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Searching for Diamonds in the Apomictic Rough: A Case Study Involving *Boechera lignifera* (Brassicaceae)

Michael D. Windham,[1,9] James B. Beck,[2,3] Fay-Wei Li,[1] Loreen Allphin,[4] John G. Carman,[5] David A. Sherwood,[5] Catherine A. Rushworth,[1] Erin Sigel,[6] Patrick J. Alexander,[7] C. Donovan Bailey,[7] and Ihsan A. Al-Shehbaz[8]

[1]Department of Biology, Duke University, Durham, North Carolina 27708, U. S. A.
[2]Department of Biological Sciences, Wichita State University, Wichita, Kansas 67260, U. S. A.
[3]Botanical Research Institute of Texas, 1700 University Drive, Fort Worth, Texas 76107, U. S. A.
[4]Department of Plant and Wildlife Sciences, Brigham Young University, Provo, Utah 84602, U. S. A.
[5]Plants, Soils and Climate Department, Utah State University, Logan, Utah 84322, U. S. A.
[6]Department of Botany, National Museum of Natural History, Smithsonian Institution, PO Box 37012, Washington, District of Columbia 20013, U. S. A.
[7]Department of Biology, New Mexico State University, Las Cruces, New Mexico 88003, U. S. A.
[8]Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U. S. A.
[9]Author for correspondence (mdw26@duke.edu)

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Abstract—The genus *Boechera* is one of the most difficult species complexes in North America, with about 70 sexual diploids and hundreds of apomictic taxa representing diverse combinations of nearly every known sexual genome. In this study, we set out to clarify the taxonomy of *Boechera lignifera*, which currently includes a small number of sexual diploid populations in addition to the widespread apomictic diploid upon which the name is based. Using data from cytological studies, microsatellite DNA analyses, geography, and morphology, we demonstrate that the apomictic populations are genetically quite divergent from the sexual diploids. We propose the name ***Boechera kelseyana*** to accommodate the sexual diploid taxon, which occurs entirely south of the geographic range of *B. lignifera*. *Boechera kelseyana* is consistently separable from *B. lignifera* based on pollen and seed morphology, the length and proximal orientation of fruiting pedicels, differences in the branching and orientation of trichomes on the lowers stems, and the number of flowers and cauline leaves on unbranched fertile stems.

Keywords—Apomixis, Cytology, microsatellites, PCO-MC, species delimitation.

Genera exhibiting widespread apomixis are anathema to many taxonomists; the mere mention of their names (e.g. *Antennaria* Gaertn., *Crataegus* L., *Crepis* L., *Hieracium* L., *Potentilla* L., *Rosa* L., *Rubus* L., and *Taraxacum* F. H. Wigg.) leaves botanists looking for ways to change the topic of conversation. The most common form of apomixis found in these groups is of the gametophytic type, in which an embryo arises directly from the megagametophyte without fertilization (Asker and Jerling 1992). This particular type of apomixis is strongly correlated with polyploidy (Carman 1997; Whitton et al. 2008; Hojsgaard et al. 2014), and can arise from within a single diploid species (autopolyploidy), through hybridization between species (allopolyploidy) or, at higher ploidy levels, a combination of the two processes (see Ramsey and Schemske 1998). In the few genera that express gametophytic apomixis at the diploid level (e.g. *Boechera* Á. Löve & D. Löve, *Erigeron* L.), diploid apomicts represent an added layer of complexity, providing a basis for the production of an even greater diversity of polyploids (Alexander et al. 2015).

As species complexes age, the sexual diploids giving rise to the apomictic taxa become increasingly hard to detect. With their genomes represented in a diversity of apomictic derivatives, the sexual diploids become more difficult to distinguish using the characters and tools commonly employed by taxonomists. Over time, successful apomictic populations often become more numerous and widespread, colonizing habitats unoccupied by their sexual diploid progenitors. This pattern is so pervasive in both plants and parthenogenetic animals that it has given rise to a substantial literature under the rubric "geographical parthenogenesis" (Vandel 1928; Lynch 1984; Haag and Ebert 2004; Kearney 2005; Hörandl 2006, 2009; Vrijenhoek and Parker 2009; Verhoeven and Biere 2013). Within regions of geographic overlap, destabilizing hybridization and competition with their apomictic relatives can lead to severe reductions in the geographic range and effective population sizes of the sexual taxa (Lynch 1984; Whitton et al. 2008), substantially increasing the risk of extinction (Lynch et al. 1995; Coron et al. 2013; Coron 2014).

The processes involved in the evolution and maturation of apomictic species complexes can greatly complicate efforts by systematists to locate and sample sexual diploids. But the importance of this task cannot be overstated. It is the sexual diploids that are the products of divergent evolution (cladogenesis); only secondarily do they give rise to apomicts through reticulate processes. Thus, the sexual diploids are the foundational "pillars" upon which apomictic complexes are built (Stebbins 1950; Hörandl et al. 2009), and even the most cursory understanding of such complexes must begin with a full accounting of sexual diploid diversity.

For the past ten years, our labs have collaborated in an NSF-funded effort to identify and describe all sexual diploids in the genus *Boechera* (Brassicaceae). As currently circumscribed, the genus includes about 70 such entities, plus hundreds of apomictic taxa involving diverse combinations of nearly every known sexual genome. A few of the sexual species, such as the widespread and well-studied *B. stricta* (Graham) Al-Shehbaz, are so distinctive morphologically that their genetic contribution to any apomictic hybrid is immediately apparent. However, most sexual diploids belong to clades containing several grossly similar species (Alexander et al. 2013, 2015), and their relationships to apomictic taxa cannot be determined on the basis of morphology alone. By combining cytological and molecular data with in-depth morphological studies, we have made substantial progress in parsing these

complexes and identifying the sexual diploids involved. Here we describe the results of our initial investigations of the *B. lignifera* (A. Nels.) W. A. Weber complex.

Boechera lignifera was described in 1899 as a new species of *Arabis* L., typified based on specimens collected by Aven Nelson near Green River, Wyoming. Most subsequent authors have treated it as a distinct species, either within *Arabis* (Rollins 1941, 1993; Mulligan 1995; Welsh 2003) or, more recently, as a member of the largely North American *Boechera* (e.g. Holmgren 2005; Al-Shehbaz and Windham 2010). Based on broad-scale morphological and palynological studies of *Boechera*, Windham and Al-Shehbaz (2006) reported notable variability among plants assigned to *B. lignifera*. First, it became clear that the two plants on the "holotype" sheet at the Rocky Mountain Herbarium (RM 12591) represented different taxa, one with sparsely pubescent fruits and the other with glabrous fruits. Overall, the plant with glabrous fruits was a better match for Nelson's (1899) protologue, and this was designated the lectotype of *A. lignifera* by Windham and Al-Shehbaz (2006). Most of the pollen remaining on the anthers of this plant was malformed, though a few ovoid-spheroid pollen grains suggestive of apomictic reproduction were present as well. Studies of microsporogenesis in plants from the type locality that are morphologically similar to the lectotype indicate that *B. lignifera* sensu stricto is an apomictic diploid (Windham and Al-Shehbaz 2006).

