INVITED REVIEW
Boechera, a model system for ecological genomics

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Abstract

The selection and development of a study system for evolutionary and ecological functional genomics (EEFG) depend on a variety of factors. Here, we present the genus *Boechera* as an exemplary system with which to address ecological and evolutionary questions. Our focus on *Boechera* is based on several characteristics as follows: (i) native populations in undisturbed habitats where current environments reflect historical conditions over several thousand years; (ii) functional genomics benefitting from its close relationship to *Arabidopsis thaliana*; (iii) inbreeding tolerance enabling development of recombinant inbred lines, near-isogenic lines and positional cloning; (iv) interspecific crosses permitting mapping for genetic analysis of speciation; (v) apomixis (asexual reproduction by seeds) in a genetically tractable diploid; and (vi) broad geographic distribution in North America, permitting ecological genetics for a large research community. These characteristics, along with the current sequencing of three *Boechera* species by the Joint Genome Institute, position *Boechera* as a rapidly advancing system for EEFG studies.

Keywords: ecological genetics, evolution of sex, life history evolution, plant mating, population genetics—empirical, quantitative genetics, systems

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The goal of evolutionary and ecological functional genomics (EEFG) is to understand phenotypic and genotypic trait variation in natural populations at a comprehensive level (Feder & Mitchell-Olds 2003; Mitchell-Olds *et al.* 2007). Ecologically important traits must be studied in the organism's natural environment, using genetic or genomic tools, and characterized by an array of molecular approaches. Study organisms selected for evolutionary and ecological functional genomics thus must be ecologically diverse, genetically tractable and amenable to laboratory conditions. Several nonmodel systems are currently being developed for use in evolutionary and ecological functional genomics. Choice among ecological model systems depends on a congruence between research goals and experimental systems, including genetic and genomic resources, related species, life history and breeding system, environmental characteristics, geographic location and the community of researchers (Anderson *et al.* 2011).

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The genus *Boechera* in the family Brassicaceae is an excellent system with which to address a variety of ecological and evolutionary questions. First, native populations of *Boechera* grow in undisturbed habitats where current environments reflect historical conditions over several thousand years. *Boechera* enjoys a broad geographic distribution in North America, growing in environments ranging from deserts to rocky scree to moist alpine meadows (Fig. 1). Its tolerance of inbreeding has enabled the development of recombinant inbred lines, near-isogenic lines, and protocols for positional cloning, while its close genetic relationship with *Arabidopsis thaliana* has facilitated the development of functional genomic tools. Although hybridization complicates taxonomic classification within *Boechera*, incomplete reproductive isolation between species and subspecies allows interspecific mapping, which permits genetic analysis of speciation. In addition, the genus contains the most thoroughly characterized instance of diploid apomixis in angiosperms, generating tremendous interest for agricultural research. Finally, research on *Boechera* is

Fig. 1 Species in the genus *Boechera* occupy diverse habitats across the western United States. These habitats include the following: (top row) serpentine (Sierra County, California), sagebrush grassland (Custer County, Idaho) and Chihuahuan desert scrub (Eddy County, New Mexico); (bottom row) subalpine meadow (Ravalli County, Montana), rocky scree (Lemhi County, Idaho) and lava flow (Carrizozo Malpais, Lincoln County, New Mexico). Photographs of habitat in California and New Mexico courtesy of P. Alexander; all others, C. Rushworth.

flourishing, with a large and international research community focused on the system.

Ecology in natural populations

Many questions in evolutionary ecology have a historical component, such as elucidating the evolutionary forces that have influenced present-day variation for complex traits or whether co-evolution has influenced patterns of host–pathogen interactions. Ideally, historical evolutionary inferences should be verified by ecological experiments in persistent environments where natural selection has operated for many generations. Some *Boechera* populations grow in relatively undisturbed environments that have existed for ~3000 years (Brunelle *et al.* 2005), where ecologically important polymorphisms have evolved for thousands of generations, in populations that have never been impacted by agriculture or forestry. Although climate change and human activities have influenced most terrestrial habitats, some experimental sites in the Northern Rocky Mountains are nearly undisturbed—in some sites, the Mitchell-Olds group has not seen other humans in nearly a decade of research. Although it is unknown whether these populations are at ecological or genetic equilibrium, they have not been impacted by habitat

destruction or introduced genotypes, which can complicate evolutionary inferences. Such undisturbed sites provide the opportunity to test historical evolutionary and ecological hypotheses in present-day field experiments.

Boechera biology

Boechera is a widespread North American genus of approximately 68 sexual diploid species (Michael Windham, personal communication). Most species have a mixed mating system, often with high levels of inbreeding and homozygosity (Song *et al.* 2006). Other genotypes reproduce asexually and display high levels of heterozygosity. *Boechera* species are short-lived perennials that are capable of several generations per year in the laboratory. The genus displays numerous variations on a rosette body plan, ranging from single rosettes with inflorescences approaching a metre in height, to tightly packed rosette clusters forming ground-hugging cushion plants. *Boechera* flowers have four white or purple petals (Fig. 2), without the floral diversity found in *Aquilegia* or *Mimulus*. Nevertheless, *Boechera* displays enormous ecological diversity, occupying habitats from deserts to mesic grasslands, montane forests and alpine meadows (Rollins 1993). In many instances, *Boechera*

Fig. 2 *Boechera* displays phenotypic diversity within the Brassicaceous bauplan. (a) leaf diversity of multiple species, left to right: *B. retrofracta*, *B. tiehmii**, *B. stricta**, *B. vivariensis**, *B. cusickii**. (b) floral diversity of multiple species and hybrids, left to right: *B.* sp., *B. parishii**, *B. constancei**, *B.* sp., *B. stricta**. (c) fruit diversity of multiple species, left to right: *B. howellii**, *B. stricta*, *B. parishii**, *B. platysperma**, *B. retrofracta*. Photographs indicated with an asterisk courtesy of P. Alexander. All others, T. Mitchell-Olds, C. Rushworth.

populations and species are locally adapted to diverse ecological characteristics among these various habitats (Knight *et al.* 2006; Anderson *et al.* 2010).

