MOLECULAR ECOLOGY

Molecular Ecology (2014) **23**, 4486–4497 doi: 10.1111/mec.12879

Mating system and environmental variation drive patterns of adaptation in *Boechera spatifolia* (Brassicaceae)

JOHN T. LOVELL,*† KELSI GROGAN,* TIMOTHY F. SHARBEL† and JOHN K. MCKAY*

**Graduate Degree Program in Ecology, Department of BioAgricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, USA, †Apomixis Research Group, Institute for Plant Genetics and Crop Plant Improvement (IPK), Gatersleben D-06466, Germany*

Abstract

Determining the relative contribution of population genetic processes to the distribution of natural variation is a major goal of evolutionary biology. Here, we take advantage of variation in mating system to test the hypothesis that local adaptation is constrained by asexual reproduction. We explored patterns of variation in ecological traits and genome-wide molecular markers in *Boechera spatifolia* (Brassicaceae), a species that contains both apomictic (asexual) and sexual individuals. Using a combination of quantitative genetics, neutral genetic (SSR) and genome-wide single nucleotide polymorphism, we assessed the hypothesis that asexual lineages should have reduced signatures of adaptation relative to sexual conspecifics. All three measures (traits, SSRs, SNPs) demonstrated that apomicts are genetically distinct from sexuals, regardless of population location. Additionally, phylogenetic clustering revealed that the apomictic group shared a single common ancestor. Across the landscape, sexual genome-wide SNP variation was strongly associated with latitude ($r^2 > 0.9$), indicating that sexual populations have differentiated across an environmental gradient. Furthermore, flowering time and growth rate, as assessed in a common garden, strongly covary with the elevation and latitude of the source population. Despite a wide geographic distribution that largely overlaps with sexual populations, there was little evidence for differentiation in molecular markers or quantitative characters among apomictic populations. Combined, these data indicated that, in contrast to asexual populations, sexual populations show evidence of local adaptation.

Keywords: apomixis, asexual, growth rate, landscape genetics, phenology, reciprocal transplants

Received 25 February 2014; revision received 22 July 2014; accepted 28 July 2014

Introduction

Adaptation to local environmental conditions is the best and longest studied manifestation of an evolutionary response to selection in nature (Turesson 1922; Clausen *et al*. 1940; Kawecki & Ebert 2004). Documented by reciprocal transplant experiments, common garden studies and ex situ experimentation (Fournier-Level *et al*. 2011; Agren & Schemske 2012), local adaptation is often viewed as ubiquitous (Kawecki & Ebert 2004). Physiological adaptation to local conditions is especially important, and well documented, in sessile organisms such as plants (Turesson 1930; Berry & Bjorkman 1980). Despite these examples, adaptation is commonly constrained by genetic and biological factors (Arnold 1992; Etterson & Shaw 2001; van Kleunen & Fischer 2005), and drift and other neutral processes may drive evolution, even under strong selection regimes. Determining the mechanisms and ecological manifestations of adaptive constraint remains a major goal of evolutionary biology.

Correspondence: John Thomson Lovell, Fax: 512-471-3878; E-mail: johntlovell@gmail.com

The relative contribution of genetic drift and selection can be inferred by comparing patterns of genomic and phenotypic differentiation with landscape and geographic variables (Manel *et al.* 2003). If genomic and/or phenotypic differentiation is largely correlated with geographic distance (isolation by distance: IBD), a balance of gene flow and genetic drift is the primary mode of evolution (Slatkin 1993). However, if differentiation is associated with environmental variables, evolution may be driven by responses to natural selection (Lasky *et al.* 2012; Lee & Mitchell-Olds 2013).

The mating system of a population can affect the relative likelihood that evolution is due to either drift or responses to natural selection (Charlesworth & Wright 2001). For example, lower effective recombination rates in self-pollinating species increase the extent of linkage disequilibria across the genome and reduce the efficacy of selection (Conway *et al.* 1999; Nordborg 2000; Qiu *et al.* 2011). Asexual lineages offer an extreme example where the entire genome is in linkage disequilibrium due to a lack of recombination (Henry *et al.* 2012). Genome-wide interference among loci in asexual lineages constrains adaptation by reducing population genetic variation and the efficacy of selection (Hill & Robertson 1966; Barton & Charlesworth 1998; Lynch & Blanchard 1998; Otto & Lenormand 2002).

Genetic constraints and reduced evolutionary potential of asexual lineages form the basis for many theories that explain the maintenance and evolution of sex, despite the fitness costs of sexual reproduction (Burt 2000; Nautiyal *et al.* 2002; Barton 2010). For example, sexually reproducing natural enemies may gain an advantage over asexual hosts ('red queen' dynamics, reviewed by Lively (2010)), or slightly deleterious mutations may not be purged by selection in asexual lineages (e.g. 'Muller's ratchet', Barton 2010). However, the adaptive constraints posed by asexual reproduction may be buffered by epigenetic variation (Bossdorf *et al.* 2008; Verhoeven *et al.* 2010).

Despite a large body of theoretical work, the manifestation of reduced responses to selection in asexual lineages has only been tested in a handful of multicellular organisms and few vascular plants (but see Pellino *et al.* 2013). This lack of experimentation is due, in part, to the fixation of hybridity and/or polyploidy in all obligately asexual plant species (Mogie 1992). These factors, which covary with mating system, confound any experimentation. *Boechera*, a close relative of the model plant, *Arabidopsis thaliana*, is unique among plants because it contains both diploid sexual and diploid apomictic (asexual reproduction through seed) lineages. As such, comparisons between mating systems can be conducted without the confounding effects of polyploidy.

Here, we analyse genomic (9126 SNPs), neutral molecular genetic (14 SSR markers) and quantitative genetic differentiation of *Boechera spatifolia* along environmental gradients in both apomictic and sexual lineages. We test the hypothesis that divergence of apomictic populations is caused primarily by drift, while that of sexual populations is dominated by natural selection. This hypothesis predicts that (i) apomictic lineages will display low genetic structure of quantitative traits among populations and little phenotypic differentiation along environmental gradients; and (ii) sexual lineages will exhibit signatures of adaptive evolution, including strong genomic and quantitative genetic structure, and covariance of phenotypic values with environmental variables.

Methods

Plant material

Boechera spatifolia (Rydb.) is a diploid ($2n = 2x = 14$), winter annual (or short lived perennial) species of the central Rocky Mountains, USA (Windham & Al-Shehbaz 2006). Small stature and a short lifespan make *B. spatifolia* a promising species for combining population genetics and field trials. We collected seeds from maternal plants from 30 populations across Colorado and southern Wyoming, USA, over the summers of 2011 and 2012 (Fig. 1, Table 1). These populations cover >90% of the geographic range of herbaria collections. To reduce the potential of collecting cryptic hybrids, where possible, populations were chosen from locations used in the systematic documentation of *Boechera* (Alexander *et al.* (2013); P. Alexander, personal cummunication). We characterized the mating system of all seed families using the flow cytometric seed screen (FCSS: Matzk *et al.* 2000) by comparing seed endosperm:embryo ploidy ratios on a Partec PAII flow cytometer (Partec GmbH Münster, Germany), following methods of both Aliyu *et al.* (2010) and Lovell *et al.* (2013a).