Beck et al. (2012) and Alexander et al. (2015) have shown that diploid apomixis in *Boechera* is strongly associated with hybridity, and thus it is quite likely that *B. lignifera* s. s. is of hybrid origin. Based on morphological and ecological similarities, previous authors have suggested a number of potential close relatives that could function as progenitors. These include *Arabis* (*Boechera*) *selbyi* (Rydb.) W. A. Weber (Rollins 1941), one or more of the sexual diploids belonging to the polyphyletic *B. holboellii* (Hornem.) Á. Löve & D. Löve complex (Welsh 2003; Windham and Al-Shehbaz 2006), or *B. cobrensis* (M. E. Jones) Dorn (Al-Shehbaz and Windham 2010). However, systematic studies of pollen morphology suggest another possible sexual diploid progenitor; *B. lignifera* itself. Plants assigned to this taxon are known to vary with respect to pollen type and reproductive mode (Windham and Al-Shehbaz 2006). Whereas most populations in the northern part of the geographic range were inferred to be apomictic in earlier pollen and/or chromosome studies, those at the southern edge (New Mexico and Arizona) produced an abundance of narrowly ellipsoidal, symmetrically tricolpate pollen grains usually associated with normal meiosis and sexual reproduction.

In this paper, we explore the taxonomic significance of the observed variation in reproductive mode within *Boechera lignifera* s. s. Based on a broad sampling of collections scattered across the geographic range of the taxon, we combine data from analyses of micro- and megasporogenesis, pollen and trichome morphology, microsatellite DNA, geography, and macromorphology to address one central question: Do the apomictic and sexual populations included within *B. lignifera* by previous authors represent a single, reproductively-variable species or two distinct taxa?

Materials and Methods

Sampling—Sixty plants were included in the microsatellite analyses that provided the genetic foundation for this effort (Appendix 1); all of these were included in the geographic and morphologic studies, with various subsets subjected to analyses of microsporogenesis (15), pollen morphology (39), and megasporogenesis (3). Samples were chosen to provide broad geographic sampling of both the presumed apomictic (northern) and sexual (southern) populations of *B. lignifera* s. l. Special emphasis was placed on sampling type specimens and vouchers for published chromosome counts. All plants are represented by voucher specimens deposited at the herbaria listed in Appendix 1.

Cytology—Microsporogenesis was studied in flower buds of wild or greenhouse-grown plants fixed in Farmer's solution (3 parts 95% ethanol: 1 part glacial acetic acid). Fixed materials were stored (for up to 15 yrs) at −20°C and transferred to 70% ethanol before preparing slides. Flower buds were dissected under 20 × magnification on a Leica MZ7.5 Stereozoom dissecting microscope; sampling targeted flowers in which the petals were ⅛-¼ the length of the enclosing sepals (a measurement providing a proxy for peak microsporogenesis in *Boechera*). Anthers from these flowers were macerated in a drop of 1% acetocarmine stain, allowed to stain for at least ten minutes (adding to the stain droplet as needed to prevent drying), then mixed 1:1 with Hoyer's solution prior to placing the cover slip and squashing. Slides were examined with a Meiji MT5310L phase contrast microscope, and representative cells were photographed using a Canon EOS Rebel T3i digital camera.

Pollen was examined from naturally dehiscing anthers of all flowering specimens included in the microsatellite study. For light microscopy, pollen samples were mounted in glycerol, and immediately examined and photographed at 400 × magnification using the phase microscope/camera set-up described in the Cytology section. For our scanning electron microscopy studies of pollen morphology, anthers shedding pollen were mounted on standard SEM stubs using double-sided tape and then sputter coated with gold using a Quorum Q-150T ES Sputter Coater. Samples were examined and photographed on a FEI/Phillips XL-30 S-FEG ESEM at an accelerating voltage of 15 kV.

For studies of megasporogenesis, clusters of floral buds at the late pre-anthesis stage and younger were fixed, either in formalin acetic acid alcohol (FAA) for 48 h or in Farmer's fixative (see Cytology section) for at least 24 h, then transferred to 70% ethanol for storage (at room temperature or at −20°C respectively depending on the fixative used). Buds were cleared in 2:1 benzyl benzoate dibutyl phthalate as in Crane and Carman (1987). Pistils were then excised, and those ranging from 0.7–3.0 mm in length were mounted such that pistils of similar length occurred in separate columns on the slides. Pistils were mounted in a small volume of clearing solution, up to 16 per slide, and covered with a coverslip. Ovaries inside the cleared pistils were studied using a BX53 microscope equipped with differential interference contrast (DIC) optics, and the pistils were photographed using an Olympus MicroFire 599809 camera.

Microsatellite Analyses—Genomic DNA was extracted from 60 air-dried herbarium specimens (Appendix 1) using a CTAB protocol modified for 96-well plates (Beck et al. 2012), Qiagen DNeasy plant mini kits (Qiagen, Germantown, Maryland), or the modified Qiagen DNeasy protocol described in Alexander et al. (2007). Microsatellite allele variation was assessed at 14 previously published loci [ICE3, ICE14 (Clauss et al. 2002); a1, a3, b6, c8, e9 (Dobeš et al. 2004); BF3, BF9 BF11, BF15, BF18, BF20, Bdru266 (Song et al. 2006)]. Forward primers for each locus were labeled with 6-FAM or HEX, and sets of three loci were simultaneously amplified using a multiplex PCR protocol. Reactions (8 ul) contained 2.5 μl 2x Qiagen Multiplex PCR master mix, 0.2 μM each primer, and approx. 20 ng DNA template. Cycling conditions included denaturing at 95°C (15 minutes), 30 cycles of 94°C denaturing (30 sec), 53°C annealing (90 sec), and 72°C extension (60 sec), followed by a final extension step at 60°C (30 minutes). Amplicons were sized using 500 ROX on an Applied Biosystems 3730xl DNA Analyzer and alleles were determined using GeneMarker 1.9 (SoftGenetics, State College, Pennsylvania). Samples exhibiting duplicate within-species multilocus genotypes were then excluded. In order to visualize major genetic contrasts among the samples, the 14-locus allele matrix was subjected to a principal coordinates analysis (PCoA) in GenAlEx 6.0 (Peakall and Smouse 2006) using a standardized covariance matrix derived from a binary genotypic genetic distance (Huff et al. 1993). The matrix was then subjected to multidimensional clustering using principal coordinates analysis with modal clustering (PCO-MC) (Reeves and Richards 2009, 2011). This approach identifies the most cohesive groups in a dataset by simultaneously considering information on all informative PCoA axes, ranking each group by a stability value that reflects the density of the group in multidimensional space.

Geography and Morphology—The distribution map for apomictic and sexual populations of *B. lignifera* s. l. was generated with QGIS v. 2.0.1-Dufour software (QGIS Development Team 2014), using state and county shape files obtained from the DIVA-GIS website (www.diva-gis.org/Data; Hijmans et al. 2004). Latitude and longitude for specific

collection localities were estimated by consensus from all relevant label information (including land survey data, distance from landmarks, elevation) using Google Earth (www.google.com/earth/) and U. S. G. S. topographic maps accessed through map-pass.mytopo.com. These coordinates were used to generate species-specific data layers, and the resulting map was modified aesthetically in Adobe illustrator v. 14.0.0 (www.adobe.com)

All specimens included in the microsatellite analyses were assessed for ≈60 qualitative and quantitative morphological characters that have proven useful for recognizing genetically divergent taxa in *Boechera* (see Species Description below). Microscopic characters were examined using a Leica MZ7.5 Stereozoom dissecting microscope and photographed using a Canon EOS Rebel T3i digital camera.

