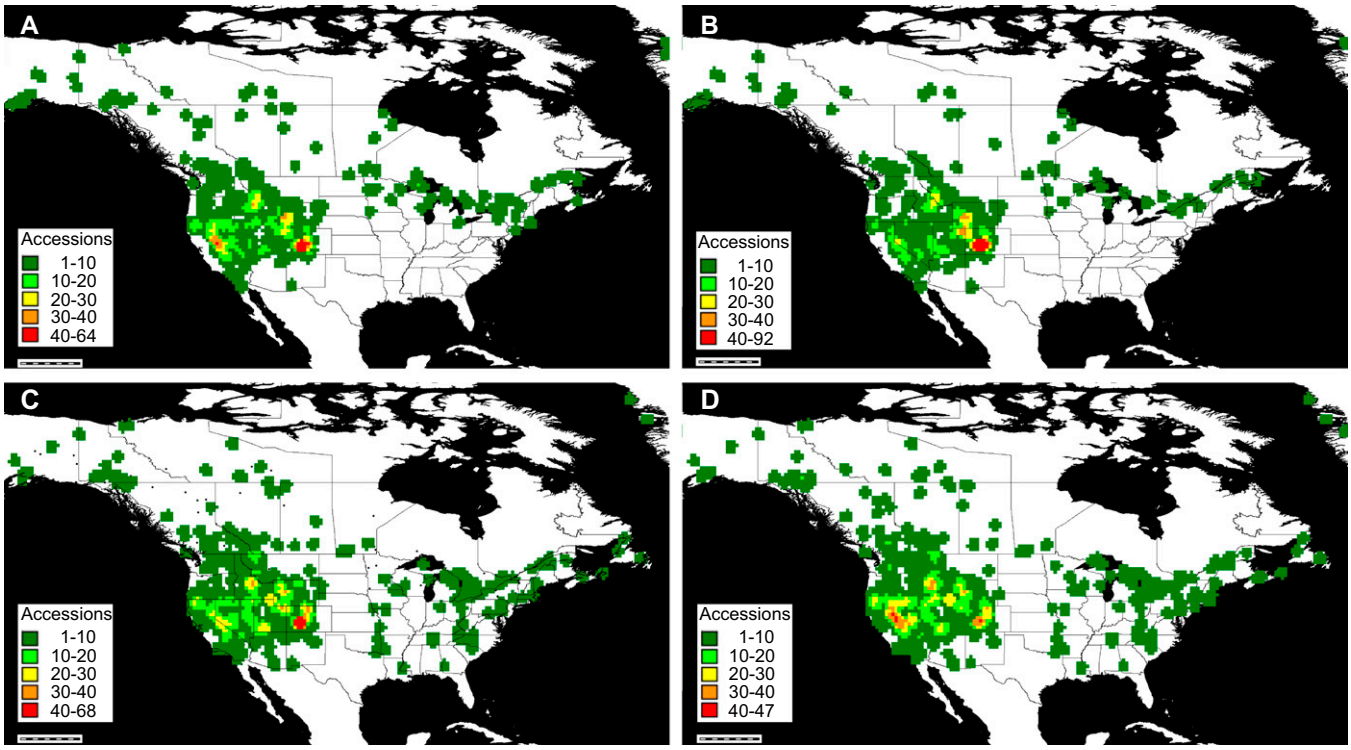

Fig. S2. Geographic distribution of *Boechera* accessions with and without apomictic alleles of the marker genes. The PCR-based screen shows similar distributional ranges of *Boechera* accessions with *APOLLO* (*A*) and with *UPGRADE2* (*B*) compared with accessions lacking *APOLLO* (*C*) or *UPGRADE2* (*D*). (Scale bars: 1,000 km).