Genetic and molecular analyses indicate that many *Boechera* species are diploid, sexual and predominantly inbreeding (Schranz *et al.* 2005; Song & Mitchell-Olds 2007). Given that most plants and animals are outcrossing and that inbreeding species may have elevated extinction rates (Takebayashi & Morrell 2001), what are the advantages of inbreeding model systems? First, tolerance of inbreeding brings major experimental benefits, such as near-isogenic lines for positional cloning and robust recombinant inbred lines for analyses of complex traits. In addition, *B. stricta* exhibits higher recombination per kilobase than *A. lyrata* (Song *et al.* 2009), facilitating fine-scale mapping of ecologically important polymorphisms. Finally, whatever the macroevolutionary fate of inbred lineages, it is clear that they can persist for millions of years, radiate into dozens of species and expand across entire continents. This is the case in the predominantly self-compatible genus *Boechera*, which has radiated into ~70 diploid sexual species over several million years, and is abundant across North America (Rollins 1993; Al-Shehbaz 2003). This genus and the 25% of plant species which are primarily self-pollinating (Takebayashi & Morrell 2001) offer many opportunities to test hypotheses in evolutionary ecology and genetics (Savolainen *et al.* 2000; Wu *et al.* 2007).

Boechera is well suited for ecological and genetic analyses in laboratory and field. Growth protocols are fully established. Individuals are propagated easily, and bolting and flowering commence after a brief vernalization period. With care, plants can easily live in greenhouse conditions for well over 1 year. In addition, although the flowers are small, crossing is straightforward, and mature fruits often yield upward of 100 seeds. Recombinant inbred lines have been developed for *B. stricta* (Anderson *et al.* 2010) and *B. retrofracta* (Colautti and Mitchell-Olds, in preparation). In addition, *Boechera* can be transported and transplanted easily, which has enabled us to plant >20 000 experimental individuals into natural populations during the past few years. This approach permits heavily replicated analyses of complex traits and components of fitness in the environments where ecologically important variation has evolved for thousands of years (Anderson *et al.* 2010).

Genomics and genetic resources

Boechera stricta and *B. retrofracta* (Fig. 2) have genome sizes of about 216 and 200 Mb, respectively (Anderson *et al.* 2011), and diploid genotypes have

seven chromosome pairs (Schranz *et al.* 2007b). Both species, as well as a diploid apomict accession, are being sequenced by the Department of Energy Joint Genome Institute (JGI), with completion expected within the next 12 months. BAC libraries from *B. stricta* and *B. retrofracta* with average insert size of more than 140 kb have been end-sequenced (JGI, unpublished), providing paired-end reads for assembly of next-generation genomic sequence. Divergence between *B. stricta* and *A. thaliana* is 13.5% at silent sites (Song *et al.* 2009), and linkage maps show large syntenic blocks among *B. stricta*, *Capsella rubella* and *A. lyrata* (Schranz *et al.* 2007a,b).

Resources for ecological genomics include hundreds of mapped molecular markers, a detailed linkage map reflecting synteny with *A. thaliana* (Schranz *et al.* 2007a,b), recombinant inbred lines in *B. stricta* (Anderson *et al.* 2010) and *B. retrofracta* (Colautti and Mitchell-Olds, in preparation), heterogeneous inbred families (HIFs, Loudet *et al.* 2005) for efficient generation of near-isogenic lines, and a diverse collection of species representing the variability within this genus. In addition, several hundred *B. stricta* accessions from across the species range are being prepared for use in genome-wide association studies.

Transformation methods have been developed for *Boechera* (Taskin *et al.* 2003). Alternatively, efficient methods for transformation in *A. thaliana* can be used to verify functionality of *Boechera* genes. Transcription profiling has been used to examine regulatory responses to herbivory, wounding and hormone treatments (Vogel *et al.* 2007). Given rapid advances in next-generation sequencing, future transcription profiling experiments will probably use sequence-based approaches to quantify gene expression (Wall *et al.* 2009), taking advantage of sequence similarity to *Arabidopsis* (Windsor *et al.* 2006). In addition, *Boechera* played a key role in the first comparative genomic analysis of conserved regulatory regions in plants (phylogenetic footprinting and phylogenetic shadowing, Koch *et al.* 2001).

Positional cloning allows researchers to identify the genes responsible for ecologically important variation. These efforts are facilitated by conserved microsynteny between genomic regions of *Boechera* and *Arabidopsis* (Schmid *et al.* 2005; Benderoth *et al.* 2006) and forthcoming *Boechera* genomic sequences. Previous experimental and theoretical analyses have shown that inbreeding species typically have higher levels of recombination per kilobase than their outcrossing relatives (Charlesworth *et al.* 1977; Roze & Lenormand 2005). This trend is also apparent in the comparison of outcrossing *A. lyrata* with inbreeding *B. stricta*, which has ~45% higher recombination per kilobase (Song *et al.* 2009). This high level of recombination facilitates fine mapping and positional cloning.