Results

All data presented in this paper point to the same, well-supported conclusion: that the northern, apomictic populations of *Boechera lignifera*, including the type collection, are not conspecific with the southern, sexual populations. For ease of reference, we hereafter refer to the northern populations as *B. lignifera* s. s. and to the southern populations as *B. kelseyana* Windham & Allphin, *sp. nov.*

***Cytology*—**Microsporogenesis was studied in ten collections of *B. lignifera* s. s., two from the type locality near Green River, Wyoming plus individuals from nine other populations in Colorado, Utah, and Wyoming (Table 1). All exhibited some variation in the number of chromosome pairs formed during microsporogenesis, but the production of well-formed pollen grains was observed only in cell lineages showing little or no pairing (i.e. those undergoing apomeiosis). These pollen mother cells (PMC) typically exhibited 14 unpaired chromosomes (Fig. 1A), which lined up individually on the metaphase plate and dissociated with 14 chromatids migrating to each of two daughter cells. These daughter cells directly gave rise to a dyad of pollen grains without further division (Fig. 1C, D). Microsporogenesis also was examined in eleven individuals representing five populations of *B. kelseyana* from Arizona and New Mexico (Table 1). Unlike *B. lignifera* s. s., no variation in chromosome pairing was observed in the hundreds of cells examined; all formed seven pairs during meiosis I (Fig. 1B). Individual chromosomes segregated into two daughter cells, which then engaged in a second division (meiosis II) to form tetrads (Fig. 1E).

Mature pollen was examined in 28 collections of *B. lignifera* s. s., all but one of which were included in the microsatellite analyses. Ten of the 28 also provided chromosome counts as a result of our studies of microsporogenesis. Among the nine individuals included in all three analyses, seven showed a preponderance of ovoid-spheroid, asymmetrically multicolpate pollen grains produced by apomeiotic microsporogenesis (Fig. 1D, G), and two yielded mostly malformed pollen with apomeiotic grains comprising 5–25% of the total sample (Table 1). Among the remaining 18 *B. lignifera* s. s. collections whose pollen was analyzed (Appendix 1), twelve had predominantly apomeiotic pollen, two had mostly malformed grains with a lower frequency of apomeiotic pollen, and four produced malformed grains almost exclusively. For *B. kelseyana,* pollen was examined for 12 of the 18 collections included in the microsatellite analyses (Appendix 1); 11 of these (from the five populations listed in Table 1) produced chromosome counts indicative of normal meiosis, and microsporogenesis resulted in a preponderance of the narrowly ellipsoid, symmetrically tricolpate pollen type associated with sexual reproduction (Fig. 1F, H). A single collection of *B. kelseyana* (*Heil 7371*: SJNM) produced mostly malformed pollen.

Based on our limited sampling, *B. lignifera* s. s. and *B. kelseyana* also showed consistent differences in megasporogenesis (Table 1). The two collections of *B. lignifera* analyzed exhibited very high frequencies of *Taraxacum*-type diplospory, in which the megaspore mother cells (MMC) produced dyads of unreduced megaspores (Fig. 1I, J). Of the 217 meiocyte-staged ovules examined, 97.2% were diplosporous and only 2.8% produced meiosis-derived tetrads. The completion of diplosporous megasporogenesis triggered degeneration of the micropylar megaspore and formation of a genetically unreduced *Polygonum*-type embryo sac from the chalazal megaspore. By contrast, the one sample of *B. kelseyana* exhibited strictly sexual megasporogenesis, in which tetrads of reduced megaspores formed from MMC (Fig. 1K, L). Completion of this process triggered degeneration of the three micropylar megaspores and formation of a genetically reduced *Polygonum*-type embryo sac from the chalazal megaspore.

Two additional features differentiated ovule development in *B. lignifera* s. s. from that observed in *B. kelseyana*. In diplosporous *B. lignifera*, the archesporial cell did not divide

Table 1. Populations of *Boechera lignifera* and *B. kelseyana* sampled for cytological analyses of sporogenesis, including basic voucher data (see Appendix for additional information), chromosome number observed during microsporogenesis, predominant pollen types (sexual, apomictic or malformed), and type of megasporogenesis observed. Asterisk (*) identifies specimen not included in microsatellite analyses. Superscripts indicate topotypes[a] and isotypes[b] of *B. lignifera* and *B. kelseyana,* respectively, and data obtained from Schranz et al. (2005)[c].

Taxon	Population	Voucher	Chrom#	Pollen	Megasporogenesis
B. lignifera	CO, Moffat Co.	*Windham 3874*	$n = 2n = 14$	MAL; APO	
	CO, Rio Blanco Co.	*Windham 3873*	$n = 2n = 14$	APO	
	UT, Summit Co.	*Windham 99–076*	$n = 2n = 14$	APO	Diplosporous[c]
	UT, Uintah Co.	*Windham 99–060*	$n = 2n = 14$	APO	
	UT, Uintah Co.	*Windham 99–066**	$n = 2n = 14$	APO	
	UT, Uintah Co.	*Windham 3876*	$n = 2n = 14$	APO	Diplosporous
	UT, Uintah Co.	*Windham 3877*	$n = 2n = 14$	APO	
	WY, Sweetwater Co.	*Windham 00–081*	$n = 2n = 14$	MAL; APO	
	WY, Sweetwater Co.	*Windham 3033*[a]	$n = 2n = 14$	APO	
	WY, Sweetwater Co.	*Windham 3805*[a]	$n = 2n = 14$	APO	
	WY, Sweetwater Co.	*Carman BB 4.59*[a]	$n = 2n = 14$		Diplosporous
B. kelseyana	AZ, Apache Co.	*Windham & Roth 2320*[b]	$n = 7$	SEX	
	AZ, Apache Co.	*Windham 3344*	$n = 7$	SEX	
	AZ, Apache Co.	*Windham 3346*	$n = 7$	SEX	
	NM, McKinley Co.	*Windham 3370*	$n = 7$	SEX	
	NM, Sandoval Co.	*Windham et al. 3950a*	$n = 7$	SEX	Haplosporous