Phenotypic analysis- glasshouse and field experimentation

We grew four replicates (sibs) from each of 190 seed families from 29 source populations ($n = 713$ plants). Plants were grown in 1″ diameter RLC-4 containers (Steuwe and Sons, Tangent, OR, USA) filled with Fafard 4P soil mix. Three seeds were placed directly on the soil and germinated following 14 days of cold stratification. After 10 days of growth, seedlings were thinned to one plant/container. Growth conditions were designed to mimic those experienced by winter annual *Boechera* species: germination in early fall (23/18 °C, 12/12 h day/

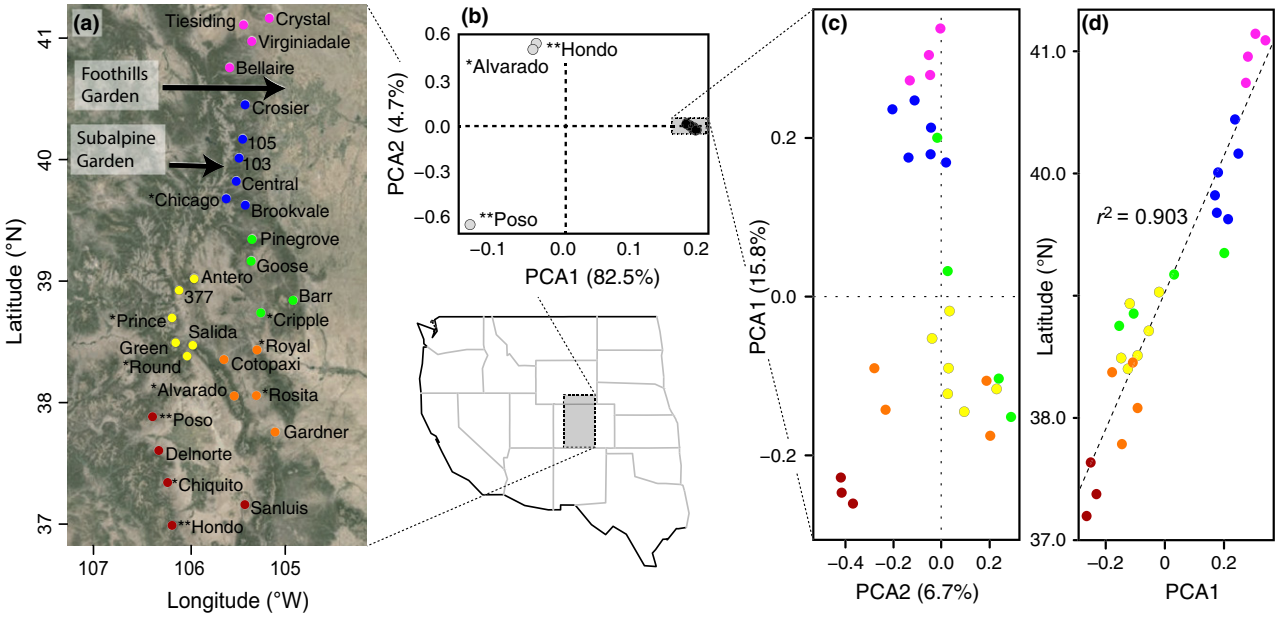

Fig. 1 Spatial and genetic distribution of sampled populations. (a) The geographic positions of seed-source populations are colour-coded by mountain range: Northern Foothills (Purple), Mt. Evans/Indian Peaks (Blue), Rampart Range (Green), Collegiate Peaks (Yellow), Sange de Cristo/Wet Mts. (Orange), San Juans Mts./San Luis Valley (Red). Other panels follow this colouring scheme. The mating system is indicated in the population label: (**) obligate apomictic, (*) mixed sexuality, (no asterisk) obligate sexual. (b) Position in genomic PC space of all 30 sampled populations. Sexual accessions are black filled circles, and apomictic accessions are highly diverged from sexuals, labelled and represented by grey filled circles. (c) Genetic PC positions of the sexual *Boechera spatifolia* populations mirror their geographic distribution. Principal component axis #1 (PCA1), which explains the most variation, is plotted along the long axis (y). (d) The correlation between genetic PCA1 and latitude is strong and positive; the linear model and reported r^2 are overlayed as a dashed line.

night), growth during the fall (18/8 °C, 12/12 h day/night), vernalization over the winter (8/4 °C, 8/16 h day/night) and then growth in the spring (23/18 °C, 12/12 h day/night). All plants were grown in a single Conviron ATC60 growth chamber at Colorado State University, Ft. Collins, CO, USA.

We assayed ten phenotypes in the growth chamber. The physiological traits, specific leaf area (*SLA*) and leaf water content (H_2O content), are related to stress tolerance, especially drought (Nautiyal *et al.* (2002)). To measure growth, we calculated the relative growth rate of both stem elongation in 'spring' conditions (*GRht*) and total rosette leaf area (*GRla*, following Lovell *et al.* 2013b). Rosette morphology, which is associated with adaptive differentiation in *Boechera* (Lee & Mitchell-Olds 2013), was assessed by the number and size of leaves and the height of the whole rosette. Finally, phenology was measured as the days to anthesis ('anthesis'), the height of the bolting structure and the days from the end of vernalization to the initiation of bolting (*FT*).

We analysed the growth chamber phenotypes to determine the extent of phenotypic divergence between mating systems. Genotypes were categorized as apomictic or sexual (from FCSS data) and analysed independent of source population. After standardizing and normalizing the growth chamber phenotypes, we calculated breeding values for each family and utilized a discriminant function analysis to determine multivariate differences between mating systems. Significance was assessed via 'Pillai's trace' multivariate tests. Multivariate analyses were conducted in JMP 10.1 PRO (SAS Corporation, Cary, NC, USA).

