Population Structure, Genetic Diversity, Geographic Distribution, and Morphology of Two Boechera (Brassicaceae) Parental Species (Boechera thompsonii and Boechera formosa) and of Their Resultant Hybrid Boechera duchesnensis

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Population Structure, Genetic Diversity, Geographic Distribution, and Morphology of Two Boechera (Brassicaceae) Parental Species (Boechera thompsonii and Boechera formosa) and of Their Resultant Hybrid Boechera duchesnensis

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A dissertation submitted to the faculty of Brigham Young University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Population Structure, Genetic Diversity, Geographic Distribution, and Morphology of Two Boechera (Brassicaceae) Parental Species (Boechera thompsonii and Boechera formosa) and of Their Resultant Hybrid Boechera duchesnensis

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Background:
Over the relatively short period of its evolutionary history, Boechera (Brassicaceae) has undergone rapid radiation that has produced 70+ morphologically distinct, sexual diploids. However, reproductive isolation has moved more slowly than morphological divergence in this group and the diploids appear to hybridize frequently where they coexist. Boechera duchesnensis appears to be the result of hybridization between its putative parents Boechera thompsonii and Boechera formosa.

Objectives:
The objectives of this study are to (i) analyze and document genetic diversity patterns in the population structure, - including allelic and heterozygosity frequencies - of B. thompsonii and B. formosa in concert with their geographic distribution to determine clustering relationships within these populations, (ii) confirm and expand the morphological characteristics of B. thompsonii and B. formosa, as initially proposed in the literature, including pollen and trichome structure using Scanning Electron Microscopy (SEM) to confirm ploidy level and to determine whether both putative parent species share morphological characteristics with their apomictic diploid offspring, and (iii) use genetic and morphologic evidence to show that B. thomposii and B. formosa are, in fact, the parents of B. duchesnensis by comparing the genetic diversity patterns, population structure, and morphological characteristics of B. duchesnensis, to those of its proposed putative parents (B. thompsonii and B. formosa) and to confirm that B. duchesnensis shares characteristics of Boechera.
Methods:
Microsatellite data from 14 loci previously identified in Boechera were used to reexamine the current classifications and taxonomic foundations of three Boechera spp. GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) was used to analyze genetic population structures of two divergent sexual diploids in the genus Boechera: B. thompsonii and B. formosa and to later compare those with the population structure of B. duchesnensis. Geographical data were plotted using ArcGIS 10.1 (Esri, 2012) to map heterozygosity distribution. Cluster analysis was run with STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) and distribution of allelic diversity and heterozygosity was subsequently compared within each taxon and correlated with geographic distribution characteristics. Resultant data were then compared with B. duchesnensis data to document genetic diversity patterns, population structure, and morphological characteristics.

Key Results:
Analysis of genetic diversity patterns, allelic distribution of the populations, and heterozygosity of B. thompsonii and B. formosa across their geographic range identified four genetically distinct clusters within B. thompsonii, and one genetically distinct cluster in B. formosa. Allelic frequencies in all four discrete population clusters of B. thompsonii and in one discrete population cluster of B. formosa were close to values found in species on the decline. Reproductive isolation, genetic variability, and allelic frequencies were determined, specimen elevations reported, and morphological characteristics reported in the literature were confirmed and expanded. A codominant genetic analysis performed for 14 different loci for B. duchesnensis against those of its parents showed that B. duchesnensis inherits alleles from both putative parents and confirms B. thompsonii and B. formosa as the parents of B. duchesnensis. Observed levels of heterozygosity of B. thompsonii and B. formosa were lower than expected levels and lower than those of other outcrossing diploids. The mean overall observed heterozygosities for each cluster were determined and documented by geographic location. A substantially higher level of observed heterozygosity in B. duchesnensis (H_o = 0.908) consistent with genetic fixation of a heterozygote and apomixis, supports hybridization as a speciation mechanism and apomixis as a mode of reproduction accounting for genotypic and phenotypic diversity.

Morphological characteristics, especially those of pollen and trichomes were confirmed, expanded, and documented with SEM imagery.

Discussion:
This study provides an analysis of the genetic diversity patterns inherent in the population structure, allelic frequencies, allelic variation among individuals of the rare sexual diploids B. thompsonii, B. formosa, and the apomictic diploid B. duchesnensis in correlation with their geographic distribution. There is an implication of a reproductive barrier, within populations of the same species, that contributes to genetic isolation
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**Keywords:** diploid apomixis, hybridization, rarity, STRUCTURE, ArcGIS, GenAlEx, Brassicaceae, microsatellite, polyploidy, triploid apomict
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LITERATURE REVIEW

Systematists and ecologists are charged with the intricate task of methodically quantifying and categorizing large genera of plants into individual species in order to establish a meaningful framework for effectively studying and proposing management and conservation strategies. This is accomplished by delineating and categorizing morphological, genetic, and reproductive diversity, including the relationship between reproductive systems and polyploidy across genera (Brochmann, 1992; Beardsly et al., 2004).

Understanding genetic diversity and the evolutionary processes contributing to varying reproductive strategies within genera is imperative in the formation of proper conservation strategies (Soltis and Gitzendanner, 1999; Beardsly et al., 2004). Through meticulous morphological research and ecological studies of model systems, the relationships between evolutionary processes and genetic variability can be related to ecological and geographical patterns (Lovell, 2011).

Comprehending population structure, dynamics, and life history characteristics is considered fundamental to the conservation of rare plant species (Baskin and Baskin, 1986; Izco, 1998). Rare species have intrinsic value to biodiversity. Boechera (Löve & Löve) is a genus comprised of many rare flowering plants with unique reproductive strategies and evolutionary histories. Environmental disturbances including climate change, desertification, and drought have recently been documented as being detrimental to Boechera, both directly and indirectly, which may further exacerbate its rarity and affect its demography (Song et al., 2006).
Boechera is generally monophyletic (Koch and Al-Shehbaz, 2002; Bailey et al., 2006; Bielstein et al., 2006; Jordan-Tayden et al., 2010) with a narrower distribution than the largely worldwide distribution (excepting the tropics and Antarctica) of other members of the Brassicaceae (Al-Shehbaz, 1984, 1987; Rollins, 1993; Cronquist, 1968). Notwithstanding, there is poor phylogenetic resolution within Boechera itself. The phylogenies are complicated by high degrees of both autoploidy and allopolyploidy, and further by hybridization (Beilstein and Windham, 2003; Bailey et al., 2006).

The study of Boechera and its evolution of speciation is significant because this genus contains a diverse set of sexual diploids that hybridize to form numerous diploid and polyploid apomicts. This unique reproductive strategy makes Boechera an ideal model system for evolutionary research in plant systematics (Beck et al., 2012). Boechera represents a monophyletic taxonomic group with a base chromosome number of $x=7$, including sexual diploids, (both euploids ($x=7$) and aneuploids ($x>7$)), apomictic diploids, and apomictic polyploids. There is also a high degree of phenotypic diversity in Boechera. One explanation for this diversity is the increased level of polymorphisms representative of polyploidy, aneuploidy, and interspecific hybridization (Kantama et al., 2007). Koch (2015) identifies the genus Boechera as one of the few available model systems of apomixis in plants and identifies that Boechera spp. are highly affected by extensive hybridization, introgression, and reticulate evolution. He proves this by using comparative chromosome painting (CCP) through published cytogenetic studies (Koch, 2015).
The transition from diploidy to polyploidy has been observed numerous times in natural populations; however detailed explanations of the evolutionary mechanisms behind reproductive strategies such as apomixis are unclear and require further examination through species-specific studies (Routley et al., 2004). Apomixis is a complex trait in plants that results in maternal clones through seed reproduction. Understanding the evolution of apomixis may be elusive, but it is also potentially revolutionary for hybrid production, creating further interest in the evolution of asexual flowering plant strategies (Akiyama et al., 2011).

*Boechera*

Commonly known as Böcher’s rock-cress, *Boechera* species are either perennial or biennial flowering mustards. Named after Tyge Wittrock Böcher, who pioneered cytogenetic studies within the Brassicaceae during his career at the University of Copenhagen (Böcher, 1951), the genus consists of 110 species with 108 of those species extant in North America (Kiefer, 2008; Windham and Al-Shehbaz, 2006, 2007a, 2007b). The two species outside of the North American continent occur in Greenland; *Boechera holboelli* (Löve and Löve) and Siberia; *Boechera falcata* (Turcz.) Al-Shehbaz).

Demographic studies show that *Boechera* species are geographically distributed from the Alaskan border through much of North America to Greenland (Kantama et al., 2007). *Boechera* species that appear throughout the Colorado Plateau occur across ecological zones defined by precipitation and dominant plant species including salt desert shrub (USDA, 2003), pinyon-juniper
(Pinus spp. - Juniperus spp.) (Springfield, 1976; Powell et al., 1994), and big sagebrush (Artemisia spp.) zones. They also appear in alpine and forest habitats at higher elevations in North America (BLM, 1985). While some species of Boechera occupy wide geographic ranges, others become specialized where they adapt to particular habitat characteristics. Habitat specificity is important in understanding variables related to evolutionary and reproductive inclination towards hybridization and polyploidization (Beck et al., 2012). Boechera species exhibit a high rate of speciation. There are approximately 70 sexual diploid species of Boechera which provide rare glimpses into the dynamics of hybridization with their morphological characteristics (Beck et al., 2011).

Reticulate evolution and divergence has resulted in the formation of over 100 species with similar ancestral roots (Al-Shehbaz, 2003).

Until recently, Boechera received little study or analysis by researchers (Löve and Löve, 1976). Following the discovery that most North American Arabis were not genetically related (Koch et al., 1999, 2000), many species were re-circumscribed into the genus Boechera. After acknowledgement that Arabis was polyphyletic in contrast with Boechera’s pronounced lineage to North American mustards within the Brassicaceae, nomenclatural adjustments were proposed (Windham and Al-Shehbaz, 2007; Koch, 2001; Mitchell-Olds et al., 2005). These adjustments were substantiated by evidence that Boechera does not share the same phylogenetic clade as Arabis (Koch, 2001; Mitchell-Olds et al., 2005). Much of the Arabis research in previous decades did not encompass true Arabis, but unintentionally included a cluster of North American species, native to
the west, that recently (Al-Shehbaz, 2003) were reassigned to *Boechera*. This reorganization exigently necessitated a thorough reexamination of *Boechera*’s current classifications and taxonomic foundations due to manifestations of widespread hybridization, polyploidy, and apomixis in this genus (Windham and Al-Shehbaz, 2007). An extensive and contemporary genus description written by Windham and Al-Shehbaz (2012) appears in the publication, *Boechera*, published in the *Flora of North America North of Mexico*.

Several areas of study have been under investigation, including research on breeding systems (Roy, 1995), geography, plant-pathogen interactions (Roy, 2001), molecular systematics (Koch et al., 1999; Koch et al., 2000; Heenan et al., 2002) hybrid speciation (Koch et al., 2003; Dobeš et al., 2004a; Dobeš et al., 2004b), phylogeography (Sharbel and Mitchell-Olds, 2001), apomixis (Roy and Rieseberg, 1989; Naumova et al., 2001; Sharbel et al., 2004a; Sharbel et al., 2004b), polyploidy (Sharbel and Mitchell-Olds, 2001), population ecology (Bloom et al, 2001), evolutionary ecology (Mitchell-Olds, 2001), phenotypic plasticity and adaptation (McKay et al., 2001), evolution of genes and gene families (Allphin, 2007; Bishop et al., 2000; Koch et al., 2000), and “concerted evolution” (Woolstenhulme, 2007; Koch et al., 2003).

*Boechera* is a prime example of a genus where hybridization and polyploidization have played key roles in its evolutionary history (Marhold and Lihova, 2006), as 37% of its species are currently recognized as polyploids and this count is predicted to increase (Warwick and Al-Shehbaz, 2006). This is based on the 232 out of 338 accepted Brassicaceae genera (68.6%), and 1558 out of the 3709 (42.0%) recognized species that
currently have chromosome counts available. Examining the allelic and habitat variability of *Boechera* apomixis and heterozygosity at varying geographical localities will help explain how interactions among *Boechera* species affect apomixis and hybrid production.

*Boechera* Morphology

*Boechera* species, as found in North America, demonstrate the following range of characteristics: herbaceous, biennial to perennial with either stalked or sessile simple or branched trichomes, with simple or branched stems. *Boechera* species feature cauline leaves that are sessile or very rarely short and petiolate while basal leaves are petiolate, simple, entire, or dentate and very occasionally lyrat-pinnatified. Inflorescences can be panicles, fruiting pedicels can be divaricate, ascending, erect, or reflexed. Petal colors include pink, purple, and white and the morphological characteristics of the flower are representative of Brassicaceae with two short and four long stamens and a fused pistil (of two carpels) surrounded by four petals over four sepals. The ovary is superior and the fruits are siliques (Windham and Al-Shehbaz, 2006, 2012) (Figure 1).

*Rarity*

A concise definition of rarity is prerequisite to any proper discussion on rarity (Bevill and Louda, 1999). Gaston (1994) defined rare species as having low abundance with or without small ranges while Rabinowitz et al. (1986) identified seven discrete kinds of rarity based on combinations of three factors: 1) habitat specificity, 2) geographical range, and 3) local population abundance. The rarest species are found in restricted geographical ranges, have narrow habitat needs, small local population sizes,
and are most vulnerable to extinction. Incongruously, rare species are rather commonplace representing nearly 75% of known species worldwide (Dinerstein, 2013). Rare forbs endemic to salt-desert shrub and pinyon-juniper zones are particularly troubling to conservationists as they include at least two of Rabinowitz’ three dimensions (Rabinowitz et al., 1986). Any species with more than one rarity characteristic is at greatest risk for extinction and merits additional research and conservation efforts.

Rarity inevitably correlates with extinction vulnerability (Davies et al., 2004) and further, will presage extinction (Darwin, 1859). Species rarity draws conservation biologists’ attention and efforts (Gabrielová et al., 2013) in equal measure to its elevated trend towards extinction (Isik, 2010). Small populations are especially vulnerable to destructive events, even those of low probability.