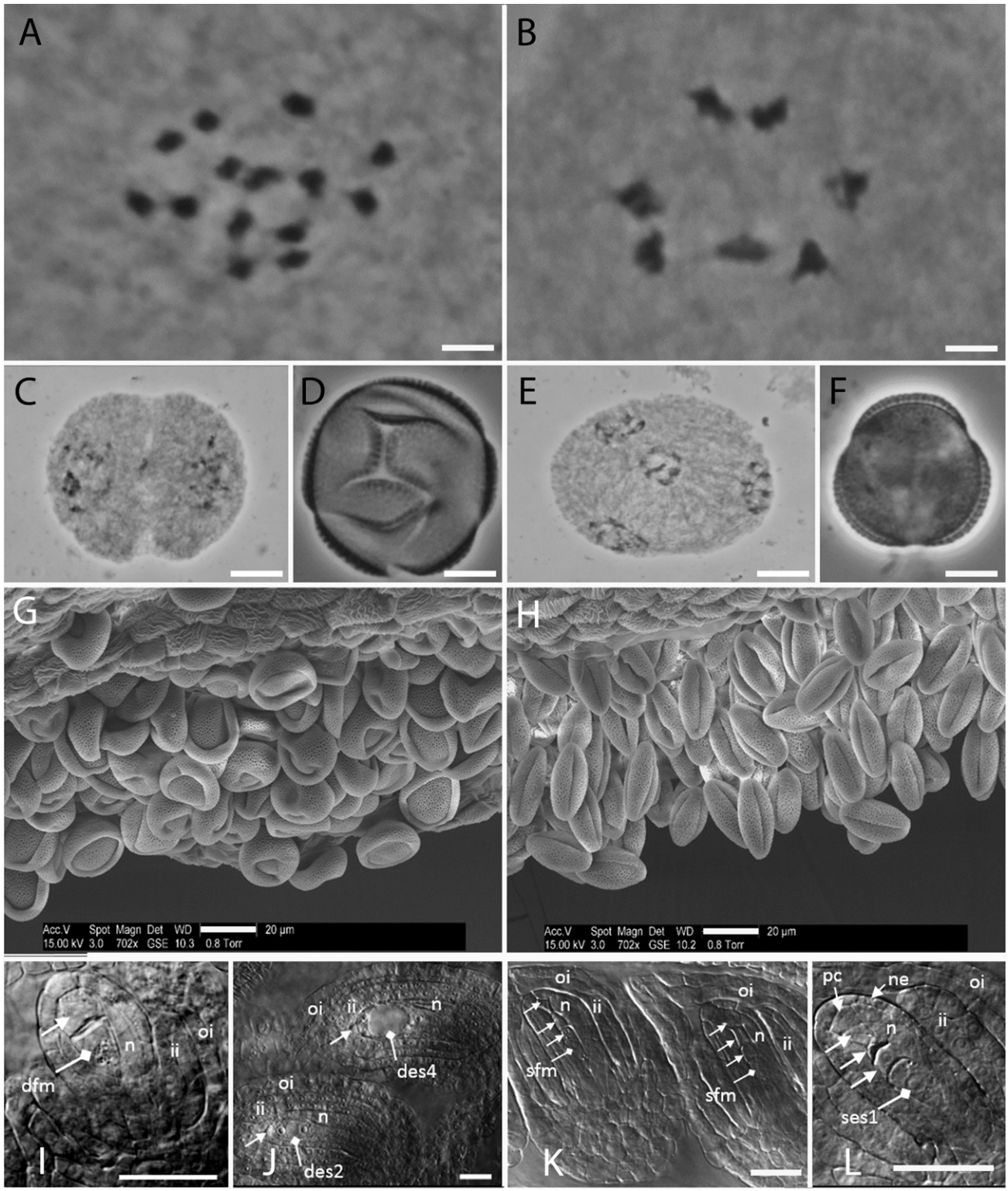

Fig. 1. Cytological comparison of *Boechera lignifera* (A, C–D, G, I–J) and *B. kelseyana* (B, E–F, H, K–L). A–B. Early microsporogenesis; scale bars = 2 µm. A. Apomeiotic late prophase in a pollen mother cell (PMC) of *B. lignifera* showing 14 unpaired chromosomes. B. Diakinesis in a PMC of *B. kelseyana* showing 7 bivalents. C–F. Later stages of microsporogenesis; scale bars = 5 µm. C–D. A PMC in *B. lignifera* forms a dyad of daughter cells (C), each of which develops into an ovoid-spheroid, asymmetrically multicolpate pollen grain (D). E–F. A PMC in *B. kelseyana* forms a tetrad of daughter cells (E), each of which develops into a narrowly ellipsoid, symmetrically tricolpate pollen grain (F = polar view). G. Scanning electron micrograph (SEM) of dehiscing anther in *B. lignifera* showing abundance of apomeiotically-derived (diplosporous) pollen. H. SEM of an anther of *B. kelseyana* showing haplosporous pollen derived from normal meiotic processes. I–L. DIC images of cleared pistils showing megasporogenesis and early embryo sac formation in ovules. Symbols used: ii = inner integument; n = nucellus; ne = nucellar epidermis; oi = outer integument; pc = parietal cell; unlabeled arrows point to degenerating megaspores; scale bars = 20 µm. I. Ovule of *B. lignifera* showing a dyad of unreduced megaspores at the diplosporous functional megaspore (dfm) stage. J. Two ovules in a single pistil of *B. lignifera* at the 2- and 4-nucleate diplosporous embryo sac (des) stages. K. Two linear tetrads of megaspores in two adjacent ovules of *B. kelseyana* at the sexual functional megaspore (sfm) stage. L. Linear tetrad in an ovule of *B. kelseyana* in which the sexual functional megaspore has matured into a 1-nucleate sexual embryo sac (ses1).

prior to MMC formation, while such division was the norm during ovule development in sexual *B. kelseyana*. When archesporial cells divided in the latter, the micropylar-most cell developed into a parietal cell that persisted between the nucellar epidermis and the differentiating MMC (Fig. 1L). Parietal cells were 4-fold more frequent in sexual *B. kelseyana* than in diplosporous *B. lignifera*, i.e. 17% versus 4%, respectively. The two species also differed in the timing of megasporogenesis relative to ovule maturity. Megasporogenesis in diplosporous *B. lignifera* s. s. occurred at an earlier stage of ovule development, before the outer integument enclosed the nucellus (Fig. 1I). By contrast, megasporogenesis in sexual *B. kelseyana* generally occurred later, while the outer integument was enclosing the nucellus and beginning to form the micropyle (Fig. 1K, L).

Microsatellite Analyses—The full 60 sample by 14-locus microsatellite dataset has been archived on the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.933v0. The 42 *B. lignifera* samples exhibited 28 unique multilocus genotypes, while the 18 *B. kelseyana* samples exhibited 15 unique multilocus genotypes. The most notable feature of these data was the striking difference in heterozygosity between *B. lignifera* s. s. and *B. kelseyana*. Among the seven loci summarized in Table 2, five exhibited predominantly (> 95%) heterozygous genotypes in *B. lignifera* s. s. And although locus Bdru266 appeared to be homozygous in this taxon, ongoing studies of the hybrid origins of *B. lignifera* s. s. (M. Windham et al. unpubl. data) have indicated that it includes a sexual diploid genome fixed for a null allele at this locus and thus should be considered heterozygous as well. In the full 14-locus dataset (not shown), the 28 multilocus genotypes encountered in this taxon were heterozygous at a minimum of eleven loci (not including Bdru266 discussed above). By contrast, *B. kelseyana* showed no detectable heterozygosity, either in the seven loci included in Table 2 or in the full 14-locus dataset.

TABLE 2 Genotypes observed at seven microsatellite loci in *Boechera lignifera* and *B. kelseyana* and their observed frequency (%) among the unique multilocus genotypes observed within each species. Presumed homozygous genotypes show a single number (allele size in bp) preceding the parentheses; heterozygotes show two alleles (e.g., 99/105). Underlined alleles are shared between the two taxa.