Systematics

The family Brassicaceae holds a unique position as a model family for answering major questions in evolutionary biology (Beilstein *et al.* 2008). However, a basic phylogenetic framework of the group has proved difficult to ascertain. Multiple taxonomic treatments have outlined varying numbers of tribes, largely based on fruit morphology, which have been unravelled by molecular work completed in the last decade. The genus *Boechera* was initially separated from *Arabis* on the basis of chromosome number—in *Boechera*, the base chromosome number is $x = 7$, one less than that of *Arabis* (Windham & Al-Shehbaz 2006). Molecular work has shown that the two taxa, members of distinct lineages within the family Brassicaceae diverged between 36.6 and 50.7 Ma (Beilstein *et al.* 2010), providing convincing support for taxonomic separation. Several phylogenetic studies have supported the family-level restructuring published by Al-Shehbaz and colleagues (2006) (Bailey *et al.* 2006; Beilstein *et al.* 2006, 2008; Franzke *et al.* 2009).

The tribe Camelineae contains several fully sequenced species, including *A. thaliana*, *A. lyrata* and *C. rubella* (Beilstein *et al.* 2008; Hu *et al.* 2011). Yet Camelineae appears in some phylogenies to be polyphyletic, as members sometimes group with other tribes including Boechereae (Beilstein *et al.* 2008). One recent study (Oyama *et al.* 2008) involving four genes supports *C. rubella* and *B. stricta* as members of the same clade, with *A. thaliana* as outlier. This result has been confirmed by a much larger analysis based on 84 loci, providing strong support for *Boechera* and *Capsella* as sister genera, with *Arabidopsis* as an isolated outgroup (Fig. 3). Consequently, for whole genome comparisons, *Boechera* and *Capsella* provide the best outgroups for each other. In addition, because *Boechera* and *Capsella* diverged long ago, the best outgroup for *Arabidopsis* is provided by the inferred ancestral state of *Boechera* + *Capsella*.

Recently, Beilstein *et al.* (2010) have inferred Brassicaceae divergence dates in the light of multiple fossil calibration points. The divergence between *Arabidopsis* and *Brassica* is estimated to have occurred approximately 43 Ma and the divergence of *A. thaliana* from its congeners ~13 Ma. Combining these dates with synonymous nucleotide divergence between *Arabidopsis* and *Brassica* ($K_s = 0.53$, Town *et al.* 2006) gives a mutation rate of 6.1×10^{-9}, close to the empirical estimate of 7×10^{-9} obtained from sequencing of mutation accumulation lines in *A. thaliana* (Ossowski *et al.* 2010). This mutation rate allows a rough calibration for the divergence of *Capsella* and *Boechera* at ~14.7 Ma ($K_s = 0.18$, Stephen Wright, personal communication), and
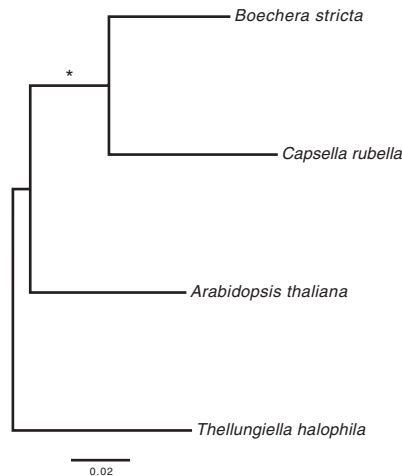
Fig. 3 Phylogenetic relationship between *B. stricta*, *C. rubella*, *A. thaliana*, and *Thellungiella halophila* (outgroup) based on 84 loci. Asterisk indicates bootstrap support of 100% for maximum likelihood, parsimony and neighbour-joining analyses and a Bayesian posterior probability of 1. Eighty four putatively orthologous loci were identified by overlapping reciprocal best BLAST from genomic sequences or amplified genes. Each locus was aligned separately using ClustalW (Thompson *et al.* 1994), and gaps were trimmed. Maximum parsimony, maximum likelihood (GTR + G + I), neighbour joining and Bayesian inference were applied to each alignment using PAUP* 4.0b10 (Swofford 2002) and MrBayes (Huelsenbeck & Ronquist 2001). Exhaustive topology searches were performed for maximum parsimony and maximum likelihood. For Bayesian inference, two independent runs were performed with 2 million generations each. Tree parameters were sampled every 1000 generations, and burn-in was performed for the first 500 sampled trees. In addition, the 84 loci were concatenated into a global alignment and analysed as earlier. Branch lengths of final consensus tree were estimated by maximum likelihood in PAUP. *Boechera stricta* sequences generated for this study: GenBank accessions JN703291-JN703309. Alignment of 84 loci in the four species and the full list of GenBank accession numbers of *Boechera stricta* loci: DRYAD entry doi:10.5061/dryad.5s3kt727.

divergence of *Arabidopsis* from the *Capsella-Boechera* ancestor about 16 Ma (K_s = 0.24, Song *et al.,* unpublished). This timescale explains the historical difficulty in resolving the *Arabidopsis*–*Boechera*-*Capsella* topology and indicates that the divergence between *A. thaliana* and *A. lyrata* is nearly as large as between the genera *Boechera* and *Capsella*.

Taxonomic treatments of *Boechera* have often overlooked minute characteristics such as trichome morphology, resulting in the lumping of sexual specimens with asexual hybrid specimens (Windham & Al-Shehbaz 2006, 2007a). Frequent hybridization among sexual species results in individuals that display morphological characters attributable to each parent. As such,

species of *Boechera* have often been falsely described as highly variable in morphology and breeding system. In addition, evolutionary relationships within *Boechera* are complex owing to reticulation and apomixis. While a robust phylogeny does not yet exist, a complete phylogenetic and taxonomic revision is currently underway, which will provide new insights into the evolutionary relationships within the genus (Patrick Alexander, personal communication). Availability of next-generation genomic sequences from multiple *Boechera* species (Prasad *et al.,* unpublished) also will improve our understanding of evolutionary relationships in this group.