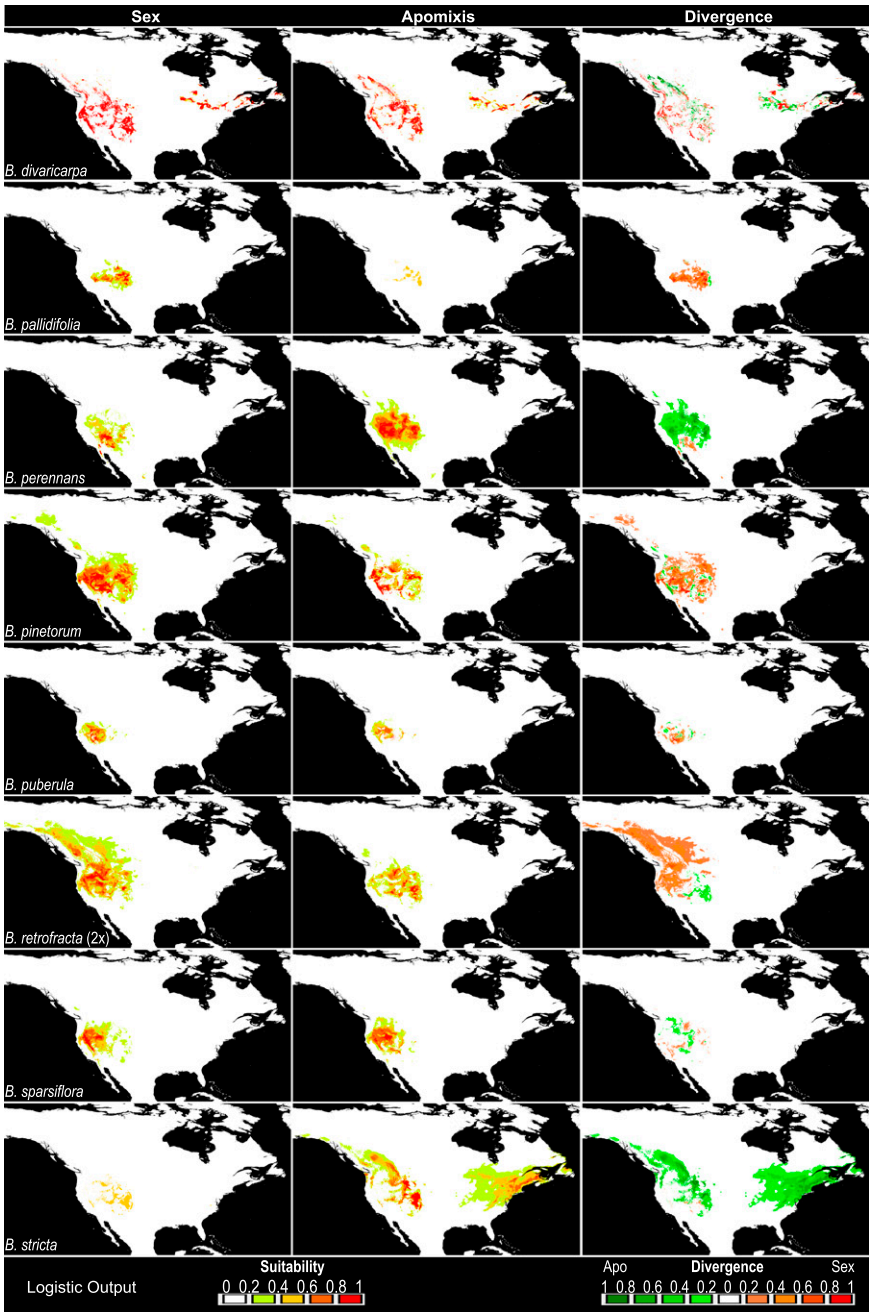

Fig. S3. Maxent predictive ecological-niche models for single *Boechera* species. Partial geographic divergences between reproductive modes on species level were observed. Statistical analysis revealed that most of the ecological divergence between sexuals and apomicts on species level is not statistically significant. Only *Boechera retrofracta* and *Boechera williamsii* (Table 1) show true geographic parthenogenesis (i.e. without interfering ploidy variation). Habitat suitability is represented using different colors from low (green) to high (red). Strength of distribution differences is displayed for the surplus of apomicts (shades of green) and the surplus of sexuals (shades of red).

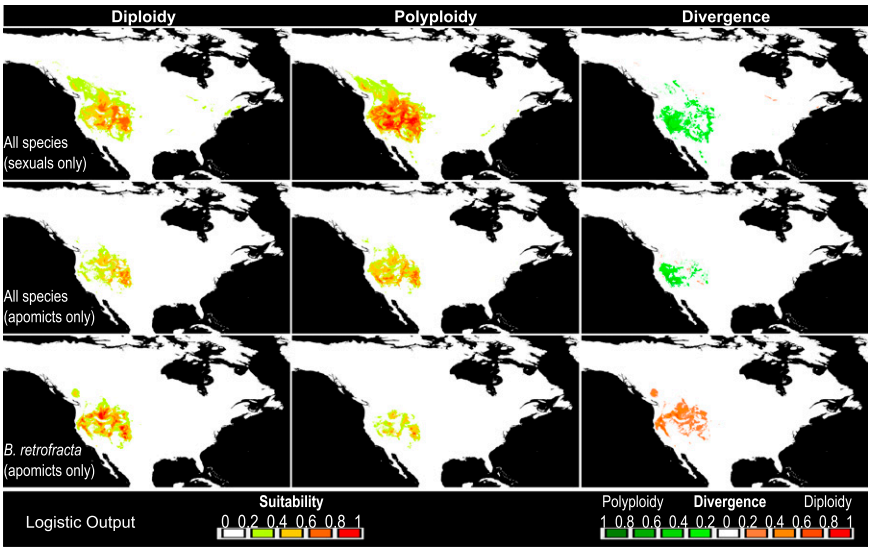

Fig. S4. Maxent predictive ecological-niche models for single *Boechera* species with ploidy as constraining variable. Statistically significant niche differentiation was observed between diploids and polyploids at genus-wide level and at species level. Habitat suitability is represented using different colors from low (green) to high (red). Strength of distribution differences is displayed for the surplus of apomicts (shades of green) and the surplus of sexuals (shades of red).