For two traits, *FT* and *GRla*, we correlated the mean phenotypic values for each population with the latitude and elevation of the site where the population was collected. We chose to focus on *FT* and *GRla* because these traits vary considerably between differentially adapted populations of other species (Angert *et al.* 2009; Wilczek *et al.* 2009; Banta *et al.* 2012; Lee & Mitchell-Olds 2013). These analyses were conducted separately for apomictic and sexual lineages in R Environment for Statistical Computing, version 2.15.1 (R Core Team 2013). Those populations with 'mixed' mating systems were split into separate subsets of sexual and apomictic genotypes. These subsets were analysed with other populations of the same mating system. We used simple mantel tests to compare phenotypic differentiation and the ecological distances among populations using the 19 bioclimatic variables (www.bioclim.org). Partial mantel tests were used to test for ecological differentiation

Table 1 Population descriptions. The 30 sampled population names, geographic position, and total sample size (apomictic/sexual individuals) are presented

ID	Latitude	Longitude	Elevation (m)	Mating system	No. Families	% Apomictic	Ploidy
103	40.0145	−105.5106	2792	Sex	8	0	2
105	40.1692	−105.4739	2596	Sex	8	0	2
377	38.9373	−106.1466	2886	Sex	8	0	2
Alvarado	38.0775	−105.5637	3031	Mixed	6	0.875	2
Antero	39.0317	−105.9862	2804	Sex	8	0	2
Barr	38.8551	−104.941	2370	Sex	6	0	2
Bellaire*	40.7507	−105.6126	2606	Sex	8	0	2
Brookvale	39.6306	−105.4464	2636	Sex	6	0	2
Central	39.8266	−105.5418	2859	Sex	8	0	2
Chicago	39.6834	−105.6487	3087	Mixed	7	0.625	2
Chiquito	37.3723	−106.2677	2663	Mixed	8	0.5	2
Cotopaxi	38.3735	−105.6705	2133	Sex	7	0	2
Cripple	38.7543	−105.281	2474	Mixed	8	0.25	2
Crosier	40.4499	−105.4468	2463	Sex	7	0	2
Crystal	41.1537	−105.1917	2159	Sex	5	0	2
Delnorte	37.6322	−106.3631	2493	Sex	7	0	2
Gardner	37.783	−105.1305	2128	Sex	6	0	2
Goose	39.1762	−105.3843	2803	Sex	8	0	2
Green	38.5108	−106.1842	2722	Mixed	5	0.75	2
Hondo	37.0234	−106.2138	2805	Apomictic	8	1	2
Pinegrove	39.3526	−105.3745	2414	Sex	6	0	2
Poso	37.9081	−106.4276	2904	Apomictic	8	1	3
Prince	38.7135	−106.2216	3294	Mixed	7	0.125	2
Rosita	38.0795	−105.3274	2714	Mixed	5	0.375	2
Round	38.4015	−106.0626	2706	Mixed	7	0.125	2
Royal	38.4531	−105.3226	1942	Mixed	3	0.67	2
Salida	38.4901	−106.0015	2770	Sex	5	0	2
Sanluis	37.1922	−105.4493	2475	Sex	8	0	2
Tiesiding	41.1001	−105.465	2419	Sex	7	0	2
Virginiadale	40.9658	−105.3761	2238	Sex	5	0	2

*Phenotypic data for the 'Bellaire' population is unavailable.

while controlling for phenotypic structure due to geographic distances.

We also analysed the extent of ecological differentiation between apomixis and sex by comparing regression coefficients and r^2 values of phenotype–environment correlations. To correct for differences in power due to sample size (9 apomictic populations and 27 sexual populations—'mixed' populations were split into apomictic and sexual subpopulations), we randomly subsampled the sexuals into sets of nine populations (with similar geographic distributions to the apomicts) and reconducted the analysis 5000 times. The relative likelihood of a true difference between the two mating systems was calculated as the proportion of subsamples that were more extreme than the apomictic r^2 of each trait and correlation coefficients (r) between *GRla* and *FT*.

To further explore ecological trait variation, we planted a subset of 12 populations in two experimental gardens, one at the upper and another at the lower elevation range margin of *B. spatifolia*. The 'subalpine' garden was located at 3022 m (40.0364°N, −105.5442°W), within 100 m of the Niwot Ridge C1 weather/climate station at the University of Colorado Mountain Research Station, Nederland, CO, USA. The 'foothills' site was located in Fort Collins, CO (40.5702°N, 105.0630°W) at 1519 m (Fig. 1a). In each site, we planted six seedlings from eight families of 12 populations (experiment wise $n = 1152$) in September 2011. These plants overwintered as rosettes and flowered after snowmelt.

Fruit (silique) length and number of seeds/silique were calculated for >10 individuals from each population and garden. For each individual, we counted the number of siliques produced and calculated total seed number (absolute fitness) by multiplying silique number by the number of seeds/fruit. These calculations were conducted separately for each garden and source population. To calculate relative fitness, the absolute fitness measure from each individual was divided by the

mean absolute fitness within each garden. Genotype means were calculated as the mean trait value of each family. We calculated linear selection coefficients of *GRla* and *FT* in both gardens following Lande & Arnold (1983) using quantile normalized genotypic means.

Population genetic analysis- neutral molecular structure and diversity

DNA from all phenotyped individuals was extracted from lyophilized leaf tissue using the ChargeSwitch gDNA plant kit (Invitrogen Corp. Carlsbad, CA, USA). We followed PCR and genotyping protocols optimized by Beck *et al.* (2012) for 14 SSR markers known to amplify well across most *Boechera* species. Basic statistics for each marker were calculated in the R package 'adegenet' (Jombart 2008) (Table S1, Supporting information).

We analysed the patterns of genetic differentiation between apomicts and sexuals by comparing the multilocus (SSR) principal component (PC) positions and phylogenetic clustering between mating systems. Like in the phenotypic analyses, genotypes in 'mixed' populations were split into apomictic and sexual subsets. A 'Bruvo' distance matrix (Bruvo *et al.* 2004), which is robust to differences in ploidy and mating systems, was used to calculate PC positions of all genotypes in the R package 'polysat' (Clark & Jasieniuk 2011). We conducted phylogenetic clustering in the 'ape' package (Paradis *et al.* 2004) by generating a neighbour-joining tree from this distance matrix rooted to an accession of *B. stricta* (42.74451°N, 106.32512°W; Natrona County, WY, USA).

Analysis of genome-wide single nucleotide polymorphism

We extracted DNA from one individual per population using the Qiagen DNEasy Plant Miniprep kit (Qiagen Corp. Germantown, MD, USA) following manufacturer protocols (www.qiagen.com). Samples were analysed at the Institute for Genomic Diversity, Cornell University, Ithaca, NY, USA, through the 'genotyping-by-sequencing' (GBS) analytical protocol (Elshire *et al.* 2011). Single nucleotide polymorphisms (SNPs) were called using the published *A. lyrata* genome (Hu *et al.* 2011) as a reference. We analysed GBS data by processing raw SNP calls in TASSEL (Bradbury *et al.* 2007). A distance matrix was constructed and used to build a neighbour-joining tree. Genetic variation was characterized with respect to environmental and geographic variables using mantel tests (via the same script used for phenotypes) in R. Partial mantel tests were used to measure environmental differentiation while controlling for geographic distance. The genomic context (exon, intron, 5′UTR, 3′UTR, intergenic) of each SNP was extracted from the published *A. lyrata* genome. SNPs found at zerofold sites, where any nucleotide change results in an amino acid substitution, were extracted using calls from Haudry *et al.* (2013).

Results

Of the 30 populations surveyed, 19 were obligately sexual, two were apomictic and nine were mixed (Table 1). Apomixis was found exclusively in the southern 3/4 of the *B. spatifolia* range, but was highly dispersed within this region (Fig. 1a).