Attention to *Boechera* taxonomy has affected the respective identities of the three species used most frequently for research. As a monophyletic, morphologically distinct diploid species, *B. stricta* (previously *Arabis drummondii*) remains intact. In contrast, the former *B. holboellii*, previously considered a highly morphologically diverse and widespread species, has been split into five species comprising *B. collinsii*, *B. pendulocarpa*, *B. retrofracta*, *B. polyantha* and the remaining *B. holboellii*, found only in Greenland. The *B. holboellii* involved in research in the Mitchell-Olds group has been identified as *B. retrofracta*. The hybrid between *B. stricta* and *B. holboellii*, *B. divaricarpa*, was previously used as a 'trashcan' classification in which hybrids between *B. stricta* and countless other species were lumped. Based on the type specimen, the name *B. divaricarpa* is currently ascribed to hybrids between *B. stricta* and *B. sparsiflora*. Hybrids between *B. stricta* and *B. retrofracta* await updated nomenclature. Circumscription of all currently recognized species is summarized in four main publications (Al-Shehbaz & Windham 2010; Windham & Al-Shehbaz 2006, 2007a,b).

Ambiguities in species nomenclature have been a historical challenge in the *Boechera* literature. In addition, natural populations consisting of both diploid sexual species and apomictic hybrids are common. For this reason, it is important to provide voucher specimens (ideally in herbaria and as viable seed collections) to frame ecological and evolutionary analyses in a clear evolutionary context. In our experience, information from a few codominant molecular markers (such as microsatellites) may be the quickest and most cost-effective way to infer breeding system, ploidy and species identification.

Genetic variation, phylogeography and speciation

Several studies have examined the phylogeography of *Boechera* species across North America (Dobeš *et al.* 2004b; Kiefer *et al.* 2009). Because of the rapid radiation of *Boechera* species (Song *et al.,* unpublished), cpDNA

polymorphisms show trans-specific polymorphisms across a number of taxa, with the exception of *B. stricta* (Kiefer *et al.* 2009). Three major cpDNA lineages were identified, with centres of diversity in the Klamath-Siskiyou region, the Colorado plateau and the southeastern Rocky Mountains. All three lineages exhibit reduced genetic diversity north of the last glacial margin, indicating northward migration and range expansion following glaciation. However, two exceptions in the Great Lakes region and the Yukon interior suggest ice-free refugia during glaciation. The former was also interpreted as a refugium in previous *Boechera* phylogeographic studies (Dobeš *et al.* 2004b) and from comparable results in butterflies (Nice *et al.* 2005).

In western North America, complex geography and repeated cycles of glaciation have generated complicated phylogeographic patterns among *Boechera* species (Kiefer *et al.* 2009), including multiple refugia along the Rocky Mountains. Patterns of microsatellite diversity offer evidence for multiple refugia for *B. stricta* in the northern and southern Rocky Mountains (Song *et al.* 2006). Analysis of species richness indicates that the highest levels of biodiversity in this genus are located in California and Nevada (Kiefer *et al.* 2009). In addition, Western North America has been home to diversification among closely related genera including *Anelsonia, Cusickiella, Halimolobos, Mancoa, Nevada, Pennellia, Phoenicaulis, Polyctenium, Sandbergia* and *Sphaerocardamum* (Bailey *et al.* 2002; Oyama *et al.* 2008).

Boechera stricta is a morphologically and genetically well-defined, predominantly inbreeding species (FIS = 0.89, Song *et al.* 2006). Several studies (Song *et al.* 2006, 2009) have found substantial genetic divergence between eastern and western subspecies, as well as isolation by distance in the eastern group (Lee & Mitchell-Olds 2011), which shows divergence between the northern and southern extremes. Depending on local and geographic sampling, genetic differentiation ranges from $F_{ST} = 0.50$ (between individuals sampled from eastern vs. western groups) to $F_{ST} = 0.44$ (comparing individuals within local populations across the species range) and to $F_{ST} = 0.07$ (comparing individuals from local populations in the northern portion of the eastern group; Mitchell-Olds, unpublished).

Genome-wide synonymous nucleotide diversity in *B. stricta* averages $\pi = 0.004$ (Song *et al.* 2009). This is lower than the estimate for most outcrossing plant species, as expected for inbreeding species (Charlesworth 2003). *Boechera stricta* is somewhat less diverse than the inbreeding *A. thaliana* ($\pi = 0.005$, Nordborg *et al.* 2005), perhaps because population sizes of *B. stricta* are generally smaller than those of the common weed *A. thaliana*. Extinction of local populations and founder effects also may have reduced genetic polymorphism in *B. stricta*.

Information on patterns and causes of linkage disequilibrium (LD) is fundamental for association studies of complex traits. Based on 86 mapped loci in *B. stricta* (Song *et al.* 2009), a species-wide sample showed that LD decays to background levels in 10 kb or less. Similar low levels of LD have been found in other inbreeding species (Morrell *et al.* 2005; Kim *et al.* 2007). However, *B. stricta* populations are genetically structured into geographic and historical lineages, and intralocus LD is about twice as high within regional samples (Song *et al.* 2009). Thus, the levels of LD depend on the reference population (Wakeley & Lessard 2003; Kim *et al.* 2007).

