2016-03-01

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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March 2016

ABSTRACT

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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. thompsoii* 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*. Geographicaldata 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

between clusters. I analyze the tendency of reduced heterozygosity to lead to genetic fixation, reproductive isolation, and how the heightened heterozygosity supports the classification of *B. duchesnensis* as an apomict. Assessing potential populations that might exist based on similar characteristics could possibly provide inferences about where future research might find similar examples of this hybridization. Reproductive isolation is hypothesized to limit gene flow between identified clusters of *B. thompsonii* and *B. formosa* exacerbating low observed heterozygosity levels and low allelic frequency levels. Population studies and cluster analysis have implications for offering future conservation strategies for both taxa.

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^2=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 15.00Kv 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 ($H_e = 1 - \Sigma p_i^2$) 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, ΔK 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 *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 *B. thompsonii* had a (H_o) range of 0.53-0.60, in Duchesne Co., Utah. *Boechera duchesnensis* has only been identified in Duchesne Co., Utah. The observed heterozygosity (H_o) frequency for *B. formosa* in Duchesne Co., Utah was zero. In Sweetwater, Wyoming *B. thompsonii* has a H_o of 0.60 while *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 oblanceolate 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 Brassicaceae 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-oblanceolate basal leaves (Figure 22), and 7-18 cauline leaves concealing the stem proximally. Flower morphology mimics the Brassicaceae 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 cauline

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. formsa*, 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 F_1 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 F_1

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-Shebaz, 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

Adams, Henry D., Guardiola-Claramonte, Maite, Barron-Gafford, Greg A., Villegas, Juan Camilo, Breshears, David D., Zou, Chris B., Troch, Peter A. Travis E. Huxman. (2009). Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *PNAS*. 106(17), 7063-7066.

Akiyama, Y., Goel, S., Conner, J. A., Hanna, W. W., Yamada-Akiyama, H., Ozias-Akins, P. (2011). Evolution of the apomixis transmitting chromosome in *Pennisetum*. *BMC Evolutionary Biology*. 11, 289.

Al-Shehbaz, I. A. (1984). The tribes of Cruciferae (Brassicaceae) in the Southeastern United States. *Journal of the Arnold Arboretum, Harvard University*. 65, 343-373.

Al-Shehbaz I. A. (1987). The genera of *Alysseae* (Cruciferae; Brassicaceae) in the southeastern United States. *Journal of the Arnold Arboretum, Harvard. University*. 68, 185-240.

Al-Shehbaz, I. A. (2003). Transfer of most North American species of *Arabis* to *Boechera* (Brassicaceae). *Novon*. 13, 381–391.

Al-Shehbaz, I. A. (2005). *Boechera falcata* (Turcz.) *Novon*. 15(4), 520.

Al-Shehbaz, I. A., Beilstein, M. A., Kellogg, E. A. (2006). Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution*. 259, 89–120.

Al-Shehbaz, I. A. (2011). *Brassicaceae (Mustard Family)*. John Wiley and Sons, Ltd.

Alberto, F., S. N. Aitken, R. Alía, S. C. González-Martínez, H. Hänninen, A. Kremer, F. Lefèvre. (2013). Potential for evolutionary responses to climate change: evidence from tree populations. *Global Change Biology.* 19, 1645–1661.

Alexander, P. J., Rajanikanth, G., Bacon, C. D., Bailey, C. D. (2007). Recovery of plant DNA using a reciprocating saw and silica-based columns. *Molecular Ecology Notes.* 7(1), 5-9.

Alexander, P. J., Windham, M. D., Beck, J. B., Al-Shehbaz, I. A., Allphin, L., Bailey, C. D. (2013). Molecular phylogenetics and taxonomy of the genus *Boechera* and related genera (Brassicaceae: Boechereae). *Systematic Botany. 38*(1), 192-209.

Alexander, P. J., Windham, M. D., Beck, J. B., Al-Shehbaz, I. A., Allphin, L., Bailey, C. D. (2015). Weaving a Tangled Web: Divergent and Reticulate Speciation in *Boechera fendleri* sensu lato (Brassicaceae: Boechereae). *Systematic Botany*. *40*(2), 572-596.

Allphin, Loreen, Windham, Michael, Harper T. Kimball. (1998). Genetic diversity and gene flow in the endangered dwarf bear poppy, *Arctomecon humilis* (Papaveraceae). *American Journal of Botany.* 85(9), 1251-1261.

Anderson, J., N. Perera, B., Chowdhury, T., Mitchell-Olds. (2015). Microgeographic patterns of genetic divergence and adaptation across environmental gradients in *Boechera stricta* (Brassicaceae). *American Naturalist.* S001-S0015.

Bailey, C. D., Koch, M. A., Mayer, M., Mummenhoff, K., O'Kane, S. L., Warwick, S. I., Al-Shehbaz, I. A. (2006). Toward a global phylogeny of the Brassicaceae. *Molecular Biology and Evolution*. 23(11), 2142-2160.

Barrett, S. C., and Shore, J. S. (1989). Isozyme variation in colonizing plants. *Isozymes in Plant Biology*. Springer. Netherlands.106-126.

Barrett, S. (2003). *Environment and Statecraft: The Strategy of Environmental Treaty-Making*. Oxford University Press.

Barringer, B.C. (2007). Polyploidy and self-fertilization in flowering plants. *American Journal of Botany.* 94, 1527–1533.

Baskin, J. M. and Baskin, C. C. (1986). Distribution and geographical/evolutionary relationships of cedar glade endemics in southeastern United States. *Association of Southeastern Biologists Bulletin.* 33, 138-154.

Beardsley, P. M., Schoenig, S. E., Whitall, J. B., Omstead, R. G. (2004). Patterns of evolution in western North American *Mimulus* (Phrymaceae). *American Journal of Botany.* 91 (3), 474-489.

Beck, J. B., Alexander, P. J., Allphin, L., Al-Shehbaz, I., Rushworth, C., Bailey, C. D., and Windham, M. D. (2012). Does hybridization drive the transition to asexuality in diploid Boechera? *Evolution.* 11, 1-11.

Beilstein, M. A., Al-Shehbaz, I. A., Kellogg, E. A. (2006). Brassicaceae phylogeny and trichome evolution. *American Journal of Botany.* 93, 607–619.

Beilstein, M. A. and M. D. Windham. (2003). A phylogenetic analysis of western North American *Draba* (Brassicaceae) based on nuclear ribosomal DNA sequences from the ITS region. *Systematic Botany.* 28,584–592.

Bennington, C., N. Fetcher, M. Vavrek, G. Shaver, K. Cummings, J. McGraw. (2012). Home site advantage in two long-lived arctic plant species: results from two 30-year reciprocal transplant studies. *Journal of Ecology*. 100, 841–851.

Bevill, R.L. and S.M. Louda. (1999). Comparisons of related rare and common species in the study of plant rarity. *Conservation Biology*. 13(3), 493-498.

Bishop, J. G., Dean, A. M., Mitchell-Olds, T. (2000). Rapid evolution in plant chitinases: molecular targets of selection in plant-pathogen coevolution. *Proceedings of the National Academy of Sciences*. 97, 5322–5327.

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

Bloom, T. C., Baskin, J. M., Baskin, C. C. (2001). Ecological life history of the facultative woodland biennial *Arabis laevigata* variety *laevigata* (Brassicaceae): survivorship. *Journal of the Torrey Botanical Society.* 128, 93-108.

Böcher, T. W. (1951). Cytological and embryological studies in the amphi-apomictic *Arabis holboellii* complex. *Det Kongelige Danske Videnskabernes Selskab.* 6, 1-59.

Böcher, T. W. (1954). Experimental taxonomic studies in the *Arabis holboellii* and allied species. *Biologiske Skrifter*. 14, 1–74.

Brennan, A. C., Harris, S. A., & Hiscock, S. J. (2013). The Population Genetics of Sporophytic Self-Incompatibility in Three Hybridizing *Senecio* (Asteraceae) Species with Contrasting Population Histories. *Evolution*. 67(5), 1347-1367.

Britton, N.L and A. Brown. (1913). An illustrated flora of the northern United States, Canada and the British Possessions. *Courtesy of Kentucky Native Plant Society.* 2, 183.

Brochmann, Christian, Soltis, Pamela, Soltis, Douglas. (1992). Recurrent formation and polyphyly of Nordic polyploids in *Draba* (Brassicaceae). *American Journal of Botany*. 79(6), 673-688.

Carman, J. G. (2015). Hot on the trail of the sex-apomixis switch in *Boechera* (Brassicaceae). *International Plant and Animal Genome XXIII Conference*. Plant and Animal Genome (PAG).

Castric, V., Bechsgaard, J., Schierup, M. H., & Vekemans, X. (2008). Repeated adaptive introgression at a gene under multiallelic balancing selection. *PLoS Genet*. 4(8), e1000168.

Cires, E., Cuesta, C., and Fernández Prieto, J. A. (2013). Genetic diversity and structure in fragmented populations of the endangered species *Ranunculus cabrerensis* (Ranunculaceae): implications for conservation. *Biologia.* 68(1), 30-40.

Clauss MJ, Mitchell-Olds T. (2006). Population genetic structure of *Arabidopsis lyrata* in Europe. *Molecular Ecology.* 15: 2753–2766.

Cronquist, A. (1968). *The Evolution and Classification of Flowering Plants.* New York Botanical Garden. New York.

Darwin, C. (1859). *On the Origin of Species*. London.

Davies, K. F., Margules C. R., and Lawrence, J. F. (2004). A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology.* 85(1), 65-271.

Davies, P. J. (2004). Plant hormones: biosynthesis, signal transduction, action. *Springer Science and Business Media*. Cornell University. Ithaca, New York.

Davis, J. I. and Nixon, K. C. (1992). Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology*. *41*(4), 421-435.

de Grammont, P., Cuarón, A. D. (2006). An evaluation of threatened species categorization systems used on the American continent. *Conservation Biology*. *20*(1), 14-27.

Dell, B., McComb, A. J. (1979). Plant resins—their formation, secretion and possible functions. *Advances in Botanical Research.* 6, 277–316.

Dinerstein, E. (2013). *The Kingdom of Rarities*. Island Press.

Dobeš, C., Mitchell-Olds, T., Koch, M. (2004a). Extensive chloroplast haplotype variation indicates Pleistocene hybridization and radiation of North American *Arabis drummondii, A. divaricarpa,* and *A. holboellii* (Brassicaceae). *Molecular Ecology.* 13, 349–370.

Dobeš, C., Mitchell-Olds, T., Koch, M. (2004b). Intraspecific diversification in North American *Boechera stricta* (=*Arabis drummondii*), *Boechera divaricarpa*, and *Boechera holboellii* (Brassicaceae) inferred from nuclear and chloroplast molecular markers-an integrative approach. *American Journal of Botany.* 91, 2087–2101.

Dobeš, C., Sharbel, T. F., Koch, M. (2007). Towards understanding the dynamics of hybridization and apomixis in the evolution of the genus *Boechera* (Brassicaceae). *Systematics and Biodiversity. 5*(03), 321-331.

Earl, D. A. and Von Holdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*. 4:2, 359-361. doi: 10.1007/s12686-011-9548-7 Core version: vA.1 March 2012 Web version: v0.6.92

Esch, J. J., Chen, M., Sanders, M., Hillestad, M., Ndkium, S., Idelkope, B., Neizer, J., Marks, M. D. (2003). A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in *Arabidopsis*. *Development.* 130, 5885–5894.

ESRI. (2012). ArcGIS Desktop: Release 10.1. Redlands, CA: Environmental Systems Research Institute.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology.* 14(8), 2611-2620.

Falk, D. A. and K. E. Holsinger. (1991). *Genetics and Conservation of Rare Plants*. Oxford University Press, New York.

Falush, D., Stephens, M., and Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics. 164*(4), 1567-1587.

Falush, D., Stephens, M., and Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular ecology notes*. *7*(4), 574-578.

Fiedler, P. L., Knapp, B., Fredericks, N. (1998). Rare plant demography: Lessons from the Mariposa Lilies (*Calochortus*: Liliaceae). In: Fiedler, P. L., Kareiva, P. M., editors. *Conservation Biology: Conservation for the Coming Decade*. Chapman and Hall. New York. 28-48.

Fischer, M. and Matthies, D. (1998). RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *American Journal of Botany*. *85*(6), 811-811.

Frankham, R., Ballou, J.D., Briscoe, D.A. (2002). Introduction to Conservation Genetics. *Cambridge University Press.* Cambridge, UK.

Fiedler, P. L., Knapp, B., Fredericks, N. (1998). Rare plant demography: Lessons from the Mariposa Lilies (*Calochortus*: Liliaceae). In: Fiedler, P. L., Kareiva, P. M., editors. *Conservation Biology: Conservation for the Coming Decade*. Chapman and Hall. New York. 28-48.

Gabrielová, J., Münzbergová, Z., Tackenberg, O., and Chrtek, J. (2013). Can we distinguish plant species that are rare and endangered from other plants using their biological traits? *Folia Geobotanica*. 48(4), 449-466.

Gaston, K. J. (1994). What is rarity? *Springer*. Netherlands. 1-21.

Gliddon, C. and Goudet, J. (1994). The genetic structure of metapopulations and conservation biology. *Conservation genetics*. 107-114. Birkhäuser Basel.

Halverson, K., Heard, S. B., Nason, J. D., and Stireman, J. O. (2008). Origins, distribution, and local co-occurrence of polyploid cytotypes in *Solidago altissima* (Asteraceae). *American Journal of Botany*. *95*(1), 50-58.

Hamrick, J. L., Linhart, Y. B., Mitton, J. B. (1979). Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics*. 173-200.

Hamrick, J. L., Godt, M. J. W., Murawski, D. A., Loveless, M. D. (1991). Correlations between species. *Genetics and Conservation of Rare Plants*. 75.

Heenan, P. B., Mitchell, A. D., Koch, M., (2002). Molecular systematics of the New Zealand *Pachycladon* (Brassicaceae) complex: generic circumscription and relationships to *Arabidopsis* sens. lat. and *Arabis* sens. lat. *New Zealand Journal of Botany*. 40, 543–562.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness tradeoffs. *American Naturalist*. 173, 579–588.

Herrera, C. M. and Bazaga, P. (2008). Population-genomic approach reveals adaptive floral divergence in discrete populations of a hawk moth-pollinated violet. *Molecular Ecology*. 17(24), 5378-5390.

Hoffmann, Matthias H., Glarb, Anne S., Tomiuk, Jurgen, Schumutts, Heike, Fritsch, Reinhard M., Konrad Bachmann. (2003). Analysis of molecular data of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) with Geographical Information Systems (GIS). *Molecular Ecology*. 12(4), 1007-1019.