Genomic variation was structured by mating system and latitude

Genotyping by sequencing of one individual from each of the 30 populations (three apomicts, 27 sexuals) resulted in *c.* 82 million barcoded high-quality reads with 9126 SNPs mapping to all eight *A. lyrata* chromosomes. The majority of SNPs are located in protein coding, exon regions (84.0%), with 899 (9.9%) in zerofold degenerate sites where any mutation causes a change in the amino acid sequence. The remaining SNPs exist in intronic (8.7%), intergenic (6.3%), 5° UTR (0.8%) and 3° UTR (0.2%) regions. While efforts were made to screen only sexual genotypes, three apomictic individuals ('Poso', 'Alvarado', 'Hondo') were also genotyped by GBS. Principal component (PC) analysis demonstrated that these three apomictic lines were genetically distinct from the sexual accessions, which formed a tight cluster (Fig. 1b). Clustering of SSR variation further supports this conclusion (Fig. S1, Supporting information). 'Poso', the only triploid individual analysed, was widely differentiated from its 2*x* apomictic counterparts.

The GBS PC scores of the sexual individuals showed extremely strong congruence with the environmental distribution of the sampled populations (Fig. 1c). In particular, latitude (the spatial axis with the greatest variance) was highly collinear with PC axis #1 ($n = 27$, $r^2 = 0.902$, $P < 0.0001$; Fig. 1d). A similar pattern ($r^2 = 0.84$, $P < 0.0001$) was observed for the 899 SNP subset that caused amino acid changes (zerofold degenerate SNPs). In addition to latitude, genomic variation was significantly correlated with six climatic variables (Fig. S2, Table S2, Supporting information). Partial mantel tests, controlling for geographic distance among populations, determined that temperature seasonality (Mantel $r = 0.196$, $P = 0.006$; BIO4) and temperature annual range (BIO7; Mantel $r = 0.266$, $P = 0.002$) were significantly associated with genomic divergence, independent of the spatial position of the population (Table S2, Supporting information).

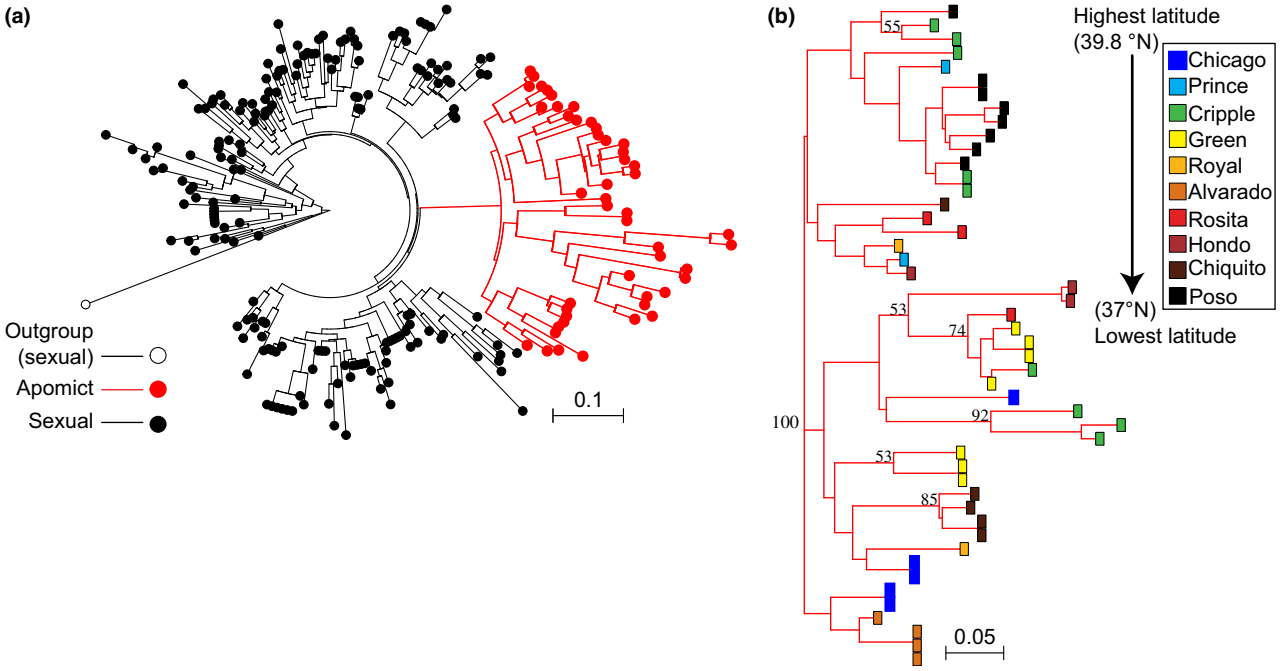

Fig. 2 Molecular genetic clustering of apomixis in *Boechera spatifolia*. (a) The neutral molecular genetic population structure (SSR) of *B. spatifolia* demonstrates sharp distinctions between apomictic and sexual individuals. These clusters are also manifested in a neighbour-joining tree rooted to a sexual *B. stricta* accession. Apomictic lineages are depicted by red edges and tip indicators. Sexuals individuals are black. The scale in Bruvo distances is presented. (b) Most apomictic populations are not monophyletic. All but the population 'Poso' contain individuals found across the genomic diversity of apomictic lineages. Bootstrap significance of edges over 50% are reported along with the Bruvo distance scale.

Across all SSR genotyped individuals, apomictic lineages formed a single group within the genetic diversity of sexual genotypes and were assigned to a genetically distinct cluster within a neighbour-joining tree, rooted to the widespread sexual relative, *B. stricta* (Fig. 2a). This pattern of genetic differentiation was also documented by principal component analyses (Fig. S1, Supporting information). There was a strong absence of population structure in apomictic lineages. All apomictic populations (or apomictic genotypes in 'mixed' populations) except 'Poso' were composed of individuals found across the genetic diversity of apomicts (Fig. 2b). The absence of structure among apomictic populations was evidenced by much lower F_{ST} (0.1661) than that among sexual populations (0.3958). Like GBS loci, SSR genetic variation was significantly correlated with geographic distance among populations ($r = 0.1727$, $P < 0.05$); however, no significant associations with any climatic variables were observed (Table S2, Supporting information).

Phenotypic divergence between mating systems

Discriminant function analysis of genotype means of the ten growth chamber phenotypes revealed highly significant differences between mating systems (Fig. 3a; Pillai test: $F_{11,180} = 45.08$, $P < 0.0001$). Phenotypic differentiation occurred along the first canonical axis and was primarily influenced by rosette architecture. *FT* and other phenological traits, which affected the second canonical axis, did not differ significantly between mating systems.