Locus	Microsatellite genotypes in *lignifera* (frequency among 28 multilocus genotypes sampled)	Microsatellite genotypes in *kelseyana* (frequency among 15 multilocus genotypes sampled)
B11	80 (96.4%)	84 (100%)
	79/80 (3.6%)	
BF3	99/105 (50.0%)	123 (40%)
	99/109 (14.3%)	125 (20%)
	99/107 (14.3%)	127 (26.7%)
	99/103 (10.7%)	121 (13.3%)
	101/105 (7.1%)	
	105 (3.6%)	
B20	205/213 (32.1%)	219 (73.3%)
	207/213 (21.4%)	221 (20%)
	213 (21.4%)	225 (6.7%)
	213/217 (14.3%)	
	213/219 (3.6%)	
	213/227 (3.6%)	
	213/231 (3.6%)	
BF15	87/105 (60.7%)	99 (73.3%)
	87/89 (14.3%)	101 (20%)
	87/97 (10.7%)	97 (6.7%)
	87/117 (7.1%)	
	87/103 (3.6%)	
	87/115 (3.6%)	
B266	143 (39.3%)	123 (33.3%)
	139 (21.4%)	135 (26.7%)
	141 (21.4%)	113 (20%)
	146 (10.7%)	121 (13.3%)
	137 (3.6%)	131 (6.7%)
	135 (3.6%)	
B9	78/84 (71.4%)	90 (40.0%)
	78/96 (14.3%)	96 (33.3%)
	78/98 (7.1%)	98 (26.7%)
	78/100 (3.6%)	
	78/80 (3.6%)	
A3	248/253 (57.2%)	255 (100%)
	248/255 (32.1%)	
	248/250 (10.7%)	

In addition to the obvious disparity in heterozygosity, *B. lignifera* s. s. and *B. kelseyana* also showed major differences in allelic composition. At two of the seven loci included in Table 2 (BF11 and BF3), the two taxa shared no alleles. Four other loci included in the seven-locus dataset exhibited one or two shared alleles, but these usually occurred at low frequency in one or both taxa and were always paired in heterozygous *B. lignifera* s. s. with a second allele not found in *B. kelseyana*. Only Bdru266 showed one allele shared between the two taxa and apparently homozygous in *B. lignifera* s. s. (Table 2; allele 135). However, as discussed previously, we believe that all Bdru266 genotypes in *B. lignifera s. s.* include a null allele. If true, the specified genotype observed in *B. lignifera* s. s. would be designated "0/135" and would not be identical to the genotype found in *B. kelseyana*.

Ordination and multivariate clustering analyses unambiguously support two groups corresponding to *B. lignifera* s. s. and *B. kelseyana*. The first axis recovered by PCoA explains 58% of the variation in the dataset, and clearly separates these two groups (Fig. 2). Axis 2 explains an additional 10% of the variation and serves to highlight the substantial genotypic diversity of *B. lignifera* s. s. Clusters corresponding to *B. lignifera* s. s. and *B. kelseyana* received strong PCO-MC stability values (92% and 89%, respectively; Fig. 2); no other clusters received stability values above 5%.

Geography and Morphology—Figure 3 shows the geographic distributions of all *B. lignifera* s. s. and *B. kelseyana* samples included in our microsatellite analysis. In this rendering, which we believe to be a fairly accurate view of the respective ranges of the two species, *B. lignifera* s. s. and *B. kelseyana* are allopatric. The closest known populations, in La Plata Co., Colorado and Sandoval Co., New Mexico respectively, are separated by ca. 100 km. At present, *B. kelseyana* is known only from Arizona and New Mexico, and *B. lignifera* s. s. appears to be restricted to Colorado, Idaho, Nevada, Utah, and Wyoming (Fig. 3).

Our morphological studies of the two groups circumscribed by microsatellite analyses identified a suite of ten characters that can be used to separate *B. lignifera* s. s. from *B. kelseyana* using the naked eye or a dissecting microscope. These characters, and their respective character states, are listed in Table 3 in approximate descending order of discriminating power. The shape and symmetry of any well-formed pollen grains was one of the strongest characters separating *B. lignifera* s. s. (Fig. 1D, G) from *B. kelseyana* (Fig. 1E, H), but required $\geq 50\times$ magnification to observe and was applicable only to plants with mature flowers (ca. 50–75% of herbarium specimens). The most useful macroscopic character was the orientation of the lowermost fruiting pedicels, which were horizontal to descending proximally in *B. lignifera* s. s. (Fig. 4A) but were usually ascending proximally in *B. kelseyana* (Fig. 4B, 5).

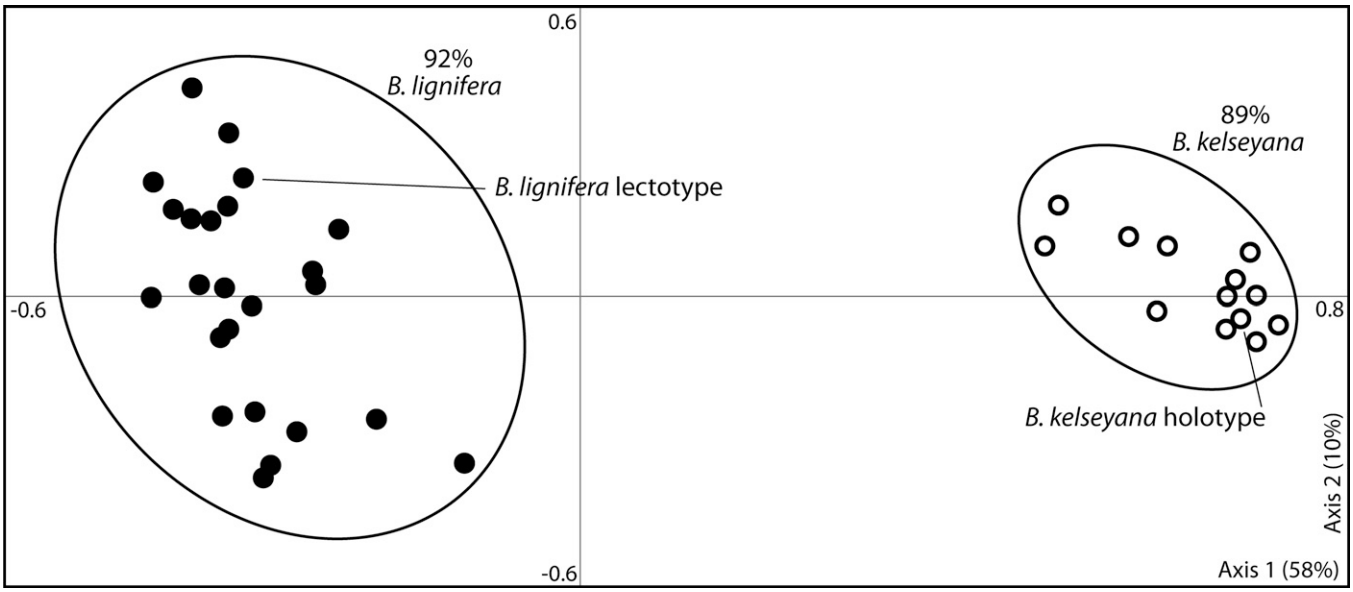

FIG. 2. Plot of scores on the first two principal coordinates from a principal coordinates analysis of 14 microsatellite loci in *Boechera lignifera* (dots) and *B. kelseyana* (circles). Stability values (%) derived from PCO-MC are shown for the two main clusters.

The morphology of the seed wing also appeared to be a useful discriminating character (Table 3), with *B. lignifera* s. s. exhibiting a narrow, poorly-developed, discontinuous wing (Fig. 4C) and *B. kelseyana* showing a broader, more continuous wing (Fig. 4D). Although the utility of this feature was limited by the rarity of specimens with seeds, the character was complementary to pollen morphology since herbarium collections lacking anthers were usually post-anthesis and often had developing or mature seeds. Two other characters involving the trichomes of the lower stems (Table 3; Fig. 4E, F) required moderate magnification but had the advantage of being present on essentially all specimens. Fruit curvature and elevation of the caudex above ground level were useful in combination with other features but often subjective. The last three characters were quantitative features that exhibited overlap at lower values (usually about 50% of the total range) but were diagnostic at higher values. *Boechera lignifera* s. s. tended to have more flowers and more cauline leaves on fertile stems, whereas *B. kelseyana* usually had longer fruiting pedicels (Table 3; Fig. 4A, B).