Because there are many rare species with vulnerable populations, rare plant distribution and their conservation persist as central issues of ecology (Gabrielová et al., 2013). Rare species that are vulnerable to extinction lack in-depth demographic studies overall (Silvertown et al., 1993; Izco, 1998). Fiedler, Knapp and Fredricks (1996) found less than 100 demographic studies for all vascular plants as of 1996, and even fewer for rare plants. Rare, localized endemic species were largely ignored while the life history strategies of most weedy and domestic species were studied extensively (Bevill and Louda, 1999; Falk and Holsinger, 1991; Gabrielová et al., 2013).
Polyploidy and Hybridization

Polyploidy is important to angiosperm speciation as evidenced by large numbers of species with polyploid origins (Stebbins, 1950, 1980; Soltis and Soltis, 2000; Halverson et al., 2008). Polyploidy is considered an evolutionary driving force for sympatric speciation appearing in 30% to 80% of all plant species (Otto and Whitton, 2000). Polyploidy as a speciation mechanism manifests temporal and species spatial origins while simultaneously accounting for higher genetic diversity levels than any other form of single-origin speciation (Marhold and Lihova, 2006).

Allopolyploidy refers to the hybridization of a polyploid. Most apomictic polyploids are allopolyploids (Beck et al., 2012). However, many polyploids that hybridize retain the same ploidy level as their parentage (homoploidy) when meiosis results in pairs between homologous chromosomes. Hybridization is generally related to polyploidy and is an important apparatus of speciation. Sources of genetic variation and atypical combinations of genes, that provide augmented fitness and sometimes prolific opportunities for radiation, include both interspecific hybridization (mostly allopolyploidization) as well as autopolyploidization variations of polyploidization (Seehausen, 2004; Marhold and Lihova, 2006). Hybridization and polyploidization equally presaged reticulate evolutionary patterns inviting additional study and consideration of their evolutionary history and patterns of speciation. The simplicity of this bifurcation can sometimes convolute phylogenetic relationships and is therefore less helpful in this regard (Marhold and Lihova, 2006). The exceedingly rare, and yet common abundance of diploid apomixis in Boechera provides a rare and unique
opportunity for potentially classifying driving factors in hybridization as it is hypothesized that *Boechera* diploid apomicts are indeed all hybrids (Beck et al., 2012).

**Apomixis**

Apomixis is a common mode of reproduction in the Brassicaceae. In *Boechera*, almost all species which reproduce by apomixis, are polyploids. Polyploidization has been thought to be the first step in transitioning to apomixis as an evolutionary process (Beck et al., 2012). Polyploidy is interrelated with mating systems, which are widely understood to improve the long-term viability of a species and actuate evolutionary trajectories (Darwin, 1859; Stebbins, 1950; Barrett, 2003; Barringer, 2007). Polyploids frequently demonstrate apomixis, even more so in fertile triploids (Windham and Al-Shehbaz, 2007). A very high percentage of uncultivated apomicts are polyploid unlike their typically diploid sexual relatives (Whitton et al., 2008).

*Boechera* reproduces both sexually via outcrossing and asexually via apomixis. Chromosomes for both *B. holboellii* and *B. stricta* relatives have been found in apomicts indicating hybrid origins (Kiefer, 2008; Rollins, 1993; Kantama et al., 2007). This indication is supported through morphological evidence (Kiefer, 2008; Windham and Al Shehbaz, 2006, 2007a, 2007b). Many other species with elevated ploidy levels are typically associated with apomixis. *Boechera* apomicts break this standard and are commonly found to be diploid, triploid or even aneuploid (Kiefer, 2008; Dobeš et al., 2006).

Diploid apomixis is a relatively new reproductive strategy documented within *Boechera*, giving researchers a rare glimpse into its role in hybridization (Beck et al.,
2012). Rather than conclude that *Boechera* transformations from sexual to asexual reproduction are surely a by-product of polyploidization, researchers must consider the prospect that hybrid individuals interacting within divergent genomes could be the cause of diploid apomixis and its resultant elevated heterozygosity (Beck et al., 2012). In sexual diploids that out-cross, each individual passes on 50% of their genes to offspring contrary to the evolutionarily adaptive apomixis and self-fertilization (Barringer, 2007). Conjointly, apomixis and self-fertilization may serve as an assurance mechanism for rare species survival, adaptation for colonization, or to maintain co-adapted genes in proximity (Barringer, 2007).
INTRODUCTION

This dissertation presents research and analysis that will support the need for additional research of *B. thompsonii* and *B. formosa*, rare, endemic species supported by phylogenetic data but lacking a complete morphological description and understanding of population structure and dynamics. Until recently, *Boechera* has been under-studied by researchers (Löve and Löve, 1976). Following the discovery that most members of North American genus *Arabis* were not genetically related (Koch et al., 1999, 2000), many species have subsequently been re-circumscribed into *Boechera* invalidating much of the *Arabis* research. Diploid apomixis provides an uncommon opportunity to potentially classify driving factors in hybridization as it is hypothesized that *Boechera* diploid apomicts are actually all hybrids (Beck et al., 2012).

Most collaborative efforts to taxonomically resolve *Boechera* follow landmark studies of *Boechera* and define species limitations using the phylogenetic species concept (PSC), an accepted approach to delimitation when rampant hybridization is determined within the genus which disallows the reproductive isolation or biological species concept from successfully detecting species (Nixon and Wheeler, 1990; Davis and Nixon, 1992). Species can be delimited by applying morphological and molecular fixation criteria. When molecular data supports delineation of individuals into a new species but morphological data has not been previously distinguished or noted, further studies that include both morphological and geographical data are recommended.

Population genetics entails measures of allele frequency variations within and between populations. Population structure measurements are most commonly derived
using Wright’s $F$ statistics (Wright, 1931). Indices are calculated by defining groups of individuals, then using their genotypes to compute allele frequency variances. Prior to inferring anything about genetic structure of populations, one must define the populations themselves as the primary step in the process (Evanno et al., 2005). Population determination can coincide geographical origins of phenotypes or samples although genetic structures of a population are not always defined by geographical proximity with other individuals in the population. Evanno et al. (2005) proposed that indiscretely distributed populations can still be genetically structured due to disturbance of gene flow due to reproductive barriers which may not be identified.

Multiple studies have assessed polymorphism using microsatellite DNA markers which are frequently used because they are both highly polymorphic and co-dominant (Jarne and Lagoda, 1996). Since groups are clusters of populations expressing differentiation, others have documented hierarchical population structure patterns including otherwise isolated populations merging in zones of contact (Trouvé et al., 2005). These scenarios can imply relative isolation genetically and occasional patterns of isolation by distance within each population group.

Sexual *Boechera* specimens have at times been mistakenly grouped together with asexual specimens because previous attempts to taxonomically define *Boechera* have not assessed minor morphological characteristics (Windham and Al-Shehbaz, 2006a; 2006b). In the past, *Boechera* have been falsely described as being highly variable in breeding system and morphology because individuals with morphological characteristics
attributable to each parent are frequently the result of hybridization among sexual species (Rushworth et al., 2011).

The study of Boechera (Brassicaceae) and speciation mechanisms within this genus are significant as the genus contains a diverse set of sexual diploids that hybridize to form numerous diploid and triploid apomicts (Windham, 2006). This relatively recent, rapid speciation expansion has occurred in part due to hybridization among sexual diploids as a mechanism of speciation (Schranz, et al., 2005).

Complicating this significance is an inadequate understanding of hybrid origins, species boundaries, and the general taxonomy of the genus. These impediments have occasioned misapplication of proper names, which has been detrimental to a complete and proper understanding of the genus as a model system (Alexander et al., 2007).

Effective conservation of rare endemic species requires a fundamental understanding of evolutionary and life history characteristics, in conjunction with genetic diversity patterns as seen in population structure and population dynamics (Izco, 1998). Appropriate studies of genetic diversity patterns need to include an elucidation of the (a) population structure and population clustering [grouping], (b) factors that drive population dynamics, and (c) factors that contribute to reproductive isolation (Cires et al., 2013; Beardsly et al., 2004; Soltis and Gitzendanner, 1999; Izco, 1998; Falk and Holsinger, 1991; Rabinowitz et al., 1986; Baskin and Baskin, 1986). Population-level genetic diversity studies that identify population structure and include clustering analysis can be used to distinguish areas of high or low allelic diversity and
inform prioritization of conservation efforts (Frankham et al., 2002; Schemske et al., 1994; Baskin and Baskin, 1986).

Gene flow or a lack thereof, caused by reproductive isolators, such as geographic barriers or elevation gradients can also be documented within populations or identified clusters and thus provide important information about management and survival strategies (Cires et al., 2013; Lovell, 2014; Bevill and Louda, 1999). One such strategy may be a proposal to place B. formosa on the IUCN Red List. This kind of additional information is generally considered essential for properly assessing species at risk of global extinction based on population and range criteria (Mace et al., 2008; de Grammont and Cuarón 2006; Rodrigues et al., 2006; Lamoreux et al. 2003). Environmental disturbances to natural landscapes like desertification and drought have recently been documented as potential threats to Boechera spp., both directly and indirectly; which may in turn exacerbate their rarity by reducing allelic availability and heterozygosity and impeding their adaptation to local environmental conditions (Lovell, 2014). Examination of environmental variations that could cause local adaptation and elicit divergent population selection is vital in the development of appropriate conversation management plans for rare endemic Boechera species (Anderson, et al., 2015; Savolainen et al., 2007; Hereford 2009; Wang et al., 2010; Bennington et al., 2012; Kalske et al., 2012; Kremer et al., 2012; Alberto et al., 2013; Vergeer and Kunin 2013).

Natural populations frequently adapt to local climatic conditions across gradients of latitudes although conditions may vary only slightly; this likely minimizes
gene flow between populations isolated by distance at different latitudes (Etterson and Shaw 2001; Ågren et al. 2013; Anderson et al., 2015). Elevation and latitude gradations offer possibilities to investigate and understand how selection and migration affect one another and also spatial variation as an aspect of adaptation (Anderson et al., 2015). The study of elevation and latitude is crucial to counteract the current unfamiliarity with how diverse gradients compounded with short spatial scales affect local adaptation and clinal fitness variances (Gould et al., 2014; Richardson et al., 2014; Wilczek et al., 2014).

Examining environmental differences, like population clusters found in close proximity but dissimilar elevations - but that may demonstrate gene flow - would likely surface predictable patterns indicating how local population clusters adapt to elevation (Anderson et al., 2015; Kim and Donohue, 2013; Storz et al., 2007; Byars et al., 2007; Clausen et al., 1940).

*Boechera thompsonii* reproduces sexually by outcrossing (Al-Shehbaz, 2010, 2013). *B. thompsonii* has been reported in Arizona, Colorado, New Mexico, Utah, and Wyoming and is found on the Colorado Plateau across several ecological zones - defined by precipitation and vegetation type including salt desert shrub (USDA, 2003) and pinyon-juniper (*Pinus edulis - Juniperus osteosperma*) zones (Springfield, 1976; Powell et al., 1994) but predominantly in sagebrush (*Artemisia tridentata* Nutt.) zones (BLM, 1985). This small, herbaceous, long-lived perennial grows in full or partial shade on fairly steep wooded slopes with minimal ground cover. *B. thompsonii* often occupies limestone chip-rock and stony areas with sagebrush on steep slopes (USFWS, 1995).
B. thompsonii could potentially be listed as threatened or endangered due to its relatively limited distribution throughout its geographic range, the limited number of identified populations and generally small population sizes (USFWS, 2004). Extinction typically threatens rare and endemic species; however, improved information about the long-term effects of population disturbances may accommodate the development of more effective conservation strategies by better understanding life history strategies and characteristics. Studies, like this one, are generating valuable data concerning genetic diversity and population structure data that could potentially improve conservation efforts by focusing on rare and transitory endemic species, such as B. thompsonii (Cires et al., 2013). The genetic diversity and allelic variation reported for B. thompsonii may prove advantageous for promoting long-term survival of the species (Frankham et al., 2002).

In sexual populations of Boechera, evolutionary responses and species limitations accommodate adaptations to local conditions which result in distinctive geographic distributions and reproductive isolation (Lovell et al., 2014). Reproductive isolation, resulting from landscape variation, elevation gradients, geographic barriers, and isolation by distance (IBD), often enhance population subdivisions. This situation can in turn produce genetically structured clusters with unique genetic variations derived in response to natural selective forces and local adaptation (Anderson et al., 2015; Lovell, et al. 2104; Mantel et al., 2003; Herrera and Bazaga, 2008). When rare populations consisting of limited and scattered individuals become reproductively isolated, genetic drift combined with environmental degradation by climate change can stimulate rapid
evolution or necessitate genetic divergence (Anderson et al., 2015). Thus, understanding evolutionary trajectories by studying population structure and allelic variability in B. thompsonii could add to the knowledge base data that could be vital to future conservation efforts (Herrera and Bazaga, 2008).

Population size significantly affects how species respond to stochastic event probabilities that can potentially negatively impact the viability of small-population species. Rare and transitory endemic species, such as Boechera, have precipitated an increase in studies over the past decade, substantiating the value of genetic diversity and population structure data (Cires et al., 2013). Genetic diversity and allelic variation within all taxa are advantageous and required for long-term survival of a species (Frankham et al., 2002). Understanding population dynamics and life history characteristics is considered fundamental to the conservation of rare plant species (Izco, 1998; Schemske et al., 1994). Still, speciation and genetics research far outstrips research on how life history and ecological traits contribute to small population persistence (Bevill and Louda, 1999; Falk and Holsinger, 1991). Falk and Holsinger (1991) have expressed concern about our inability to conserve that which we do not understand, yet our understanding of the population dynamics of rarity is still lacking (Bevill and Louda, 1999).

Hybridization

Hybridization has played a primary role in the evolutionary history and evolutionary trajectory of Boechera (Marhold and Lihová, 2006). Apomixis is a complex trait in plants that results in maternal clones through asexual seed formation without
fertilization. Understanding the evolution of apomixis can be elusive, but it is also potentially revolutionary for hybrid production, creating further interest in the evolution of asexual flowering plant strategies (Akiyama et al., 2011), and laying a foundation for an appreciation of this unique type of speciation. Examining allelic diversity patterns as correlated with the habitat variability of Boechera apomicts and observed heterozygosity at geographical sites will help explain how interactions with other Boechera species affect apomixis and hybrid production within geographic ranges.

Apomixis makes Boechera an ideal model system for evolutionary research in plant systematics with implications for conservation (Beck et al., 2012). Future conservation efforts should focus on the hybridizing sexual diploid parents and particularly on populations in contact with other sexual diploids that have potential for hybridization because allelic fixation may be an evolutionary driving force towards speciation.