Humphrey, L. D. and Schupp, E. W. (2001). Seed banks of *Bromus tectorum*–dominated communities in the Great Basin. *Western North American Naturalist*. 85-92.

Isik, K. (2011). Rare and endemic species: Why are they prone to extinction? *Turk Journal of Botany*. 35, 411-417.

Izco, J. (1998). Types of rarity of plant communities. Journal of Vegetation Science. 9(5), 641-646.

Jarne, P., & Lagoda, P. J. (1996). Microsatellites, from molecules to populations and back. Trends in ecology & evolution, 11(10), 424-429.

Johnston, J. S., Pepper, A. E., Hall, A. E., Chen, Z. J., Hodnett, G., Drabek, J., Lopez, R., Price, H. J. (2005). Evolution of genome size in Brassicaceae. Annals of Botany. 95, 229–235.

Kalske, A., A. Muola, L. Laukkanen, P. Mutikainen, R. Leimu. (2012). Variation and constraints of local adaptation of a long-lived plant, its pollinators and specialist herbivores. *Journal of Ecology*. 100, 1359–1372.

Kane, N. C., King, M. G., Barker, M. S., Raduski, A., Karrenberg, S., Yatabe, Y., Rieseberg, L. H. (2009). Comparative genomic and population genetic analyses indicate highly porous genomes and high levels of gene flow between divergent *Helianthus* species. *Evolution*. 63(8), 2061-2075.

Kantama, Laksana, Sharbel, Timothy F., Schranz, M. Eric, Mitchell-Olds, Thomas, de Vries, Sacco, de Jong, Hans. (2007). Diploid apomicts of the *Boechera holboellii* complex display large-scale chromosome substitutions and aberrant chromosomes. *Proceedings of the National Academy of Sciences*. 104, 35-41.

Kiefer, Christiane. (2008). Evolution and phylogeography of the North American genus *Boechera* (Brassicaceae) and the evolution of apomixis. *Dissertation*. Ruperto-Carola University of Heidelberg, Germany.

Kim, M., Cui, M. L., Cubas, P., Gillies, A., Lee, K., Chapman, M. A., Coen, E. (2008). Regulatory genes control a key morphological and ecological trait transferred between species. *Science*. 322(5904), 1116-1119.

Klich, M. G. (2000). Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. *Environmental and Experimental Botany*. 44, 171–183.

Knight, C., Vogel, H., Kroymann, K. (2006). Expression profiling and local adaptation of *Boechera holboellii* populations for water use efficiency across a naturally occurring water stress gradient. *Molecular Ecology*. 15, 1229–1237.

Koch, Marcus. (2015). A new chromosome was born: comparative chromosome painting in *Boechera. Trends in Plant Science*. 20(9).

Koch, M. and Al-Shehbaz, I. A. (2002). Molecular data indicate complex intra-and intercontinental differentiation of American *Draba* (Brassicaceae). *Annals of the Missouri Botanical Garden*. 88-109.

Koch, M., Al-Shehbaz, I. A., Mummenhoff, K. (2003). Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). *Annals of the Missouri Botanical Garden*. 90, 151–171.

Koch, M., Bishop, J., Mitchell-Olds, T. (1999). Molecular systematics and evolution of *Arabidopsis* and *Arabis*. *Plant Biology*. 1, 529–537.

Koch, M., Haubold, B., Mitchell-Olds, T. (2000). Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis, Arabis*, and related genera (Brassicaceae). *Molecular biology and evolution*. *17*(10), 1483-1498.

Koch, M., Haubold, B., Mitchell-Olds, T. (2001). Molecular systematics of the Brassicaceae: evidence from coding plastidic matK and nuclear Chs sequences. *American Journal of Botany*. 88, 534–544.

Koch, M., Dobeš, C., Mitchell-Olds, T. (2003). Multiple hybrid formation in natural populations: concerted evolution of the internal transcribed spacer of nuclear ribosomal DNA (ITS) in North American *Arabis divaricarpa* (Brassicaceae). *Molecular Biology and Evolution*. *20*(3), 338-350.

Kremer, A., O. Ronce, J. Robledo-Arnuncio, F. Guillaume, G. Bohrer, R. Nathan, J. Bridle. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters.* 15, 378–392.

Lamoreux, J., Akçakaya, H. R., Bennun, L., Collar, N. J., Boitani, L., Brackett, D., Stuart, S. (2003). Value of the IUCN red list. *Trends in Ecology and Evolution*. *18*(5), 214-215.

Leimu, R., Mutikainen, P. I. A., Koricheva, J., Fischer, M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology.* 94(5), 942-952.

Looney, Christopher E. Sullivan, Benjamin W., Kolb, Thomas E., Kane Jeffrey M., Stephen C. Hart. (2012). Pinyon pine (*Pinus edulis*) mortality and response to water addition across a three million year substrate age gradient in Northern Arizona, USA. *Plant Soil*. 357, 89-102.

Lovell, J. T. (2011). *Boechera* Summit 2011. *Molecular Ecology.* 20(23), 4840-4842.

Lovell, J. T., Grogan, K., Sharbel, T. F., & McKay, J. K. (2014). Mating system and environmental variation drive patterns of adaptation in *Boechera spatifolia* (Brassicaceae). *Molecular ecology*. *23*(18), 4486-4497.

Löve, Á., Löve, D. (1976). Nomenclatural notes on Arctic plants. *Botanical Garden and Botanical Museum Berlin-Dahlem.*128, 497-523.

Loveless, M. D. and Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual review of ecology and systematics.* 65-95.

MacArthur, R. H. and Wilson, E. O. (1967). *The theory of island geography* (Vol. 1). Princeton University Press.

Mace, G. M., Collar, N. J., Gaston, K. J., Hilton-Taylor, Craig, Akçakaya, H. R., Leader-Williams, Nigel, Stuart, S. N. (2008). Quantification of extinction risk: IUCN's system for classifying threatened species. *Conservation Biology*. *22*(6), 1424-1442.

Magurran, A. E. and Henderson, P. A. (2003). Explaining the excess of rare species in natural species abundance distributions. *Nature*. 422(6933), 714-716.

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

Manetas, Y. (2003). The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. *New Phytologist*. 158, 503–508.

Marhold K. and Lihova, J. (2006). Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae. *Plant Systematics and Evolution.* 259, 143–174.

Marks, M. D., Wenger, J. P., Gilding, E., Jilk, R., Dixon, R. A. (2009). Transcriptome analysis of *Arabidopsis* wild-type and gl3-sst sim trichomes identifies four additional genes required for trichome development. *Molecular Plant.* 2, 803–822.

McKay, J. K., Bishop, J. G., Lin, J. Z., (2001). Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. *Proceedings of the Royal Society of London - Series B: Biological Sciences*. 268, 1715–1721.

Martin, N. H., Bouck, A. C., Arnold, M. L. (2006). Detecting adaptive trait introgression between *Iris fulva* and *I. brevicaulis* in highly selective field conditions. *Genetics*. 172(4), 2481-2489.

McNeill, J., F. R. Barrie, H. M. Burdet, V. Demoulin, D. L. Hawksworth, K. Marhold, D. H. Nicolson, J. Prado, P. C. Silva, J. E. Skog, J. H. Wiersema, and N. J. Turland, (2006). *International Code of Botanical Nomenclature*. (Vienna Code). Koeltz Scientific Books. Königstein, Germany.

McNeill, J., Barrie, F. R., Buck, W. R., Demoulin, V., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Marhold, K., Prado, J., Prud'homme van Reine, W. F., Smith, G. F., Wiersema, J. H., Turland, N. J. (2011). International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), *adopted by the Eighteenth International Botanical Congress.* Melbourne, Australia.

Mitchell-Olds, T. (2001). *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. *Trends in Ecology and Evolution*. 16, 693-700.

Mitchell-Olds, T., Al-Shehbaz, I. A., Koch, M., Sharbel, T. (2005). Crucifer evolution in the post-genomic era. In: *Diversity and Evolution of Plants — Genotype and Phenotype Variation in Higher Plants* (ed. Henry R). 119 –137. CABI Press. Cambridge, Massachusetts.

Mitchell-Olds, T. and Schmitt, J. (2006). Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature.* 441(7096), 947-952.

Mueller, Rebecca C., Scudder, Crescent M., Porter, Marianne E., Trotter, R. Talbot III, Gehring, Catherine A., Thomas G. Whitham. (2005). Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. *Journal of Ecology*. 93, 1085-1093.

Nixon, K. C. and Wheeler, Q. D. (1990). An amplification of the phylogenetic species concept. *Cladistics*. *6*(3), 211-223.

Nybom H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology.* 13:1143 – 1155.

O'Kane, S. L. Jr. and Al-Shehbaz, I. A. (2003). Phylogenetic position and generic limits of *Arabidopsis* (Brassicaceae) based on sequences of nuclear ribosomal DNA. *Annals of the Missouri Botanical Garden.* 90, 603-615.

Otto S. P. and J. Whitton. (2000). Polyploid incidence and evolution. *Annual Review of Genetics.* 34, 401–437.

Oyama, Ryan K., Clauss, Maria J., Formanova, Natasa, Kroymann, Jurgen, Schmid, Karl J., Vogel, Heiko, Weniger, Kerstin, Windsor, Aaron J., Mitchell-Olds, Thomas. (2008). The shrunken genome of *Arabidopsis*. *Plant System Evolution*. 273, 257–271.

Peakall, R. and Smouse P.E. (2006). GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6, 288-295.

Peakall, R. and Smouse P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics.* 28, 2537-2539.

Powell, D. S., Faulkner, J. L., Darr, D. R., Zhu, Z., MacCleery, D. W. (1994). Forest resources of the United States, 1992. *Fort Collins, CO. USDA Forest Service General Techincal Report*. RM-234 (Revised). 133.

Preston, F. W. (1948). The commonness, and rarity, of species. *Ecology*. *29*(3), 254-283.

Pritchard, J. K., Stephens, M., Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics. 155*(2), 945-959.

Putnam, E. R. S. (2013). Ecology, Phylogenetics, and Conservation of *Draba asterophora* Complex: A Rare, Alpine, Endemic from Lake Tahoe, USA. *Disseration*. Brigham Young University, Provo, UT.

Rabinowitz, D., Cairns, S., Dillon, T. (1986). Seven forms of rarity and their frequency in the flora of the British Isles. *Conservation Biology, The Science of Scarcity and Diversity.* Sinauer. 182-204.

Rieseberg, L. H., Raymond, O., Rosenthal, D. M., Lai, Z., Livingstone, K., Nakazato, T., Lexer, C. (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science*. 301(5637), 1211-1216.

Rodrigues, A. S., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M., & Brooks, T. M. (2006). The value of the IUCN Red List for conservation. *Trends in Ecology and Evolution*. *21*(2), 71-76.

Rollins, R. C. (1993). *The Cruciferae of Continental North America: Systematics of the Mustard Family from the Arctic to Panama.* Stanford University Press. Stanford, CA

Rondeau, R., K. Decker, J. Handwerk, J. Siemers, L. Grunau, and C. Pague. (2011). The state of Colorado's biodiversity 2011. *Prepared for The Nature Conservancy*. Colorado Natural Heritage Program, Colorado State University, Fort Collins, Colorado.

Routley, M. B., Bertin, R. I., Husband, B. C. (2004). Correlated evolution of dichogamy and self-incompatibility: a phylogenetic perspective. *International Journal of Plant Sciences*. *165*(6), 983-993.

Roy, B. A. (1995). The breeding systems of six species of *Arabis* (Brassicaceae). *American Journal of Botany.* 82(7), 869-877.

Roy, B. A. (2001). Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. *Evolution*. 55(1), 41-53.

Roy, B. A. and Rieseberg, L. H. (1989). Evidence for apomixes in *Arabis Journal of Heredity.* 80, 506-509.

Rushworth, C. A., Song, B. H., Lee, C. R., Mitchell-Olds, Thomas (2011). *Boechera*, a model system for ecological genomics. *Molecular Ecology*. 20(23), 4843-4857.

Savolainen, O., T. Pyhajarvi, T. Knurr. (2007). Gene flow and local adaptation in trees. *Annual Review of Ecology Evolution and Systematics*. 38, 595–619.

Schaal, B. A., and Levin, D. A. (1976). The demographic genetics of Liatris cylindracea Michx. (Compositae). *American Naturalist.* 191-206.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., Bishop, J. G. (1994). Evaluating approaches to the conservation of rare and endangered plants. *Ecology*. *75*(3), 584-606.

Schranz, M. Eric, Dobeš, Christoph, Loch, Marcus A., Mitchell-Olds, Thomas. (2005). Sexual reproduction, Hybridization, apomixis, and polyploidization in the genus *Boechera* (Brassicaceae). *American Journal of Botany.* 92(11), 1997- 1810.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution*. 19(4), 198-207.

Sharbel, T. F., Voigt, M. L., Mitchell-Olds, T., Kantama, L., de Jong, H. (2004). Is the aneuploid chromosome in an apomictic *Boechera holboellii* a genuine B chromosome? *Cytogenetic and Genome Research.* 106, 173–183.

Shaw, John D., Steed, Brytten E., Larry T. DeBlander. (2005). Forest Inventory and Analysis (FIA) Annual Inventory Answers the Question: What Is Happening to Pinyon-Juniper Woodlands? *Journal of Forestry.* 9, 280-285.

Silvertown, J., Franco, M., Pisanty, I., Mendoza, A. (1993). Comparative plant demography--relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. *Journal of Ecology*. 465-476.

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

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

SoftGenetics, L. L. C. (2007). GeneMarker Operation Manual. Pennsylvania.

Soltis, P. S. and Gitzendanner, M. A. (1999). Molecular systematics and the conservation of rare species. *Conservation Biology*. 13(3), 471-483.

Soltis, P. S. and Soltis, D. E. (2000). The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences*. 97(13), 7051-7057.

Song, B. H., Clauss, M. J., Pepper, A., Mitchell-Olds, Thomas (2006). Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of Arabidopsis. *Molecular Ecology*. 15(2), 357-369.

Springfield, H. W. (1976). Characteristics and Management of Southwestern Pinyon-juniper Ranges: The Status of our Knowledge. *USDA Forest Service Research Paper.* RM- 160.

Stebbins Jr., C. L. (1950). *Variation and Evolution in Plants*. Columbia University Press. New York.

Stebbins, G. L. (1980). Polyploidy in plants: unsolved problems and prospects in polyploidy. *Springer.* US. 495-520.

Suo, B., Seifert, S., Kirik, V. (2013). *Arabidopsis* GLASSY HAIR genes promote trichome papillae development. *Journal of Experimental Botany*. 1-11.