Discussion

Cytology—The published literature contains just four original chromosome counts attributed to *Arabis* (*Boechera*) *lignifera*; counts reported by Mulligan (1995) and Dobeš et al. (2006) simply reiterate earlier results. We have observed the cytogenetic details of microsporogenesis in ten individuals of *B. lignifera* s. s. (Table 1), greatly increasing the number of chromosome counts for the species. As mentioned earlier, the production of well-formed (potentially viable) pollen grains was observed only in cell lineages showing little or no pairing of the 14 somatic chromosomes. In these cell lineages, there was no reduction division, the resultant spores were diplospores, and the gametophytes and sporophytes had the same chromosome number (reported as $n = 2n = 14$ in Table 1). Comparisons of our results to the published data provide a good illustration of the difficulties associated with *Boechera* taxonomy/cytogenetics and the pitfalls of accepting previous identifications and chromosome counts at face value.

The first two chromosome counts attributed to *Arabis* (*Boechera*) *lignifera* were published by Rollins (1941). He reported "$2n = 14$, $n = 7$?" based on a collection from Uinta Co., Wyoming (*Rollins 2308*; voucher deposited at GH) and $n = 7$ from a population in Montrose Co., Colorado (*Rollins 2129*; also at GH). Both localities fall within the geographic range of *B. lignifera* s. s. documented herein (Fig. 3). Although Rollins (1941) did not explicitly discuss apomixis in the case of his Uinta Co. collection, the format of his report ($2n = 14$, $n = 7$?) suggests that he was observing the meiotic irregularities associated with apomeiosis. The multilocus genotype of the voucher specimen for this count exhibits the high levels of heterozygosity (11/14 loci) characteristic of apomicts, and the specific alleles observed confirm that the collection is, indeed, *B. lignifera* s. s.

Rollins' (1941) Montrose Co. count is problematic given that the reported number ($n = 7$) was observed at very low frequencies (< 0.1%) in our sampling of *B. lignifera* s. s. This number implies that Rollins observed cells that were engaged in (or had already experienced) a reduction division, suggesting that most cells in the buds analyzed were undergoing normal meiosis rather than apomeiosis. Microsatellite results from the purported voucher specimen for this count at GH add a further complication ; the plant we sampled (one of two on the sheet) is a triploid hybrid between *B. lignifera* s. s. and *B. gracilipes* (Greene) Dorn. Furthermore, both plants mounted on this herbarium sheet were too mature to have provided flower buds for the meiotic chromosome studies conducted by Rollins (1941). Thus, phenology and DNA concur that this collection cannot be the voucher for the reported count. We suspect that the count actually originated from an unvouchered and unidentified sexual diploid co-occurring at the collection site.

The third count attributed to *Arabis* (*Boechera*) *lignifera* was published by Roy (1995) based on a collection from Gunnison Co., Colorado (*Roy 1183*; voucher deposited at RSA). The

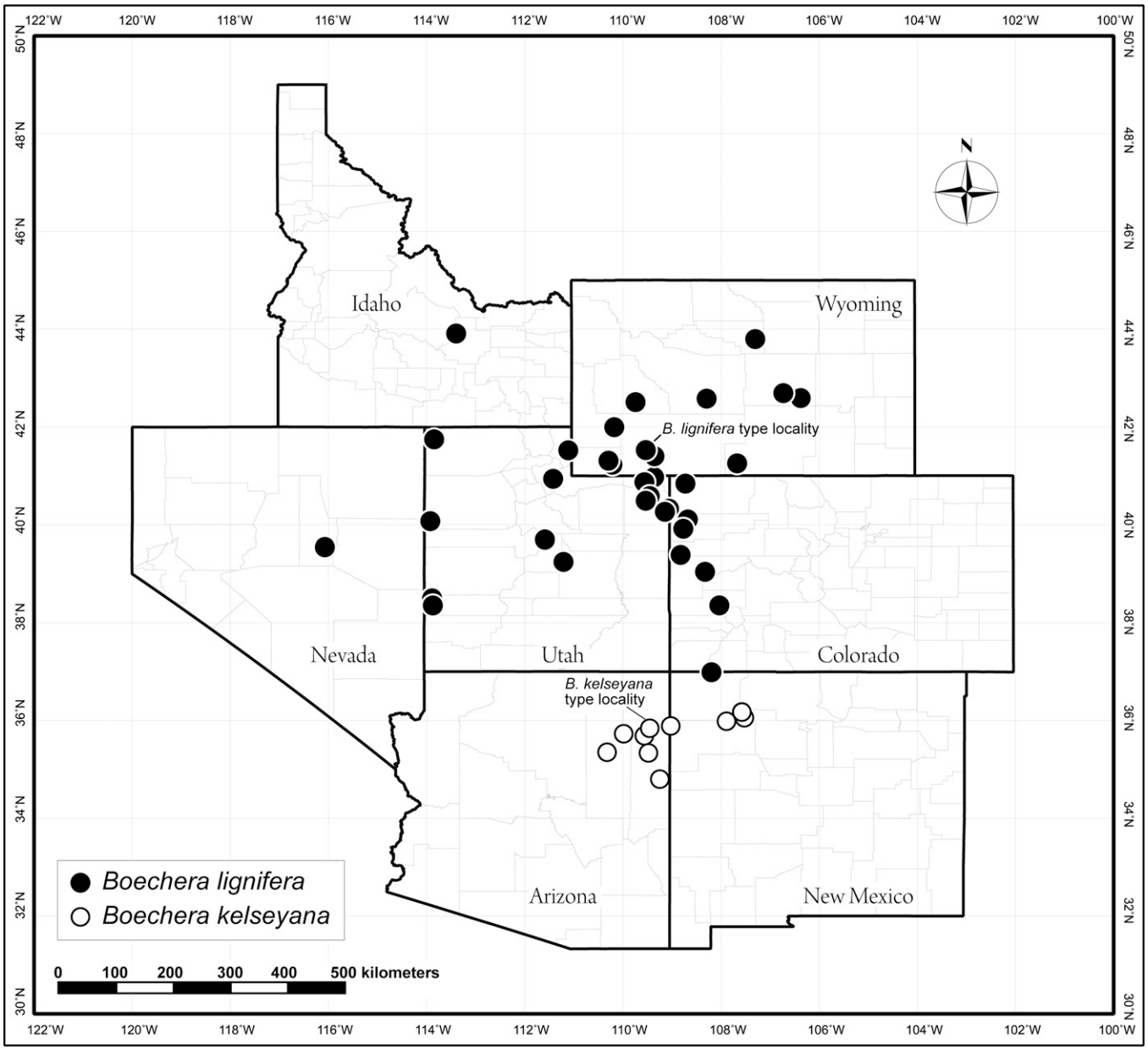

FIG. 3. Geographic distribution of *Boechera lignifera* (dots) and *B. kelseyana* (circles) samples included in the microsatellite and morphological analyses.