Increased levels of heterozygosity are found to promote longevity in perennial outcrossing species, such as Boechera (Allphin et al., 1998; Schaal and Levin, 1976; Hamrick et al., 1979). Observations of diploid apomixis as a mode of reproduction have demonstrated elevated heterozygosity suggesting that the incorporation of divergent genomes within a hybrid individual may enable the transition from sexuality to asexuality in Boechera and not necessarily as a result of polyploidization (Beck et al., 2012). Diploid apomixis provides a unique opportunity to further understand mechanisms resulting in hybridization. Currently all Boechera diploid apomicts are thought to be hybrids (Beck et al., 2012). One such example is B. duchesnensis which was
formerly classified as *Arabis pulchra* var. *duchesnensis* (Windham et al., 2007), and which has, thus far, only been found at a single location 1,676.4 meters above sea level (asl) in Duchesne County, Utah.

Big sagebrush, *Artemisia tridentata* (Nutt.) and yellow rabbitbrush, *Chrysothamnus viscidiflorus* (Hook.) Nutt.) are the dominant – although sparse - plant species occupying the bench tops where *B. duchesnensis* has been found in close proximity to *B. thompsonii* and *B. formosa*. The plant communities observed in and around the Duchesne site also include various species of *Atriplex* and black greasewood, *Sarcobatus vermiculatus* (Hook.) Torr.) consistent with plant communities in a salt-desert shrub habitat. The immediate area where *B. duchesnensis* occurs is almost entirely devoid of vegetation compared with locations occupied by other *Boechera* spp. which typically have well-developed vascular plant communities. *B. formosa* and *B. thompsonii* have been observed throughout the Colorado Plateau syntopically and in habitats consistent with salt-desert shrub, sagebrush, and pinyon-juniper zones. However, to date, these two species have only shown evidence of hybridization at the Duchesne site. (Figure 2).

Threats to rare *B. duchesnensis* populations within salt-desert shrub communities are the same as with all plants in this zone and include drought and resultant wind erosion and desertification. *B. formosa* and *B. thompsonii* are also threatened by climate change, desertification, drought, and wind erosion in Duchesne County (Anderson et al., 2011). Within sagebrush and pinyon-juniper ranges on the Colorado Plateau *Boechera* populations are also threatened by drought and erosion (Looney et al., 2012; Adams et al., 2009; Mueller et al., 2005).
Objectives

My first objective was to determine the genetic diversity patterns in the population structure of the rare sexual diploids *B. thompsonii* and *B. formosa* and define their geographic distribution; including allelic and heterozygosity frequencies to identify possible genetic clusters within each taxon. I hypothesized that genetically distinct clusters would be observed across the entire sampled parental population.

My second objective was to confirm and expand the known morphological and cytogenetic characteristics of *B. thompsonii* and *B. formosa* within the literature. I examined the morphology and cytology of *B. thompsonii* and *B. formosa* including trichome and pollen structures with Scanning Electron Microscopy (SEM) imagery. I hypothesized that the morphological traits viewed with SEM would support previous molecular studies that classified *B. thompsonii* and *B. formosa* as sexual diploids.

My third objective was to compare the genetic diversity patterns, population structure and morphological characteristics of *B. duchesnensis*, with those of its proposed putative parents (*B. thompsonii* and *B. formosa*). I hypothesized that the genetic diversity patterns and allelic frequencies of *B. duchesnensis* would confirm molecular studies that support *B. duchesnensis* as the hybrid of *B. thompsonii* and *B. formosa* – and as a hybrid diploid apomict. I further hypothesized that the inherited morphological traits viewed with SEM would confirm previous molecular studies (Beck et al., 2012; Koch, 2015) that classified *B. duchesnensis* as a diploid apomict.
MATERIALS AND METHODS

Sampling Protocol

Samples totaled 105 individuals, consisting of 53 B. thompsonii, 45 B. formosa, and seven B. duchesnensis. These samples were collected with scattered distribution occupying a range including Utah, Wyoming, New Mexico, Colorado, and Arizona. All samples of B. thompsonii and B. formosa outside of Duchesne Co., Utah were obtained from herbarium vouchers while the seven B. duchesnensis individuals were collected in the field in limited numbers to avoid excessive impact on the few individuals remaining. Specifically, tissue samples of both leaves and reproductive structures (flowers) were collected in addition to a record of each sample’s geographic coordinates. Tissue samples were dried with silica gel in preparation for DNA isolation. To maintain consistency with collaborative studies of Boechera, I collected samples from herbarium vouchers that were molecularly identified as individuals of genetically confirmed species which also had reliable geographic coordinates.

Sample Selection

Samples were selected from herbarium vouchers stored in prestigious North American herbariums only to maintain consistency with large-scale collaborative Boechera research. Additionally, vouchers were only used when microsatellite analysis was able to be done on the specimen to confirm that the species matched its label and description. This step was included to carefully avoid any bias that might have been introduced by misclassification of the voucher specimens or improper herbarium procedures or storage parameters. Vouchers that were validated by this process
temporally span over 150 years and, due to the stringent requirements, were very well preserved. All specimens were originally collected from the field, pressed and dried, sampled for microsatellite analysis to confirm species, genetic diversity, and SEM analysis.

The final step in the sample selection was a thorough examination of all samples under a dissecting microscope to find pristine reproductive structures. I identified samples with unopened flowers to improve my chances of finding undisturbed pollen. From these samples, I then selected the best specimens based on the quality of the samples as measured by damage caused by collection and handling and chose representative samples to be used in my study.

*Microsatellite Analysis*

Microsatellite analysis is invaluable in hybrid origin assessments, especially in groups where speciation has recently occurred (Beck et al., 2012). A microsatellite study was conducted by multiple researchers throughout North America, in collaboration with Duke University, using a protocol outlined by Beck et al., (2012), in which 15 unique microsatellite loci were identified for *Boechera*. All microsatellites included in this study were run at Duke University in the Windham Labs, as part of a large-scale project focused on *Boechera* morphological identification (Alexander et al., 2013, Alexander et al., 2015; Rushworth et al., 2011). All DNA extractions were processed at a lab at Duke University in order to ensure data consistency across all *Boechera* research projects. Following the Beck et al., (2012) protocol – our team identified 1,393 diploid *Boechera* (Brassicaceae) individuals as being genetically and geographically distinct.
Beck et al. (2012) categorized and assembled a genus-wide data set. In addition, a core set of microsatellite loci were developed for 69 diploid Boechera species obtained from North American herbarium-vouchered specimens continent-wide. These loci demonstrated a significant relationship ($r=0.055$, $P<0.01$) between genomic consistency and variation in the age of the collections (~150 years). This decreases the likelihood of an ascertainment bias (Beck et al., 2012) while showing that limited phylogenetic divergence is optimal for comparative studies by allowing for correlative analyses of highly variable loci that show phenotypic plasticity across a range of Boechera lineages.

Fourteen previously published loci were used to assess variation in microsatellite alleles (Alexander et al., 2007; Beck et al., 2012). A multiplex PCR protocol was used to amplify sets of two or three loci simultaneously. The 500 ROX Standard was applied for species-comparative analyses using an Applied Biosystems 373xl DNA Analyzer to size amplicons. GeneMarker version 1.9 was used to identify alleles (Alexander et al., 2007; Beck et al., 2012; SoftGenetics, State College, PA).

In the study of B. duchesnensis, genetic diversity statistics were analyzed based on microsatellite variation at 14 of the 15 unique loci identified by Beck et al., (2012). Each sample site for B. thompsonii, B. formosa, and B. duchesnensis was studied to determine the distribution of genetic diversity within and among populations, in conjunction with propensities for hybridization. Further study was performed to determine the extent of shared genetics and to reconfirm that B. thompsonii and B. formosa are the diploid parents of B. duchesnensis. Specifically, the fourteen microsatellite regions of B. thompsonii, B. formosa, and B. duchesnensis were compared using STRUCTURE 2.3.3
(Pritchard et al., 2000; Falush et al., 2003, 2007) to determine the extent of shared
 genetics and to reconfirm whether *B. thompsonii* and *B. formosa* are the diploid parents of
*B. duchesnensis*. GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) was used to analyze
allelic frequencies and heterozygosity at each locus.

**Ploidy-Level Determination**

The ploidy level of all individuals was calculated based on the maximum
number of alleles per microsatellite locus. Individuals with a maximum of two alleles
per the 15 loci were identified as diploid. Ploidy counts of the specimens used in this
study were correlated with ploidy levels of the total genome-wide studied individuals
previously confirmed with chromosomal counts by Beck et al., (2012). Microsatellite
analyses of *B. thompsonii*, *B. formosa*, and *B. duchesnensis* successfully amplified and
produced 99%, 98%, and 93% readable results respectively; thus 14 of the 15 available
loci data-sets developed by Duke University for use with *Boechera* were analyzed for
genetic diversity patterns and population structure.

**Genetic Diversity and Population Structure Analyses**

The model-based clustering software STRUCTURE 2.3.3 developed by Falush et
al. (2003) was used to estimate the number of genetically unique population clusters (K)
using an admixture model with correlated allele frequencies (Pritchard et al., 2000;
Falush et al., 2003, 2007). STRUCTURE was run 10 times (10 runs) for each cluster (K)
ranging from 2 - 10 (K = 2 - K = 10) using a burn-in of 1,000 and 10,000 Markov Chain
Monte Carlo (MCMC) repetitions (Pritchard et al., 2000; Falush et al., 2003, 2007). Data
from STRUCTURE was then used to determine K using the largest ΔK (rate of change in
the log probability of all data between consecutive K values) (Evanno et al., 2005). The Evanno method (Evanno et al., 2005) was implemented using STRUCTURE HARVESTER (Earl and von Holdt, 2012, 2011) to perform iterative sampling in order to distinguish distinct clusters (K), using an ad hoc statistic ΔK based on rate of change in log probability of data between successive K values, I found that STRUCTURE accurately detects the uppermost hierarchical level of structure for the scenarios tested.

GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) was used to analyze observed and expected heterozygosity, percentage of polymorphisms and allelic diversity for each taxon (Peakall and Smouse, 2006, 2012). Heterozygosity and documentation of outcrossing were included for all samples, along with hybridization. This analysis was performed regardless of the geographical origin of each population’s information.

A matrix consisting of allelic data was input into the software program STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007). An admixture model accounting for ancestry of individuals was used employing an algorithm that assigns each individual to fixed K clusters, allowing for each individual to have partial ancestry in each K cluster. All Boechera individuals were assumed to have shared ancestry, based on previous microsatellite analyses by Beck et al. (2012) demonstrating monophyly in Boechera; discrete clusters were then simulated.

SEM Analysis

Samples were prepared by taking sampled voucher materials, mounting them on SEM specimen stubs, sputter-coating them using an 80/20 gold-palladium mix for SEM study. A heavier application of sputter coating was employed to reduce charging effects
because reproductive structures caused a higher degree of charging than non-reproductive structures; also some microscopy was done in a low-vacuum environment in the SEM.

An Environmental Scanning Electron Microscope (ESEM) was used in a high-vacuum environment for most samples with an accelerating voltage ranging from 1.00 kV to 5.00 kV, and spot sizes ranging from 1.0 to 3.0 based on the most appropriate settings for the sample being observed. Samples that included only pollen grains sometimes required a low-vacuum environment at 0.8 Torr., an accelerating voltage of 15.00 Kv and spot size of 3.0. This SEM analysis was used to confirm morphological characteristics and distribution of glandular trichomes on stems, basal leaves, and fruiting pedicels between B. thompsonii and B. formosa. Stalked and rayed glandular trichomes, a type of capitate hair, are commonly found on aerial plant structures. Correlations between reproductive mode and ploidy level vs. pollen shape and size were morphologically corroborated with trichome formation and anatomy in an effort to determine species limitation and range of B. thompsonii and B. formosa and to increase available information on all Boechera sexual diploids. B. duchesnensis was examined for intermediate morphological trichomes traits inherited from B. thompsonii and B. formosa and differences were characterized between 1) apomictic diploid pollen and 2) sexual diploid pollen; further, all diploid pollen was characterized against the more common apomictic triploid pollen.
Geography

Genetic diversity of the Duchesne site, or in relation to the site with the hybridization occurrence, was subsequently analyzed using geographical technologies accomplished by integrating allelic frequency and heterozygosity data using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) and cluster analysis data using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) into ArcGIS 10.1 (Esri, 2012). These results provided an opportunity to examine how genetic diversity in Boechera populations is distributed over a geographic range. Areas of higher diversity within each population were mapped and analyzed for existing and contributing variables towards speciation. ArcGIS 10.1 (Esri, 2012) provided visualization and analysis of geographical population structures and genetic diversity patterns based on molecular data (Hoffmann et al., 2003). Individuals of all taxa were assigned to K = 6 clusters, assigned a unique color and then identified geographically using ArcGIS 10.1 (Esri, 2012) mapping applications.

RESULTS

Analysis of the genetic and morphological characteristics of the populations of B. thompsonii and B. formosa across their geographic range provided three primary pieces of information that supported my objectives: 1) identify four genetically distinct clusters within B. thompsonii, and one genetically distinct cluster in B. formosa through a comparison of allelic variation among individuals, 2) define the geographic distribution for each of the genetic clusters, and 3) confirm the morphological characteristics of B. thompsonii and B. formosa reported in the literature.
Microsatellite data results of *Boechera* species showed genetic diversity patterns that could be effectively separated with STRUCTURE 2.3.3 cluster analysis (Pritchard et al., 2000; Falush et al., 2003, 2007). Delta K values and log-likelihood values for each were calculated (Evanno et al., 2005) using STRUCTURE HARVESTER (Earl and Von Holdt, 2011) and identified as belonging to six distinct clusters (K = 6) that were mapped to document geographical patterns (Figure 4). Bayesian clustering was used to identify the highest likelihood value (Ln P_rX/K) occurring at K = 6 within all three species and resulted in four genetically distinct populations of *B. thompsonii* spread across its geographical range (K = 4). *B. formosa* maintained one uniformly genetically distinct population across its geographical range (K = 1). *B. duchesnensis* also maintained one uniformly genetically distinct population across its geographical range (K = 1). Individuals of each taxon were designated into K = 6 clusters, assigned a unique color and then identified geographically for distribution using ArcGIS 10.1 (Esri, 2012) mapping applications (Figure 6).

*Boechera formosa* (Greene) is typically found in the native sagebrush and pinyon pine habitats of Arizona, Colorado, New Mexico, Utah, and Wyoming (Figure 3). *Boechera thompsonii*, occurs in a geographical range spanning five states; Arizona, Colorado, New Mexico, Utah, and Wyoming. The clusters spread across multiple counties and states and transverse other clusters (Figure 5). Individuals from the genetic cluster 1 report in Utah, Wyoming, Colorado, and New Mexico. Individuals from cluster 2 are found along the 100th and 110th meridians west from north-eastern Arizona, up the eastern Utah border, and into Wyoming. (Figure 6). Individuals from
cluster 3 occur east of Utah with four near the border between New Mexico and Colorado and one member over 100 miles north. Individuals from cluster 4 are found east of the 108th meridian west either in Colorado or northern New Mexico.