In addition to the growth chamber analysis, we also surveyed phenotypes in two experimental gardens. Two populations of the 12-population subset analysed in the field experiment contained both sexual and apomictic lineages. Of the 87 genotypes, seven were apomictic (8%). Comparisons of genotypic means between mating systems after controlling for variation among populations and gardens were consistent with growth chamber analyses: mating system variation was associated with significant differences between rosette growth traits (*GRla*: $F_{1,29} = 23.67$, $P < 0.0001$), but not phenology (*FT*: $F_{1,29} = 0.244$, $P = 0.63$). Interestingly, apomicts have greater *GRla*, but similar *FT*, which leads to slightly elevated fitness ($F_{1,29} = 3.88$, $P = 0.058$).

Selection on FT and GRla was conserved across populations and mating systems

To measure genetic correlations and the strength of selection, we compared correlation structures of the 80

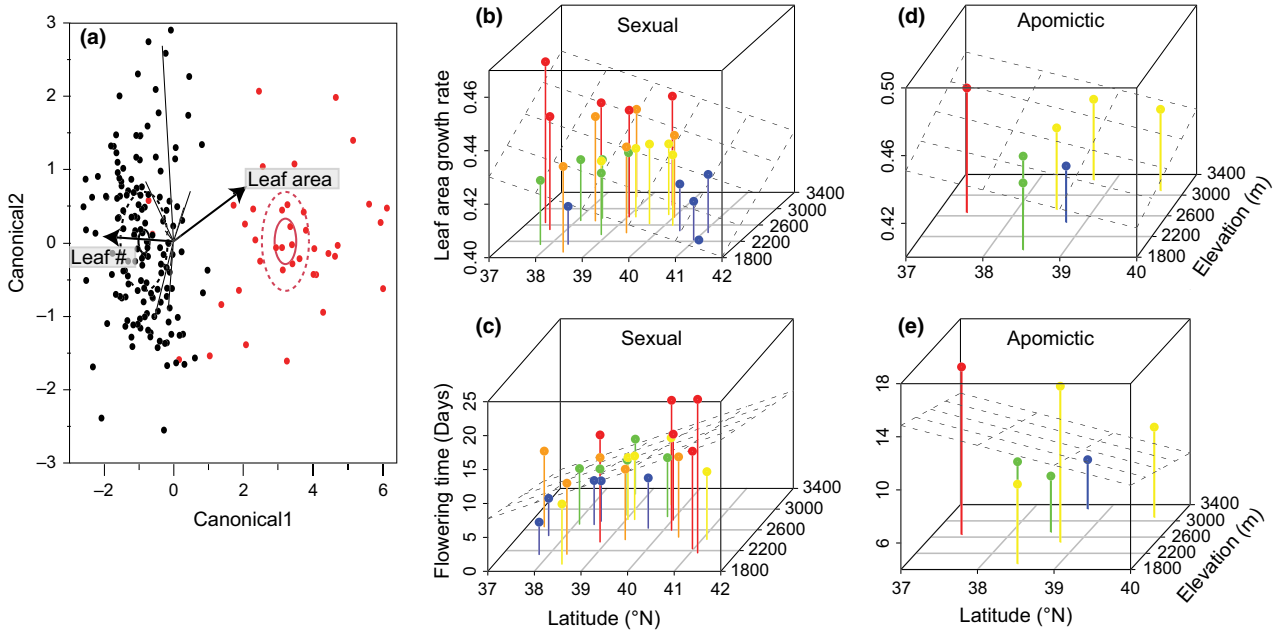

Fig. 3 Phenotypic variation of sexual and apomictic lineages across the landscape. (a) Discriminant function analysis reveals strong multivariate phenotypic differentiation between apomictic (red points) and sexual (black) individuals. Vectors from the origin depict the loading of each of the 10 phenotypes. Of these, only the arrowed and labelled vectors are significantly associated with mating system divergence. (b–e) Correlations of population breeding values for phenotypes with latitude and elevation for sexual (b–c) and apomictic (d–e) populations. The geographic variables latitude and elevation are plotted on the x and z axes. Population-level least square means for flowering time (c, e) and growth rate (b, d) are plotted on the respective y-axes. Populations are ranked by breeding values for each trait and colour-coded: top 20%-Red, 80–61%-Orange, 60–41%-Yellow, 40–21%-Green, bottom 20%-Blue. A linear model (phenotype=latitude + elevation) was fit to the data and plotted as the dashed line plane.

sexual genotypic means of *FT*, *GRla* and relative fitness measured in the experimental gardens. We detected strong directional selection on *GRla* and *FT* in both field sites, as evidenced by highly significant linear selection gradients (Table 2, Fig. S3, Supporting information). We estimated the selection gradient of *GRla* in the foothills site to be more than twice as strong as that found in the subalpine site (Table 2). Alternately, selection on *FT* was slightly weaker in the subalpine site than the foothills garden (Table 2). Despite differences in the strength of selection between sites, the direction and linear shape of the selection gradients are strongly conserved: early flowering and high growth rate lines were favoured in both environments. Nonlinear selection gradients were not significant for any site–trait combination. Apomictic phenotypes were also subjected to these selection gradients as evidenced by similar residual variance to that of sympatric sexuals in both gardens (subalpine: $t_{14} = 1.25$, $P > 0.1$, foothills: $t_{12} = 0.47$, $P > 0.1$).

Table 2 Genotypic selection gradients for flowering time (*FT*) and relative growth rate of leaf area (*GRla*) are presented for each garden. Statistical significance of the whole model is reported

Phenotype (site)	Estimate	*t*	*P*
FT (Foothills)	−0.2994	−3.427	>0.001
FT (Subalpine)	−0.3841	−3.829	>0.001
GRla (Foothills)	0.5707	6.548	>0.001
GRla (Subalpine)	0.2794	2.792	>0.001

Sexual, but not apomictic, phenotypic variance was highly correlated with environmental variables

We associated population-level breeding values (growth chamber) of *FT* and *GRla* with two geographic variables, latitude and elevation, separately for each mating system. Among sexual populations, flowering time was strongly correlated with the latitude of the source population ($r = 0.721$, $P < 0.0001$), but not elevation ($P = 0.202$; Fig. 3b). Growth rate was driven by latitude of the source population ($r = -0.434$, $P = 0.0235$) and was also significantly associated with elevation ($r = 0.395$, $P = 0.024$; Fig. 3c). These correlations held, even when population structure was controlled through partial mantel tests (*FT*: $r = 0.41$, $P = 0.0019$; *GRla*: $r = 0.34$, $P = 0.0075$; Table S3, Supporting information). Growth rate and flowering time were negatively

correlated ($n = 26$, $r = -0.526$, $P = 0.0058$). With the exception of seasonality (BIO6), other climatic variables were not significantly associated with phenotypic differentiation (Table S3, Supporting information).