A number of evolutionary studies have begun to elucidate genetic and ecological mechanisms contributing to speciation. Most genetic studies have examined intrinsic genetic incompatibilities, which can be studied in the laboratory using genomic tools in model organisms. In contrast, the role of ecological selection in shaping species divergence has been less clear. Recently, with the publication of several examples, it has become clear that ecological selection plays an important role in incipient speciation (Sobel *et al.* 2010). Ample genetic and ecological tools make *Boechera* a promising system for testing the importance of ecological speciation, and work in this area is ongoing. Fine-scale sampling of more than 200 *B. stricta* populations has identified a hybrid zone between two subspecies in the Rocky Mountains, an area previously glaciated (Porter *et al.* 1983) and thus representing a zone of secondary contact. Within the contact zone, distance to the nearest stream is the most significant difference between habitats of eastern and western genotypes; thus, these subspecies have diverged ecologically into groups that are predominantly montane or riparian (Lee & Mitchell-Olds 2011). This result emphasizes the importance of natural selection based on local environmental conditions during the process of speciation.

Ecological influences on speciation are also evident in an interspecific comparison of *B. stricta*, *B. retrofracta* and *B. fecunda*. Demographic estimates (Song *et al.*, unpublished) suggest that these species diverged very recently (~1 Ma), with little evidence for gene flow among these species (Song *et al.*, unpublished). Furthermore, niche modelling has identified significant environmental differentiation among these species, again showing the importance of ecological adaptation in evolving *Boechera* lineages (Song *et al.*, unpublished).

Apomixis

Apomixis is defined in flowering plants as asexual reproduction via seed. Nearly all apomicts are

polyploid, but *Boechera* provides the only well-documented example of diploid apomixis in angiosperms (Dobeš *et al.* 2007; Ozias-Akins & van Dijk 2007). Study of apomictic *Boechera* thus provides a unique opportunity to examine evolutionary questions relating to asexuality, disentangled from the effects of polyploidy, in relatively undisturbed habitats.

Most studies of apomixis in *Boechera* have focused on the species *B. holboellii*, which has since been recognized as a species complex. Early cytological investigations of *B. holboellii* showed that apomixis occurs at the triploid, aneuploid and diploid level (Böcher 1951). Tetraploids and higher polyploids occur infrequently (Dobes *et al.* 2006). While the majority of apomictic angiosperm lineages are derived from self-incompatible parents, apomictic *Boechera* lineages are often derived from self-compatible progenitors (Asker & Jerling 1992). However, apomixis in *Boechera* is associated with hybridization (Schranz *et al.* 2005; Kantama *et al.* 2007; Sharbel *et al.* 2009, 2010), which is widespread and recurrent (Dobeš *et al.* 2004a,b; Windham & Al-Shehbaz 2006). Apomictic accessions contain variable numbers of chromosomes contributed from each parent, as well as aberrant chromosomes that are inherited by subsequent generations (Kantama *et al.* 2007). Apomictic individuals are highly heterozygous and capable of outcrossing with sexual individuals (Roy & Rieseberg 1989; Schranz *et al.* 2005, 2006; Voigt *et al.* 2007), although experimental crosses between sexuals and apomicts have thus far not produced apomictic offspring (Schranz *et al.* 2005, 2006). Apomixis is facultative in *Boechera*, and both diploid and triploid apomicts have been shown to reproduce sexually (Schranz *et al.* 2005; Kantama *et al.* 2007). The combination of high heterozygosity and the potential for both periodic sexual reproduction and outcrossing offers substantial opportunity for the production of myriad novel genotypes in mixed sexual and asexual populations of *Boechera*.

Apomixis in *Boechera* is *Taraxacum*-type diplospory, wherein the megaspore mother cell fails to complete meiosis I and undergoes normal meiosis II (Böcher 1951; Naumova *et al.* 2001; Corral *et al.* 2009). Chromosomal synapsis occurs with varying success during meiosis, which may lead to some recombination events (Kantama *et al.* 2007). Endosperm formation can initiate autonomously or via the more common method of pseudogamy, in which pollen fertilizes the endosperm but not the embryo (Naumova *et al.* 2001; Voigt *et al.* 2007). Meiosis during microsporogenesis is disturbed in apomictic accessions, and although apomictic *Boechera* accessions exhibit greater variability in pollen size than sexual accessions, apomicts are capable of producing a stable quantity of viable pollen (Voigt *et al.* 2007). Apomeiosis (abnormal meiosis) occurs at a low level in many *Boechera*

species and may represent an ancestral proclivity for the production of unreduced gametes (Aliyu *et al.* 2010). This has been shown in sexual accessions, which occasionally produce unreduced pollen (Kantama *et al.* 2007).

Many evolutionary and ecological theories have been proposed to explain the establishment and spread of apomixis in natural populations. For example, one significant drawback to asexual reproduction is the loss of recombination, which stifles production of new genotypes while leading to an accumulation of deleterious mutations in existing lineages (Muller's ratchet). This theory resulted in the historical view of apomixis as an evolutionary dead end (Stebbins 1950; Maynard Smith 1978). Indeed, studies in multiple systems have shown that apomicts exhibit less genetic diversity than their sexual counterparts (Paun *et al.* 2006; Loomis & Fishman 2009; Lo 2010). However, apomictic *Boechera* exhibit considerable genetic diversity that may be due to hybridization, tandem duplications or allelic sequence divergence (Corral *et al.* 2009). Concurrent with numerous unique and rare genotypes, putatively identical *Boechera* clones have been identified in locations up to approximately 60 km apart within their natural habitat (Rushworth and Mitchell-Olds, in preparation). This suggests that environmental variation may play a key role in the persistence of apomictic lineages.