Trouvé, S., Degen, L., & Goudet, J. (2005). Ecological components and evolution of selfing in the freshwater snail Galba truncatula. *Journal of Evolutionary Biology*, *18*(2), 358-370.

USDA. Natural Resources Conservation Service. (2003). Major Land Resource Area 28A: Great Salt Lake Area; Nevada Ecological Site Descriptions. 028AY003NV, 028AY012NV, 028AY014NV, 028AY016NV, 028AY018NV.

USFWS. (1995). Migratory non-game birds of management concern in the United States: The 1995 list. Office of migratory bird management. US Fish and Wildlife Service. Washington, DC.

USFWS. (2004). Recovery report to Congress: fiscal years 2001-2002. US Fish and Wildlife Service. Arlington, VA.

Vergeer, P., and W. E. Kunin. (2013). Adaptation at range margins: common garden trials and the performance of *Arabidopsis lyrata* across its northwestern European range. *New Phytologist.* 197, 989–1001.

Wang, T., G. A. O'Neill, S. N. Aitken. (2010). Integrating environmental and genetic effects to predict responses of tree populations to climate. *Ecological Applications*. 20, 153–163.

Warwick, S. I. and Al-Shehbaz, I.A. (2006). Brassicaceae: chromosome number index and database on CD-Rom. *Plant Systematics and Evolution.* 259, 237–248.

Weiss-Schneeweiss, H., Greilhuber, J, Schneeweiss, G.M. (2006). Genome size evolution in holoparasitic *Orobanche* (Orobanchaceae) and related genera. *American Journal of Botany.* 93, 148–156.

Whitney, K. D., Randell, R. A., Rieseberg, L. H. (2006). Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. *The American Naturalist*. 167(6), 794-807.

Whitton, J., Sears, C. J., Baack, E. J., Otto, S. P. (2008). The dynamic nature of apomixis in the angiosperms. *International Journal of Plant Sciences*. 169(1), 169-182.

Windham, M. D. and Al-Shehbaz, I. A. (2006). New and noteworthy species of *Boechera* (Brassicaceae) I: Sexual diploids. *Harvard Papers in Botany.* 11(1), 61–88.

Windham, M. D. and Al-Shehbaz, I. A. (2007a). New and noteworthy species of *Boechera* (Brassicaceae) II: apomictic hybrids. *Harvard Papers in Botany*. 11(2), 257-274.

Windham, M. D. and Al-Shehbaz, I. A. (2007b). New and noteworthy species of *Boechera* (Brassicaceae) III: additional sexual diploids and apomictic hybrids. *Harvard Papers in Botany*. 12(1), 235-257.

Windham, M. D. and Al-Shehbaz, I. A. (2012). *Boechera*. *Flora of North America north of Mexico.* Oxford University Press. New York

Woolstenhulme, Loreen. (2007). NSF Grant Proposal.

Yan, J., Chu, H.-J., Wang, Heng-Chang, L., Jian-Qiang, Sang, T. (2008). Population genetic structure of two *Medicago* species shaped by distinct life form, mating system and seed dispersal. *Annals of Botany*. 1-10.

Young, J. A. and Evans, R. A. (1975). Germinability of seed reserves in a big sagebrush community. *Weed Science*. 358-364.

TABLES

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.

Taxon	**Percentage of Polymorphic Loci**
Boechera thompsonii	92.86%
Boechera formosa	78.57%
Boechera duchesnensis	92.86%
Mean	88.10%
SE	4.76%

Table 2. Observed and expected heterozygosity at 14 unique loci were examined using GenAlEx 6.501 for *B. thompsonii* based on codominant data.

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

N= number of individuals; Na= number of different alleles; Ne= number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; uHe= unbiased expected heterozygosity $(2N / (2N-1)) * H_e$; F= Fixation index $(H_e - H_o) / H_e = 1 - (H_o / H_e)$.

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.

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

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)) * H_e; F= Fixation index (H_e - H_o) / H_e = 1 - (H_o / H_e).

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).

Taxon		N	Na	Ne	Ho	He	uHe	F
B. thompsonii	Mean	51.92	7.786	3.663	0.223	0.533	0.538	0.540
	SE	0.412	1.491	0.801	0.033	0.080	0.081	0.045
B. formosa	Mean	42.64	7.071	3.804	0.166	0.430	0.436	0.545
	SE	0.507	1.675	1.038	0.038	0.100	0.101	0.069
B. duchesnensis	Mean	6.857	2.286	2.032	0.908	0.482	0.520	-0.905
	SE	0.097	0.304	0.116	0.071	0.039	0.043	0.058

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.

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

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)) * H_e; F= Fixation index (H_e - H_o) / H_e = 1 - (H_o / H_e).

Table 6. *B. thompsonii* cluster (K) 1 geographical data for individual specimens – with coordinates, elevation, observed heterozygosity statistics, and county/state location information.

K	ID	Latitude	Longitude	Elevation (m)	H_o	County/St
1	43	38.6733	-108.3211	1643.90	0.40	Delta/CO
1	53	37.8075	-108.7919	2422.56	0.00	Dolores/CO
1	38	39.6257	-107.1123	1894.51	0.00	Eagle/CO
1	27	36.7323	-107.2237	2271.95	0.00	Garfield/CO
1	52	38.9686	-108.4669	1436.58	0.00	Mesa/CO
1	31	39.0317	-108.6895	1958.84	0.33	Mesa/CO
1	19	37.1722	-108.5000	2002.13	0.00	Montezuma/CO
1	39	38.2161	-108.6058	1675.91	0.07	Montrose/CO
1	44	38.1633	-109.0040	2099.08	0.40	Montrose/CO
1	23	39.9447	-108.7569	1763.71	0.60	Rio Blanco/CO
1	28	39.9447	-108.7569	1763.71	0.13	Rio Blanco/CO
1	8	39.6902	-108.9986	1936.89	0.47	Rio Blanco/CO
1	48	36.7426	-105.6815	2219.51	0.08	Taos/NM
1	3	40.1706	-110.3319	1685.06	0.53	Duchesne/UT
1	7	40.1808	-110.4983	1771.34	0.60	Duchesne/UT
1	4	37.5489	-112.0471	1777.13	0.53	Garfield/UT
1	9	39.0356	-109.7024	1745.42	0.07	Grand/UT
1	21	37.2855	-112.5375	1928.96	0.47	Kane/UT
1	6	37.4431	-112.4925	2235.97	0.73	Kane/UT
1	17	38.0135	-109.3496	1927.74	0.07	San Juan/UT
1	51	37.9181	-109.9453	2190.85	0.73	San Juan/UT
1	30	40.3667	-109.1197	2464.93	0.20	Uintah/UT
1	29	41.0426	-107.7130	1985.36	0.47	Carbon/WY
1	10	41.0157	-108.2598	2116.46	0.00	Sweetwater/WY

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H_o = 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.

K	ID	Latitude	Longitude	Elevation (m)	H_o	County/St
2	41	36.5316	-109.2173	2110.74	0.53	Apache/AZ
2	42	36.4431	-109.1797	2213.46	0.60	Apache/AZ
2	24	36.8124	-112.0854	1819.66	0.00	Coconino/AZ
2	33	38.3496	-109.0326	2075.99	0.27	Montrose/CO
2	2	38.0066	-108.9338	2140.61	0.07	San Miguel/CO
2	49	40.8972	-109.7069	2319.83	0.00	Daggett/UT
2	50	38.1365	-110.1442	1967.18	0.53	Garfield/UT
2	11	37.3076	-109.5585	1369.16	0.60	San Juan/UT
2	25	37.9181	-109.9453	2190.29	0.53	San Juan/UT
2	32	37.6028	-110.0261	1823.31	0.00	San Juan/UT
2	36	37.9181	-109.9453	2190.29	0.54	San Juan/UT
2	5	41.4259	-109.3472	1955.90	0.60	Sweetwater/WY

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H_o = 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.

K	ID	Latitude	Longitude	Elevation (m)	H_o	County/St
3	1	36.8022	-107.9036	1945.23	0.00	San Juan/NM
3	16	39.5484	-107.3182	1829.11	0.07	Rio Arriba/NM
3	26	36.9103	-106.9989	2125.37	0.00	Rio Arriba/NM
3	40	37.0694	-107.8287	2011.37	0.07	La Plata/CO
3	45	37.32	-108.6783	1766.31m	0.00	Montezuma/CO

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.

K	ID	Latitude	Longitude	Elevation (m)	H_o	County/St
4	14	38.8796	-107.5349	2127.50	0.07	Delta/CO
4	37	39.6747	-107.0951	2051.61	0.53	Eagle/CO
4	20	38.4828	-107.1864	2313.13	0.40	Gunnison/CO
4	15	38.4589	-107.2986	2416.15	0.29	Gunnison/CO
4	13	38.5646	-107.7909	2085.44	0.07	Montrose/CO
4	18	38.5014	-107.7308	2142.74	0.00	Montrose/CO
4	35	38.6172	-107.5805	2236.93	0.00	Montrose/CO
4	22	36.2064	-107.5603	2287.83	0.00	Sandoval/NM
4	46	35.9803	-105.9126	1936.09	0.00	Santa Fe/NM
4	12	35.9523	-105.9106	2034.24	0.07	Santa Fe/NM
4	34	36.6740	-105.6871	2081.48	0.00	Taos/NM
4	47	36.6740	-105.6871	2081.48	0.07	Taos/NM

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.

K	ID	Latitude	Longitude	Elevation	H_o	County/St
1	70	38.5008	-108.0265	1824.85m	0.07	Apache/AZ
1	86	37.8514	-110.6536	1841.01m	0.40	Apache/AZ
1	72	36.7855	-107.9814	1816.93m	0.20	Coconino/AZ
1	74	36.8622	-107.9798	1749.87m	0.07	Mohave/AZ
1	93	38.4613	-109.7850	1402.40m	0.07	Navajo/AZ
1	78	36.8401	-108.0127	1739.51m	0.00	Navajo/AZ
1	96	40.5173	-109.5240	1684.95m	0.40	Delta/CO
1	54	36.9148	-109.3382	1741.95m	0.38	Garfield/CO
1	92	37.2486	-109.6605	1346.92m	0.29	Mesa/CO
1	94	37.2486	-109.6605	1346.92m	0.29	Mesa/CO
1	61	38.7628	-108.2433	1574.31m	0.09	Mesa/CO
1	58	36.8814	-112.8950	1585.58m	0.33	Mesa/CO
1	95	40.5173	-109.5240	1684.95m	0.29	Mesa/CO
1	62	38.9678	-108.4658	1480.73m	0.00	Moffat/CO
1	79	39.5472	-110.6486	1713.91m	0.13	Montezuma/CO
1	98	41.5514	-109.5233	1870.88m	0.00	Montrose/CO
1	60	36.6845	-110.5175	1948.61m	0.31	Montrose/CO
1	88	38.8331	-109.2882	1269.81m	0.25	San Juan/NM
1	91	37.0383	-112.3311	1579.79m	0.27	San Juan/NM
1	57	36.6353	-111.6319	1655.38m	0.20	San Juan/NM
1	69	38.2164	-108.6050	1661.78m	0.11	San Juan/NM
1	73	36.8622	-107.9798	1749.87m	0.07	San Juan/NM
1	56	36.1543	-109.4439	1763.28m	0.27	San Juan/NM
1	76	36.8695	-107.9472	1770.60m	0.20	San Juan/NM
1	97	41.0395	-107.8490	1917.52m	0.20	San Juan/NM
1	59	35.8115	-110.1895	1957.75m	0.13	San Juan/NM
1	80	40.8642	-109.0750	1722.44m	0.13	Carbon/UT
1	83	40.1797	-110.4969	1752.92m	0.00	Daggett/UT
1	65	39.0158	-108.4147	1521.88m	0.00	Duchesne/UT
1	81	40.1561	-110.2552	1673.98m	0.00	Duchesne/UT
1	77	36.8336	-107.9751	1766.03m	0.00	Emery/UT
1	90	37.1083	-112.0064	1819.67m	0.29	Emery/UT
1	63	39.1365	-108.7554	1451.17m	0.29	Grand/UT
1	85	39.1974	-110.3863	1530.41m	0.29	Grand/UT
1	68	37.3430	-108.8172	1687.39m	0.50	Grand/UT
1	89	38.7042	-109.3869	1273.77m	0.00	Kane/UT
1	75	36.8695	-107.9472	1770.60m	0.13	Kane/UT
1	87	38.5744	-109.5270	1364.91m	0.07	San Juan/UT
1	84	39.1936	-110.3936	1549.31m	0.36	San Juan/UT
1	71	36.8191	-107.9908	1736.46m	0.14	San Juan/UT
1	64	39.1184	-109.0419	1320.40m	0.07	Uintah/UT
1	82	40.1708	-110.3292	1673.06m	0.00	Uintah/UT
1	55	36.9283	-109.1619	1647.46m	0.14	Wayne/UT
1	67	40.7410	-108.8406	1805.35m	0.00	Carbon/WY
1	66	38.9333	-108.5678	1988.53m	0.14	Sweetwater/WY

K = assigned cluster; ID = individual number; Latitude; Longitude; Elevation; H_o = observed heterozygosity; County/State.

Table 11. Morphological characteristics of *B. thompsonii*.

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

Table 12. Morphological characteristics of *B. formosa*.

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

Table 13. Morphological characteristics of *B. duchesnensis*.

Locus	***Boechera duchesnensis***
Perennial	Long lived, apomictic, caudex woody
Stems/Caudex	1 per caudex from center of rosette
Basal Leaves	Blade linear-oblanceolate
Cauline Leaves	3-8 not concealing stem
Racemes	11-22 flowered, unbranched
Fruiting Pedicels	Horizontal, straight
Stem Trichomes	Short-stalked, 2-7 rayed
Basal Leaf Trichomes	Densely pubescent, short stalked, 4-8 rayed
Fruiting Pedicel Trichomes	Appressed, branched
Flowers	Divaricate-ascending, petals whitish to pale lavender
Fruits	Erect to ascending, straight
Seeds	Sub-biseriate, wing continuous

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.

ID	I3	A1	B20	B11	C8	I14	B9	E9	B18	BF3	B6	BF19	A3	B266
99	89-91	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-308	155-157	248-253	123-139
100	89-91	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-308	155-157	248-253	139-143
101	89-91	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-308	155-157	248-253	139-143
102	89-91	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-308	155-157	248-253	139-143
103	91-0	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-310	155-159	248-253	139-0
104	89-91	238-0	199-205	80-84	230-252	217-227	72-84	193-205	112-117	95-105	299-308	155-157	248-253	139-141
105	89-91	238-0	199-205	80-84	230-252	0-0*	72-84	193-205	112-117	95-105	0-0*	155-157	248-253	140-142

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.