In contrast, among apomictic populations, no significant associations between phenotypes and elevation or latitude (*FT*-elevation: $P = 0.719$; *FT*-latitude: $P = 0.574$; *GRla*-elevation: $P = 0.393$; *GRla*-latitude: $P = 0.261$, Fig. 3d,e) or any of the 19 bioclimatic variables were found (Table S4, Supporting information). Furthermore, the two phenotypes were positively, but not significantly correlated ($P = 0.404$). To overcome the difference in power between sexual and apomictic regressions, we subsampled 5000 nine-population sets from the sexual distribution and recalculated the model statistics. These subsamples were spatially constrained to correct for the smaller geographic distribution of apomicts. For >99.9% of the *FT* and >82.4% of *GRla* subsamples, sexual r^2 values exceeded apomicts. Correlation coefficients between *FT* and *GRla* were negative in >98.6% of all sexual subsamples (Fig. S4, Supporting information). These data indicated that the observed differences between mating systems were not a statistical artefact.

These correlations were also found among sexual populations planted in the field gardens. In the subalpine site, elevation of the source population was negatively associated with *FT* (higher populations flower earlier) but positively with *GRla* (higher populations grow faster; Table 3). Latitude only marginally affected *FT* and was nonsignificantly associated with *GRla*. In the foothills site, latitude strongly affected *FT* (more northern populations flower later) and marginally affects *GRla* (northern populations grow faster). Elevation was strongly associated with *GRla* (higher elevation populations grow faster) but not *FT* (Table 3).

Table 3 Statistical analyses of relationships among phenotypes and the source latitude/elevation of populations. *F*-statistics are generated from a mixed effect model controlling for variation within families. The direction of effect is reported, (+) or (−), from associated *t*-test of all significant relationships

Site (Phenotype)	Latitude (effect direction)	Elevation (effect direction)
Foothills (FT)	$F_{1,82.81} = 8.92$, $P = 0.0037$ (+)	$F_{1,81.9} = 0.123$, $P = 0.7265$
Foothills (*GRla*)	$F_{1,85.77} = 3.045$, $P = 0.0845$ (+)	$F_{1,84.3} = 6.39$, $P = 0.0133$ (+)
Subalpine (FT)	$F_{1,69.6} = 2.617$, $P = 0.11$ (−)	$F_{1,75.8} = 6.196$, $P = 0.0150$ (−)
Subalpine (*GRla*)	$F_{1,81.6} = 0.304$, $P = 0.583$	$F_{1,84.01} = 4.36$, $P = 0.039$ (+)

Discussion

Experimental evolution of rapidly cycling organisms and in silico inquiry (Otto & Lenormand 2002) has demonstrated that evolution in asexual lineages is in fact less affected by selection than sexual lineages (Engelstädter 2008); however, these comparisons are not normally possible in higher plants or most multicellular taxa because polyploidy or other confounding factors are fixed in asexual relatives of sexual diploids (Carman 1997; Paun *et al.* 2006; Robertson *et al.* 2010). Here, we presented ecological genomic and quantitative genetic comparisons between asexual and sexual lineages of *Boechera spatifolia*, a species that contains both diploid sexual and diploid apomictic lineages. This situation is rare among angiosperms and may provide an opportunity to investigate the causes and consequences of the evolution of asexual reproduction.

By collecting populations from across the genetic and geographic distribution of *B. spatifolia*, we were able to (i) compare the degree of genetic and physiological divergence among mating systems; and (ii) assess what factors drive population differentiation. Our data showed strong evidence of molecular and quantitative differentiation among sexual populations across environmental gradients, implicating adaptation as the primary cause of population structure. In contrast, apomictic populations were not structured in a consistent manner across environmental gradients, had high within population diversity and demonstrated nonadaptive phenotypic correlations. Combined, these data indicate that apomictic lineages were relatively less affected by selection.

Biogeography and evolutionary history of apomixis

Geographic parthenogenesis, where asexual, polyploid lineages exhibit divergent ecological characteristics and geographic ranges compared to sexual relatives, is nearly omnipresent across plants (Kearney 2005; Hörandl 2006; Mráz *et al.* 2009). In contrast, our comparison of diploid apomictic and diploid sexual populations showed limited evidence of geographic parthenogenesis. The geographic range of apomictic *B. spatifolia* was broad and overlapped with approximately 70% of the sexual distribution (Fig. 1). While apomixis was on average found in higher elevation and lower latitude sites than sexuals (Fig. 1, Table 1), apomixis was still widely distributed across elevation (>1100 m) and geographic area (>20 000 km^2). Taken together with evidence for a single or ancient origin of apomixis (Figs 1b and 2a), this implies a geographic spread of apomixis over much of the range of sexuals. Ecological differentiation, and the resulting pattern of geographic parthenogenesis, has not accompanied the

geographic spread of diploid apomictic *B. spatifolia*. This observation raises the possibility that the ubiquity of geographic parthenogenesis in other taxa may be driven primarily by the confounding factor of ploidy, not apomixis per se. Analyses of ecological niche characteristics among ploidy levels and reproductive modes across *Boechera* species are needed to confirm this hypothesis.

Across the genus *Boechera*, it has been hypothesized that apomictic lineages were recurrently derived by independent hybridization events (Dobeš *et al.* 2007; Beck *et al.* 2012). While we cannot rule out this possibility in *B. spatifolia*, our phylogenetic and phenotypic analyses showed strong clustering of apomictic genotypes within the diversity among sexual populations. Genome-wide genotyping indicated that apomictic lineages were genetically distinct from their sexual relatives. This observation was confirmed by multilocus SSR data, which indicated that apomictic lineages were a monophyletic group within the diversity of sexual *B. spatifolia* (Figs 1b and 2a). Despite geographic proximity to sexual conspecifics, and wide distances among populations, all apomictic populations clustered together.

Many studies have documented phenotypic and ecological differentiation between asexual and sexual lineages (Hörandl 2006; Mráz *et al.* 2009). Here, apomictic *B. spatifolia* lineages also displayed ecological trait divergence from sexuals, especially in rosette morphological and growth rate phenotypes (Fig. 3a). While on a broad geographic scale these phenotypic shifts did not coincide with altered ecological niches, microclimatic differentiation may have accompanied the divergent phenotypes of apomicts.

Relative impacts of selection and drift in apomictic and sexual lineages

Selection gradients, inferred by correlating genotypic trait means with relative fitness, demonstrated that selection is acting in a similar fashion on populations across a wide range of environments (Table 2, Fig. S3, Supporting information). At both the high and low elevation extremes of the *B. spatifolia* range, those lines that flowered early and/or grew vegetative tissue most quickly displayed the greatest relative fitness. *GRla* was under stronger selection in the more benign 'foothills' site, while *FT* was under the strongest selection in the highly stressful subalpine site. Despite a small sample size, genotype means of apomicts followed these correlations as strongly as sexuals. Given this result and the highly overlapping distributions of the two mating systems, it appeared that apomicts were subject to similar selection regimes as sexual population.