In recent years, the prospect of implementing apomixis in agriculture has stimulated research in many molecular biology laboratories. Hybrid or genetically modified crop plants lose their carefully constructed genomes through one cycle of sexual reproduction, but an apomictic crop plant would clonally reproduce the desired genotype for generations (Asker & Jerling 1992; Carman 1997; Grimanelli *et al.* 2001). However, the genetic mechanism of apomixis in *Boechera* remains elusive. The presence of a B chromosome has often been noted in apomictic genotypes (Sharbel *et al.* 2004, 2005; Kantama *et al.* 2007). Several researchers have theorized that apomixis is simply transmitted through the B chromosome (Sharbel *et al.* 2005; Dobeš *et al.* 2007), although this seems unlikely (Schranz *et al.* 2006). Also, unresolved is the origin of the B chromosome; it may be of ancient origin or derived through new hybridizations (Sharbel *et al.* 2005; Dobeš *et al.* 2007). It still remains possible that the genetic control of apomixis is complex and multigenic (Schranz *et al.* 2005). Recent work, however, has identified differential expression of mRNA tags in sexual and apomictic ovules, along with apomixis- and sex-specific gene expression (Sharbel *et al.* 2009, 2010), suggesting that apomixis might be a result of asynchronous gene expression caused by hybridization or gene duplication (the hybridization-derived floral asynchrony or HFA hypothesis, Carman 1997, 2001).

Interactions with herbivores and pathogens

Boechera is commonly attacked by several species of *Puccinia* rust fungi, producing a striking pseudoflower in early spring (Fig. 4). This is an elongated axis of modified yellow leaves (Farrar 1999), producing both nectar and volatile fragrance which attract insect visitors (Roy 1993a; Raguso 2004), which then spread fungal spores to other host plants. *B. holboellii* (sensu lato) genotypes show differential susceptibility to fungal infection, and host plant fitness is reduced by *P. monoica* under greenhouse conditions (Roy 1993b; Roy & Bierzychudek 1993).

Because pathogens may influence the maintenance of sexual reproduction in host populations (Maynard Smith 1978), several studies have examined ecological interactions between *Puccinia* and *Boechera*. Apomictic

Fig. 4 Biotic interactions with *Boechera*. (a) *Anthocharis* (Pieridae) Lepidopteran larva feeding on *B. retrofracta*. (b) *Puccinia*-induced fungal pseudoflowers on *Boechera puberula*. Photographs, T. Mitchell-Olds and P. Alexander.

populations of *B. holboellii* are genetically diverse and display heritable differences in susceptibility to rust infection. Evidence for frequency-dependent selection has been found in some studies (Siemens & Roy 2005), but not others (Roy 1993b, 1998; Roy & Bierzychudek 1993). Frequency-dependent selection may be unlikely because of broad susceptibility to pathogen attack across multiple host genotypes, or complex communities of pathogen and insect enemies. Roy (2001) examined possible co-evolution between *Puccinia* pathogens and their *Boechera* hosts over evolutionary time, identifying pathogen lineages using ITS sequences and host lineages using cpDNA and ITS sequences. The phylogenies of host and pathogen were largely incongruent, providing little support for co-evolution or cospeciation between plant and fungus. Instead, most observed transitions were to geographically associated *Boechera* lineages, suggesting that host usage was determined primarily by geographical proximity. However, because studies to date have provided little phylogenetic resolution among *Boechera* species, it may be useful to revisit this relationship using genome-scale data.

Ecological effects of pseudoflowers have also been examined, comparing insect visitation rates in single species plots and mixed populations of *Boechera* pseudoflowers and buttercups (*Ranunculus*), which display early-season yellow flowers of similar size. Insect visitation rates were higher in mixed populations than in single species plots (Roy 1994). Across populations and years, patterns of insect visitation were influenced primarily by the proportion of flies and bees among the floral visitors (Roy 1996). These experiments demonstrate the importance of long-term studies in multiple populations to understand the variation in insect abundance and patterns of natural selection on both plant species.

Natural *Boechera* populations are frequently attacked by mammalian and insect herbivores (Fig. 4). Susceptibility to herbivore damage is genetically variable and experiences strong natural selection favouring resistant genotypes (Carmona *et al.* 2011). Studies of resistance in *Boechera* have benefitted from its close relationship to *Arabidopsis*, providing information on molecular and chemical mechanisms of resistance to insect damage (Vogel *et al.* 2007; Schranz *et al.* 2009). Ecological processes that could maintain genetic variation for resistance include trade-offs between resistance and resource allocation or other components of fitness (Anderson & Mitchell-Olds 2010) or tolerance to damage, which could mitigate the fitness consequences of herbivory (Manzaneda *et al.* 2010). In addition, herbivore damage and defensive physiology may interact with the levels of competition (Jones *et al.* 2006) or availability of water and nutrients (Haugen *et al.* 2008; Siemens *et al.* 2009).

Several studies have examined these questions in *Boechera*. Siemens *et al.* (2010) identified a significant cost of defence when resistant plants were grown in the absence of herbivores. This cost of defence was independent of resource availability, and glucosinolate chemical defences did not vary across a resource gradient. In addition, Manzaneda *et al.* (2010) analysed genetic variation for tolerance to herbivory. Significant genetic variation was found for tolerance to leaf damage, but the ability to tolerate herbivore damage carried no detectable cost. Genetic variation was also significant for glucosinolate composition and for basal and induced concentrations of these metabolites, although chemical defences were not correlated with tolerance.

Glucosinolates, biologically active secondary compounds that occur in the Capparales, have been studied extensively in *Brassica*, *Arabidopsis* and *Boechera* (Kliebenstein *et al.* 2005a; Halkier & Gershenzon 2006). These metabolites play a defensive role against generalist insect herbivores and some microbial pathogens (Kliebenstein *et al.* 2005b; Brader *et al.* 2006; Bednarek *et al.* 2009; Stotz *et al.* 2011). However, specialist insects can detoxify or sequester glucosinolates, using them as attractants for feeding and stimulants for oviposition. Therefore, the fitness consequences of glucosinolate chemistry depend on the herbivores and pathogens present in different environments, as well as on the quantity and side-chain composition of these compounds.