Table S1. PCR screen of apomixis marker allele presence and absence in 84 species of *Boechera*, three species each of *Polytectenium* and *Sandbergia*, two species of *Cusickiella*, and one species of *Schoenocrambe*

	APOLLO				*UPGRADE2*								Source		
Taxa	Pres	Abs	*N*	%	Pres	Abs	*N*	%	*ITS*	*N*	*TrnL-F*	*N*	MOR	Ref. 1	Ref. 2	FNA
Boechera arcuata	2	2	4	50.00	2	2	4	50.00			kd, ci	2				
Boechera atrorubens	0	1	1	0.00	0	1	1	0.00								
Boechera breweri	1	9	10	10.00	0	10	10	0.00	gx, ha, kn	3	fy, fe, ci, if, gt, fz, bu, ci	8				
Boechera breweri var. shastaensis	0	1	1	0.00	0	2	2	0.00	pf	1						
Boechera californica	3	0	3	100.00	3	0	3	100.00	ev ,og	2	ci, bu	2				
Boechera canadensis	1	16	17	5.88	0	17	17	0.00	ko, kp, ma, mb, mc, md, sc, sd	8	fu, fv, fw, fx, ek, ei	6				
Boechera cobrensis	1	21	22	4.55	0	22	22	0.00	ad, gy, hk, hl, hm, kr, ks	7	ci, ig	2				
Boechera collinsii	9	8	17	52.94	9	9	18	50.00	ae, co, h, I	4	CG, CF, BY	3				
Boechera consanguinea	1	0	1	100.00	1	0	1	100.00								
Boechera constancei	0	1	1	0.00	0	1	1	0.00	ph	1						
Boechera crandallii	4	12	16	25.00	15	1	16	93.75	f, hn, ln, lo, lp, pk, sb ,sl	8	cl, ci	2				
Boechera cusickii	1	4	5	20.00	0	6	6	0.00	b, h, mi	3	k, m	2				
Boechera davidsonii	1	1	2	50.00	0	8	8	0.00	pl	1	b, KF, kg, KC, kh	5				
Boechera dentata	0	2	2	0.00	0	2	2	0.00								
Boechera dispar	0	2	2	0.00	0	2	2	0.00								
Boechera divaricarpa	158	46	204	77.45	128	83	211	60.66	cj, ad, ay, az, ba, bn, bs, bt, bw, bx, by, c, cw, dk, dr, ds, e, eb, ec, ed, ee, ef, eg, ek, en, fl, fm, fp, fs, fu, fy, gm, gp, h, i, k, l, r, s, t, v, y	42	A, AF, AH, AJ, AO, AS, AV, AW, AZ, B, BA, BC, CI, BD, BJ, BO, BR, bs, BT, F, BY, CA, cc, AL, CG, CI, CP, CX, D, id, K, Z	32				
Boechera divaricarpa × *Boechera retrofracta*	2	1	3	66.67	0	3	3	0.00	h	1	X, Y, CS	3				
Boechera falcatoria	1	0	1	100.00	1	1	2	50.00	ee	1						
Boechera falcifructa	1	0	1	100.00	0	1	1	0.00	pm	1						
Boechera fecunda	0	1	1	0.00	0	1	1	0.00			m	1				
Boechera fendleri	9	8	17	52.94	13	4	17	76.47	ev, f, g, is, kd, ke, nm, sn	8	ed, ec, en, f, hr, eb, ci, gf	8				
Boechera fernaldiana ssp. fernaldiana	3	7	10	30.00	1	9	10	10.00	il, op, pc, ok	4	hi, hk, ci, c	4				
Boechera fernaldiana ssp. vivariensis	0	3	3	0.00	0	3	3	0.00	f, pn	2						
Boechera formosa	1	8	9	11.11	1	8	9	11.11	ad, lc, po, pp, pq	5	br, gs	2				

Table S1. Cont.