Allelic data, from the 105 total individuals of *B. thompsonii* (53), *B. formosa* (45), and the resulting diploid apomictic hybrid *B. duchesnensis* (7) were distinguishable for scoring. *B. thompsonii* loci were 92.86% polymorphic, *B. formosa* loci were 78.57% polymorphic, and *B. duchesnensis* loci were 92.86% polymorphic. The mean percentage of polymorphic loci across all taxa was 88.10% with a standard error (SE) of 4.76% (Table 1).

Ninety-nine percent of the microsatellite data at 14 loci yielded distinguishable results for 53 individuals of *B. thompsonii* accounting for 109 alleles. The mean observed heterozygosity in *B. thompsonii* ranged from 0.000-0.404 compared to the mean expected heterozygosity range of 0.000-0.902. The mean range of the number of effective alleles across the entire population of *B. thompsonii* was 1-17, with 6 loci having 10 or more alleles identified. Additionally, one locus had a mean of 1.000 effective alleles (N.e). Genetic diversity patterns and population structure for the 53 *B. thompsonii* individuals and 109 alleles showed locus B18 to be fixed and homozygous across all five states. Six loci had a higher degree of allelic variability, with ≤ 10 alleles identified (Table 2).

Ninety-eight percent of microsatellite data at 14 loci yielded distinguishable results for 45 individuals of *B. formosa* accounting for 101 alleles. The mean observed heterozygosity for *B. formosa* ranged from 0.000-0.349 compared to the mean expected heterozygosity range of 0.000-0.919 (Table 10). The number of effective alleles present
per loci across the of *B. formosa* population ranged from 1-12, with 4 loci having 10 or more alleles identified. Additionally, 6 loci had a combined mean of 1.428 effective alleles (*N_e*). *B. formosa* individuals also had fixed homozygosity at loci A1, B20, and I14 across all five states (Table 3).

The mean observed heterozygosity value for *B. thompsonii*, *B. formosa*, and *B. duchesnensis* was 0.223, 0.166, and 0.908, respectively. The mean expected heterozygosity (*H_e* = 1 − Σ*p_i^2*) value for *B. thompsonii*, *B. formosa*, and *B. duchesnensis* was 0.533, 0.430, and 0.039, respectively. (Table 4).

Genetic diversity patterns and population structure differed substantially between the *B. thompsonii* and *B. formosa* populations. Genetic diversity patterns and population structure also differed intra-specifically between the *B. thompsonii* population clusters. For *B. thompsonii* 53 individuals were analyzed and 109 alleles were distinguished at 14 microsatellite loci. For *B. formosa* 45 individuals were analyzed and 82 alleles were distinguished at 14 microsatellite loci. For *B. duchesnensis* seven rare individuals were analyzed and 32 alleles were distinguished at 14 microsatellite loci (Table 5).

**STRUCTURE 2.3.3** (Pritchard et al., 2000; Falush et al., 2003, 2007) and **STRUCTURE HARVESTER** (Earl and Von Holdt, 2011) analyses of *B. thompsonii* identified four distinct genetic clusters (K) across the five state localities (Figure 7). Therefore, ∆K is highest for K = 4 based on the hierarchical structure determined by the data set (Figure 8). The mean elevation measured for cluster 1 was 1954.4 m with an elevation spread of 1643.5-2464.3 m. The mean elevation for cluster 2 was 2014.7 m
with an elevation spread of 1369.2-2421.9 m. The mean elevation for cluster 3 was 1935.5 m with an elevation spread being 1766.3-2125.4 m. The mean elevation for cluster 4 was 2149.5 m with an elevation spread of 1936-2416.2 (Figure 9).

STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) and STRUCTURE HARVESTER (Earl and Von Holdt, 2011) analyses of B. formosa identified a single genetically distinct cluster (K = 1) across the five states. This genetic cluster had a mean elevation of 1660.86 m with an elevation spread of 1269.8-1988.52 m (Figure 10). Microsatellite data of genetic variability were analyzed using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012). B. thompsonii and B. formosa both showed a mean allele number (N_a) of 7.786 and 7.071, respectively, while B. duchesnensis, the resulting apomictic hybrid, showed an actual mean allele number of 2.286. The actual mean allele number for the parental taxa B. thompsonii and B. formosa was significantly higher than the expected mean number (N_e) of alleles at 3.7 and 3.8, respectively. Hybridization was verified using a STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) analysis of population dynamics while allowing for assignment of individuals to populations in documented zones of hybridization based on the mean F-value for B. thompsonii and B. formosa respectively, which were 0.540 and 0.545. In contrast, the mean F-value for B. duchesnensis was -0.905 (Table 4).

Allelic frequency divergence among populations or net nucleotide distance was computed using point estimates of P. Vector P contains allelic frequencies characterized in clusters at each of the 14 individual loci. B. duchesnensis had specific inherited alleles.
at all loci from each of its putative parents, except at locus A1 which is fixed and homozygous and with 6 and 3 (variable) alleles that appear at the B266 and B6 loci, respectfully, in *B. duchesnensis* (Table 5).

A codominant genetic analysis was performed for 14 different loci for *B. duchesnensis*. The total population sampled was N = 7. Two individual specimens each had missing data from loci I14 and B6 where microsatellite analysis did not produce readable results due to age and condition of the specimens. The number of alleles at a given locus was identified to determine whether *B. duchesnensis* has a high level of fixation. Eleven loci showed only two allele options with 100% uniformity of the first allele coming from *B. thompsonii* and the second from *B. formosa*. Only two loci (B266 and B6) had a higher degree of allelic variability, with 6 and 3 alleles identified, respectively (Table 5).

Allelic frequencies were determined for each of the 14 unique loci analyzed for *B. duchesnensis* as well as its putative parents *B. thompsonii* and *B. formosa*. *B. duchesnensis* was homozygous at locus A1 and heterozygous at loci I3, B20, B11, C8, I14, B9, E9, B18, BF3, B6, BF 15, A3, and B266 (Figure 11). At locus I3 *B. duchesnensis* is heterozygous with allele “89” inherited from *B. formosa* and allele “91” inherited from *B. thompsonii*. At locus B20 *B. duchesnensis* is heterozygous with allele “199” inherited from *B. formosa* and allele “205” from *B. thompsonii*. Additionally, all seven *B. duchesnensis* individuals showed a pattern of inheriting a single specific allele from *B. thompsonii* and *B. formosa*, at each of the remaining loci (B11, C8, I14, B9, E9, B18, BF3, B6, BF 15, A3). Lastly, loci B266 and B6, showed allelic variation between the *B. duchesnensis* individuals
demonstrating that crossing had occurred between *B. thompsonii* and *B. formosa* on more than one occasion (Figures 11 and 14).

In cluster 1, 12 out of 25 individuals had a mean observed heterozygosity level of zero ($H_o = 0.00$) and 11 occurred in 5 Colorado counties (Dolores, Eagle, Garfield, Mesa and Montezuma). The remaining individual (specimen 10) was collected in Sweetwater Co., Wyoming. Among the five states, the highest mean observed heterozygosity levels for cluster 1 were recorded in Utah and ranged from $H_o = 0.07-0.73$, with an overall mean of $H_o = 0.44$. Among the counties, the highest mean observed heterozygosities were observed in Duchesne Co., Utah with a recorded mean of $H_o = 0.57$ and in Kane Co., Utah with a recorded mean of $H_o = 0.60$. The mean overall observed heterozygosity for cluster 1 was $H_o = 0.28$ (Table 6).

In cluster 2, 3 out of 12 individuals had an observed heterozygosity level of $H_o = 0.00$ and were recorded in Coconino Co., Arizona and in San Juan Co., Utah. The highest levels of observed heterozygosity overall were reported for individuals from Apache Co., Arizona and Sweetwater Co., Wyoming with recorded frequencies of $H_o = 0.57$ and $H_o = 0.60$, respectively. The mean overall observed heterozygosity for cluster 2 was $H_o = 0.36$ (Table 7).

In cluster 3, 3 out of 5 individuals had an observed heterozygosity level of zero ($H_o = 0.00$) and occurred in Montezuma Co., Colorado; Rio Arriba Co., New Mexico; and San Juan Co., New Mexico. The highest levels of heterozygosity ($H_o = 0.07$) were reported for individuals from La Plata Co., Colorado and Rio Arriba Co., New Mexico. The mean overall observed heterozygosity for cluster 3 was $H_o = 0.03$ (Table 8).
In cluster 4, 5 out of 12 individuals had an observed heterozygosity level of zero \( (H_o = 0.00) \). Two specimens were from Montrose Co., Colorado; and one each from Sandoval Co., New Mexico; Santa Fe Co., New Mexico; and Taos Co., New Mexico. Individuals from Gunnison, Colorado has a significant mean observed heterozygosity ranging from \( H_o = 0.29-0.40 \), with a mean of \( H_o = 0.35 \). The mean overall observed heterozygosity for cluster 4 was \( H_o = 0.13 \) (Table 9).

Within the \textit{B. formosa} population, 9 out of 45 individuals had an observed heterozygosity frequency of zero \( (H_o = 0.00) \). These \( H_o = 0 \) specimens occurred in: 1 Arizona county (Navajo), 2 Colorado counties (Moffat and Montrose), and 4 Utah counties (Daggett, Duchesne, Emery, Kane, and Uintah). Two of the nine Utah specimens with an observed heterozygosity of 0 occurred in Duchesne County, Utah. There were no individuals in New Mexico with a mean observed heterozygosity of zero \( (H_o = 0.00) \). Among the 5 states, the highest overall observed heterozygosity frequency was recorded for two individuals \( (H_o = 0.40) \) in Arizona Cos. (Apache and Delta Cos.). The mean overall observed heterozygosity across all five states was \( (H_o = 0.166) \).

Calculated observed heterozygosity \( (H_o) \) frequencies plotted geographically showed \textit{B. thompsonii} had a \( (H_o) \) range of 0.53-0.60, in Duchesne Co., Utah. \textit{Boechera duchesnensis} has only been identified in Duchesne Co., Utah. The observed heterozygosity \( (H_o) \) frequency for \textit{B. formosa} in Duchesne Co., Utah was zero. In Sweetwater, Wyoming \textit{B. thompsonii} has a \( H_o \) of 0.60 while \textit{B. formosa} has a \( H_o \) of zero.
Morphological Findings

*Boechera thompsonii* (Al-Shehbaz, 2013) is distinguished by 2-5 caudices arising from the margin of rosettes of ob lanceolate to obovate-basal leaf blades, and 3-8 cauline leaves that do not conceal the stem. Flower morphology in *B. thompsonii* mimics that of other Brassicacea flowers with four sepals and 4-15 unbranched inflorescent pedicels that are ascending to divaricate-ascending or straight. Individuals of *B. thompsonii* produce lavender flowers, ascending straight fruits, and uniseriate winged nearly continuous seeds (Windham and Al-Shehbaz, 2006; Al-Shehbaz, 2010) (Table 11).

*Boechera formosa* reproduces via outcrossing (Al-Shehbaz, 2011) and is morphologically identified by a single caudex from the center of the leaf fruit, with linear to linear-ob lanceolate basal leaves (Figure 22), and 7-18 cauline leaves concealing the stem proximally. Flower morphology mimics the Brassicacea flower with four sepals and 6-26 unbranched inflorescent pedicels that are horizontal or descending. Individuals have either white or pale lavender flowers, divaricate descending to reflexed fruits, and bi-seriate winged continuous seeds (Table 12). Leaf trichomes are short-stalked, simple or 2-rayed (Figure 14) and have specialized surface papillae (Suo et al, 2013; Marks et al, 2009, Esch et al., 2003). Environmental scanning electron microscopy (ESEM) of *B. formosa* anthers confirmed the existence of symmetrical ellipsoid pollen grains typically over 20 µm in length and 12-17 µm wide consistent with *Boechera* sexual diploids (Figures 15a; 15b) demonstrating that they are remarkably different than the typical asymmetrical spheroid pollen grains of apomictic triploid individuals (Figures 27a; 27b). Trichome anatomy of *B. formosa* was confirmed with
SEM analysis and demonstrated distinctive papillae formation on individual cauline leaf trichomes (Figure 14).

*Boechera duchesnensis*, an apomictic diploid, tends to coexpress several of the morphological characteristics of its sexual diploid parents, most notable are the presentation of trichomes in *Boechera* hybrids. For example, *B. duchesnensis* produces short-stalked trichomes of 2-7 rays (Figure 17); the result of additive genetic effects of simple trichomes of 2 rays in *B. thompsonii* and short-stalked or simple trichomes of 2 rays in *B. formosa*. *Boechera duchesnensis* also produces an intermediate count of 11-22 flowers, while *B. thompsonii* and *B. formosa* produce 4-15 flowers and 6-26 flowers respectively (Windham and Al-Shehbaz, 2006. *Boechera duchesnensis* demonstrated the mechanism of additive genetic effect by inheriting characteristics and exhibited the combined effects of different alleles (at two or more gene loci) equal to the sum of their individual effects. Traits from both parents coexpress in *Boechera duchesnensis* consistent with hybrid formation. Another example of intermediate character integration in *B. duchesnensis* is flower-color expression with the inheritance of both lavender (*B. thompsonii*) and white (*B. formosa*) alleles which is expressed phenotypically as pale lavender colored flowers in *B. duchesnensis* (Table 13).

*Analysis of Pollen Grains Demonstrated*

There is a reliable correlation between 1) the mode of reproduction and ploidy level and 2) the morphology of *Boechera* pollen grains (Beck et al., 2012). This allows researchers to differentiate apomicts and polyploids from sexual diploids in *Boechera* (Windham and Al-Shehbaz 2007a, 2007b). The pollen grains produced by most *Boechera*
sexual diploids are 13-20 µm in diameter (relatively small), have three symmetrical colpi and ellipsoid pollen grain shape when dehydrated (Figures 18; 19). Apomictic triploid individuals produce significantly larger 20-30 µm in diameter grains with asymmetrical colpi and spheroid grain shape when dehydrated (Figures 27a; 27b).