FIGURES

Morphological Characteristics

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

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)*.

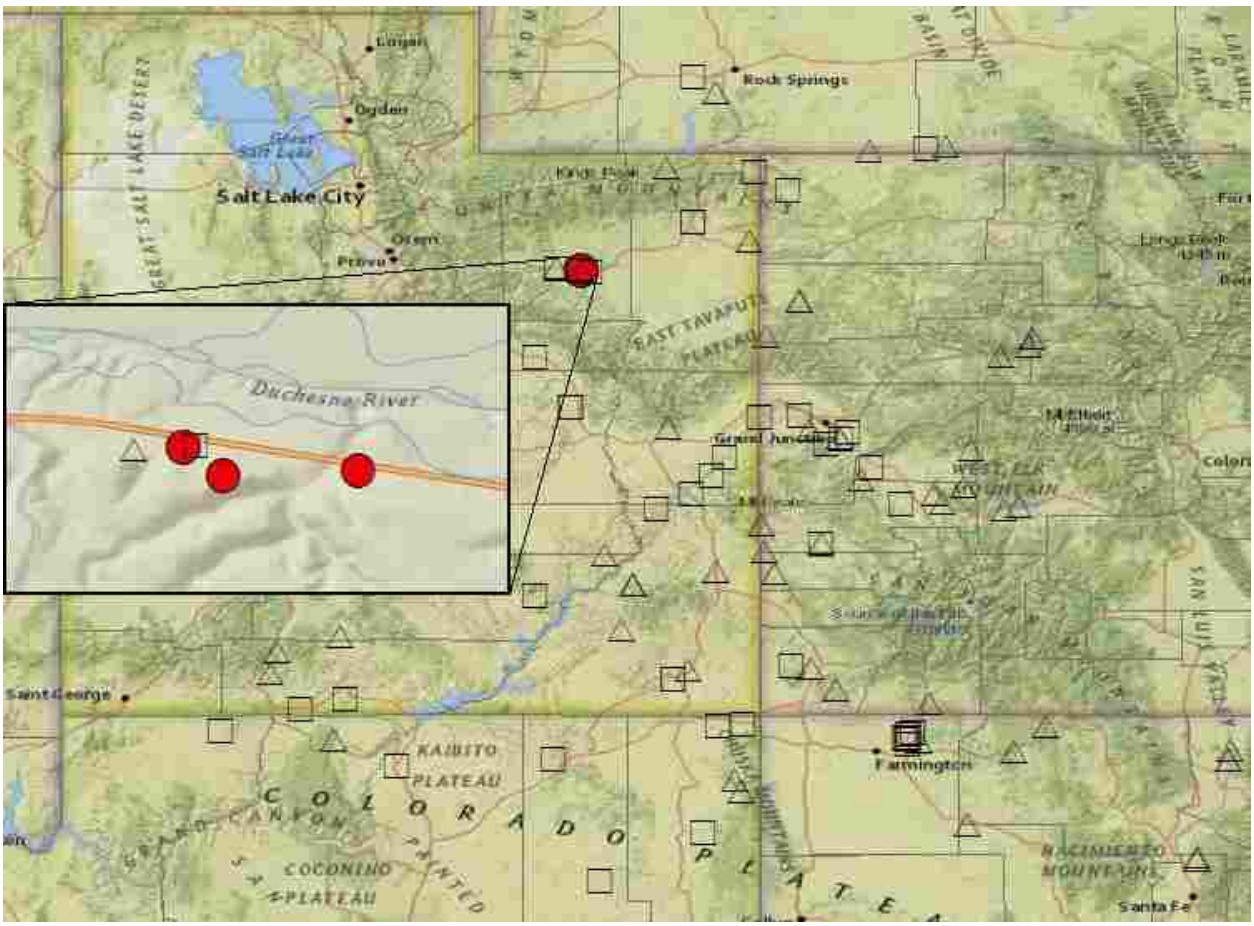

Figure 2. Distribution of the studied *Boechera* spp. individuals across their geographic range a) *B. thompsonii* indicated with triangles, b) *B. formosa* indicated with squares, c) *B. duchesnensis* indicated with red circles. Greater detail shown at Duchesne site highlights *B. duchesnensis*.

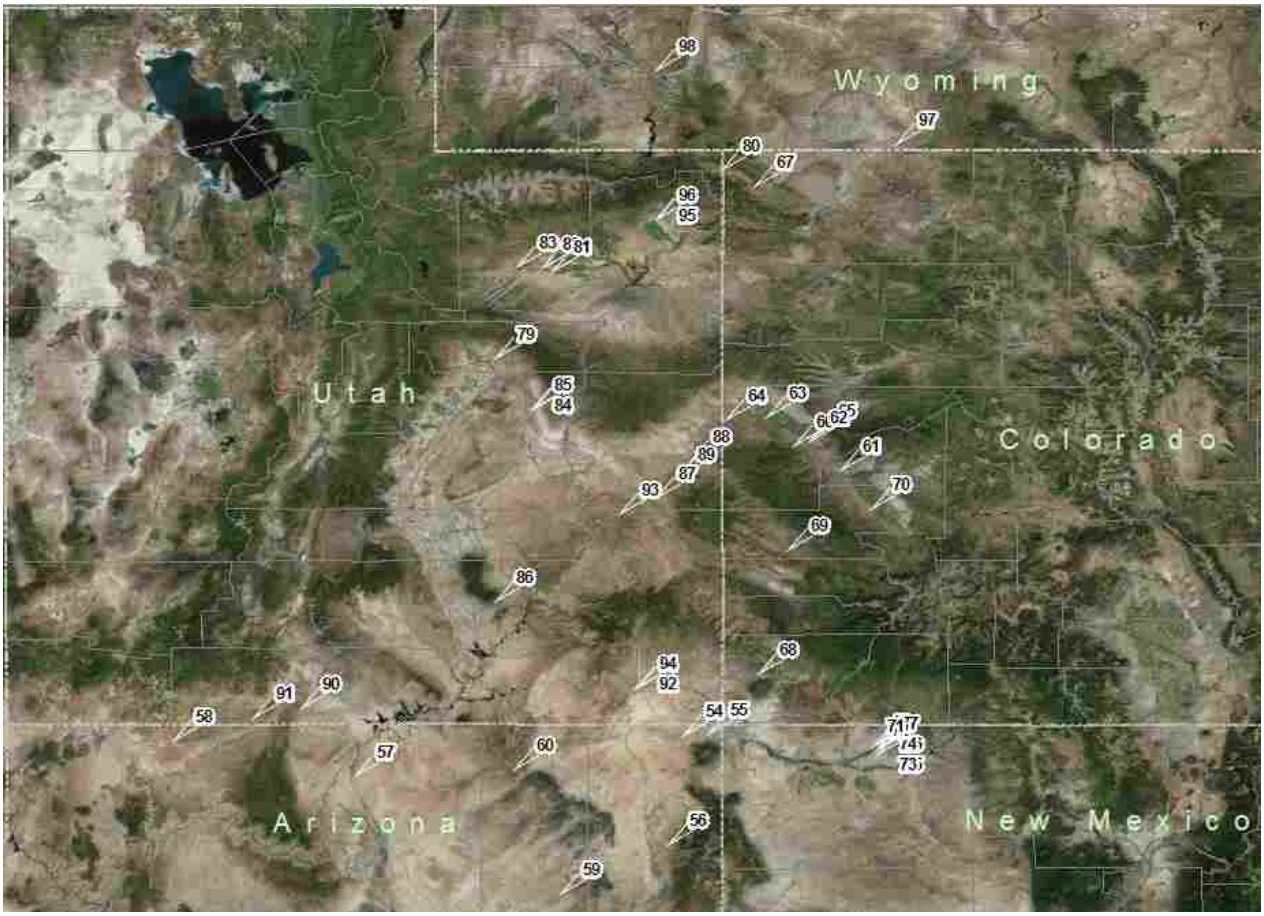

Figure 3. Geographic range distribution of *B. formosa* individuals, identified by species ID number, across 18 counties of 5 states demonstrating their placement in relation to physical characteristics and possible physical boundaries.

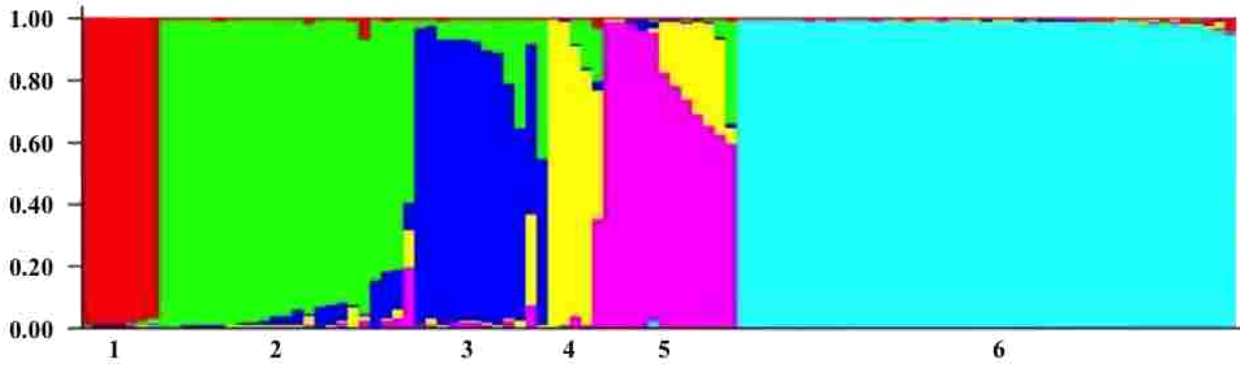

Figure 4. Genotypic population clustering graph based on 105 *Boechera* individuals and displaying 6 genetically distinct clusters (K = 6). STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) was used to produce graph showing *B. duchesnensis* (1) in red; *B. thompsonii* (2, 3, 4, 5) in green, blue, yellow, and fuchsia; and *B. formosa* (6) in light blue.

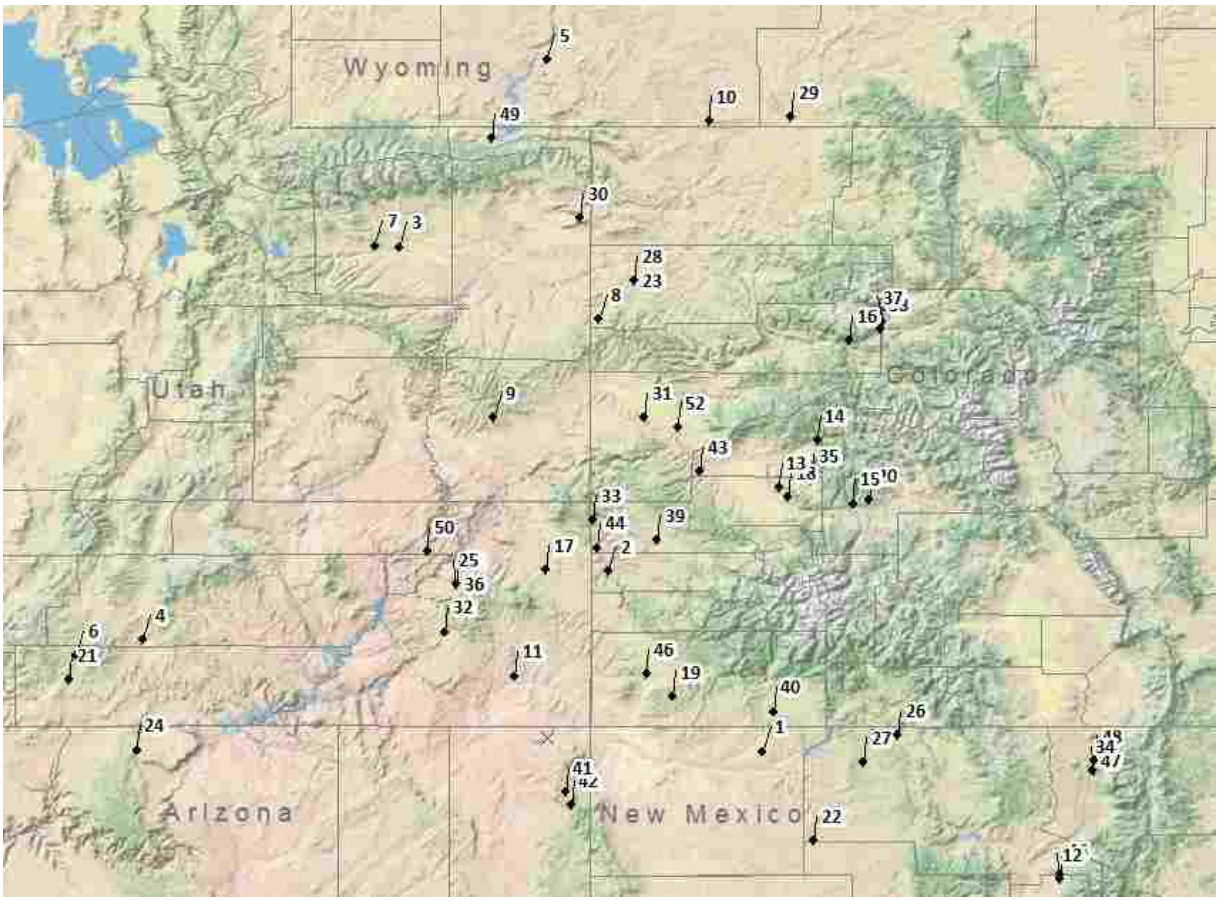

Figure 5. Geographic distribution of *B. thompsonii* individuals identified by species ID number, across 27 counties in 5 states demonstrating their placement in relation to physical characteristics and possible physical boundaries..