	APOLLO				*UPGRADE2*								Source		
Taxa	Pres	Abs	*N*	%	Pres	Abs	*N*	%	*ITS*	*N*	*TrnL-F*	*N*	MOR	Ref. 1	Ref. 2	FNA
Boechera glareosa	2	0	2	100.00	2	1	3	66.67	g	1	cl	1				
Boechera glaucovalvula	0	5	5	0.00	0	6	6	0.00	nh, sp	2	hb, hc	2				
Boechera gracilipes	1	5	6	16.67	1	5	6	16.67	f	1						
Boechera gunnisoniana	1	1	2	50.00	1	1	2	50.00	pk, ad	2	ds	1				
Boechera hastatula	1	0	1	100.00	1	0	1	100.00								
Boechera holboellii	2	2	4	50.00	1	3	4	25.00								
Boechera howellii	1	2	3	33.33	0	6	6	0.00	pr	1	er	1				
Boechera inyoensis	6	1	7	85.71	6	1	7	85.71	ad, ar, i, mk, ml	5	ci, aa	2				
Boechera johnstonii	0	1	1	0.00	0	1	1	0.00	ev	1						
Boechera koehleri	0	8	8	0.00	0	8	8	0.00	ac ,kn ,mm ,sa	4	ee, gb, ci, gc	4				
Boechera laevigata	1	20	21	4.76	0	22	22	0.00	kf, kh, ki, kk, no, np, nr, ns, nt, nu, or, ot	12	ib, fl, ef, fg	4				
Boechera lasiocarpa	0	2	2	0.00	1	2	3	33.33	ad	1	ah	1				
Boechera lemmonii	8	24	32	25.00	6	26	32	18.75	er, gx ,kl, km, ly, lz, ps, pt	8	dm, br, bu, ci, eh, fs, m, cw	8				
Boechera lignifera	24	5	29	82.76	20	9	29	68.97	ac, ad, ee, er, gz, h, i, ib, ic, id, ie, iw, ix	13	u, by, b, ci, dt, ab, bu	7				
Boechera lincolnensis	3	1	4	75.00	3	2	5	60.00	ev, pw, mu, mx, so	5	gq, gr	2				
Boechera lyallii	6	14	20	30.00	10	11	21	47.62	ab, ad, iy, iz, l, py, v	7	m, fh, ah, as, dv, ft, bt	7				
Boechera macounii	8	3	11	72.73	8	3	11	72.73	iw, ad, h, il, lt	5						
Boechera microphylla	27	8	35	77.14	30	7	37	81.08	ik, il, im, in, h, ka, kb, op	8	b, m, dy, c, ci, fo, dw, dx, dz, dy	10				
Boechera missouriensis	0	7	7	0.00	0	7	7	0.00	ll, lr, ls, nn, sg	5	fl, fg, hm	3				
Boechera nevadensis	0	1	1	0.00	0	1	1	0.00	px	1						
Boechera ophira	0	1	1	0.00	0	1	1	0.00								
Boechera oxylobula	0	8	8	0.00	0	8	8	0.00	hc, pz, ra,g	4	c, ds, ci	3				
Boechera pallidifolia	19	16	35	54.29	33	2	35	94.29	ad, rb, gz, lf	4	ds, ci, fb, bw, gd, do	6				
Boechera parishii	0	2	2	0.00	0	2	2	0.00	f	1	ci	1				
Boechera patens	0	2	2	0.00	0	3	3	0.00	st	1						
Boechera pauciflora	18	6	24	75.00	13	12	25	52.00	ar, h , hf	3	b, cc, ci, cl, iw, ix, iy, kb, L, o, u	11				
Boechera paupercula	0	3	3	0.00	0	3	3	0.00	rc ,v	2						
Boechera pendulina	4	26	30	13.33	5	27	32	15.63	ev ,f, g ,kt, ni, rd	6	en, hq, el, bk, ci, ho, em, c, hp, hd, he	11				

Table S1. Cont.

	APOLLO				*UPGRADE2*								Source		
Taxa	Pres	Abs	*N*	%	Pres	Abs	*N*	%	*ITS*	*N*	*TrnL-F*	*N*	MOR	Ref. 1	Ref. 2	FNA
Boechera pendulocarpa	8	32	40	20.00	10	32	42	23.81	ab, bt, bw, bx, eo, ep, eq ,ff, fg, g, h	11	U, BM, BN, BP, BU, BW, CK, CL, CO, CR, K, M, Q ,S ,U	15				
Boechera perennans	12	20	32	37.50	22	11	33	66.67	ad, ev, f, ku, ne, nf, pb, re, rf	9	ab, ci, dr, ep, eq, f, ha	8				
Boechera perstellata	0	3	3	0.00	0	3	3	0.00	se	1	fl					
Boechera pinetorum	61	21	82	74.39	44	38	82	53.66	ac, ad, aj, ar, au, cg, ch, ci, ck, dy, f, fk, ga, gh, h, i, z	17	AH, AS, AT, B, BM, BP, BY, C, CG, CI, CM, CQ, CX, F,J ,K, N, O, S, U	20				
Boechera pinzliae	0	2	2	0.00	0	2	2	0.00	rg	1						
Boechera platysperma	4	5	9	44.44	0	11	11	0.00	kv, my, mz, na, nb, rh	6	es, er, gv, gw, gx, gy	6				
Boechera polyantha	2	6	8	25.00	2	10	12	16.67	h, ri	2	ci	1				
Boechera polyantha × *Boechera retrofracta*	2	0	2	100.00	2	0	2	100.00								
Boechera puberula	17	30	47	36.17	1	47	48	2.08	au, eu, it, iu, kx, ol, om, on, pa, rj, rk, sf, sw	13	cj, h, hs, ht, hu, m, o, s, u, y	10				
Boechera puberula × *Boechera retrofracta*	1	0	1	100.00	1	0	1	100.00								
Boechera pulchra	2	7	9	22.22	1	16	17	5.88	eu, h, ky, la, lb, lc, ld, nk, nl, rl, su	11	ci, s, hg, et, eu, ev, ew	7				
Boechera pusilla	2	0	2	100.00	2	0	2	100.00	f	1						
Boechera pygmaea	0	2	2	0.00	0	3	3	0.00			EX	1				
Boechera rectissima	2	6	8	25.00	0	9	9	0.00	le, mp, z	3	ey, gl, gi, gk	4				
Boechera repanda	0	6	6	0.00	0	6	6	0.00	mr, rm, rn	3	ln, gm	2				
Boechera retrofracta	158	97	255	61.96	139	116	255	54.51	aa, ac, ad, au, bj, bt, ck, cl, cr, du, dx, dy, dz, er, et, eu, ev, ey, ez, f, fa, fb, fc, fd, fe, fk, gy, h, ha, i, r, y, z	33	AA, AC, AH, AG, B, BJ, BU, BX, BY, BZ ,CB, CC, CD, CE, CG, CI, CJ, CN, CQ, CS, CT, CU, CV, CW, CX, CY, E, hz, I, M, N, O, P, R, S, T, U, V, Y	39				
Boechera rigidissima	1	1	2	50.00	0	1	1	0.00	kv	1	bd	1				
Boechera schistacea	0	3	3	0.00	0	5	5	0.00	ro, rp, sh	3	s, fa	2				
Boechera shockleyi	0	5	5	0.00	0	5	5	0.00	mn, mo	2	ge, ab	2				
Boechera shortii	0	5	5	0.00	0	6	6	0.00	lg	1	gh, z	2				