*Boechera duchesnensis*, inherits the symmetrical colpi and ellipsoid pollen grain shape from *B. formosa* (Figures 15a; 15b) and *B. thompsonii* (Figure 16). While molecular studies have identified *B. duchesnensis* as an apomictic diploid, SEM imagery provides further evidence that pollen grain shape is congruent with mode of reproduction in *Boechera*. Despite these similarities to its parent species, there are also telling contrasts in *B. duchesnensis*. Pollen concentration on anthers is sparse and, of the fewer pollen grains in existence, abortive and malformed pollen is common on the few available, but representative, samples viewed (Figure 20). While samples of BD are very limited due to rarity, the sparse nature of pollen formation found is consistent with reports by Windham and Al-Shehbaz (2007a, 2007b).

*Analysis of Trichomes Demonstrated*

The presence of trichomes on plant surfaces provide various benefits to the plant especially in higher elevations where frost is typical. In areas like the Colorado Basin trichomes are adaptive for plants and can act as a barrier to catch the frost and maintain its distance from living cells on the surface from which the trichomes emanate. In windy areas like the Duchesne site, trichomes can reduce evapotranspiration by disrupting air flow across the plant surface. Densely pubescent trichomes like those on the cauli...
leaves of *B. formosa* reflect sunlight and protect delicate tissues in hot, dry environments (Figure 21).

Trichome anatomy of *B. thompsonii*, *B. formosa*, and *B. duchesnensis* were analyzed and confirmed with SEM analysis. *Boechera thompsonii* trichome characteristics differ from *B. formosa* and *B. duchesnensis* in that there are stem trichomes (Figure 26) and basal leaf trichomes, but there are no trichomes present on fruiting pedicels (Figure 22). On *B. thompsonii* stems, trichomes are short-stalked, simple and 2-rayed and glabrous (Figure 23) or sparsely pubescent distally (Figure 24). On basal leaves, simple and 2-rayed trichomes appear moderately pubescent and can be short-stalked, 4-8 rayed and measure between 0.1 to 0.3 mm in length (Table 11).

*Boechera formosa* trichome morphology analysis shows short-stalked 4-7 rayed stem trichomes of 0.1 to 0.4 mm in length, pubescent distally (Figure 26). The basal leaf trichomes are relatively longer at 0.8 mm in length with densely pubescent surfaces covered in 4-8 rayed, short-stalked trichomes with individual rays measuring 0.1 to 0.3 mm in length. *B. formosa* fruiting pedicels are appressed and branched (Table 12).

*Boechera duchesnensis* trichome morphology demonstrates interesting mixtures of the morphological characteristics of its putative parents, *B. thompsonii*, and *B. formosa*. As mentioned previously, *B. duchesnensis* produces short-stalked stem trichomes of 2-7 rays; the result of additive genetic effects of simple trichomes of 2 rays in *B. thompsonii* and short-stalked trichomes of 2 rays in *B. formosa*. These stem trichomes are between 0.2 to 0.5 mm in length and are sometimes found mixed with larger trichomes proximally and sparsely pubescent distally and can appear almost stellate (star-shaped)
due to their short-stalked with many rays characteristics (Figure 25). Basal leaf trichome surfaces densely pubescent were found bearing short-stalked, 4-8 rayed trichomes 0.15-0.4 mm in length. *Boechera duchesnensis* has fruiting pedicel trichomes that are appressed and branched (Table 13).

DISCUSSION

*Allelic Frequencies and Heterozygosity*

This study provides an analysis of the genetic diversity patterns inherent in the population structure of the rare sexual diploids *B. thompsonii*, and *B. formosa* in correlation with their geographic distribution. My study: 1) compared allelic frequencies among all sampled individuals of *B. thompsonii*, *B. formosa* and *B. duchesnensis*, and 2) compared allelic variation among individuals within four newly-identified genetically distinct clusters of *B. formosa* and and determined that there is a single population of *B. thompsonii* that is not separated by any apparent reproductive barriers. My research supported my objective that I would find decreased allelic diversity areas throughout the range and that allelic frequency distribution is different in clusters than it is across the population as a whole. My findings suggest that the reproductive barriers, within populations of *B. formosa*, are contributing to genetic isolation among the clusters. The heterozygosity data from GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) also show that *B. thompsonii* and *B. formosa* have low allelic frequencies and low heterozygosity throughout their ranges consistent with genetic data for rare species.
My hypothesis that allelic distribution would differ between clusters and the entire population is evidenced by my STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) analysis of *B. thompsonii* which, when joined with geographic distributions, found that there were four genetically unique clusters including unique alleles and unique allelic frequencies that I speculate were separated by reproductive barriers which on a map which I inferred as distance. However, with *B. formosa*, the same geographic range yielded no clustering and the same alleles present and at the same frequencies were found throughout the entire range. These data show that individuals from cluster 1 of *B. thompsonii* hybridize with *B. formosa* and is consistent with the Brennan et al., (2013) study of rare plants; that one cluster can have advantages over another and that further studies of genomic regions that show reduced divergence are suggested in *Boechera* (Kane et al., 2009; Castric et al., 2008; Kim et al., 2008; Whitney et al., 2006; Reisenberg et al., 2003; Marin et al., 2006). Future conservation efforts should assess whether there is a genetic driving force towards hybridization, supporting the conclusion of Dobeš et al. (2007) that outcrossing is an ongoing and frequent process in *Boechera* spp. (Koch, 2003).

The processes of gene flow and genetic drift are primarily driven by reproductive isolation (Anderson et al., 2015; Lovell, 2014; Slatkin 1987, 1993), and may have also promoted various adaptations, as previously documented within the genus, and as seen in *B. thompsonii*; a pattern which is also consistent with other *Boechera* species that reproduce sexually through outcrossing (Rushworth, 2011). Reproductive isolation is also influenced by genetic variation for some of the following underlying
traits: abiotic factors, pollinators, self-incompatibility, flowering time, and disease resistance (Brennan, 2013; Castric et al., 2008). Apomictic species of Boechera did not show this adaption trend and were relatively less influenced by selection in these apomictic lineages (Lovell et al., 2014).

**Taxonomy of B. duchesnensis**

These data establish the taxonomic status, genetic diversity patterns, and population structure of three Boechera species. Microsatellite data were used to effectively determine parentage in a hybridization occurrence in Duchesne Co., Utah which resulted in the diploid apomict hybrid, B. duchesnensis (Beck et al., 2012). Microsatellite data also provided further documentation of allelic diversity patterns, hybridization, and diploid apomixis in the context of evolutionary patterns and the phylogenetic history of Boechera (Marhold and Lihová, 2006).

Tendency towards overall reduced heterozygosity appears to lead to genetic fixation. Complete fixation (H\(_O\) = 0.0) was seen in B. formosa individuals sampled in Duchesne Co., Utah. These data suggest an evolutionary propensity that physical proximity plays a role in hybridization as demonstrated by B. thompsonii and B. formosa when B. formosa with low heterozygosity is in close proximity to B. thompsonii. High observed heterozygosity frequencies at each locus in B. duchesnensis are consistent with allelic fixation of B. formosa and indicative of organisms that utilize selfing as a mode of reproduction (Roy, 1995). These data showing that B. duchesnensis exhibits characteristics consistent with fixation as a heterozygote suggest the possible need for conservation efforts and further research. Additionally, B. formosa is a sexual diploid
and is not known to self-fertilize in natural populations but rather utilizes outcrossing as a mode of reproduction, along with a higher level of inbreeding. Evidence of heterozygotes in the field strongly suggests that outcrossing occurs in natural populations (Roy and Rieseberg, 1989). These data are significant and suggest the need for active conservation efforts focused primarily on *B. formosa*.

In a normal population autogamous (selfing) individuals have a lower observed heterozygosity level (Roy, 1995). This is inconsistent with *B. duchesnensis* individuals which have a mean polymeric loci prevalence of (92.86%). Another inconsistency demonstrated by *B. duchesnensis* is high genetic diversity, given the standard cutoff of 95% for the most common allele (Roy, 1995). However, while all *B. duchesnensis* individuals in this study had a high level of heterozygosity consistent with accepting a single allele at each locus from *B. thompsonii* and *B. formosa*, all studied individuals exhibit a propensity for a specific allele at each of the 14 loci, making *B. duchesnensis* appear to be low in allelic diversity. According to Roy (1995) a multilocus heterozygous presentation at individual loci that remain heterozygous in the progeny is defined as having reached fixation and suggestive of apomixis as a breeding system. Barrett and Shore (1989) found that heterozygous patterns seen in hybrid apomictic individuals typically result from gene duplication and not from true-heterozygosity as previously defined in the literature. *B. duchesnensis* received a greater diversity of alleles from *B. thompsonii* than from *B. formosa*, which is closer to fixation. However, *B. duchesnensis* still has a higher level of heterozygosity than *B. formosa* because different alleles from each
of the putative parents supplied a fixed heterozygosity at thirteen loci (I3, B20, B11, C8, I14, B9, E9, B18, BF3, B6, BF 15, A3, and B266) of the 14 loci analyzed and recorded. 

Morphological Characteristics

My SEM imagery of morphological characteristics of B. thompsonii, B. formosa, and B. duchesnensis supports my hypotheses from the second and third objectives; that 1) morphological traits would support previous classifications of B. thompsonii and B. formosa as sexual diploids, and that 2) inherited morphological traits would confirm previous classification of B. duchesnensis as a hybrid diploid apomict (Beck et al., 2012).

I confirmed that B. thompsonii and B. formosa pollen grains each share the characteristic symmetrical ellipsoid pollen shape indicative of Boechera sexual diploids (Windham and Al-Shehbaz 2007a, 2007b; Beck, et al., 2012) and confirmed and expanded on other observable morphological characteristics to add to and support foundational literature concerning the morphological identification of these three species of Boechera. I confirmed that B. duchesnensis shares morphological features with B. thompsonii, and B. formosa including stem, leaf, trichome, pollen, flower color, and seed characteristics. The patterns of intermediate morphological traits inherited from both B. thompsonii and B. formosa are consistent with B. duchesnensis being their produced hybrid. Boechera duchesnensis pollen shape being consistent with the characteristic diploid pollen shape further leads us to conclude that B. duchesnensis is indeed a diploid. Based on my extensive and thorough examination of all available samples of B. duchesnensis I confirmed that its pollen is extremely sparse, misshapen and appears abortive. These observations and analysis support previous cytogenetic
and molecular findings supporting apomixis as a mode of reproduction and B. duchesnensis as a diploid apomict (Koch, 2015).

The genetic diversity patterns and allelic frequencies of B. duchesnensis confirm my hypothesis, and previous molecular studies by Beck et al., (2012) and Koch (2015), that B. duchesnensis is a hybrid diploid apomict. While we cannot conclude from the available data in this study the total number of times B. duchesnensis has been produced through the hybridization of B. thompsonii and B. formosa, the results do support that B. duchesnensis is not the product of a single hybridization event. While the sample size of B. duchesnensis is limited due to rarity, small sample sizes in rare plants are common and are broadly considered to be valid by those who regularly study rare plants. This is evidenced by at least four separate hybridization events within my limited sample set, as four of the sampled individuals show slightly different genetic diversity (Table 14). The remaining three of the seven B. duchesnensis individuals in this data set have identical genetic markers and thus indicate clonal reproduction. Apomixis is the only mechanism of asexual reproduction that allows for this type of F₁ clonal reproduction (Table 14; Dobeš et al., 2007). This supports my hypothesis that B. duchesnensis is a hybrid diploid apomict (Table 14) as recently proven cytogenetically through chromosome painting by Koch (2015). Therefore, the B. duchesnensis individuals sampled in this study are, directly or indirectly, the product of multiple hybridization events between B. thompsonii and B. formosa (Figure 11). Direct hybridization events yielded offspring by sexual crossing of B. thompsonii and B. formosa; these results also showed indirect hybridization through asexual apomictic clonal reproduction of F₁.
hybrids in subsequent generations as evidenced by the three clonal individuals in this sample pool.

Mating systems play a key role in shaping genetic diversity patterns, population structure, and geographical distributions (Yan et al., 2008; Clauss and Mitchell-Olds, 2006; Otto and Whitton, 2000; Loveless and Hamrick, 1984). The high level of observed heterozygosity detected in *B. duchesnensis* (H_o 0.908) supports cytogenetic evidence cited by Carman (2015) identifying *B. duchesnensis* as an apomict, and supports molecular data identifying apomixis as a mode of reproduction in *Boechera* hybrids (Beck et al., 2012; Schranz et al., 2005). While extensive sampling was from all major North American herbaria, only one location of *B. duchesnensis* has been recorded so all conclusions are based on a small sample size from a single location in Duchesne County Utah. Hybridization as a mechanism for speciation accounts for a portion of this genotypic and phenotypic diversity. *Boechera* species demonstrate a high degree of genetic diversity and can be found in a variety of ecosystems throughout western North America (Rushworth et al., 2011). These data are also significant when discussing genetic diversity patterns in flowering plants in general. Trends based on microsatellite data suggest that a mean expected heterozygosity (H_E) value of 0.41 is typical of inbred plant populations with a mean expected heterozygosity (H_E) of 0.65 for outcrossed plant populations (Yan et al., 2008; Nybom, 2004), documenting outcrossing as a mechanism of increased heterozygosity in apomicts that have reached allelic fixation.
Future Implications

Despite that species abundance factors, rare plant distribution and their conservation persist as central issues of ecology (Gabrielová et al., 2013), rare species that are vulnerable to extinction represent a minimal quantity of in-depth demographic studies overall (Silvertown et al., 1993; Izco, 1998). Fiedler, Knapp and Fredricks (1996) found less than 100 demographic studies for all vascular plants as of 1996, and even fewer for rare plants. Rare, localized endemic species were largely ignored while simultaneously most weedy and domestic species’ life history strategies were studied extensively (Bevill and Louda, 1999; Falk and Holsinger, 1991; Gabrielová et al., 2013). Since many rare endemic species suffer from high extinction risk, it is imperative to predict the long-term effects of population disturbances and establish effectual conservation strategies through understanding their life history strategies and characteristics.

Rabinowitz et al. (1986) identified variations of rarity based on combinations of three dimensions: habitat specificity, geographical range, and local population abundance. Any species with more than one characteristic is at greatest risk for extinction and merits additional research and conservation efforts. One species, *Boechera formosa*, manifests all three rarity factors and is at high risk of extinction and therefore merits focused conservation efforts. Regrettably, conservation strategies for *B. formosa* are complicated by misidentification issues and mistaken circumscription of the entire genus into *Arabis*. Re-circumscription into *Boechera* has occurred; however, reexamination of taxonomic foundations and population structure of *B. formosa* is
essential for development of effective management approaches (Windham and Al-Shehbaz, 2007). Results from this study, showing low overall genetic diversity, in conjunction with outcrossing as a reproductive mechanism, add to the literature and may support consideration for *B. formosa* warranting further research and conservation efforts generally afforded to rare species with the possible consideration of adding *B. formosa* to the ICUN Red List (de Grammont and Cuarón, 2006; Lamoreux et al., 2003; Mace et al., 2008; Rodrigues et al., 2006).