TABLE 3. Morphological characters useful in separating *Boechera kelseyana* from *B. lignifera*

Character	*Boechera lignifera*	*Boechera kelseyana*
Well-formed pollen grains	Ovoid-spheroid, asymmetrically multicolpate	Narrowly ellipsoid, symmetrically tricolpate
Fruiting pedicel orientation	Divaricate to descending proximally	Mostly ascending proximally
Seed wing	Mostly discontinuous, < 0.1 mm wide	Nearly continuous, 0.1–0.2 mm wide
Lower stem trichomes	Mostly appressed, ± uniform in orientation and size	Loosely appressed to spreading, with mixture of orientations and sizes
Largest trichomes of lower stems	Usually the most branched (5–7-rayed)	Usually the least branched (2–4-rayed)
Fruit curvature	Mostly straight	Mostly curved
Caudices	Often elevated above ground level	Usually at or slightly below ground level
# of flowers on main axis of inflorescence	(5–)8–25(–40)	5–15
# of cauline leaves on unbranched fertile stems	4–21	4–13
Length of lowermost fruiting pedicels	6–13 mm	(5–)8–21 mm

locality lies east the confirmed geographic range of *B. lignifera* s. s. (Fig. 3), but is close enough that the species could potentially occur there. Through allozyme studies of genetic segregation in progeny arrays, coupled with observations of microsporogenesis, Roy (1995) established that the plants she identified as *Arabis lignifera* were apomictic (non-segregating) diploids. However, examination of the voucher specimen at RSA revealed that the collection is misidentified. The fruiting

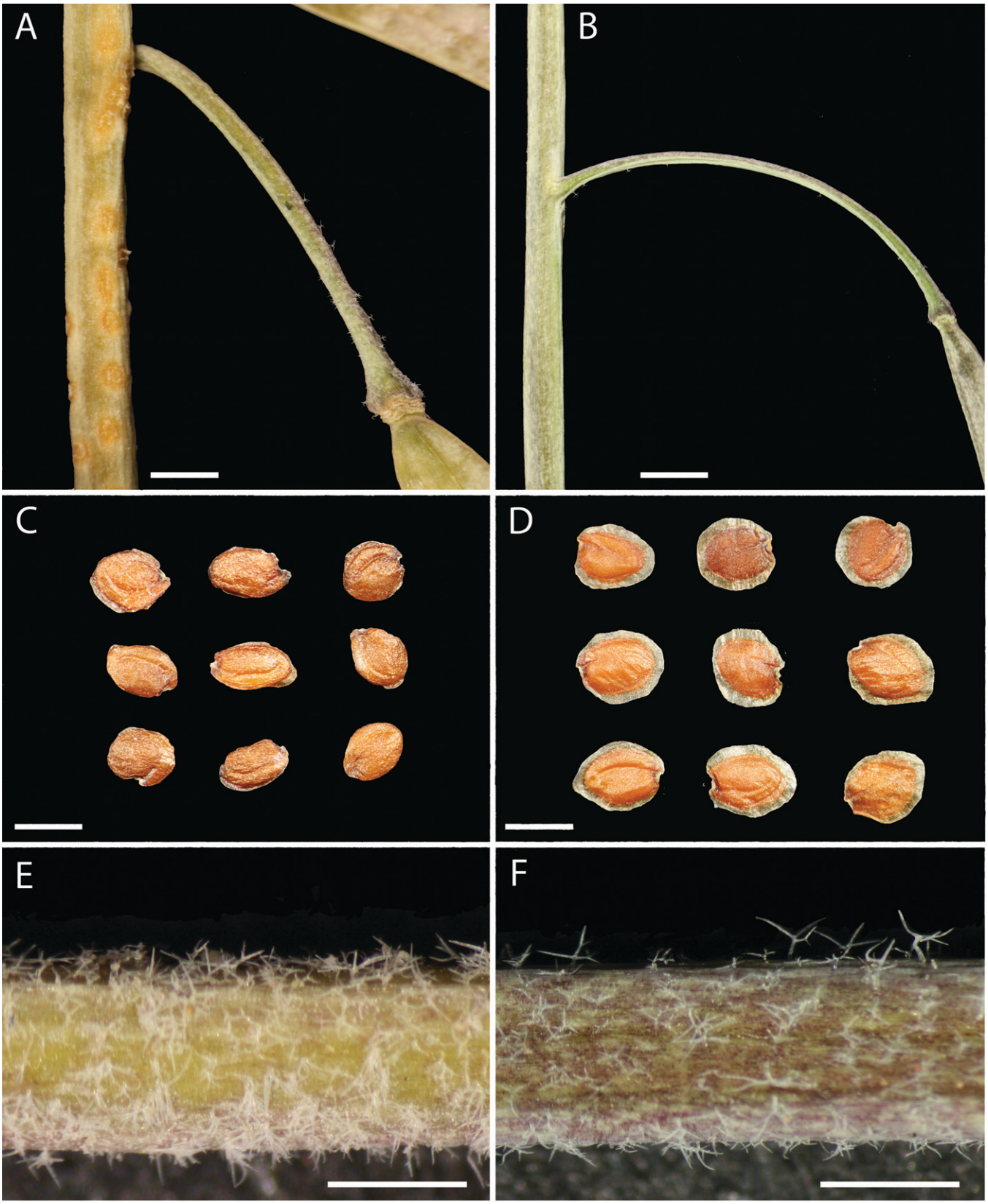

FIG. 4. Morphological comparison of *Boechera lignifera* (A, C, E) and *B. kelseyana* (B, D, F). A. Lowermost fruiting pedicel on lectotype of *B. lignifera*; scale bar = 1 mm. B. Lowermost fruiting pedicel on ASU isotype of *B. kelseyana*; scale bar = 2 mm. C–D. Seeds obtained from extant populations at type localities; scale bars = 1 mm. C. *B. lignifera*. D. *B. kelseyana*. E–F. Lower stems; scale bars = 1 mm. E. Lectotype of *B. lignifera*. F. ASU isotype of *B. kelseyana*.

FIG. 5. Holotype of *Boechera kelseyana*.

pedicels are ascending proximally and too long (see Table 3) and the basal leaves are dentate (compared to entire in *B. lignifera*; Al-Shehbaz and Windham 2010). Although her voucher was not included in our DNA studies, we are convinced that it is not *B. lignifera* s. s. and that Roy's (1995) chromosome count and genetic data should not be associated with this species.

The last published count attributed to *Arabis* (*Boechera*) *lignifera* comes from Koch et al. (1999) based on a collection from Rich Co., Utah (*Marler s. n.*; voucher location not provided). Although the reported count of $2n = 14$ is compatible with our results and the collection locality falls within the known range of *B. lignifera* s. s. (Fig. 3), we have been unable to locate a voucher specimen. Given the taxonomic complexity of *Boechera* (amply demonstrated by the previous paragraphs), we are hesitant to accept this count without seeing a voucher.

The preceding discussion has important implications for our understanding of *B. lignifera* s. s. Three of the four previously published chromosome counts must be set aside, either because the vouchers were misidentified (*Rollins 2129*; *Roy 1183*) or untraceable (*Marler s. n.*). Mulligan's (1995) summary of published chromosome counts in *Arabis* s. l. combined the two counts by Rollins (one valid, one not) to incorrectly conclude that $n = 7$, $2n = 14$ for this species. Our analyses, combined with the one confirmed count from Rollins (1941), tell a different story. These data make it clear that the production of viable male gametophytes in *B. lignifera* s. s. depends, perhaps exclusively, on an apomeiotic process that results in unreduced diplospores. Thus, gametophytes and sporophytes have the same chromosome numbers, and counts for *B. lignifera* s. s. are more appropriately reported as $n = 2n = 14$. This is a critical feature distinguishing the species from *B. kelseyana*, which has not been studied previously and thus far has yielded only sexual diploid chromosome counts of $n = 7$ (Table 1).

