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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 = $(1$ Cmaternal $) + (1$ Cpaternal $)$] 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 $MgCl₂$, 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) cpDNAhaplotype designations based on $trnL-F$ sequence data (EU154066– EU154341; GenBank Nucleotide database, [www.ncbi.nlm.nih.](http://www.ncbi.nlm.nih.gov/nuccore) [gov/nuccore\)](http://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 [\(www.diva-gis.org/](http://www.diva-gis.org/)) to calculate the geographic range area for species with at least five accessions in each of the two reproductive classes. For the geographic range of each reproductive class per species, we created a minimum convex polygon, clipped these polygons to North America (i.e., excluding accessions in Greenland), removed oceanic coverage, and calculated the area of each polygon in square kilometers (13). We used minimum convex polygons to estimate species-specific reproductive-mode geographic range because this approach provides a way to consistently calculate range across taxa. Calculations of species-specific niche models for apomicts and sexuals were performed with Maxent version 3.3.3 (default settings, replicates = 15, random seed, training set = 80% , test set = 20% , regularization multiplier = 1; convergence threshold = 0.00001 , maximum iterations $= 5,000$ (14). For reasons of model stability only species with at least 10 observations in each reproductive-mode class were considered for Maxent niche models using the 2.5 arcminute (\sim 5 km²) climate and elevation grids including all climatic layers ($n = 19$) from the WorldClim database (15). Maxent generated a threshold-independent, continuous output for climatic suitability range $(0-1)$ of each sample subset based upon its biogeographic abundance. The model performance was then evaluated using the receiver operating characteristic (ROC) analysis (16) with the area under ROC curve (AUC) index (17). An AUC value of 0.5 indicates that the performance of the model meets randomness whereas values closer to 1.0 indicate better model performance. Map reconstructions were performed with DIVA GIS v7.5 ([www.diva-gis.org/\)](http://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) eco-

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logical 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 $\alpha = 0.05$ 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 PCRbased 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).

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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).

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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).

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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

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

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Table S1. Cont.

Abs, absent; FNA, Flora of North America website [\(www.efloras.org/browse.aspx?flora_id](http://www.efloras.org/browse.aspx?flora_id=1&start_taxon_id=104152)=1&start_taxon_id=104152); ITS, internal transcribed spacer; MOR, mode of reproduction as indicated by presence and אם, advertic rim, riora or inorin America website (www.euioras.org/u/wsecaspx?riora_ua= ושפחה המאסו_ua= ישראל, ווא
absence of the apomictic APOLLO allele; pres, present. Red cells, apomictic taxa; yellow cells, sexual/ap 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.

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

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 Table S3. Species-specific habitat distribution variation

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meets randomness whereas values closer to 1.0 indicate better model performance. —, species with n ≤ 10 observations in each reproductive-mode class were not considered for Maxent niche models. $^{\tt t}$ Bold letters denote significant differences between reproductive modes (Student's t test, $\alpha=$ 0.05). ‡Bold letters denote significant differences between reproductive modes (Student's t test, α = 0.05).

^sBiome 4, temperate broadleaf and mixed forests; biome 5, temperate conifer forests; biome 6, boreal foreststaiga; biome 8, temperate grasslands, savannas, and shrublands; biome 11, tundra; biome 12,
Mediterranean fores §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

Bio1, annual mean temperature; bio3, isothermality; bio4, temperature seasonality; bio12, annual precipitation; bio18, precipitation of warmest quarter; median (lower quartile, upper quartile). Bold letters {Bio1, annual mean temperature; bio3, isothermality; bio4, temperature seasonality; bio12, annual precipitation; bio18, precipitation of warmest quarter; median (lower quartile, upper quartile). Bold letters between sexuals and apomicts (Fisher's exact test). - no WWF biome information available. between sexuals and apomicts (Fisher's exact test). —, no WWF biome information available.

denote significant differences between reproductive modes (Student's t test, a = 0.05). denote significant differences between reproductive modes (Student's t test, α = 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

Other Supporting Information Files

[Dataset S1 \(XLSX\)](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1423447112/-/DCSupplemental/pnas.1423447112.sd01.xlsx)