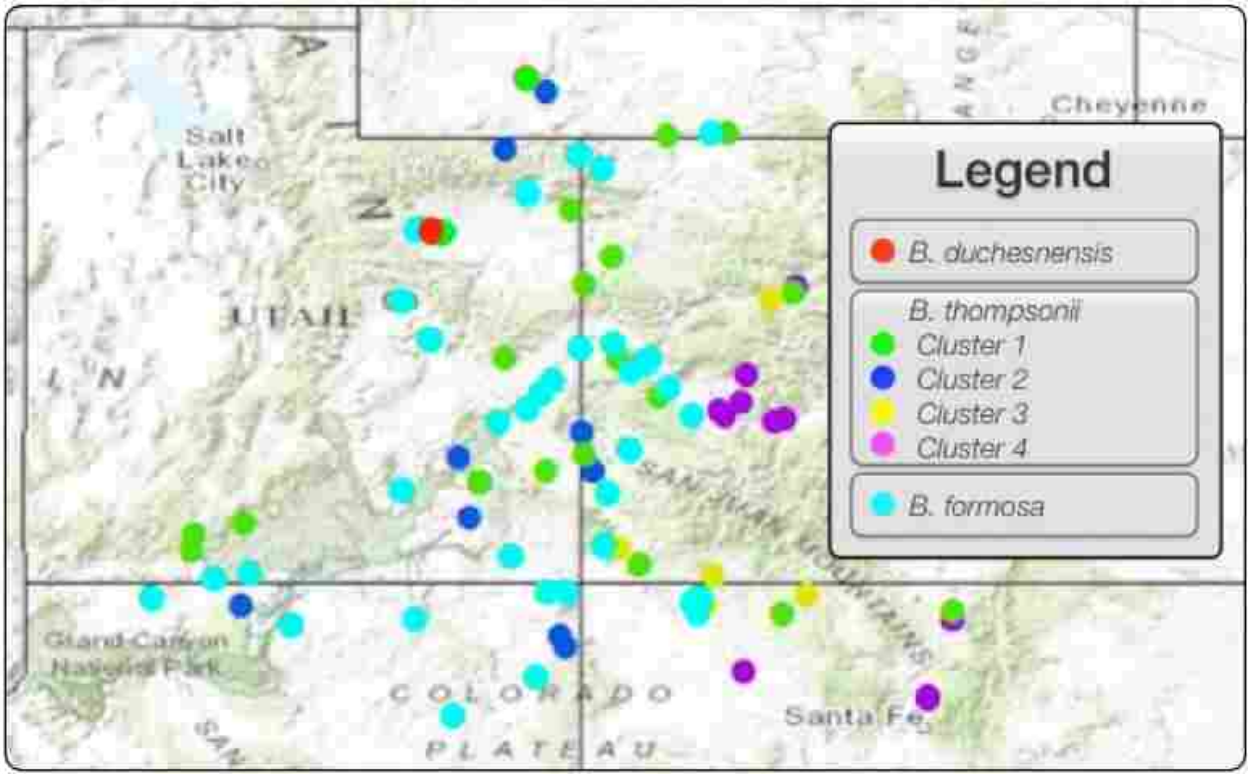

Figure 6. Distribution of 105 *Boechera* spp. individuals using data mapped with ArcGIS 10.1 (ESRI, 2012) and displaying the 6 genetic clusters' (K = 6) grouping, also using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007). *B. duchesnensis* is represented by red circles; *B. thompsonii* is represented by green, blue, yellow, and green circles and; *B. formosa* is represented by turquoise circles.

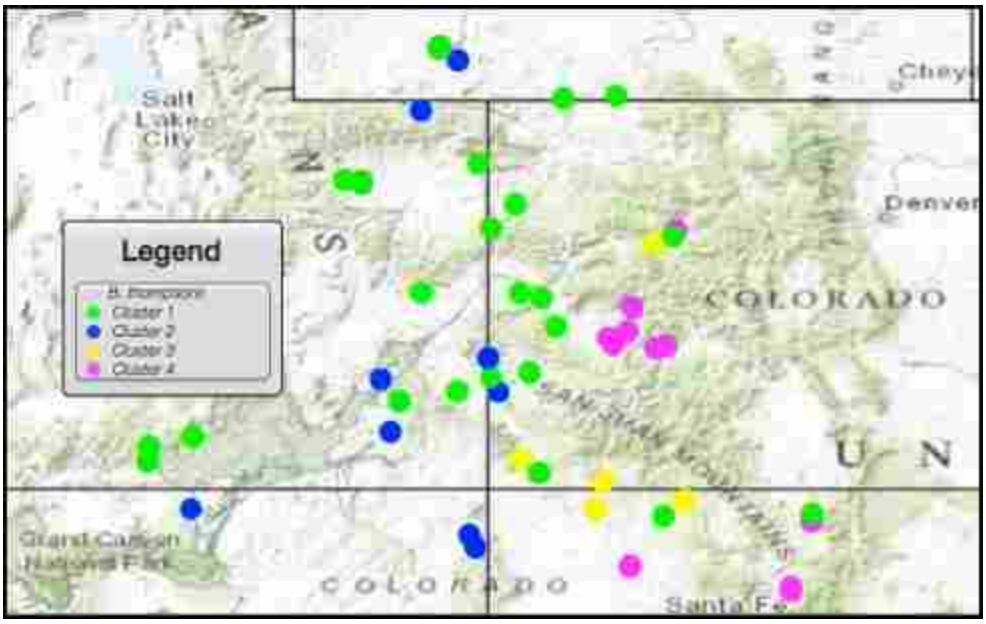

Figure 7. Distribution of 53 *B. thompsonii* individuals using data mapped with ArcGIS 10.1 (ESRI, 2012) and displaying 4 genetically distinct clusters' (K = 4) grouping, also using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007). Cluster 1 is represented by green circles, cluster 2 is represented by blue circles, cluster 3 is represented by yellow circles, and cluster 4 is represented by fuchsia circles.

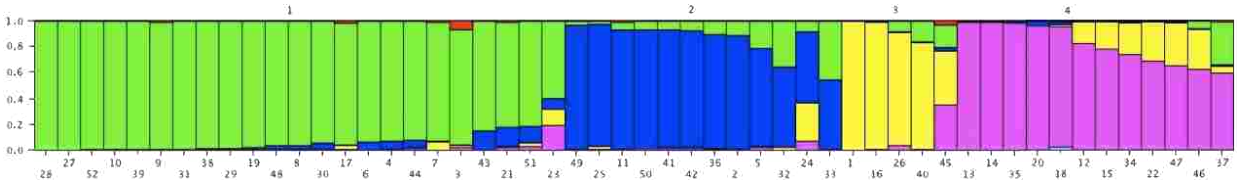

Figure 8. Genotypic clustering graph for 52 *B. thompsonii* individuals at 14 loci with four distinct clusters identified (K = 4). STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) was used to produce this graph showing frequency of allelic similarity on the X axis and identified individuals by ID number on the Y axis.

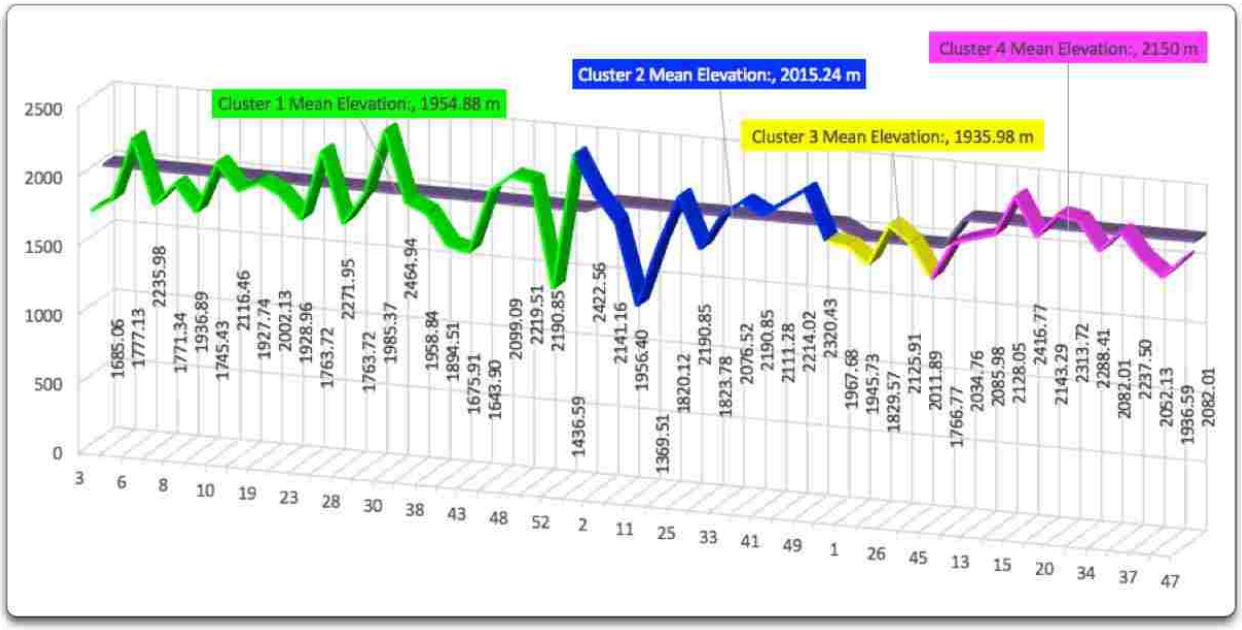

Figure 9. Elevation of each individual by Specimen ID and the mean elevation for each of the 4 identified clusters within the population of *B. thompsonii*. Altitudes from lowest to highest: cluster 3, cluster 1, cluster 2, then cluster 4.

X axis = Specimen ID Number; Y axis = Elevation; Green = Cluster (K) 1; Blue = Cluster (K) 2; Yellow = Cluster (K) 3; Fuchsia = (K) 4.

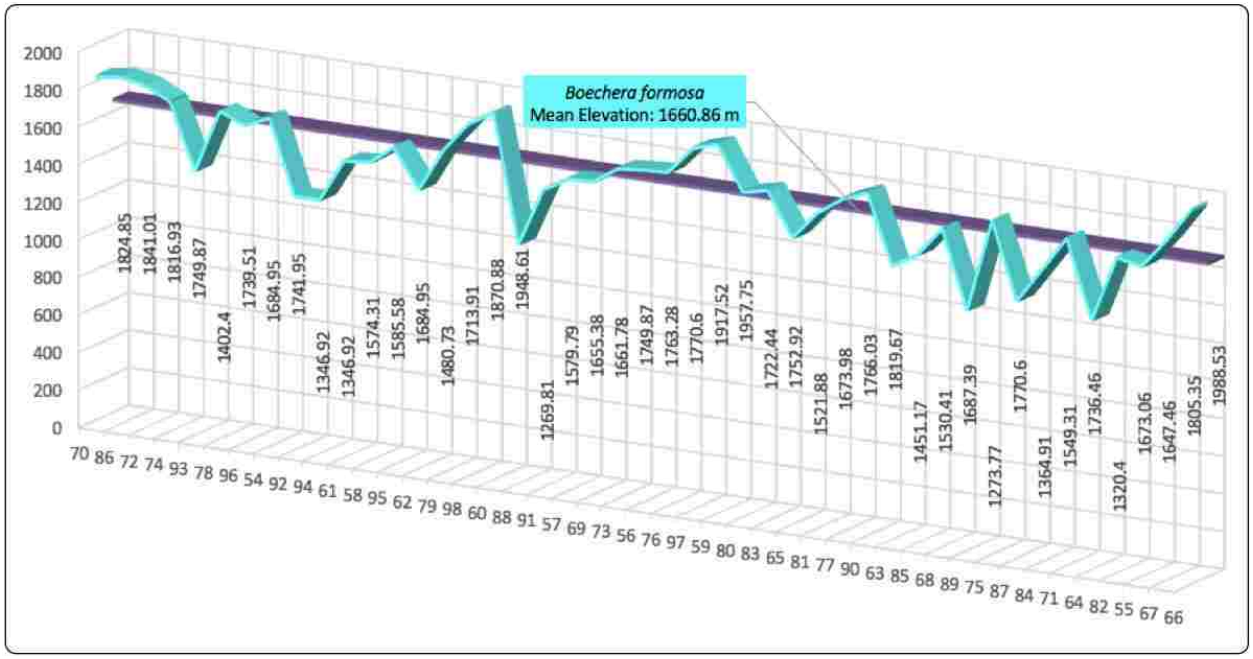

Figure 10. Elevation of each individual by Specimen ID and the mean elevation for the single population cluster of *B. formosa*. Assigned color is turquoise. X axis = Specimen ID Number; Y axis = Elevation.

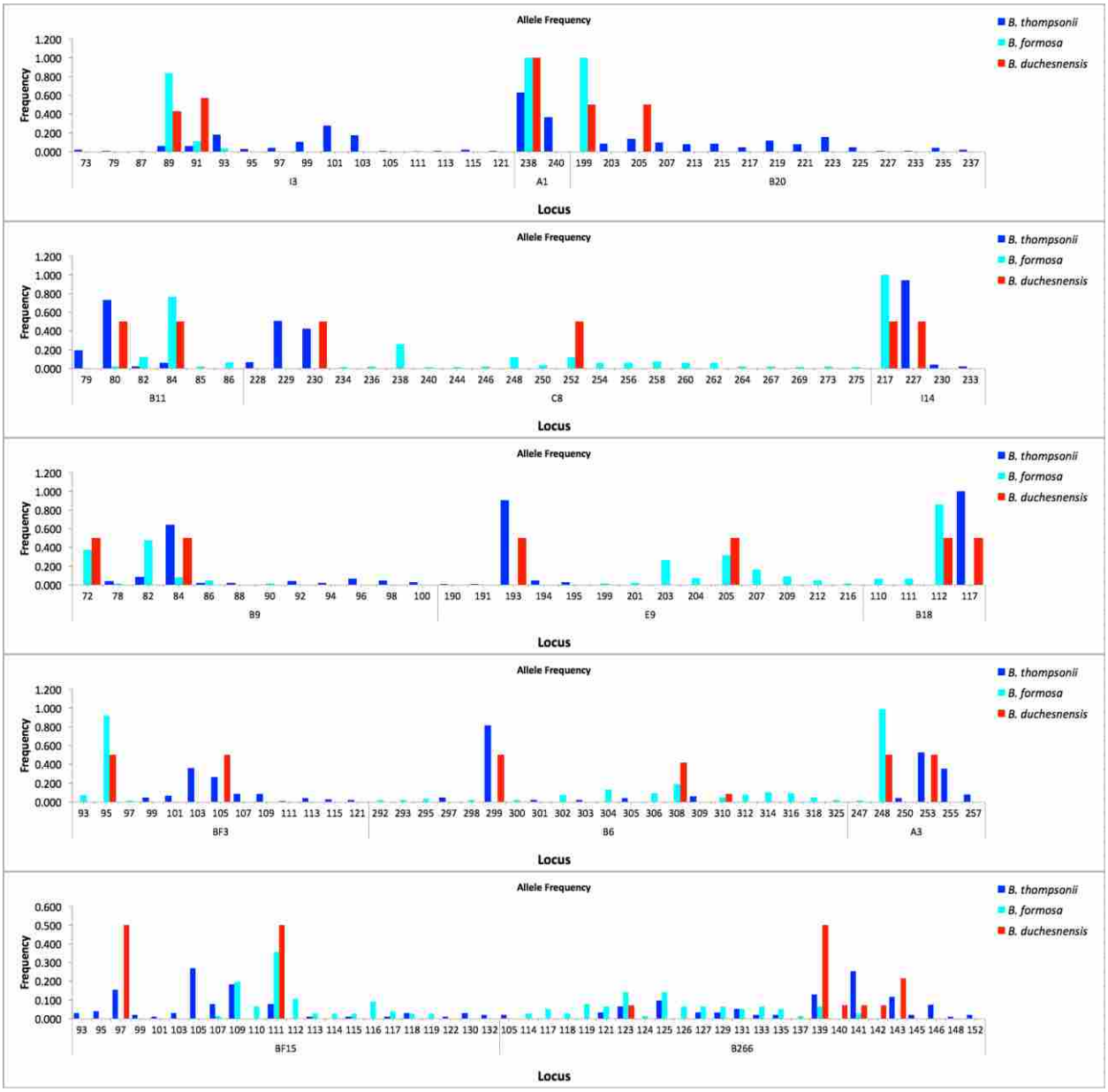

Figure 11. Percentage of allelic presence per taxon from 14 independently assorting loci across 114 total individuals of *B. thompsonii*, *B. formosa*, and *B. duchesnensis* calculated using GenAlEx 6.501 (Peakall and Smouse, 2006, 2012). Locus B266 shows six available alleles and locus B6 shows 3 available alleles in *B. duchesnensis* showing hybridization from *B. thompsonii* and *B. formosa* has occurred more than one time by crossing.