Table S1. Cont.

	APOLLO				*UPGRADE2*								Source		
Taxa	Pres	Abs	*N*	%	Pres	Abs	*N*	%	*ITS*	*N*	*TrnL-F*	*N*	MOR	Ref. 1	Ref. 2	FNA
Boechera sparsiflora	20	27	47	42.55	12	35	47	25.53	gx, h, ii ,lm , ou,c, f, i, lv, mf ,og ,rr, u	13	ci, b, iz, u, ka, p, ke, fd, fi cy, fk, bu, ga, as, fz, hh, fc	17				
Boechera sparsiflora × ?	1	0	1	100.00	1	0	1	100.00								
Boechera spatifolia	11	8	19	57.89	11	8	19	57.89								
Boechera species	19	8	27	70.37	15	12	27	55.56	az, bw, bx, h	4	AB, AH, AS, BJ, BU, BY, CC, ci, CI, dl, dn, hl, ie, L, M, U	16				
Boechera stricta	29	149	178	16.29	10	173	183	5.46	e, az, c, cm, cn, cq, d, dj, e, gz, l, m, r, rs, s, t, v	17	AD, ah, AI, AJ, AK, AM, AN, AO, AP, AQ, AR, as, AU, av, AY, BA, BB, BC, BD, BE, bf, bh, BJ, BK, BL, bs, CW, df, dp, hy, ic	31				
Boechera stricta × *Boechera retrofracta*	1	0	1	100.00	1	1	2	50.00			U	1				
Boechera stricta × *Boechera spatifolia*	15	0	15	100.00	14	1	15	93.33								
Boechera subpinnatifida	1	3	4	25.00	0	4	4	0.00	ac, li, lw, lx	4	m, fe	2				
Boechera suffrutescens	3	3	6	50.00	0	7	7	0.00	gx, lk, me	3	db, fq, ff, fe	4				
Boechera tiehmii	1	0	1	100.00	0	1	1	0.00	er	1	hw	1				
Boechera williamsii	3	3	6	50.00	3	3	6	50.00	ao, bt, iv	3	hx, as	2				
Boechera xylopoda	0	1	1	0.00	1	0	1	100.00	mw	1	gq	1				
Cusickiella douglasii	0	4	4	0.00	1	4	5	20.00								
Cusickiella quadricostata	1	1	2	50.00	0	2	2	0.00								
Polyctenium fremontii	0	9	9	0.00	1	8	9	11.11								
Polyctenium fremontii var. confertum	0	1	1	0.00	0	1	1	0.00								
Polyctenium williamsiae	0	2	2	0.00	0	2	2	0.00								
Sandbergia perplexa	0	1	1	0.00	0	1	1	0.00								
S. perplexa var. lemhiensis	0	1	1	0.00	0	1	1	0.00								
Sandbergia whitedii	0	1	1	0.00	0	1	1	0.00								
Schoenocrambe linifolia	0	7	7	0.00	0	22	22	0.00								

Abs, absent; FNA, Flora of North America website (www.efloras.org/browse.aspx?flora_id=1&start_taxon_id=104152); ITS, internal transcribed spacer; MOR, mode of reproduction as indicated by presence and absence of the apomictic *APOLLO* allele; pres, present. Red cells, apomictic taxa; yellow cells, sexual/apomictic taxa; blue cells, sexual taxa; white cells, no designation.

1. Dobes CH, Mitchell-Olds T, Koch MA (2004) Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii*, *A. x divaricarpa*, and *A. holboellii* (Brassicaceae). *Mol Ecol* 13(2):349–370.
2. Kiefer C, Koch MA (2012) A continental-wide perspective: The genepool of nuclear encoded ribosomal DNA and single-copy gene sequences in North American *Boechera* (Brassicaceae). *PLoS ONE* 7(5):e36491.