Given comparable selection regimes, the distribution of genetic variance across the landscape can permit inference about the relative effect of selection and neutral process on the evolution of populations. Here, we conducted two analyses to assess the hypothesis that selection is the primary evolutionary force in sexual, but not apomictic populations. First, apomictic genotypes deviated from a strong ecological correlation among traits. Selection in nature causes a negative ecological correlation between flowering time and growth rate (Angert *et al.* 2009; Lovell *et al.* 2013b). This adaptive negative correlation was strongly present in sexuals; however, the *FT–GRla* correlation was positive, but not significantly so, in apomicts. The nonadaptive sign of the correlation was indicative of a deviation from a selectively advantageous suite of trait values. Second, while sexual population breeding values were highly correlated with the environmental characteristics of the local habitat, the same is not true of apomicts. These comparisons were partially affected by differential power of regressions within apomicts ($n = 9$) and sexuals ($n = 27$). However, our results held true, even after subsampling the sexuals into groups of nine (with similar ranges of latitude and elevation). These results indicated that, even when controlling for sample size, phenotypes of apomicts showed a weaker association with environmental factors than those of sexuals.

Population and landscape genomics of B. spatifolia

Despite strong divergence between mating systems, there was very little genetic structure among apomictic populations (Fig. 2b). The cause of decreased genetic structure, even across wide environmental gradients, was not clear. Recent gene flow or high rates of dispersal may have geographically distributed diverse genotypes. Alternatively, it was possible that the diverse genotypes in apomictic population were maintained by a relative lack of evolutionary responses to directional or purifying selection. The latter scenario seems more likely because the rate of inconspicuous sexual reproduction in apomictic *Boechera* is thought to be very low (Aliyu *et al.* 2010), and there was no obvious difference in dispersal capability between mating systems.

Across our study system, latitude was a major driver of climatic variation. Given our 470 km (~4° latitude) sampling gradient, it was not surprising that we observed a very strong genomic cline across latitudes. This cline was observed with both the full 9126 SNP panel and an 899 SNP subset of zerofold degenerate sites that cause amino acid changes. In fact, latitude explained >46% of the total genomic variance from GBS. However, latitude was also a predictor of geographic distance. Isolation by distance, which is driven primarily by neutral processes of drift and gene flow (Slatkin 1987, 1993), may have also promoted this

cline. In addition to latitude, we also found strong associations between sexual GBS genetic distance and environmental variables that are not correlated with geographic distance. In particular, overall temperature variance and differences in temperature across seasons were highly correlated with GBS genetic distance and phenotypic variation even after correcting for geographic distance among populations. As the majority of GBS SNPs are in coding regions, these correlations may indicate adaptive differentiation in response to diverse environmental conditions. It is important to note that these analyses cannot rule out epigenetic variation as a possible driver of adaptive evolution in apomictic lineages. Analyses of methylation patterns are currently underway to connect epigenetics to adaptive responses to the environment.

Conclusions

Apomixis has played a major role in the evolution of *Boechera* (Beck *et al*. 2012; Lovell *et al*. 2013a). Our data indicated a single or ancient phylogenetic origin of apomixis in *B. spatifolia*. Since divergence, apomictic lineages experienced relatively less quantitative and molecular genetic differentiation among populations than sexuals. More importantly, apomictic population divergence was not correlated with environmental variation, and covariation among traits was in the opposite direction of that shown to be adaptive in other species. Conversely, genomic structure and quantitative traits of sexual lineages were highly correlated with latitude, climactic variables and to a lesser extent elevation. The negative growth rate-flowering time correlation is adaptive in *A. thaliana* and is conserved in sexual *B. spatifolia*, but is not present among apomictic lineages. Combined, these data pointed to a lack of adaptive evolution in apomictic relative to sexual *B. spatifolia* lineages.

We presented data on a previously unstudied species that displays remarkable physiological and ecological diversity. Data transfer from related model systems (*Arabidopsis thaliana, A. lyrata, Capsella grandiflora*), field manipulation and quantitative genetic analyses are simple in this species, making it an ideal system with which to answer questions related to adaptive evolution in nature. Furthermore, the presence of sympatric diploid apomictic and sexual lineages in *B. spatifolia* provides a unique opportunity to understand the formative processes and consequences of apomixis.

Acknowledgements

We thank S. Roberts, A. Lovell and K. Guilbert for their assistance with laboratory, greenhouse and field work, L. Boerner for assistance with FCSS, and A. Angert, S. Sheth, C. Ghalambor, B. Bauerle and M. Pellino for their comments on early drafts of this manuscript and advice about statistical analyses. We also thank P. Alexander for assistance with seed collections and J. Beck, D. Bailey, M. Windham and I. Al-Shehbaz for specimen identification and systematics advice. S. Wright and W. Wang provided SNP genomic information. This research was funded by a μMorph Training Fellowship to JTL, and NSF grant DEB-1022196 to JKM and DFG grant no. SH337/7-1 to TFS. JTL was supported by an NSF PRFB (no. IOS-1402392). The authors declare no conflicts of interest.

References

Agren J, Schemske DW (2012) Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. *New Phytologist*, **194**, 1112–1122.

Alexander PJ, Windham MD, Beck JB *et al*. (2013) Molecular phylogenetics and taxonomy of the genus *Boechera* and related genera (Brassicaceae: Boechereae). *Systematic Botany*, **38**, 192–209.

Aliyu OM, Schranz ME, Sharbel TF (2010) Quantitative variation for apomictic reproduction in the genus *Boechera* (Brassicaceae). *American Journal of Botany*, **97**, 1719–1731.

Angert AL, Huxman TE, Chesson P, Venable DL (2009) Functional tradeoffs determine species coexistence via the storage effect. *Proceedings of the National Academy of Sciences USA*, **106**, 11641–11645.

Arnold SJ (1992) Constraints on phenotypic evolution. *American Naturalist*, **140**, S85–S107.

Banta JA, Ehrenreich IM, Gerard S *et al*. (2012) Climate envelope modelling reveals intraspecific relationships among flowering phenology, niche breadth and potential range size in *Arabidopsis thaliana*. *Ecology Letters*, **15**, 769–777.

Barton NH (2010) Mutation and the evolution of recombination. *Philosophical Transactions of the Royal Society of London B*, **365**, 1281–1294.

Barton NH, Charlesworth B (1998) Why sex and recombination? *Science*, **281**, 1986–1990.

Beck JB, Alexander PJ, Allphin L *et al*. (2012) Does hybridization drive the transition to asexuality in diploid *Boechera*? *Evolution*, **66**, 985–995.

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher-plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **31**, 491–543.

Bossdorf O, Richards CL, Pigliucci M (2008) Epigenetics for ecologists. *Ecology Letters*, **11**, 106–115.

Bradbury PJ, Zhang Z, Kroon DE *et al*. (2007) TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, **23**, 2633–2635.

Bruvo R, Michiels NK, D'Souza TG, Schulenburg H (2004) A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology*, **13**, 2101–2106.

Burt A (2000) Perspective: sex, recombination, and the efficacy of selection—was weismann right? *Evolution*, **54**, 337–351.

Carman JG (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biological Journal of the Linnean Society*, **61**, 51–94.

Charlesworth D, Wright SI (2001) Breeding systems and genome evolution. *Current Opinion in Genetics and Development*, **11**, 685–690.