Boechera shows genetic variation in types and quantities of glucosinolates within and among populations. Schranz *et al.* (2009) mapped a QTL controlling glucosinolate profile and showed that insect resistance maps to this same chromosomal region. More recently, the Mitchell-Olds group has identified the responsible locus by positional cloning and verified functional and fitness effects in the field (Prasad *et al.*, in preparation). These experiments illustrate the potential for the *Boechera* system to advance our understanding of evolutionary and ecological functional genomics.

Flowering and life history variation

Age at first reproduction and timing of flowering during the growing season are fundamental aspects of an organism's life history that are genetically variable in many plant species (Metcalf & Mitchell-Olds 2009). Experiments with *Arabidopsis* have identified genes and pathways controlling flowering time, enabling molecular studies of ecology and evolution of life history variation (e.g. Kuittinen *et al.* 2008; Wellmer & Riechmann 2010). Timing of reproduction can experience strong natural selection, favouring genotypes that reproduce during optimal conditions (Anderson *et al.* 2011;

Munguía-Rosas *et al.* 2011). However, recent experiments in *Arabidopsis* and *Boechera* have found surprisingly weak correlations between genotype performance in the laboratory and field (Wilczek *et al.* 2009; Anderson *et al.* 2010; Brachi *et al.* 2010), suggesting that the signals that control flowering in the field have not been modelled effectively under laboratory conditions. Ecological model systems such as *Boechera* are ideal for such experiments in ecological genetics.

Anderson and colleagues (unpublished) examined genetic variation and natural selection influencing age at first reproduction in the short-lived perennial *B. stricta* using >8000 individuals from a recombinant inbred line cross between Colorado and Montana genotypes, comparing the results under laboratory and field conditions. Strong directional selection favoured early flowering in the field (Anderson *et al.* 2010). One large effect QTL influenced probability of flowering during the first summer season under field conditions, and age and plant size at first reproduction in the laboratory. In *Arabidopsis*, this chromosomal region contains *flowering locus T*, a central integrator of floral signalling pathways. In *B. stricta*, this *nFT* QTL ('near *FT*') experiences strong natural selection in the field. Furthermore, *nFT* alleles show significant genetic trade-offs between the parental environments in Montana and Colorado—local alleles show a home-field advantage, with higher probability of reproduction at their home site. Such trade-offs may cause balancing selection, maintaining genetic polymorphisms for complex trait variation among natural populations.

Physiological ecology and drought tolerance

Because water availability is an important factor in plant adaptation to natural environments (Arntz & Delph 2001), the ecology of drought tolerance has been a focus of several studies in *Boechera* (McKay *et al.* 2001; Knight *et al.* 2006). In many species, mechanisms for dealing with water stress include escape from drought (typically by flowering before water availability becomes limiting, Franks *et al.* 2007) or dehydration avoidance (if changes in root system architecture enable improved access to soil moisture, Bernier *et al.* 2009). These mechanisms are ecologically important in *Boechera*, which may enable identification of the genes and pathways responsible for local adaptation to drought, as well as mechanisms of ecological speciation (Lee & Mitchell-Olds 2011).

Using common garden experiments, McKay *et al.* (2001) demonstrated local adaptation to climatic conditions in *B. fecunda*, a rare endemic from Montana. They found significant differences in climatic variables and soil water availability between a high and low elevation

garden, with greater drought stress at the low elevation site. Genotypes from these populations showed significant differences in instantaneous water use efficiency, as well as rosette morphology, root mass ratio and carbon isotope indicators of water use efficiency. In addition, they showed that genetic differentiation among populations for drought related traits (Q_{ST}) was significantly higher than population differences for neutral molecular markers (F_{ST}), providing independent evidence supporting natural selection causing local adaptation.

Similar results were found by Knight *et al.* (2006), who compared two *Boechera* populations from locations differing in elevation, temperature and soil water availability. Reciprocal transplants showed evidence for local adaptation, with viability selection favouring local genotypes in their home environment. The low elevation site was significantly hotter and drier, and genotypes from this site had higher water use efficiency owing to reduced transpirational water loss, as well as to thicker leaves and greater root/shoot ratios.

Haugen *et al.* (2008) examined genetic variation between two species, *B. holboellii* and *B. stricta*. They found interspecific differences in the effects of drought on glucosinolate metabolites, as well as on genetic variation within *B. stricta* for several indicators of drought tolerance. They also found genetic correlations between glucosinolate defences, herbivore resistance, transpiration rates and plant growth rates. Subsequently, a field study across a local environmental gradient showed that drought and water use efficiency were important factors in plant performance across the patch boundary (Siemens *et al.* 2009).

Opportunities for future work

Boechera offers the opportunity for research on a genetically tractable perennial plant that grows in relatively undisturbed environments. Environmental context is central to *Boechera* research, a system that provides opportunities for ecological genetics in native habitats for researchers. For realistic analysis of traits and fitness, it is important that experiments take place in the natural environment and incorporate existing vegetation (Fig. 5), which may have large effects on competition, herbivory levels, flowering time and other quantitative traits (Anderson *et al.* 2011). Future work can also examine ecologically important variation using QTL mapping and genome-wide association studies in natural populations. Although few studies have examined QTLs and local adaptation across multiple populations, these experiments are now feasible in *Boechera* and several other ecological model systems (Anderson *et al.* 2011).