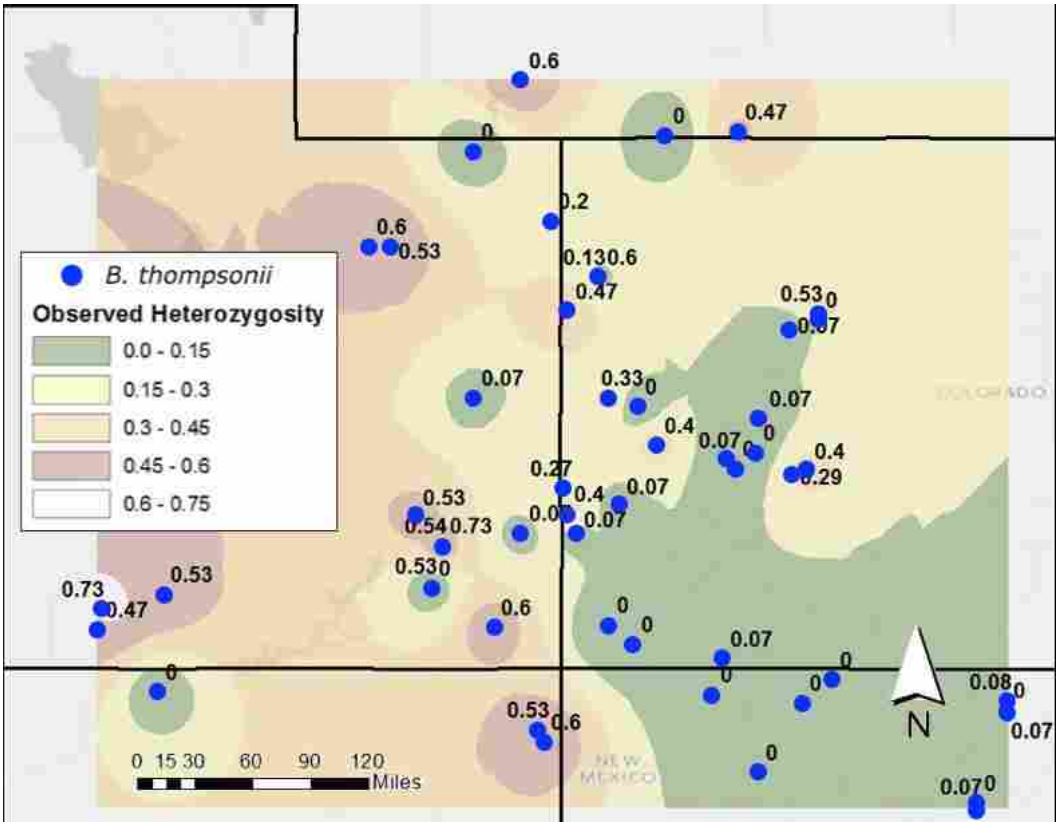

Figure 12. Observed heterozygosity (H_o) of *B. thompsonii* throughout its geographic range.

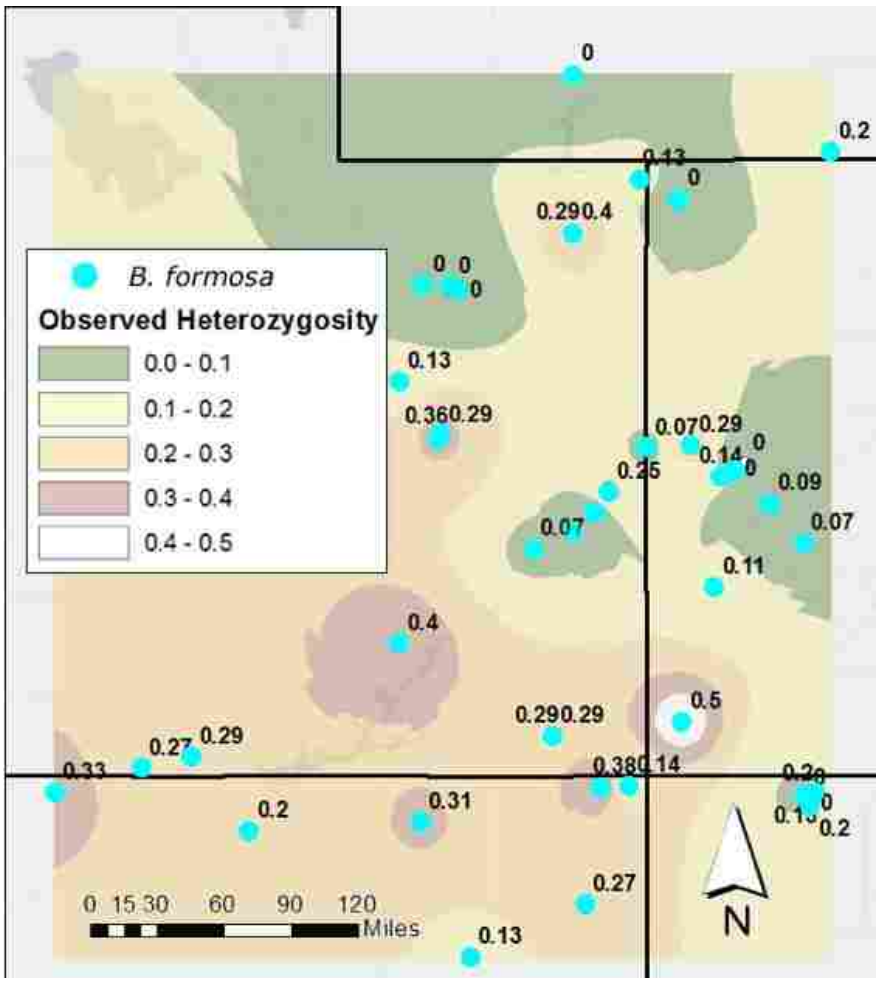

Figure 13. Observed heterozygosity (H_o) of *B. formosa* throughout its geographic range.

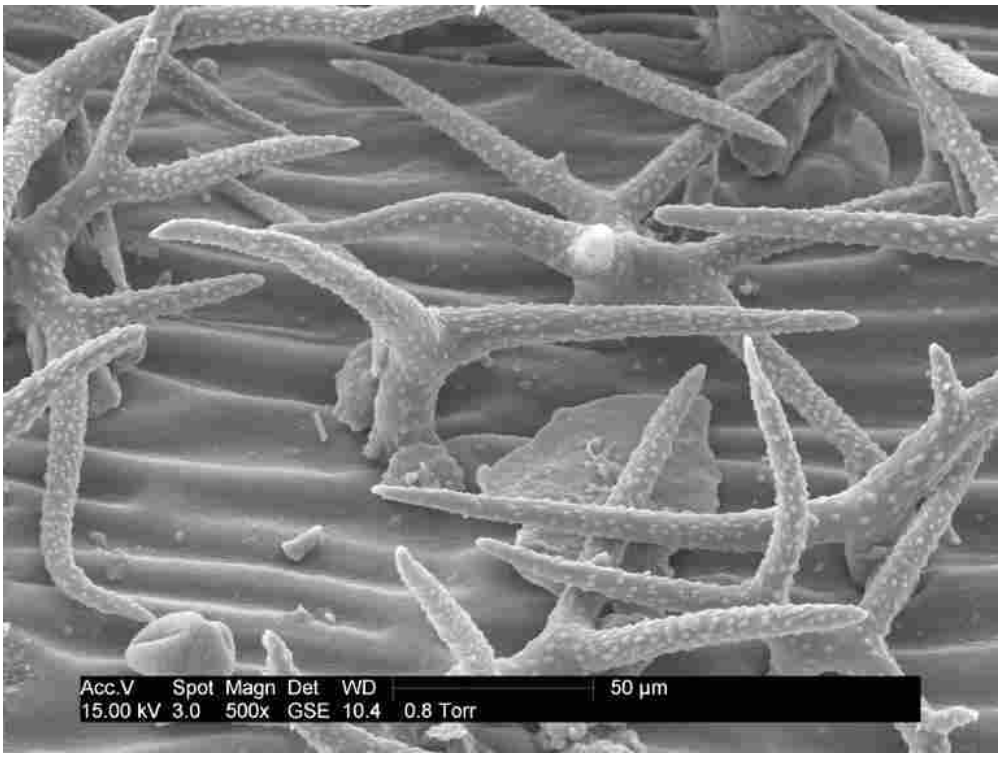

Figure 14. This image shows morphological characteristics of *B. formosa* at medium magnification – including the existence of papillae on individual basal leaf trichomes which are short-stalked and simple or 2-rayed. Image also shows broken trichome tips and debris due to sample handling and mounting.

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.

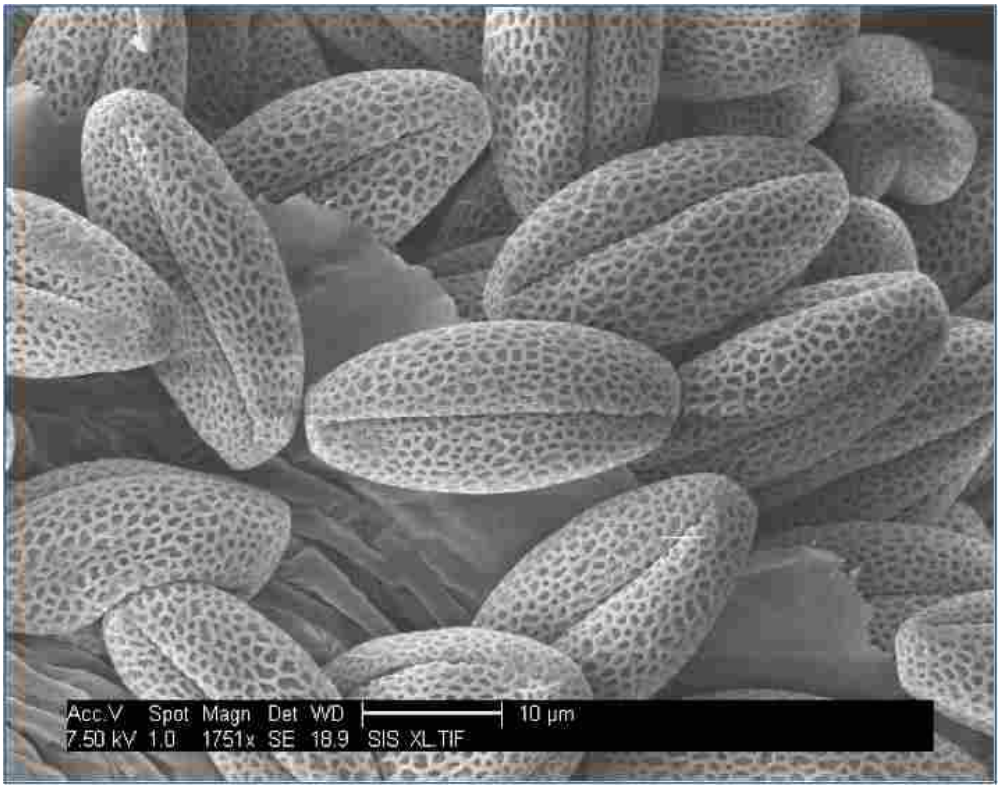

Figure 15b. This image shows morphological characteristics of *Boechera formosa* pollen at high magnification – especially the existence of symmetrically-shaped ellipsoid pollen grains and their three colpi. Image also shows portions of the anther.

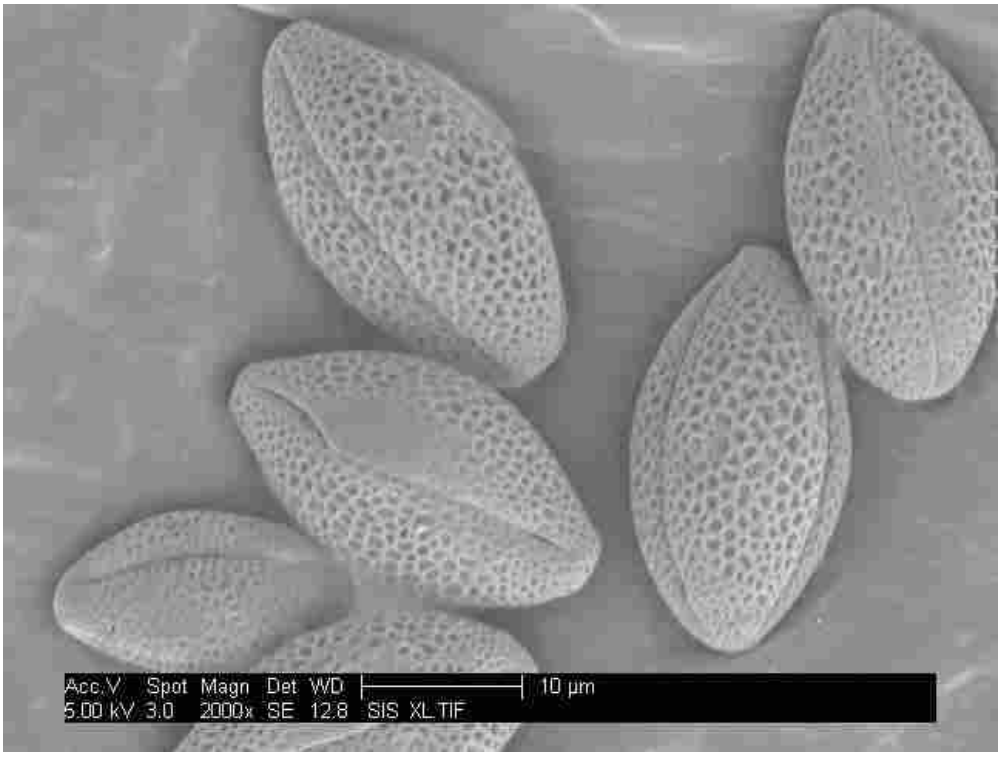

Figure 16. This image shows morphological characteristics of *B. thompsonii* pollen at high magnification – especially the existence of symmetrically-shaped ellipsoid pollen grains and their three colpi.