Dobeš (2007) stated that hybridization is an ongoing and frequent process in *Boechera* spp. This study supports the hypothesis that hybridization may occur when populations of *Boechera* spp. have: 1) lower allelic variation, 2) less ability to adapt to environmental conditions, and 3) trend towards low observed heterozygosity levels. *Boechera thompsonii*, as documented in previous studies, contributes to hybridization events that produce new *Boechera* spp. (Windham and Al-Shebazz, 2007; Carman, 2015). Microsatellite data for *B. thompsonii* provides further confirmation of allelic diversity patterns and hybridization in the context of evolutionary patterns and the phylogenetic history of *Boechera* (Marhold and Lihova, 2006).

*Boechera duchesnensis* allows researchers the ability to investigate *Boechera* as a model system of apomixis based on the previously established *Boechera* apomixis model (Koch 2015). This model system of hybridization could be used to evaluate other species in relatively pristine habitats in areas affected by climate change and potentially impacted by desertification and drought.

*Conservation Implications*
One outcome of this study could be to encourage future researchers to identify and propose conservation strategies including the possible addition of *B. formosa* to the IUCN Red List. The IUCN Red List of Threatened Species requires assessors to provide data based on the following information categories: taxonomy, assessment, geographic range, population, habitat and ecology, threats, and conservation actions.

Population structure studies that include genetic diversity patterns and population dynamics for distinct taxa, such as *B. formosa* offer insights into possible conservation strategies. This kind of population-level data offers a baseline read on gene flow and allelic variability, allowing for future long-term studies consistent with the IUCN Red List criteria for rare endemics like *Boechera* species. Conservation efforts should also focus on habitats affected by drought, desertification, and human disturbances such as recreational sports and other potential threats. One threat to *B. formosa* identified by Rondeau et al. (2011) are roads that intersect with rare plant populations. Total habitat loss or partial habitat reduction and other extrinsic factors such as recreational activities and grazing may be threats of particular concern and should be considered. There is also a critical need for field-based studies to carefully examine impact levels of grazing and invasive species on *Boechera* species in order to effectively document the need for conservation efforts.

A lack of field studies and limited historical data on new individuals and locations for *B. thompsonii* contribute to the general lack of awareness and capacity for assessing population conditions. Long-term field-based studies are required to better
understand: 1) pollination dynamics, 2) clustering outcomes, 3) depletion of allelic variation in clusters, and 4) low heterozygosity ($H_o$) levels.

Data from this study will be useful in the furtherance of understanding for the future development of a conservation plan for the rare and endangered species $B. thompsonii$ (Cires et al., 2013). Conservation of previously identified clusters of $B. thompsonii$ is supported by Fischer and Matthies’ (1998) conclusions that proximity between clusters with the potential for gene flow has a higher potential of maintaining genetic variability than a large deme and therefore is less susceptible to genetic drift while being more susceptible to demographic stochasticity (Cires et al., 2013; Gliddon and Goudet, 1994). These findings support the need for increased efforts to further assess existing clusters of $B. thompsonii$ by generating additional data about genetic diversity and the status of progeny in situ. In addition, concurrent studies examining demographic patterns, anthropogenic disruption, long-term consequences of climate change, and other potential impacts on $B. thompsonii$ populations will provide a solid foundation for future management decisions.
LITERATURE CITED


BLM (US), USDA SCS, OR, Agricultural Experiment Station, and US Forest Service. (1985).


Table 1. Percentage of polymorphic loci per taxon from 14 independently assorting loci across 105 total individuals of *B. thompsonii*, *B. formosa*, and *B. duchesnensis* calculated using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) to show prevalence of polymorphic loci.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Percentage of Polymorphic Loci</th>
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<tbody>
<tr>
<td><em>Boechera thompsonii</em></td>
<td>92.86%</td>
</tr>
<tr>
<td><em>Boechera formosa</em></td>
<td>78.57%</td>
</tr>
<tr>
<td><em>Boechera duchesnensis</em></td>
<td>92.86%</td>
</tr>
</tbody>
</table>

Mean 88.10%  
SE 4.76%
Table 2. Observed and expected heterozygosity at 14 unique loci were examined using GenAlEx 6.501 for \textit{B. thompsonii} based on codominant data.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>uHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>I3</td>
<td>52</td>
<td>14.000</td>
<td>6.159</td>
<td>0.404</td>
<td>0.838</td>
<td>0.846</td>
<td>0.518</td>
</tr>
<tr>
<td>A1</td>
<td>53</td>
<td>2.000</td>
<td>1.870</td>
<td>0.208</td>
<td>0.465</td>
<td>0.470</td>
<td>0.554</td>
</tr>
<tr>
<td>B20</td>
<td>52</td>
<td>14.000</td>
<td>10.223</td>
<td>0.365</td>
<td>0.902</td>
<td>0.911</td>
<td>0.595</td>
</tr>
<tr>
<td>B11</td>
<td>52</td>
<td>4.000</td>
<td>1.740</td>
<td>0.154</td>
<td>0.425</td>
<td>0.429</td>
<td>0.638</td>
</tr>
<tr>
<td>C8</td>
<td>52</td>
<td>3.000</td>
<td>2.256</td>
<td>0.135</td>
<td>0.557</td>
<td>0.562</td>
<td>0.758</td>
</tr>
<tr>
<td>I14</td>
<td>51</td>
<td>3.000</td>
<td>1.126</td>
<td>0.078</td>
<td>0.112</td>
<td>0.113</td>
<td>0.301</td>
</tr>
<tr>
<td>B9</td>
<td>53</td>
<td>10.000</td>
<td>2.325</td>
<td>0.321</td>
<td>0.570</td>
<td>0.575</td>
<td>0.437</td>
</tr>
<tr>
<td>E9</td>
<td>53</td>
<td>5.000</td>
<td>1.214</td>
<td>0.151</td>
<td>0.177</td>
<td>0.178</td>
<td>0.145</td>
</tr>
<tr>
<td>B18</td>
<td>53</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>BF3</td>
<td>53</td>
<td>10.000</td>
<td>4.505</td>
<td>0.396</td>
<td>0.778</td>
<td>0.785</td>
<td>0.491</td>
</tr>
<tr>
<td>B6</td>
<td>52</td>
<td>6.000</td>
<td>1.480</td>
<td>0.135</td>
<td>0.324</td>
<td>0.327</td>
<td>0.585</td>
</tr>
<tr>
<td>BF15</td>
<td>52</td>
<td>17.000</td>
<td>6.785</td>
<td>0.327</td>
<td>0.853</td>
<td>0.861</td>
<td>0.617</td>
</tr>
<tr>
<td>A3</td>
<td>52</td>
<td>4.000</td>
<td>2.418</td>
<td>0.192</td>
<td>0.586</td>
<td>0.592</td>
<td>0.672</td>
</tr>
<tr>
<td>B266</td>
<td>47</td>
<td>16.000</td>
<td>8.181</td>
<td>0.255</td>
<td>0.878</td>
<td>0.887</td>
<td>0.709</td>
</tr>
</tbody>
</table>

N= number of individuals; Na= number of different alleles; Ne= number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; uHe= unbiased expected heterozygosity \((2N / (2N-1)) \times He\); F= Fixation index \((He - Ho) / He = 1 - (Ho / He)\).
Table 3. Observed and expected heterozygosity at 14 unique loci were examined using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) for *B. formosa* based on codominant data.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>uHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>I3</td>
<td>45</td>
<td>5.000</td>
<td>1.412</td>
<td>0.133</td>
<td>0.292</td>
<td>0.295</td>
<td>0.543</td>
</tr>
<tr>
<td>A1</td>
<td>43</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>B20</td>
<td>44</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>B11</td>
<td>45</td>
<td>5.000</td>
<td>1.644</td>
<td>0.178</td>
<td>0.392</td>
<td>0.396</td>
<td>0.546</td>
</tr>
<tr>
<td>C8</td>
<td>43</td>
<td>19.000</td>
<td>8.660</td>
<td>0.326</td>
<td>0.885</td>
<td>0.895</td>
<td>0.632</td>
</tr>
<tr>
<td>I14</td>
<td>43</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>B9</td>
<td>44</td>
<td>6.000</td>
<td>2.652</td>
<td>0.273</td>
<td>0.623</td>
<td>0.630</td>
<td>0.562</td>
</tr>
<tr>
<td>E9</td>
<td>43</td>
<td>9.000</td>
<td>4.693</td>
<td>0.349</td>
<td>0.787</td>
<td>0.796</td>
<td>0.557</td>
</tr>
<tr>
<td>B18</td>
<td>44</td>
<td>3.000</td>
<td>1.324</td>
<td>0.000</td>
<td>0.245</td>
<td>0.248</td>
<td>1.000</td>
</tr>
<tr>
<td>BF3</td>
<td>43</td>
<td>3.000</td>
<td>1.178</td>
<td>0.116</td>
<td>0.151</td>
<td>0.153</td>
<td>0.231</td>
</tr>
<tr>
<td>B6</td>
<td>43</td>
<td>15.000</td>
<td>10.104</td>
<td>0.349</td>
<td>0.901</td>
<td>0.912</td>
<td>0.613</td>
</tr>
<tr>
<td>BF15</td>
<td>38</td>
<td>12.000</td>
<td>5.148</td>
<td>0.316</td>
<td>0.806</td>
<td>0.816</td>
<td>0.608</td>
</tr>
<tr>
<td>A3</td>
<td>40</td>
<td>2.000</td>
<td>1.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>-0.013</td>
</tr>
<tr>
<td>B266</td>
<td>39</td>
<td>17.000</td>
<td>12.416</td>
<td>0.2560</td>
<td>0.919</td>
<td>0.931</td>
<td>0.721</td>
</tr>
</tbody>
</table>

N= number of individuals; Na= number of different alleles; Ne= number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; uHe= unbiased expected heterozygosity (2N / (2N-1) * He); F= Fixation index (He - Ho) / He = 1 - (Ho / He).
Table 4. Observed and expected heterozygosity were calculated using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) allowing the assignment of individuals to populations in documented zones of heterozygosity (Pritchard et al., 2000).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>uHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. thompsonii</td>
<td>Mean</td>
<td>51.92</td>
<td>7.786</td>
<td>3.663</td>
<td>0.223</td>
<td>0.533</td>
<td>0.538</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.412</td>
<td>1.491</td>
<td>0.801</td>
<td>0.033</td>
<td>0.080</td>
<td>0.081</td>
</tr>
<tr>
<td>B. formosa</td>
<td>Mean</td>
<td>42.64</td>
<td>7.071</td>
<td>3.804</td>
<td>0.166</td>
<td>0.430</td>
<td>0.436</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.507</td>
<td>1.675</td>
<td>1.038</td>
<td>0.038</td>
<td>0.100</td>
<td>0.101</td>
</tr>
<tr>
<td>B. duchesnensis</td>
<td>Mean</td>
<td>6.857</td>
<td>2.286</td>
<td>2.032</td>
<td>0.908</td>
<td>0.482</td>
<td>0.520</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.097</td>
<td>0.304</td>
<td>0.116</td>
<td>0.071</td>
<td>0.039</td>
<td>0.043</td>
</tr>
</tbody>
</table>

N= number of individuals; Na= number of different alleles; Ne= number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; uHe= unbiased expected heterozygosity (2N / (2N-1)) * He; F= Fixation index (He - Ho) / He = 1 - (Ho / He).
Table 5. Observed and expected allelic heterozygosity at 14 unique loci were examined using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012) for *B. duchesnensis* based on codominant data.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>Ne</th>
<th>Ho</th>
<th>He</th>
<th>uHe</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI3</td>
<td>7</td>
<td>2.000</td>
<td>1.960</td>
<td>0.857</td>
<td>0.490</td>
<td>0.527</td>
<td>-0.750</td>
</tr>
<tr>
<td>A1</td>
<td>7</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>B20</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>B11</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>C8</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>I14</td>
<td>6</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.545</td>
<td>-1.000</td>
</tr>
<tr>
<td>B9</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>E9</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>B18</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>BF3</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>B6</td>
<td>6</td>
<td>3.000</td>
<td>2.323</td>
<td>1.000</td>
<td>0.569</td>
<td>0.621</td>
<td>-0.756</td>
</tr>
<tr>
<td>BF15</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>A3</td>
<td>7</td>
<td>2.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.500</td>
<td>0.538</td>
<td>-1.000</td>
</tr>
<tr>
<td>B266</td>
<td>7</td>
<td>6.000</td>
<td>3.161</td>
<td>0.857</td>
<td>0.684</td>
<td>0.763</td>
<td>-0.254</td>
</tr>
</tbody>
</table>

N= number of individuals; Na= number of different alleles; Ne= number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; uHe= unbiased expected heterozygosity (2N / (2N-1)) * He; F= Fixation index (He - Ho) / He = 1 - (Ho / He).
Table 6. *B. thompsonii* cluster (K) 1 geographical data for individual specimens - with coordinates, elevation, observed heterozygosity statistics, and county/state location information.