Table S2. Frequencies of sexual and apomictic *Boechera* across recent and ancient cpDNA haplotypes

		UPGRADE2					*APOLLO*				
cpDNA haplotype	Age, Mya	Noncarriers, *N*	Carriers, *N*	Noncarriers, %	Carriers, %	Ratio	Noncarriers, *N*	Carriers, *N*	Noncarriers, %	Carriers, %	Ratio
AB	0.7–2	2	2	0.010	0.012	0.8	3	1	0.018	0.005	3.5
B	0.7–1	8	12	0.039	0.072	0.5	6	14	0.035	0.071	0.5
BR	0.35–1	1	2	0.005	0.012	0.4	1	2	0.006	0.010	0.6
AH	0.35–1	48	46	0.236	0.277	0.9	38	54	0.224	0.274	0.8
BU	0.25–0.7	19	19	0.094	0.114	0.8	18	20	0.106	0.102	1.0
AS	0.25–0.7	87	49	0.429	0.295	1.5	70	66	0.412	0.335	1.2
CG	0.12–0.3	12	21	0.059	0.127	0.5	13	20	0.076	0.102	0.8
BY	Tip	26	15	0.128	0.090	1.4	21	20	0.124	0.102	1.2
Total no.	—	203	166	1.000	1.000	—	170	197	1.000	1.000	—
r^2	—	—	—	—	—	0.281	—	—	—	—	0.499

The age estimations corresponding to the various cpDNA haplotypes were calculated in ref. 1. Mya, million years ago; Tip, cpDNA haplotypes at the tip of a strict consensus phylogenetic tree that is assembled from 10,000 maximum parsimonious trees.

1. Dobes CH, Mitchell-Olds T, Koch MA (2004) Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii*, *A. x divaricarpa*, and *A. holboellii* (Brassicaceae). *Mol Ecol* 13(2):349–370.