Methods developed for human genetics (e.g. Coop *et al.* 2010; Hancock *et al.* 2011) will enable identification of SNPs that are correlated with environmental conditions owing to historical selection for local adaptation. Next-generation sequencing will provide the nucleotide polymorphism data necessary for application of these methods to *Boechera* ecological genetics. Declining costs for genomic analysis will also enable transcriptomic studies of ecologically important genes and pathways. Characterization of phylogenetic relationships and reproductive compatibility among different *Boechera* species is ongoing, although future work is needed (Donovan Bailey and Patrick Alexander, personal communication).

Several characteristics facilitate analyses of speciation in *Boechera*. Many studies of speciation have focused on intrinsic mechanisms of reproductive isolation, which can be readily studied under laboratory conditions. Because of ecological differentiation among *Boechera* species and ready access to natural environments, *Boechera* has great potential to contribute to our understanding of ecological speciation. In addition, *Boechera* may permit tests of the evolutionary importance of chromosomal rearrangements, which may play an important role in
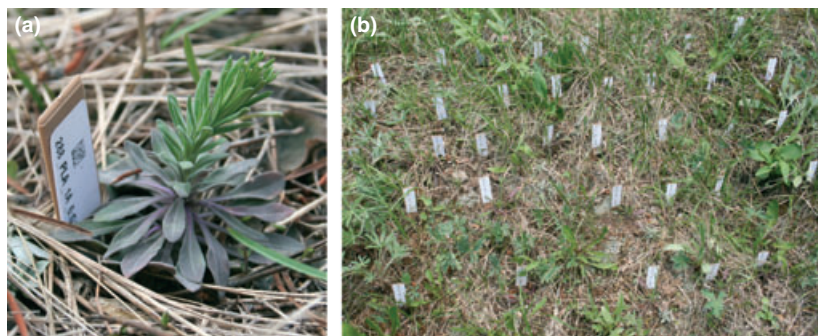
Fig. 5 *Boechera* transplants growing in undisturbed native vegetation. Fine-scale patterns of background vegetation have important impacts on growth rate, herbivore damage, flowering time and other ecologically important traits. Photographs, T. Mitchell-Olds.

local adaptation and speciation (Kirkpatrick & Barton 2006; Lowry & Willis 2010). Schranz *et al.* (2009) mapped a large chromosomal inversion on linkage group 1, which predicts ecologically important trait variation in natural populations. The inversion's breakpoints have been identified precisely, and the geographic range of the derived haplotype has been determined.

Boechera will also contribute to evolutionary and ecological analyses of biotic interactions. For example, herbivory in the field may influence life history variation. Herbivory on young rosettes often results in increased rosette number in subsequent years, which may influence tolerance and compensation to herbivory, as well as life history responses from multiple flowering rosettes.

Genomics approaches and similarity to *Arabidopsis* will enable functional understanding of *Puccinia*-induced pseudoflower production in *Boechera*, and whether co-evolutionary interactions are occurring between these two species. For example, we still have limited understanding of whether polymorphism within populations is maintained by frequency-dependent selection or whether local populations are genetically monomorphic, with differences reflecting adaptation between different populations (Mitchell-Olds *et al.* 2007).

Many possibilities also exist for metagenomic analysis of microbial communities associated with *Boechera* in the field. Metagenomic analyses indicate that microbial associates are influenced by glucosinolate composition in *Arabidopsis* (Bressan *et al.* 2009), and improvements in genomic technology can address these issues in undisturbed populations that have been unaffected by agriculture or many human disturbances.

Studies of apomixis present opportunities for molecular, ecological and evolutionary analyses. JGI is currently sequencing a diploid apomictic *Boechera* accession, and work is ongoing in several laboratories to understand genetic control of this agriculturally important trait. In parallel, ecological genomic analyses are characterizing molecular polymorphism in mixed sexual and asexual populations, and reciprocal transplants are being performed to compare environmental responses of genotypes with contrasting mating systems (Rushworth, unpublished data). These approaches can infer the possible importance of balancing selection in the maintenance of asexual populations. In addition, genomic approaches can determine whether epigenetic changes contribute to phenotypic divergence among clone mates. Furthermore, after the molecular determinants of apomixis are identified, it should be feasible to test the prediction that deleterious polymorphisms accumulate in nonrecombining regions.

Ecological genomic analyses can begin to infer the evolutionary processes that influence natural genetic variation for phenotypic traits. For example, what is the genetic architecture of adaptation or the relative importance of genetic drift and natural selection in the wild? What proportion of adaptive changes arise from new mutations or from previously neutral standing variation that becomes favoured because of environmental change? How does gene expression influence genotype-by-environment interactions in the field? As sequencing costs decline, it will soon be feasible to conduct genome-wide association studies on fully sequenced accessions from across the species range and to examine expression of ecologically important traits across a range of natural environments. Finally, genomic sequence information will allow us to follow dispersal of adaptive alleles from their point of origin across the species landscape.

Finally, the international community of researchers working with *Boechera* is collaborative and supportive. In July 2011, the first *Boechera* community meeting was held at the University of Colorado's Mountain Research Station (Lovell 2011), and future meetings are planned. This vibrant network of researchers offers support and established protocols for new members of the *Boechera* community. For these many reasons, the genus *Boechera* is uniquely poised as an ideal system with which to answer fundamental questions in evolutionary biology.

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Data accessibility

Boechera stricta sequences generated for this study: GenBank accessions JN703291-JN703309.

Alignment of 84 loci in the four species and the full list of GenBank accession numbers of *Boechera stricta* loci: DRYAD entry doi:10.5061/dryad.5s3kt727.