<table>
<thead>
<tr>
<th>K</th>
<th>ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>H&lt;sub&gt;o&lt;/sub&gt;</th>
<th>County/St</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>38.6733</td>
<td>-108.3211</td>
<td>1643.90</td>
<td>0.40</td>
<td>Delta/CO</td>
</tr>
<tr>
<td>1</td>
<td>53</td>
<td>37.8075</td>
<td>-108.7919</td>
<td>2422.56</td>
<td>0.00</td>
<td>Dolores/CO</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>39.6257</td>
<td>-107.1123</td>
<td>1894.51</td>
<td>0.00</td>
<td>Eagle/CO</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>36.7323</td>
<td>-107.2237</td>
<td>2271.95</td>
<td>0.00</td>
<td>Garfield/CO</td>
</tr>
<tr>
<td>1</td>
<td>52</td>
<td>38.9686</td>
<td>-108.4669</td>
<td>1436.58</td>
<td>0.00</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>39.0317</td>
<td>-108.6895</td>
<td>1958.84</td>
<td>0.33</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>37.1722</td>
<td>-108.5000</td>
<td>2002.13</td>
<td>0.00</td>
<td>Montezuma/CO</td>
</tr>
<tr>
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<td>39</td>
<td>38.2161</td>
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<td>1675.91</td>
<td>0.07</td>
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</tr>
<tr>
<td>1</td>
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<td>38.1633</td>
<td>-109.0040</td>
<td>2099.08</td>
<td>0.40</td>
<td>Montrose/CO</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>39.9447</td>
<td>-108.7569</td>
<td>1763.71</td>
<td>0.60</td>
<td>Rio Blanco/CO</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>39.9447</td>
<td>-108.7569</td>
<td>1763.71</td>
<td>0.13</td>
<td>Rio Blanco/CO</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>39.6902</td>
<td>-108.9986</td>
<td>1936.89</td>
<td>0.47</td>
<td>Rio Blanco/CO</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>36.7426</td>
<td>-105.6815</td>
<td>2219.51</td>
<td>0.08</td>
<td>Taos/NM</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>40.1706</td>
<td>-110.3319</td>
<td>1685.06</td>
<td>0.53</td>
<td>Duchesne/UT</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>40.1808</td>
<td>-110.4983</td>
<td>1771.34</td>
<td>0.60</td>
<td>Duchesne/UT</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>37.5489</td>
<td>-112.0471</td>
<td>1777.13</td>
<td>0.53</td>
<td>Garfield/UT</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>39.0356</td>
<td>-109.7024</td>
<td>1745.42</td>
<td>0.07</td>
<td>Grand/UT</td>
</tr>
<tr>
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<td>21</td>
<td>37.2855</td>
<td>-112.5375</td>
<td>1928.96</td>
<td>0.47</td>
<td>Kane/UT</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>37.4431</td>
<td>-112.4925</td>
<td>2235.97</td>
<td>0.73</td>
<td>Kane/UT</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>38.0135</td>
<td>-109.3496</td>
<td>1927.74</td>
<td>0.07</td>
<td>San Juan/UT</td>
</tr>
<tr>
<td>1</td>
<td>51</td>
<td>37.9181</td>
<td>-109.9453</td>
<td>2190.85</td>
<td>0.73</td>
<td>San Juan/UT</td>
</tr>
<tr>
<td>1</td>
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<td>40.3667</td>
<td>-109.1197</td>
<td>2464.93</td>
<td>0.20</td>
<td>Uintah/UT</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>41.0426</td>
<td>-107.7130</td>
<td>1985.36</td>
<td>0.47</td>
<td>Carbon/WY</td>
</tr>
<tr>
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<td>10</td>
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<td>-108.2598</td>
<td>2116.46</td>
<td>0.00</td>
<td>Sweetwater/WY</td>
</tr>
</tbody>
</table>

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H<sub>o</sub> = observed heterozygosity; County/State.
Table 7. *B. thompsonii* cluster (K) 2 geographical data for individual specimens – with coordinates, elevation, observed heterozygosity statistics, and county/state location information.

<table>
<thead>
<tr>
<th>K</th>
<th>ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>H₀</th>
<th>County/St</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>-109.2173</td>
<td>2110.74</td>
<td>0.53</td>
<td>Apache/AZ</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>36.4431</td>
<td>-109.1797</td>
<td>2213.46</td>
<td>0.60</td>
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</tr>
<tr>
<td>2</td>
<td>24</td>
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<td>-112.0854</td>
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<td>0.00</td>
<td>Coconino/AZ</td>
</tr>
<tr>
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<td>33</td>
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<td>-109.0326</td>
<td>2075.99</td>
<td>0.27</td>
<td>Montrose/CO</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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<td>2140.61</td>
<td>0.07</td>
<td>San Miguel/CO</td>
</tr>
<tr>
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<td>-109.7069</td>
<td>2319.83</td>
<td>0.00</td>
<td>Daggett/UT</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
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<td>-110.1442</td>
<td>1967.18</td>
<td>0.53</td>
<td>Garfield/UT</td>
</tr>
<tr>
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<td>-109.5585</td>
<td>1369.16</td>
<td>0.60</td>
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</tr>
<tr>
<td>2</td>
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<td>0.53</td>
<td>San Juan/UT</td>
</tr>
<tr>
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<td>37.6028</td>
<td>-110.0261</td>
<td>1823.31</td>
<td>0.00</td>
<td>San Juan/UT</td>
</tr>
<tr>
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<td>36</td>
<td>37.9181</td>
<td>-109.9453</td>
<td>2190.29</td>
<td>0.54</td>
<td>San Juan/UT</td>
</tr>
<tr>
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<td>5</td>
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<td>-109.3472</td>
<td>1955.90</td>
<td>0.60</td>
<td>Sweetwater/WY</td>
</tr>
</tbody>
</table>

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H₀ = observed heterozygosity; County/State.
Table 8. *B. thompsonii* cluster (K) 3 geographical data for individual specimens – with coordinates, elevation, observed heterozygosity statistics, and county/state location information.

<table>
<thead>
<tr>
<th>K</th>
<th>ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>H_o</th>
<th>County/St</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
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<td>-107.9036</td>
<td>1945.23</td>
<td>0.00</td>
<td>San Juan/NM</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>39.5484</td>
<td>-107.3182</td>
<td>1829.11</td>
<td>0.07</td>
<td>Rio Arriba/NM</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>36.9103</td>
<td>-106.9989</td>
<td>2125.37</td>
<td>0.00</td>
<td>Rio Arriba/NM</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>37.0694</td>
<td>-107.8287</td>
<td>2011.37</td>
<td>0.07</td>
<td>La Plata/CO</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>37.32</td>
<td>-108.6783</td>
<td>1766.31m</td>
<td>0.00</td>
<td>Montezuma/CO</td>
</tr>
</tbody>
</table>

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H_o = observed heterozygosity; County/State.
Table 9. *B. thompsonii* cluster (K) 4 geographical data for individual specimens - with coordinates, elevation, observed heterozygosity statistics, and county/state location information.

<table>
<thead>
<tr>
<th>K</th>
<th>ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>$H_o$</th>
<th>County/State</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>14</td>
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<td>-107.5349</td>
<td>2127.50</td>
<td>0.07</td>
<td>Delta/CO</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>39.6747</td>
<td>-107.0951</td>
<td>2051.61</td>
<td>0.53</td>
<td>Eagle/CO</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>38.4828</td>
<td>-107.1864</td>
<td>2313.13</td>
<td>0.40</td>
<td>Gunnison/CO</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>38.4589</td>
<td>-107.2986</td>
<td>2416.15</td>
<td>0.29</td>
<td>Gunnison/CO</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>38.5646</td>
<td>-107.7909</td>
<td>2085.44</td>
<td>0.07</td>
<td>Montrose/CO</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>38.5014</td>
<td>-107.7308</td>
<td>2142.74</td>
<td>0.00</td>
<td>Montrose/CO</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>38.6172</td>
<td>-107.5805</td>
<td>2236.93</td>
<td>0.00</td>
<td>Montrose/CO</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>36.2064</td>
<td>-107.5603</td>
<td>2287.83</td>
<td>0.00</td>
<td>Sandoval/NM</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>35.9803</td>
<td>-105.9126</td>
<td>1936.09</td>
<td>0.00</td>
<td>Santa Fe/NM</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>35.9523</td>
<td>-105.9106</td>
<td>2034.24</td>
<td>0.07</td>
<td>Santa Fe/NM</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>36.6740</td>
<td>-105.6871</td>
<td>2081.48</td>
<td>0.00</td>
<td>Taos/NM</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>36.6740</td>
<td>-105.6871</td>
<td>2081.48</td>
<td>0.07</td>
<td>Taos/NM</td>
</tr>
</tbody>
</table>

*K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; $H_o$ = observed heterozygosity; County/State.*
Table 10: *B. formosa* cluster (K) 1 geographical data including coordinates, elevation, observed heterozygosity statistics, and location by individual.

<table>
<thead>
<tr>
<th>K</th>
<th>ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation</th>
<th>H_o</th>
<th>County/St</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>38.5008</td>
<td>-108.0265</td>
<td>1824.85m</td>
<td>0.07</td>
<td>Apache/AZ</td>
</tr>
<tr>
<td>1</td>
<td>86</td>
<td>37.8514</td>
<td>-110.6536</td>
<td>1841.01m</td>
<td>0.40</td>
<td>Apache/AZ</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>36.7855</td>
<td>-107.9814</td>
<td>1816.93m</td>
<td>0.20</td>
<td>Coconino/AZ</td>
</tr>
<tr>
<td>1</td>
<td>74</td>
<td>36.8622</td>
<td>-107.9798</td>
<td>1749.87m</td>
<td>0.07</td>
<td>Mohave/AZ</td>
</tr>
<tr>
<td>1</td>
<td>93</td>
<td>38.4613</td>
<td>-109.7850</td>
<td>1402.40m</td>
<td>0.07</td>
<td>Navajo/AZ</td>
</tr>
<tr>
<td>1</td>
<td>78</td>
<td>36.8401</td>
<td>-108.0127</td>
<td>1739.51m</td>
<td>0.00</td>
<td>Navajo/AZ</td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>40.5173</td>
<td>-109.5240</td>
<td>1684.95m</td>
<td>0.40</td>
<td>Delta/CO</td>
</tr>
<tr>
<td>1</td>
<td>54</td>
<td>36.9148</td>
<td>-109.3382</td>
<td>1741.95m</td>
<td>0.38</td>
<td>Garfield/CO</td>
</tr>
<tr>
<td>1</td>
<td>92</td>
<td>37.2486</td>
<td>-109.6605</td>
<td>1346.92m</td>
<td>0.29</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>94</td>
<td>37.2486</td>
<td>-109.6605</td>
<td>1346.92m</td>
<td>0.29</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>61</td>
<td>38.7628</td>
<td>-108.2433</td>
<td>1574.31m</td>
<td>0.09</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
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<td>36.8814</td>
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<td>0.33</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>40.5173</td>
<td>-109.5240</td>
<td>1684.95m</td>
<td>0.29</td>
<td>Mesa/CO</td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>38.9678</td>
<td>-108.4658</td>
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<td>Moffat/CO</td>
</tr>
<tr>
<td>1</td>
<td>79</td>
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<td>0.13</td>
<td>Montezuma/CO</td>
</tr>
<tr>
<td>1</td>
<td>98</td>
<td>41.5514</td>
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<td>1870.88m</td>
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</tr>
<tr>
<td>1</td>
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<td>36.6845</td>
<td>-110.5175</td>
<td>1948.61m</td>
<td>0.31</td>
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<tr>
<td>1</td>
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<tr>
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<td>37.0383</td>
<td>-112.3311</td>
<td>1579.79m</td>
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<td>San Juan/NM</td>
</tr>
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<td>57</td>
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<td>-111.6319</td>
<td>1655.38m</td>
<td>0.20</td>
<td>San Juan/NM</td>
</tr>
<tr>
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<td>69</td>
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<td>0.11</td>
<td>San Juan/NM</td>
</tr>
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<td>1749.87m</td>
<td>0.07</td>
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</tr>
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</tr>
<tr>
<td>1</td>
<td>76</td>
<td>36.8695</td>
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</tr>
<tr>
<td>1</td>
<td>97</td>
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<td>San Juan/NM</td>
</tr>
<tr>
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<td>40.1797</td>
<td>-110.4969</td>
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<td>Daggett/UT</td>
</tr>
<tr>
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<td>65</td>
<td>39.0158</td>
<td>-108.4147</td>
<td>1521.88m</td>
<td>0.00</td>
<td>Duchesne/UT</td>
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<tr>
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<td>-110.2552</td>
<td>1673.98m</td>
<td>0.00</td>
<td>Duchesne/UT</td>
</tr>
<tr>
<td>1</td>
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<td>-107.9751</td>
<td>1766.03m</td>
<td>0.00</td>
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</tr>
<tr>
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<td>90</td>
<td>37.1083</td>
<td>-112.0064</td>
<td>1819.67m</td>
<td>0.29</td>
<td>Emery/UT</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>0.50</td>
<td>Grand/UT</td>
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<td>38.7042</td>
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<td>1273.77m</td>
<td>0.00</td>
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<td>75</td>
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<td>-107.9472</td>
<td>1770.60m</td>
<td>0.13</td>
<td>Kane/UT</td>
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<tr>
<td>1</td>
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<td>38.5744</td>
<td>-109.5270</td>
<td>1364.91m</td>
<td>0.07</td>
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</tr>
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<td>-110.3936</td>
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</tr>
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<td>64</td>
<td>39.1184</td>
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<td>1320.40m</td>
<td>0.07</td>
<td>Uintah/UT</td>
</tr>
<tr>
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<td>82</td>
<td>40.1708</td>
<td>-110.3292</td>
<td>1673.06m</td>
<td>0.00</td>
<td>Uintah/UT</td>
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<td>-109.1619</td>
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<td>-108.8406</td>
<td>1805.35m</td>
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<td>Carbon/WY</td>
</tr>
<tr>
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<td>66</td>
<td>38.9333</td>
<td>-108.5678</td>
<td>1988.53m</td>
<td>0.14</td>
<td>Sweetwater/WY</td>
</tr>
</tbody>
</table>

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H_o = observed heterozygosity; County/State.
Table 11. Morphological characteristics of *B. thompsonii*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Boechera thompsonii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Perennial</td>
<td>Short-lived, sexual, caudex not woody</td>
</tr>
<tr>
<td>Stems/Caudex</td>
<td>2-5 per caudex from rosette margins</td>
</tr>
<tr>
<td>Basal Leaves</td>
<td>Blade oblanceolate to obovate</td>
</tr>
<tr>
<td>Cauline Leaves</td>
<td>3-8 not concealing stem</td>
</tr>
<tr>
<td>Racemes</td>
<td>4-15 flowered, unbranched</td>
</tr>
<tr>
<td>Fruiting Pedicels</td>
<td>Ascending to divaricate-ascending, straight</td>
</tr>
<tr>
<td>Stem Trichomes</td>
<td>Short stalk, 2-6 rayed, glabrous distally</td>
</tr>
<tr>
<td>Basal Leaf Trichomes</td>
<td>Simple, 2-rayed, short stalk</td>
</tr>
<tr>
<td>Flowers</td>
<td>Ascending at anthesis, petals lavender</td>
</tr>
<tr>
<td>Fruits</td>
<td>Ascending, straight</td>
</tr>
<tr>
<td>Seeds</td>
<td>Uniseriate, wing nearly continuous</td>
</tr>
</tbody>
</table>
Table 12. Morphological characteristics of *Boechera formosa*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Boechera formosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perennial</td>
<td>Long-lived, sexual, woody caudex</td>
</tr>
<tr>
<td>Stems/Caudex</td>
<td>1 per caudex from center of leaf tuft</td>
</tr>
<tr>
<td>Basal Leaves</td>
<td>Blade linear to linear-oblanceolate</td>
</tr>
<tr>
<td>Cauline Leaves</td>
<td>7-18 concealing stem proximally</td>
</tr>
<tr>
<td>Racemes</td>
<td>6-26 flowered, unbranched</td>
</tr>
<tr>
<td>Fruiting Pedicels</td>
<td>Horizontal to descending</td>
</tr>
<tr>
<td>Stem Trichomes</td>
<td>Short-stalk, 4-7 rayed</td>
</tr>
<tr>
<td>Basal Leaf Trichomes</td>
<td>Simple or 2-rayed, dense</td>
</tr>
<tr>
<td>Fruiting Pedicel Trichomes</td>
<td>Short-stalked, simple or 2-rayed</td>
</tr>
<tr>
<td>Flowers</td>
<td>Divaricate at anthesis, petals white to pale lavender</td>
</tr>
<tr>
<td>Fruits</td>
<td>Divaricate descending to reflexed</td>
</tr>
<tr>
<td>Seeds</td>
<td>Biseriate, wing continuous</td>
</tr>
</tbody>
</table>
Table 13. Morphological characteristics of *B. duchesnensis*.