Table S3. Species-specific habitat distribution variation

				Geographic variables		WWF biome occupation, %[§]							Selection of biovariables influential on habitat distribution[¶]					
Species	MOR*	AUC[†] (training)	AUC[†] (test)	Range, $\log_{10}$km^2	Latitude[‡]	4	5	6	8	11	12	13	Elevation	Bio1	Bio3	Bio4	Bio12	Bio18
B. collinsii	Sex	—	—	6.11	49 (47, 50)	16.7	0.0	16.7	66.7	0.0	0.0	0.0	541 (345, 871)	3 (3, 4)	26 (25, 31)	1,103 (1,043, 1,200)	445 (401, 714)	208 (163, 235)
	Apo	—	—	6.61	48 (48, 48)	66.7	11.1	11.1	11.1	0.0	0.0	0.0	66 (66, 309)	3 (3, 3)	24 (24, 24)	1,051 (1,051, 1,051)	925 (448, 925)	254 (171, 254)
B. crandallii	Sex	—	—	3.44	**39 (38, 39)**	0.0	33.3	0.0	0.0	0.0	0.0	66.7	2,591 (2,462, 2,660)	2 (2, 3)	41 (41, 41)	974 (935, 981)	386 (370, 388)	119 (112, 124)
	Apo	—	—	2.85	**38 (38, 38)**	0.0	33.3	0.0	0.0	0.0	0.0	66.7	2,732 (2,386, 2,732)	2 (2, 3)	42 (42, 42)	889 (865, 916)	501 (397, 519)	141 (115, 141)
B. divaricarpa	Sex	0.979	0.977	6.96	44 (41, 47)	18.0	66.7	5.1	5.1	2.6	0.0	5.1	1,738 (216, 2,428)	4 (2, 6)	37 (26, 43)	865 (726, 1,026)	526 (413, 783)	113 (73, 180)
	Apo	0.988	0.960	6.96	45 (42, 47)	11.9	66.4	7.0	4.9	0.7	0.0	9.8	1,850 (334, 2,304)	3 (1, 5)	38 (27, 42)	842 (781, 1,023)	554 (456, 788)	136 (102, 216)
B. fendleri	Sex	—	—	5.63	36 (35, 37)	0.0	14.3	0.0	14.3	0.0	0.0	71.4	2,010 (1,875, 2,718)	9 (7, 11)	43 (41, 44)	772 (734, 783)	375 (314, 436)	100 (90, 141)
	Apo	—	—	5.36	37 (36, 38)	0.0	25.0	0.0	0.0	0.0	0.0	75.0	2,236 (1,691, 2,308)	8 (8, 9)	41 (38, 42)	818 (770, 840)	372 (307, 405)	94 (83, 114)
B. lemmonii	Sex	—	—	6.19	42 (38, 49)	0.0	100.0	0.0	0.0	0.0	0.0	0.0	**2,998 (2,230, 3,425)**	−1 (−2, 1)	39 (34, 42)	788 (608, 810)	667 (606, 755)	143 (101, 185)
	Apo	—	—	5.58	50 (47, 51)	0.0	75.0	5.0	0.0	0.0	0.0	20.0	**2,138 (1,559, 2,282)**	1 (0, 3)	32 (31, 38)	802 (706, 822)	685 (673, 929)	171 (101, 179)
B. lyallii	Sex	—	—	5.89	49 (47, 51)	0.0	85.7	0.0	0.0	0.0	0.0	14.3	2,037 (1,702, 2,299)	2 (−1, 3)	35 (32, 37)	791 (779, 807)	693 (656, 757)	178 (170, 194)
	Apo	—	—	5.00	47 (47, 49)	0.0	100.0	0.0	0.0	0.0	0.0	0.0	2,132 (1,918, 2,295)	1 (−1, 2)	32 (32, 34)	741 (699, 814)	586 (519, 1,370)	123 (122, 126)
B. microphylla	Sex	—	—	4.84	42 (41, 42)	0.0	66.7	0.0	0.0	0.0	0.0	33.3	2,551 (2,326, 2,664)	2 (2, 3)	37 (37, 37)	902 (860, 926)	364 (309, 511)	88 (74, 111)
	Apo	—	—	5.63	42 (41, 44)	0.0	22.2	0.0	0.0	0.0	0.0	77.8	1,957 (1,772, 2,318)	5 (4, 6)	41 (40, 42)	787 (766, 843)	391 (353, 529)	77 (67, 102)
B. pallidifolia	Sex	0.999	1.000	4.96	39 (39, 41)	25.0	0.0	0.0	0.0	0.0	0.0	75.0	2,069 (1,929, 2,233)	6 (5, 7)	41 (37, 41)	914 (906, 916)	313 (266, 378)	81 (74, 103)
	Apo	1.000	1.000	5.02	39 (39, 40)	16.7	0.0	0.0	0.0	0.0	0.0	83.3	1,980 (1,778, 2,514)	6 (3, 7)	39 (37, 41)	896 (881, 992)	331 (271, 359)	102 (74, 131)
B. pauciflora	Sex	—	—	5.30	41 (41, 44)	0.0	66.7	0.0	0.0	0.0	0.0	33.3	1,553 (1,423, 1,874)	6 (5, 7)	43 (40, 45)	725 (715, 769)	446 (405, 462)	66 (47, 87)
	Apo	—	—	5.44	41 (39, 42)	0.0	60.0	0.0	0.0	0.0	0.0	40.0	1,911 (1,550, 2,169)	6 (5, 7)	44 (42, 45)	704 (690, 737)	444 (346, 547)	55 (48, 61)
B. pendulocarpa	Sex	—	—	6.07	**45 (43, 51)**	0.0	31.3	31.3	12.5	0.0	0.0	25.0	**1,912 (914, 2,507)**	2 (0, 4)	37 (33, 38)	852 (813, 900)	441 (350, 535)	106 (97, 132)
	Apo	—	—	5.36	**41 (39, 43)**	0.0	37.5	0.0	0.0	0.0	0.0	62.5	**2,637 (2,176, 2,794)**	2 (2, 5)	38 (37, 43)	825 (762, 919)	427 (334, 498)	87 (75, 115)
B. perennans	Sex	1.000	0.962	5.77	37 (35, 38)	0.0	7.7	0.0	0.0	0.0	0.0	92.3	1,708 (1,269, 1,970)	13 (9, 15)	42 (41, 44)	823 (772, 862)	312 (251, 403)	86 (62,105)
	Apo	0.991	0.989	5.34	37 (36, 38)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1,371 (1,184, 1,833)	13 (9, 14)	40 (40, 42)	800 (782, 825)	238 (213, 277)	62 (50, 66)
B. pinetorum	Sex	0.980	0.975	6.47	39 (37, 43)	0.0	41.7	16.7	16.7	0.0	0.0	58.3	1,911 (1,233, 2,172)	5 (4, 9)	42 (41, 42)	791 (763, 835)	377 (305, 471)	88 (58, 112)
	Apo	0.992	0.989	6.67	39 (38, 42)	0.0	50.0	2.1	4.2	0.0	0.0	50.0	2,015 (1,821, 2,420)	6 (4, 8)	42 (39, 44)	791 (698, 840)	384 (279, 548)	74 (50, 108)
B. puberula	Sex	0.999	0.998	5.45	41 (39, 42)	0.0	15.4	0.0	0.0	0.0	0.0	84.6	1,927 (1,707, 2,142)	7 (6, 8)	43 (42, 45)	772 (734, 807)	292 (246, 337)	57 (47, 64)
	Apo	0.999	0.999	5.14	41 (40, 42)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	1,922 (1,716, 2,103)	7 (6, 7)	43 (42, 44)	747 (715, 782)	348 (295, 435)	55 (50, 61)
B. retrofracta	Sex	0.991	0.975	6.77	**47 (43, 55)**	6.2	56.9	**16.9**	1.5	6.2	0.0	**18.5**	**1,034 (622, 1,701)**	4 (0, 7)	36 (28, 41)	880 (810, 1,002)	430 (347, 675)	108 (87, 154)
	Apo	—	—	6.78	**42 (39, 45)**	1.0	55.1	**4.1**	5.1	2.0	0.0	**37.8**	**2,180 (1,604, 2,593)**	3 (1, 5)	40 (38, 42)	850 (775, 887)	412 (346, 529)	102 (68, 132)
B. sparsiflora	Sex	0.996	0.995	6.13	41 (38, 42)	0.0	31.8	0.0	4.6	0.0	13.6	50.0	1,568 (1,341, 2,038)	7 (6, 9)	43 (42, 45)	737 (708, 815)	356 (277, 406)	50 (39, 64)
	Apo	0.997	0.987	5.88	42 (40, 43)	0.0	33.3	0.0	0.0	0.0	5.6	61.1	1,688 (1,193, 2,081)	6 (5, 10)	43 (38, 45)	749 (709, 842)	421 (328, 511)	64 (45, 76)
B. spatifolia	Sex	—	—	3.83	39 (39, 41)	—	—	—	—	—	—	—	2,774 (2,438, 2,804)	2 (2, 4)	41 (40, 42)	820 (814, 877)	398 (338, 422)	147 (145, 157)
	Apo	—	—	3.96	38 (38, 39)	—	—	—	—	—	—	—	2,774 (2,722, 2,774)	2 (2, 3)	41 (41, 41)	820 (820, 851)	495 (379, 495)	188 (140, 188)
B. stricta	Sex	0.998	0.999	6.21	42 (40, 45)	**11.7**	64.0	**0.9**	3.6	0.9	0.0	19.8	**2,499 (2,025, 2,899)**	2 (0, 3)	39 (37, 41)	829 (782, 870)	538 (434, 670)	137 (109, 170)
	Apo	0.986	0.970	6.82	43 (41, 46)	**44.4**	42.9	**16.7**	0.0	0.0	0.0	5.6	**1,848 (200, 2,708)**	3 (0, 7)	38 (28, 40)	906 (789, 995)	650 (495, 873)	181 (138, 261)
B. williamsii	Sex	—	—	3.92	43 (43, 43)	0.0	100.0	0.0	0.0	0.0	0.0	0.0	**2,290 (2,143, 2,544)**	1 (0, 1)	40 (38, 41)	889 (841, 908)	445 (379, 461)	112 (94, 115)
	Apo	—	—	3.47	43 (43, 43)	0.0	100.0	0.0	0.0	0.0	0.0	0.0	**2,586 (2,580, 2,686)**	1 (1, 2)	37 (37, 38)	894 (880, 908)	383 (356, 390)	95 (84, 97)
All species	Sex	0.959	0.957	7.06	41 (39, 44)	6.3	49.9	5.8	4.3	1.5	0.8	33.1	1,974 (1,126, 2,469)	5 (2, 7)	40 (37, 42)	832 (771, 916)	440 (336, 643)	103 (68, 147)
	Apo	0.965	0.951	7.04	42 (39, 45)	7.0	52.4	4.6	3.3	1.5	0.2	32.5	2,027 (1,402, 2,520)	4 (2, 6)	41 (37, 42)	829 (758, 908)	438 (356, 603)	101 (67, 141)