Clark LV, Jasieniuk M (2011) POLYSAT: an R package for polyploid microsatellite analysis. *Molecular Ecology Resources*, **11**, 562–566.

Clausen JC, Keck DD, Hiesey WM (1940) *Experimental Studies on the Nature of Species. I. Effects of Varied Environments on Western North American plants*. Carnegie Institute of Washington Publication 520, Washington, DC.

Conway DJ, Roper C, Oduola AM *et al.* (1999) High recombination rate in natural populations of *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences USA*, **96**, 4506–4511.

Dobeš C, Sharbel TF, Koch M (2007) Towards understanding the dynamics of hybridization and apomixis in the evolution of the genus *Boechera* (Brassicaceae). *Systematics and Biodiversity*, **5**, 321–331.

Elshire RJ, Glaubitz JC, Sun Q *et al.* (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, **6**, e19379.

Engelstädter J (2008) Constraints on the evolution of asexual reproduction. *BioEssays*, **30**, 1138–1150.

Etterson JR, Shaw RG (2001) Constraint to adaptive evolution in response to global warming. *Science*, **294**, 151–154.

Fournier-Level A, Korte A, Cooper MD *et al.* (2011) A map of local adaptation in *Arabidopsis thaliana*. *Science*, **334**, 86–89.

Haudry A, Platts AE, Vello E *et al.* (2013) An atlas of over 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions. *Nature genetics*, **45**, 891–898.

Henry L, Schwander T, Crespi BJ (2012) Deleterious mutation accumulation in asexual *Timema* stick insects. *Molecular Biology and Evolution*, **29**, 401–408.

Hill W, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genetical Research*, **8**, 269–294.

Hörandl E (2006) The complex causality of geographical parthenogenesis. *New Phytologist*, **171**, 525–538.

Hu TT, Pattyn P, Bakker EG *et al.* (2011) The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. *Nature Genetics*, **43**, 476–481.

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Kearney M (2005) Hybridization, glaciation and geographical parthenogenesis. *Trends in Ecology and Evolution*, **20**, 495–502.

van Kleunen M, Fischer M (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist*, **166**, 49–60.

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.

Lasky JR, Des Marais DL, McKay J *et al.* (2012) Characterizing genomic variation of *Arabidopsis thaliana*: the roles of geography and climate. *Molecular Ecology*, **21**, 5512–5529.

Lee CR, Mitchell-Olds T (2013) Complex trait divergence contributes to environmental niche differentiation in ecological speciation of *Boechera stricta*. *Molecular Ecology*, **22**, 2204–2217.

Lively CM (2010) A review of Red Queen models for the persistence of obligate sexual reproduction. *Journal of Heredity*, **101**, S13–S20.

Lovell JT, Aliyu O, Mau M *et al.* (2013a) On the origin and evolution of apomixis in *Boechera*. *Plant Reproduction*, **26**, 309–315.

Lovell JT, Juenger TE, Michaels SD *et al.* (2013b) Pleiotropy of *FRIGIDA* enhances the potential for multivariate adaptation. *Proceedings of Royal Society of London B: Biological Sciences*, **280**, 20131043.

Lynch M, Blanchard JL (1998) Deleterious mutation accumulation in organelle genomes. *Genetica*, **102**, 29–39.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution*, **18**, 189–197.

Matzk F, Meister A, Schubert I (2000) An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *The Plant Journal*, **21**, 97–108.

Mogie M (1992) *The evolution of asexual reproduction in plants*. Chapman and Hall, London.

Mráz P, Chrtek J, Šingliarová B (2009) Geographical parthenogenesis, genome size variation and pollen production in the arctic-alpine species *Hieracium alpinum*. *Botanica Helvetica*, **119**, 41–51.

Nautiyal P, Rachaputi NR, Joshi Y (2002) Moisture-deficit-induced changes in leaf-water content, leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. *Field Crops Research*, **74**, 67–79.

Nordborg M (2000) Linkage disequilibrium, gene trees and selfing: an ancestral recombination graph with partial self-fertilization. *Genetics*, **154**, 923–929.

Otto SP, Lenormand T (2002) Resolving the paradox of sex and recombination. *Nature Reviews Genetics*, **3**, 252–261.

Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Paun O, Stuessy TF, Hörandl E (2006) The role of hybridization, polyploidization and glaciation in the origin and evolution of the apomictic *Ranunculus cassubicus* complex. *New Phytologist*, **171**, 223–236.

Pellino M, Hojsgaard D, Schmutzer T *et al.* (2013) Asexual genome evolution in the apomictic *Ranunculus auricomus* complex: examining the effects of hybridization and mutation accumulation. *Molecular Ecology*, **22**, 5908–5921.

Qiu S, Zeng K, Slotte T, Wright S, Charlesworth D (2011) Reduced efficacy of natural selection on codon usage bias in selfing *Arabidopsis* and *Capsella* species. *Genome Biology and Evolution*, **3**, 868.

R Core Team (2013) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Robertson A, Rich TC, Allen AM *et al.* (2010) Hybridization and polyploidy as drivers of continuing evolution and speciation in *Sorbus*. *Molecular Ecology*, **19**, 1675–1690.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science*, **15**, 787–792.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Turesson G (1922) The species and the variety as ecological units. *Hereditas*, **3**, 100–113.

Turesson G (1930) The selective effect of climate upon the plant species. *Hereditas*, **14**, 99–152.
Verhoeven KJF, Jansen JJ, van Dijk PJ, Biere A (2010) Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytologist*, **185**, 1108–1118.
Wilczek AM, Roe JL, Knapp MC *et al.* (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science*, **323**, 930–934.
Windham MD, Al-Shehbaz IA (2006) New and noteworthy species of *Boechera* (Brassicaceae) I: sexual diploids. *Harvard Papers in Botany*, **11**, 61–88.

All authors contributed extensively to this manuscript. J.T.L., T.F.S. and J.K.M. designed the project and wrote the manuscript. J.T.L. and K.A.G. conducted the experiment. J.T.L. and J.K.M. analysed the data.

Data accessibility

All genotype and trait data, the tree file and the custom script to conduct spatially constrained mantel tests have been electronically archived in Dryad (http://datadryad.org/) DOI: 10.5061/dryad.gp495.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 SSR clustering of apomictic and sexual individuals.

Fig. S2 Clustered heatmap of the correlations among bioclimatic and geographic variables.

Fig. S3 Linear selection surfaces for FT and *GRla*.

Fig. S4 Distribution of correlation statistics following subsampling of sets of nine sexual populations.

Table S1 Descriptive statistics of 14SSR markers.

Table S2 Mantel correlation statistics for genetic and environmental distances.

Table S3 Mantel correlation statistics for phenotypic and environmental distances.

Table S4 Correlation statistics between environmental variables and the phenotypes flowering time (FT) and leaf area growth rate (GRLA) for a subset of sexual populations sympatric with the apomictic populations.