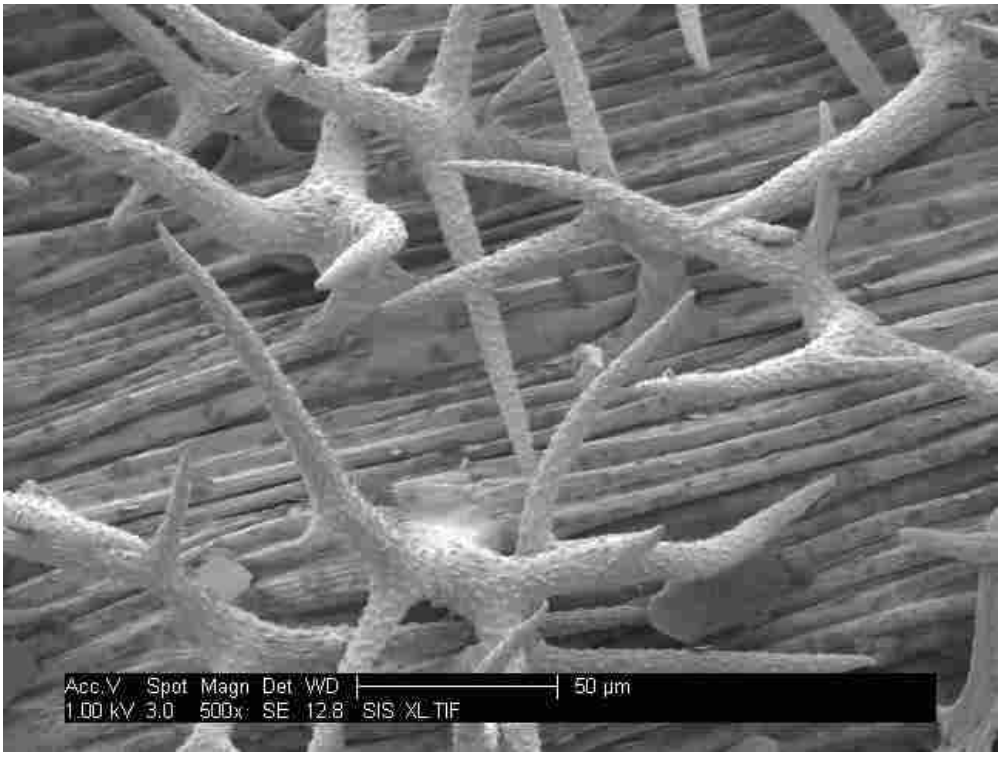

Figure 17. This image shows morphological characteristics of *B. duchesnensis* trichomes at medium magnification – notably the existence of short-stalked 2-7 rayed trichomes on a caudex stem presenting with sparsely intermediate characteristics of distally glabrous *B. thomspsonii* and pubescent *B. formosa*. Image also shows grains of dust or debris either from the field or from handling and preparation.

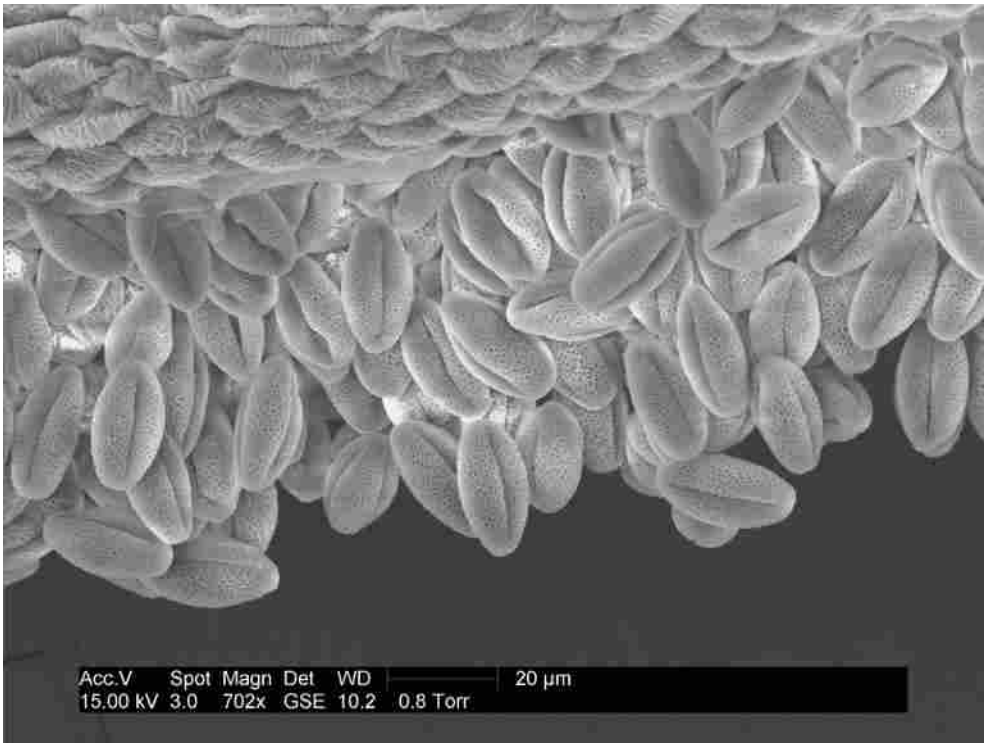

Figure 18. This image shows typical morphological characteristics of pollen from the genus *Boechera* at medium magnification – notably the typical symmetrical ellipsoid pollen grains with three colpi of sexual diploid individuals which are relatively smaller - 13-20 µm - in diameter than the three species studied herein. Image also shows portions of the petal.

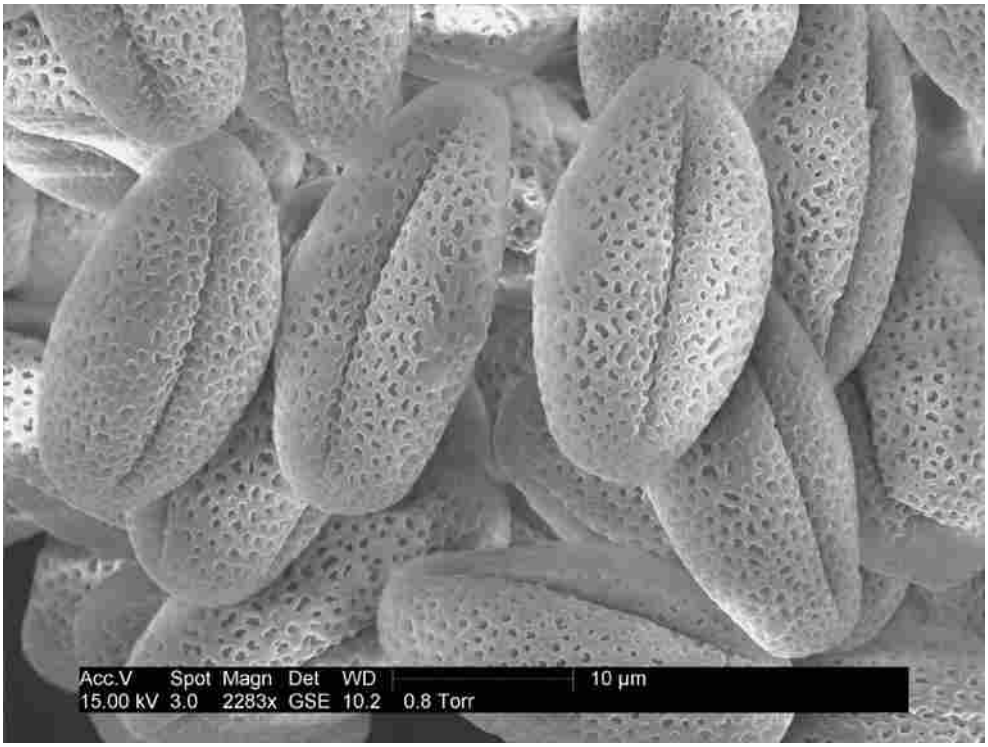

Figure 19. This image shows typical morphological pollen characteristics of the genus *Boechera* at high magnification – notably the typical symmetrical ellipsoid pollen grains with three colpi of sexual diploid individuals which are relatively smaller - 13-20 µm - in diameter than the three species studied herein. Image also shows portions of the petal.

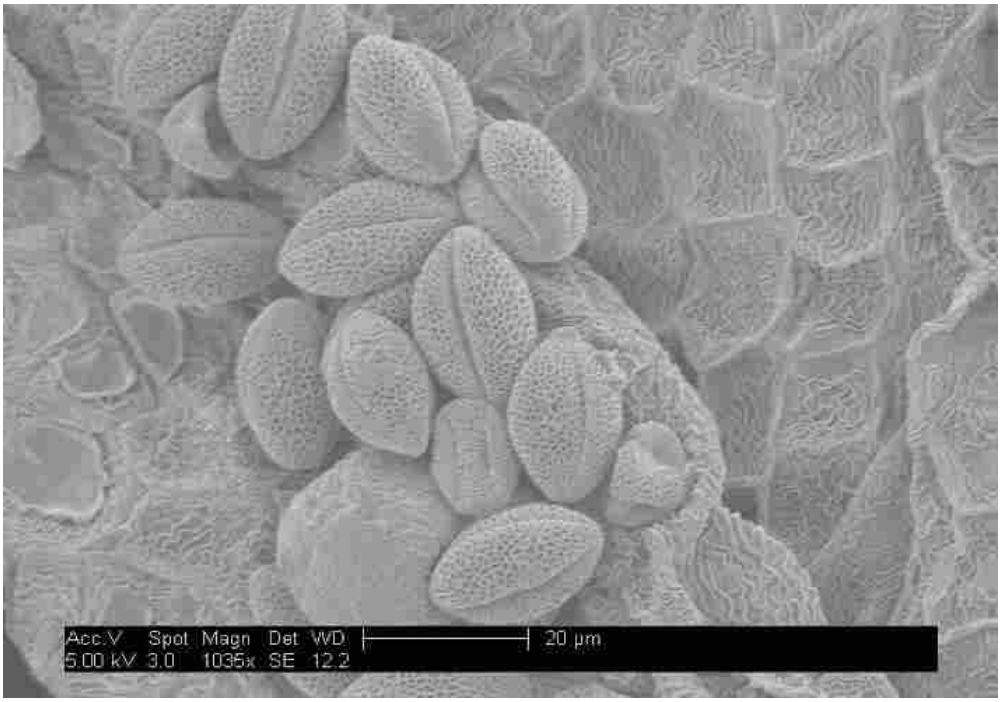

Figure 20. This image demonstrates representative morphological characteristics of *B. duchesnensis* pollen at medium magnification – most notably low pollen count and abortive and malformed pollen grains. Image also shows portions of the petal and ellipsoid pollen grain shape (where not malformed).

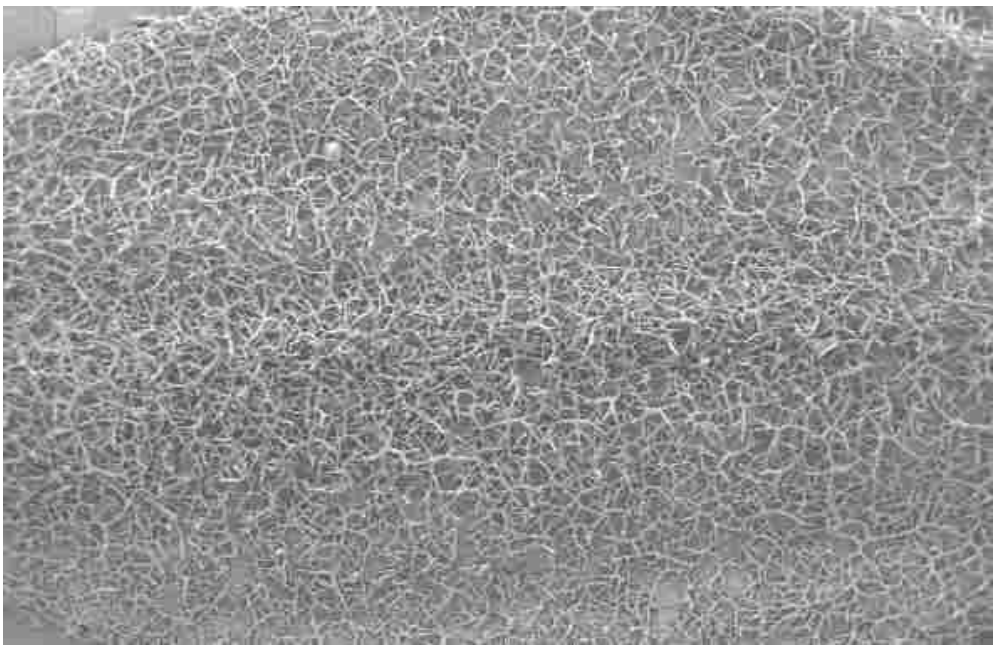

Figure 21. This image demonstrates representative morphological characteristics of *B. formosa* at low magnification – most notably densely pubescent trichomes covering a cauline leaf. This morphological characteristic helps to reflect sunlight and protect delicate tissues in hot, dry environments.