*MOR, mode of reproduction.

[†]The model performance was evaluated using the receiver operating characteristic (ROC) analysis with the area under ROC curve (AUC) index. An AUC value of 0.5 indicates that the performance of the model meets randomness whereas values closer to 1.0 indicate better model performance. —, species with $n \leq 10$ observations in each reproductive-mode class were not considered for Maxent niche models.

[‡]Bold letters denote significant differences between reproductive modes (Student's t test, $\alpha = 0.05$).

[§]Biome 4, temperate broadleaf and mixed forests; biome 5, temperate conifer forests; biome 6, boreal forests/taiga; biome 8, temperate grasslands, savannas, and shrublands; biome 11, tundra; biome 12, Mediterranean forests, woodlands, and scrub; biome 13, deserts and xeric shrublands. Grey blocks denote major habitat for each species (≥50%). Bold letters mark significant differences in WWF biome affiliation between sexuals and apomicts (Fisher's exact test). —, no WWF biome information available.

[¶]Bio1, annual mean temperature; bio3, isothermality; bio4, temperature seasonality; bio12, annual precipitation; bio18, precipitation of warmest quarter; median (lower quartile, upper quartile). Bold letters denote significant differences between reproductive modes (Student's t test, $\alpha = 0.05$).

Table S4. Comparison of genetic diversity (number of cpDNA haplotypes per number of individuals) among sexual and apomictic accessions per species illustrating variation between reproductive mode on species level and similar distribution ranges across species

	No. of individuals		No. of genotypes		Genetic diversity	
Taxa	Apo	Sex	Apo	Sex	Apo	Sex
B. collinsii	8	5	1	3	0.13	0.60
B. divaricarpa	144	40	28	13	0.19	0.33
B. lemmonii	7	17	5	6	0.71	0.35
B. microphylla	20	5	9	4	0.45	0.80
B. pauciflora	12	6	7	6	0.58	1.00
B. pendulocarpa	7	26	4	12	0.57	0.46
B. perennans	8	10	4	4	0.50	0.40
B. pinetorum	52	15	18	10	0.35	0.67
B. puberula	14	23	6	7	0.43	0.30
B. retrofracta	109	67	27	20	0.25	0.30
B. sparsiflora	16	18	8	9	0.69	0.50
B. stricta	24	133	8	29	0.33	0.22
Mean	—	—	—	—	0.43	0.49
SE	—	—	—	—	0.05	0.07
Student's *t* test (*P*)	—	—	—	—	0.478	0.478

Other Supporting Information Files

Dataset S1 (XLSX)