<table>
<thead>
<tr>
<th>Locus</th>
<th><em>Boechera duchesnensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Perennial</td>
<td>Long lived, apomictic, caudex woody</td>
</tr>
<tr>
<td>Stems/Caudex</td>
<td>1 per caudex from center of rosette</td>
</tr>
<tr>
<td>Basal Leaves</td>
<td>Blade linear-oblanceolate</td>
</tr>
<tr>
<td>Cauline Leaves</td>
<td>3-8 not concealing stem</td>
</tr>
<tr>
<td>Racemes</td>
<td>11-22 flowered, unbranched</td>
</tr>
<tr>
<td>Fruiting Pedicels</td>
<td>Horizontal, straight</td>
</tr>
<tr>
<td>Stem Trichomes</td>
<td>Short-stalked, 2-7 rayed</td>
</tr>
<tr>
<td>Basal Leaf Trichomes</td>
<td>Densely pubescent, short stalked, 4-8 rayed</td>
</tr>
<tr>
<td>Fruiting Pedicel Trichomes</td>
<td>Appressed, branched</td>
</tr>
<tr>
<td>Flowers</td>
<td>Divaricate-ascending, petals whitish to pale lavender</td>
</tr>
<tr>
<td>Fruits</td>
<td>Erect to ascending, straight</td>
</tr>
<tr>
<td>Seeds</td>
<td>Sub-biseriate, wing continuous</td>
</tr>
</tbody>
</table>
Table 14. *Boechera duchesnensis* microsatellite-identified alleles at 14 unique loci showing clonal and sexual inheritance through hybridization. Each locus shows diploid allele inheritance. These data support three clonal individuals (ID 100, 101, and 102) of *B. duchesnensis* and 4 outcrossed individuals (ID 99, 103, 104, and 105) from independent hybridization events.

<table>
<thead>
<tr>
<th>ID</th>
<th>I3</th>
<th>A1</th>
<th>B20</th>
<th>B11</th>
<th>C8</th>
<th>I14</th>
<th>B9</th>
<th>E9</th>
<th>B18</th>
<th>BF3</th>
<th>B6</th>
<th>BF19</th>
<th>A3</th>
<th>B266</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>89-91</td>
<td>238-0</td>
<td>199-205</td>
<td>80-84</td>
<td>230-252</td>
<td>217-227</td>
<td>72-84</td>
<td>193-205</td>
<td>112-117</td>
<td>95-105</td>
<td>299-308</td>
<td>155-157</td>
<td>248-253</td>
<td>139-143</td>
</tr>
<tr>
<td>102</td>
<td>89-91</td>
<td>238-0</td>
<td>199-205</td>
<td>80-84</td>
<td>230-252</td>
<td>217-227</td>
<td>72-84</td>
<td>193-205</td>
<td>112-117</td>
<td>95-105</td>
<td>299-308</td>
<td>155-157</td>
<td>248-253</td>
<td>139-143</td>
</tr>
<tr>
<td>103</td>
<td>91-0</td>
<td>238-0</td>
<td>199-205</td>
<td>80-84</td>
<td>230-252</td>
<td>217-227</td>
<td>72-84</td>
<td>193-205</td>
<td>112-117</td>
<td>95-105</td>
<td>299-310</td>
<td>155-159</td>
<td>248-253</td>
<td>139-0</td>
</tr>
<tr>
<td>105</td>
<td>89-91</td>
<td>238-0</td>
<td>199-205</td>
<td>80-84</td>
<td>230-252</td>
<td>0-0*</td>
<td>72-84</td>
<td>193-205</td>
<td>112-117</td>
<td>95-105</td>
<td>0-0*</td>
<td>0-0*</td>
<td>155-157</td>
<td>248-253</td>
</tr>
</tbody>
</table>

ID = individual number; I3-B266 = Loci
* Microsatellites identification for ID 105 could not be read at loci I14 and B6, however these readability limitations do not change the final outcome.
### Morphological Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>B. formosa</th>
<th>B. thompsonii</th>
<th>B. duchesnensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perennial</td>
<td>Long lived, sexual, woody caudex</td>
<td>Short-lived, sexual, caudex not woody</td>
<td>Long lived, apomictic, caudex woody</td>
</tr>
<tr>
<td>Stems/Caudex (stem structure)</td>
<td>1 per caudex from center of leaf tuft</td>
<td>2-5 per caudex from margin of rosette</td>
<td>1 per caudex from center of rosette</td>
</tr>
<tr>
<td>Basal Leaves (from base of plant)</td>
<td>Blade linear to linear-oblongate</td>
<td>Blade oblongate to obovate</td>
<td>Blade linear-oblongate</td>
</tr>
<tr>
<td>Cauline Leaves (from stem, not stalk)</td>
<td>7-18 concealing stem proximally</td>
<td>3-8 not concealing stem</td>
<td>3-8 not concealing stem</td>
</tr>
<tr>
<td>Racemes (unbranched inflorescent pedicels)</td>
<td>6-26 flowered, unbranched</td>
<td>4-15 flowered, unbranched</td>
<td>11-22 flowered, unbranched</td>
</tr>
<tr>
<td>Fruiting Pedicels (short floral stalk)</td>
<td>Horizontal to descending</td>
<td>Ascending to divaricate-ascending, straight</td>
<td>Horizontal, straight</td>
</tr>
<tr>
<td>Trichomes (hairs)</td>
<td>Short-stalked, simple, or 2-rayed</td>
<td>Simple and 2-rayed</td>
<td>Short-stalked, 2-7 rayed</td>
</tr>
<tr>
<td>Flowers</td>
<td>Divaricate at anthesis, petals white to pale lavender</td>
<td>Ascending at anthesis, petals lavender</td>
<td>Divaricate-ascending, petals whitish to pale lavender</td>
</tr>
<tr>
<td>Fruits</td>
<td>Divaricate descending to reflexed</td>
<td>Ascending, straight</td>
<td>Erect to ascending, straight</td>
</tr>
<tr>
<td>Seeds</td>
<td>Biseriate, wing continuous</td>
<td>Uniseriate, wing nearly continuous</td>
<td>Sub-biseriate, wing continuous</td>
</tr>
</tbody>
</table>

Figure 1. Comparison of morphological features of a) Boechera formosa b) Boechera thompsonii, and c) Boechera duchesnensis showing trait inheritance from parent species to offspring (B. duchesnensis).
Figure 2. Distribution of the studied *Boechera* spp. individuals across their geographic range
a) *B. thompsonii* indicated with triangles, b) *B. formosa* indicated with squares, c) *B. duchesnensis* indicated with red circles. Greater detail shown at Duchesne site highlights *B. duchesnensis*. 
Figure 3. Geographic range distribution of *B. formosa* individuals, identified by species ID number, across 18 counties of 5 states demonstrating their placement in relation to physical characteristics and possible physical boundaries.
Figure 4. Genotypic population clustering graph based on 105 Boechera individuals and displaying 6 genetically distinct clusters (K = 6). STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) was used to produce graph showing B. duchesnensis (1) in red; B. thompsonii (2, 3, 4, 5) in green, blue, yellow, and fuchsia; and B. formosa (6) in light blue.
Figure 5. Geographic distribution of *B. thompsonii* individuals identified by species ID number, across 27 counties in 5 states demonstrating their placement in relation to physical characteristics and possible physical boundaries.
Figure 6. Distribution of 105 Boechera spp. individuals using data mapped with ArcGIS 10.1 (ESRI, 2012) and displaying the 6 genetic clusters’ (K = 6) grouping, also using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007). B. duchesnensis is represented by red circles; B. thompsonii is represented by green, blue, yellow, and green circles and; B. formosa is represented by turquoise circles.
Figure 7. Distribution of 53 *B. thompsonii* individuals using data mapped with ArcGIS 10.1 (ESRI, 2012) and displaying 4 genetically distinct clusters’ (K = 4) grouping, also using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007). Cluster 1 is represented by green circles, cluster 2 is represented by blue circles, cluster 3 is represented by yellow circles, and cluster 4 is represented by fuchsia circles.
Figure 8. Genotypic clustering graph for 52 *B. thompsonii* individuals at 14 loci with four distinct clusters identified (K = 4). STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) was used to produce this graph showing frequency of allelic similarity on the X axis and identified individuals by ID number on the Y axis.
Figure 9. Elevation of each individual by Specimen ID and the mean elevation for each of the 4 identified clusters within the population of *B. thompsonii*. Altitudes from lowest to highest: cluster 3, cluster 1, cluster 2, then cluster 4.

X axis = Specimen ID Number; Y axis = Elevation; Green = Cluster (K) 1; Blue = Cluster (K) 2; Yellow = Cluster (K) 3; Fuchsia = (K) 4.
Figure 10. Elevation of each individual by Specimen ID and the mean elevation for the single population cluster of *B. formosa*. Assigned color is turquoise. X axis = Specimen ID Number; Y axis = Elevation.
Figure 11. Percentage of allelic presence per taxon from 14 independently assorting loci across 114 total individuals of *B. thompsonii*, *B. formosa*, and *B. duchesnensis* calculated using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012). Locus B266 shows six available alleles and locus B6 shows 3 available alleles in *B. duchesnensis* showing hybridization from *B. thompsonii* and *B. formosa* has occurred more than one time by crossing.
Figure 12. Observed heterozygosity ($H_o$) of *B. thompsonii* throughout its geographic range.
Figure 13. Observed heterozygosity ($H_o$) of $B. \text{formosa}$ throughout its geographic range.
Figure 14. This image shows morphological characteristics of *B. formosa* at medium magnification – including the existence of papillae on individual basal leaf trichomes which are short-stalked and simple or 2-rayed. Image also shows broken trichome tips and debris due to sample handling and mounting.
Figure 15a. This image shows morphological characteristics of *Boechera formosa* pollen at medium magnification – especially the existence of symmetrically-shaped ellipsoid pollen grains and their three colpi. Image also shows portions of the anther.
Figure 15b. This image shows morphological characteristics of *Boechera formosa* pollen at high magnification – especially the existence of symmetrically-shaped ellipsoid pollen grains and their three colpi. Image also shows portions of the anther.
Figure 16. This image shows morphological characteristics of *B. thompsonii* pollen at high magnification – especially the existence of symmetrically-shaped ellipsoid pollen grains and their three colpi.
Figure 17. This image shows morphological characteristics of *B. duchesnensis* trichomes at medium magnification – notably the existence of short-stalked 2-7 rayed trichomes on a caudex stem presenting with sparsely intermediate characteristics of distally glabrous *B. thompsontii* and pubescent *B. formosa*. Image also shows grains of dust or debris either from the field or from handling and preparation.
Figure 18. This image shows typical morphological characteristics of pollen from the genus *Boechera* at medium magnification – notably the typical symmetrical ellipsoid pollen grains with three colpi of sexual diploid individuals which are relatively smaller - 13-20 µm - in diameter than the three species studied herein. Image also shows portions of the petal.
Figure 19. This image shows typical morphological pollen characteristics of the genus *Boechera* at high magnification – notably the typical symmetrical ellipsoid pollen grains with three colpi of sexual diploid individuals which are relatively smaller - 13-20 µm - in diameter than the three species studied herein. Image also shows portions of the petal.
Figure 20. This image demonstrates representative morphological characteristics of *B. duchesnensis* pollen at medium magnification – most notably low pollen count and abortive and malformed pollen grains. Image also shows portions of the petal and ellipsoid pollen grain shape (where not malformed).
Figure 21. This image demonstrates representative morphological characteristics of *B. formosa* at low magnification – most notably densely pubescent trichomes covering a cauline leaf. This morphological characteristic helps to reflect sunlight and protect delicate tissues in hot, dry environments.
Figure 22. This image demonstrates representative morphological characteristics of *B. thompsonii* at low magnification – most notably a glabrous (free from trichomes) fruiting pedicel.
Figure 23. This image demonstrates typical morphological characteristics of the genus *Boechera* at low magnification – most notably a glabrous (free from trichomes) basal leaf with linear-ob lanceolate shape.
Figure 24. This image shows two specimens of *B. thompsonii* from the same individual exhibiting morphological characteristics at low magnification and on the same stub. The specimen on the left side shows basal leaves that are distally pubescent (trichomes present on the outer edges of the leaf). The specimen on the right shows a glabrous (lacking trichomes) fruiting pedicel.
Figure 25. This image demonstrates typical morphological characteristics of *B. duchesnensis* at medium magnification – most notably a stem that is sparsely pubescent distally with a relatively larger trichome that appears almost stellate (star-shaped) due to its short-stalked, many rays characteristic.
Figure 26. This image demonstrates morphological characteristics of *B. formosa* at medium magnification – most notably the distal end of a stem that is densely pubescent (covered with trichomes).
Figure 27a. This image shows morphological characteristics of typical Boechera apomictic triploid spp. pollen (Windham and Al-Shehbaz, 2007a, 2007b) at medium magnification – especially the existence of asymmetrically-shaped spheroid pollen grains. Image also shows portions of the petal.
Figure 27b. This image shows morphological characteristics of typical *Boechera* apomictic triploid spp. pollen (Windham and Al-Shehbaz, 2007a, 2007b) at high magnification – especially the existence of asymmetrically-shaped spheroid pollen grains. Image also shows portions of the petal.