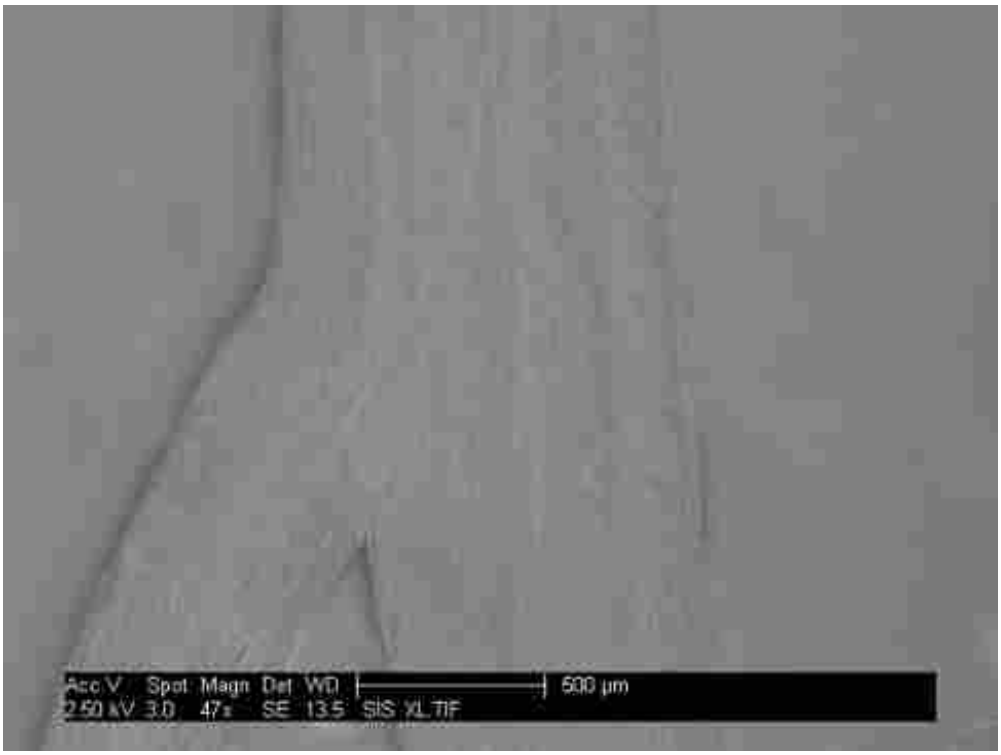

Figure 22. This image demonstrates representative morphological characteristics of *B. thompsonii* at low magnification – most notably a glabrous (free from trichomes) fruiting pedicel.

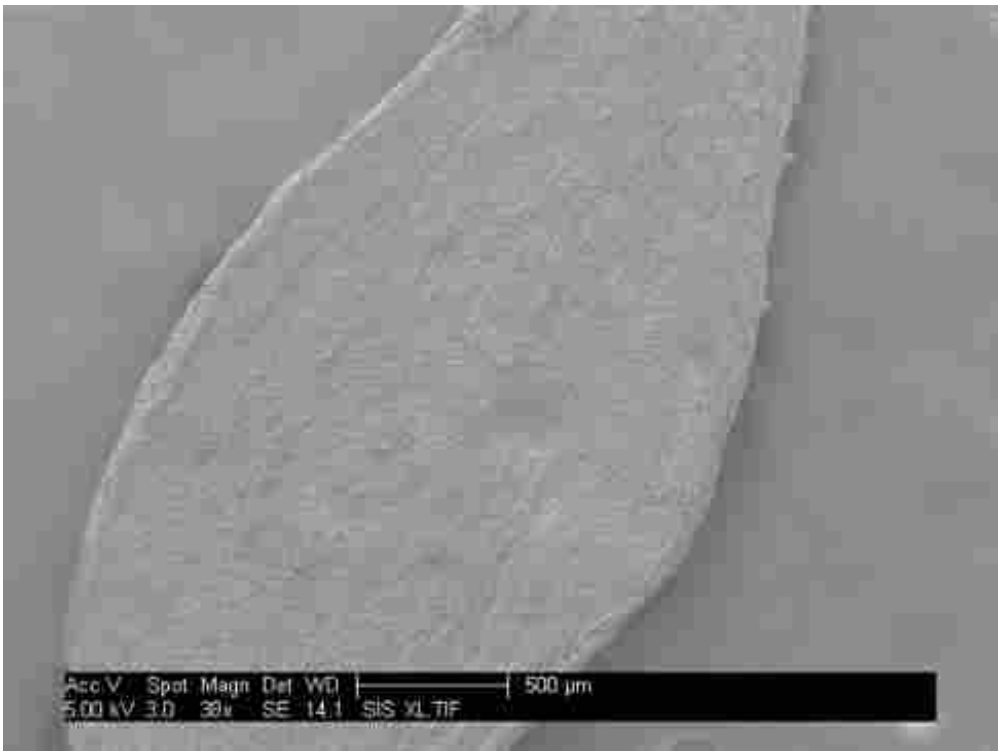

Figure 23. This image demonstrates typical morphological characteristics of the genus *Boechera* at low magnification – most notably a glabrous (free from trichomes) basal leaf with linear-oblanceolate shape.

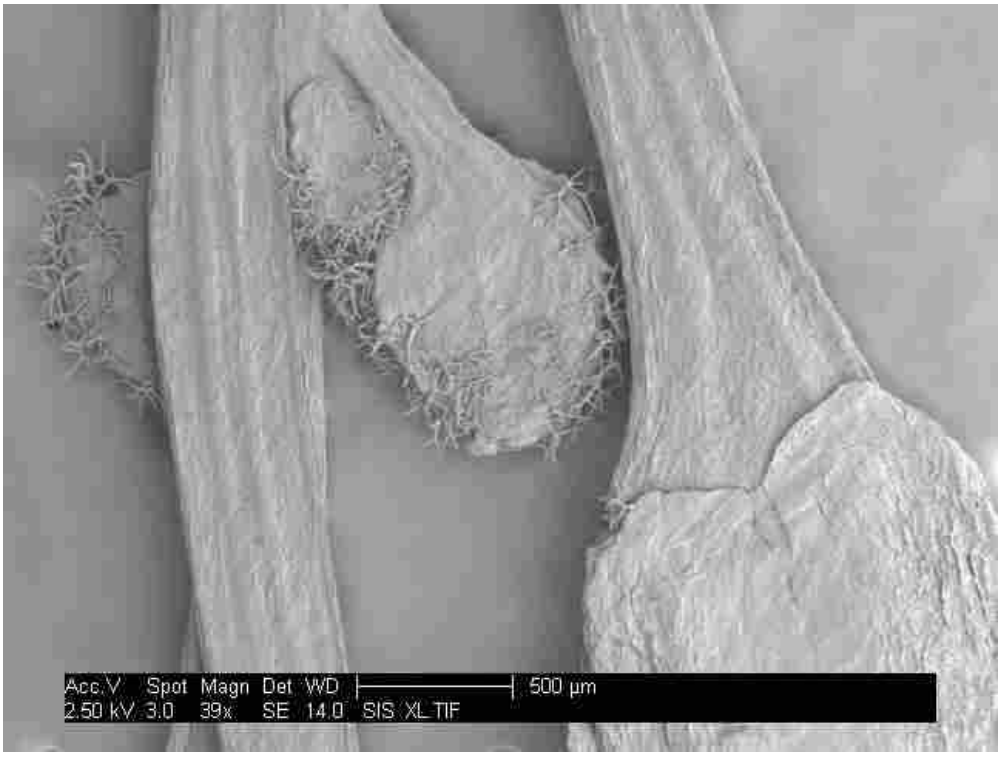

Figure 24. This image shows two specimens of *B. thompsonii* from the same individual exhibiting morphological characteristics at low magnification and on the same stub. The specimen on the left side shows basal leaves that are distally pubescent (trichomes present on the outer edges of the leaf). The specimen on the right shows a glabrous (lacking trichomes) fruiting pedicel.

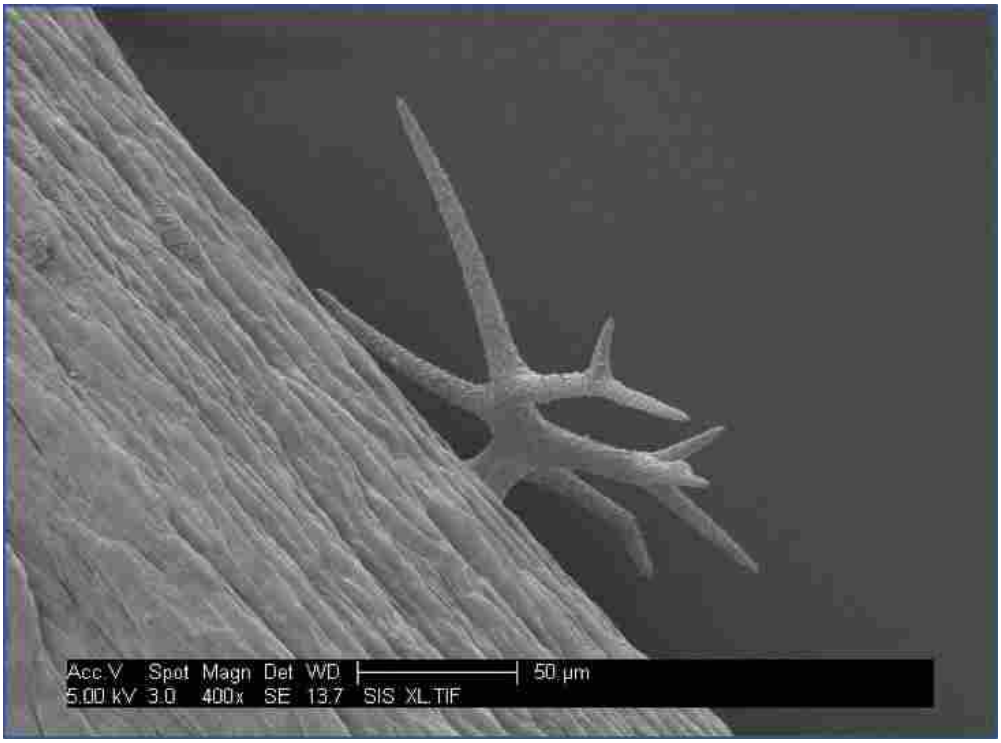

Figure 25. This image demonstrates typical morphological characteristics of *B. duchesnensis* at medium magnification – most notably a stem that is sparsely pubescent distally with a relatively larger trichome that appears almost stellate (star-shaped) due to its short-stalked, many rays characteristic.

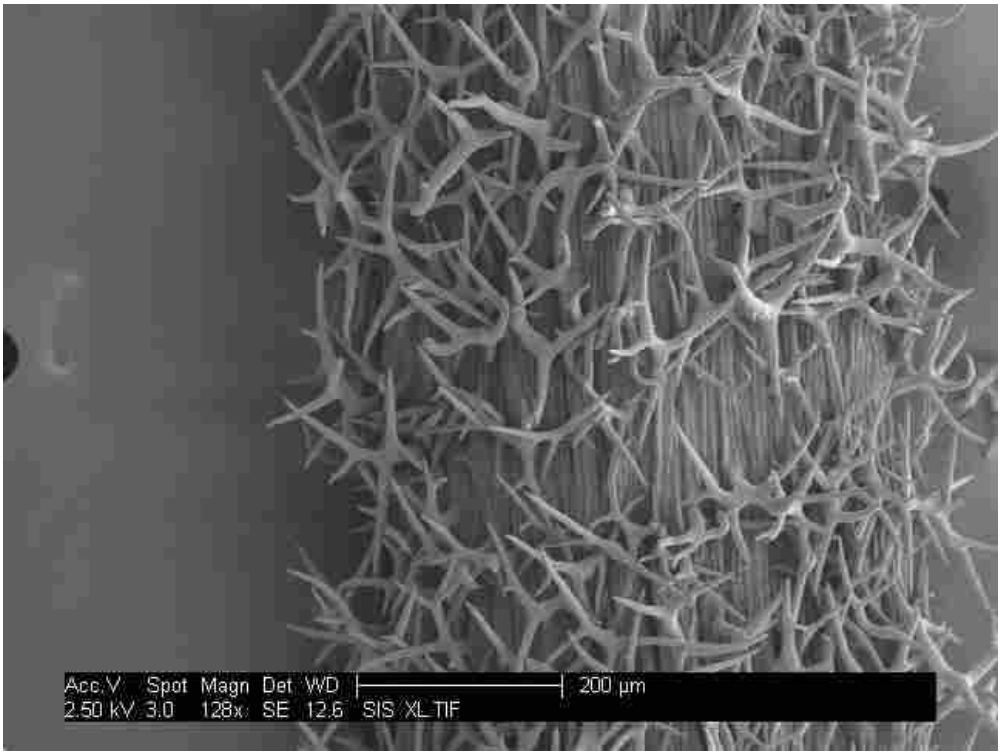

Figure 26. This image demonstrates morphological characteristics of *B. formosa* at medium magnification – most notably the distal end of a stem that is densely pubescent (covered with trichomes).

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.

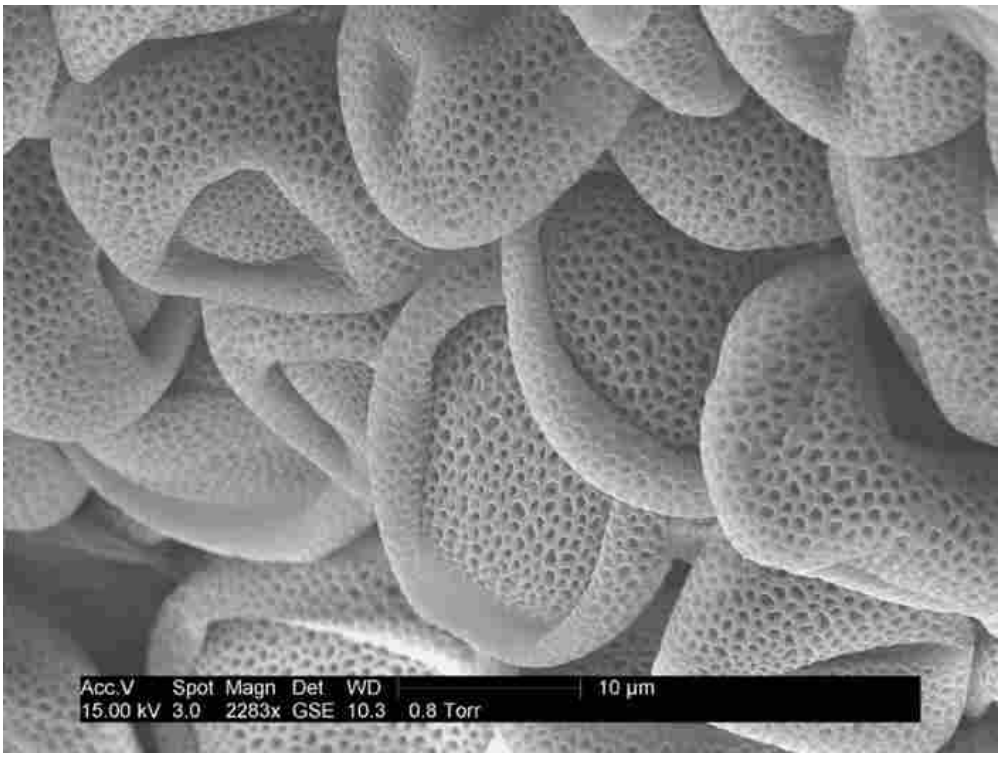

Figure 27b. This image shows morphological characteristics of typical *Boechera* apomictic triploid spp. pollen (Windham and Al-Shehbaz, 2007a, 2007b) at high magnification – especially the existence of asymmetrically-shaped spheroid pollen grains. Image also shows portions of the petal.