As has been documented in several previous studies of *Boechera* (Koch et al. 2003; Sharbel et al. 2005; Windham and Al-Shehbaz 2006; Beck et al. 2012), pollen morphologies were strongly correlated with the type of microsporogenesis observed in a particular plant (Table 1). Mature pollen grains generally fell into one of three phenotypic classes: 1) small, malformed grains; 2) larger, ellipsoid grains that are symmetrically tricolpate; and 3) even larger, ovoid-spheroid or triangular grains with multiple, asymmetric colpi. Irregular chromosome pairing, and the resultant uneven distribution of chromosomes among daughter cells appear to be the primary sources of malformed pollen grains in *Boechera* (M. Windham et al. unpubl. data). The underlying causes could be genetic, environmental, or some combination of the two (Raghavan 1997), and malformed pollen occurred sporadically in both *B. kelseyana* and *B. lignifera* s. s., though it is much more common in the latter.

Well-formed, viable pollen grains in apomictic *Boechera* serve a vital purpose beyond their practical value for taxonomists trying to determine mode of reproduction. Though not participating directly in the formation of the embryo, they can provide sperm to fertilize the central cell of the megagametophyte, thus contributing to the formation the nutritive endosperm. This process (pseudogamy) is nearly universal among apomictic taxa in *Boechera* (Böcher 1951; Naumova et al. 2001; Taskin et al. 2004; Aliyu et al. 2010) and may help explain the trends observed in *B. lignifera* s. s. toward the stabilization of male apomeiosis (Fig. 1A, C) and the concomitant production of well-formed unreduced microspores (Fig. 1D, G). Progeny of one of the *B. lignifera* s. s. plants analyzed here (Table 1; *Windham 99–076*) were used as males in crossing studies by Schranz et al. (2005). When crossed to sexual diploid females producing haploid gametes, the resultant F_1 offspring were uniformly triploid, reinforcing our observations that the functional pollen grains of *B. lignifera* s. s. are apomeiotic diplospores.

Apomixis in flowering plants is defined as asexual reproduction through seeds (i.e. reproduction without union of egg and sperm nuclei; see Asker and Jerling 1992; Hand and Koltunow 2014). As such, the strongest inferences regarding this process are based on cytological studies of female functionality. However, the technical challenges of working with megagametophytes imbedded in ovules enclosed in carpels have, so far, limited the number of researchers exploring this topic. In our study, megasporogenesis was examined in two collections of *B. lignifera* s. s. (Table 1), both of which produced a preponderance of diplospores (>95%) that developed into unreduced megagametophytes. Progeny of a third *B. lignifera* s. s. plant included in our study (Table 1; *Windham 99–076*) were used as females in the crossing studies of Schranz et al. (2005). When crossed to sexual diploid males producing haploid pollen, the resultant F_1 embryos were consistently triploid. This supports our conclusion that the megaspores of *B. lignifera* s. s. are, like the microspores, diplosporous. Interestingly, the studies of Schranz et al. (2005) demonstrate that the gametophytes produced by these megaspores are not locked into autochthonous development but can, under certain circumstances, form outcrossed embryos.

In addition to *B. lignifera* s. s., female diplospory has now been documented in diploid and triploid accessions of *B. holboellii* s. s. (Böcher 1951; Naumova et al. 2001), in diploid accessions of *B. retrofracta* (Sharbel et al. 2010) and *B. duchesnensis* (Carman et al. 2015), and in diploid or triploid interspecific hybrids involving *B. gunnisoniana* (Taskin et al. 2004), *B. formosa*, *B. lincolnensis*, *B. retrofracta,* and *B. stricta* (Carman et al. 2015).

We observed a 4-fold higher frequency of parietal cells in sexual *B. kelseyana* (Fig. 1L) coupled with a delayed megasporogenesis (compare Fig. 1I–L). This is consistent with findings of Böcher (1951) who also observed a correlation between occurrence of parietal cells and lateness of megasporogenesis. Böcher reasoned that low frequency parietal cell formation in *Boechera* was caused by diplosporous megasporogenesis occurring precociously, i.e. before archesporial cells could divide to form a parietal cell plus the MMC. Precocious megasporogenesis and embryo sac formation has also been observed in aposporous *Sorghum* (Carman et al. 2011) and diplosporous *Tripsacum* (Peel et al. 1997a) and *Elymus* (Peel et al. 1997b).

Microsatellite Analyses—The clear differences in heterozygosity and allelic composition documented in Table 2 are indicative of a major genetic discontinuity between *B. lignifera* s. s. and *B. kelseyana*. Specimens of the latter show no discernable heterozygosity at the 14 microsatellite loci analyzed, a situation commonly observed among largely-inbreeding, sexual diploid species of *Boechera* (e.g. *B. stricta*; Song et al. 2006). By contrast, our samples of *B. lignifera* s. s. are heterozygous at a minimum of 11 loci (Table 2). High levels of heterozygosity are characteristic of apomictic taxa in *Boechera*, which are primarily of hybrid origin (Beck et al. 2012). Such taxa

obtain different alleles from different sexual diploids, and the resultant heterozygosity is passed to subsequent generations through non-segregating apomeiosis (Roy and Rieseberg 1989; Schranz et al. 2006; Hojsgaard et al. 2013). Interestingly, though the overwhelmingly heterozygous genotypes observed in *B. lignifera* s. s. leave little doubt that this taxon is of hybrid origin, our data suggest that *B. kelseyana* probably was not involved. Although divergence estimates based on rapidly mutating microsatellite alleles can be problematic (see Paun and Hörandl 2006), the low frequency of shared alleles (Table 2) seems to argue against the idea that *B. lignifera* s. s. originated through hybridization between *B. kelseyana* and some other species of *Boechera*. Certainly there is no evidence of recent gene flow, as demonstrated by the occurrence of two widely separated sample clusters in the PCO-MC analysis (Fig. 2). The possible parentage of *B. lignifera* s. s. is currently under investigation based on a much larger sampling of sexual *Boechera* species. For now, we have strong evidence that *B. lignifera* s. s. and *B. kelseyana* are genetically divergent and isolated to a degree compatible with their recognition as separate species. The only other major criterion to be addressed is diagnosability (Reeves and Richards 2011), which, in this case, rests on a combination of geography and morphology.

Geography and Morphology—No specimens were found to validate the reports of *B. lignifera* from Arizona (Rollins 1941) or New Mexico (Martin and Hutchins 1980; Windham 2013); all collections labeled as such were either *B. kelseyana*, hybrids involving that species, or specimens that were clearly misidentified. However, the southernmost confirmed population of *B. lignifera* s. s. lies just 2.3 km north of the Colorado/New Mexico state line, and thus it is likely that this species eventually will be found in New Mexico as well. Although *Arabis* (*Boechera*) *lignifera* has been reported from Montana (Dorn 1984) and western Canada (Mulligan 1995), we have seen no specimens of *B. lignifera* s. s. from these areas despite having examined most of the *Boechera* collections housed at CAN, DAO, MONTU, and UBC. Thus, the documented range of the species still closely matches the distribution cited by Rollins (1993). *Boechera kelseyana* currently is known only from Arizona and New Mexico (Fig. 3), but seemingly appropriate habitats exist in adjacent parts of Colorado and Utah and the species eventually may be found in these states as well.

Although Windham and Al-Shehbaz (2006) were unable to draw clear morphological distinctions between the apomictic and sexual populations herein called *B. lignifera* s. s. and *B. kelseyana* respectively, the clarity of the genetic separation between the two (Fig. 2) both prompted and guided a critical reexamination of the available herbarium specimens. This effort has identified ten morphological features that are highly correlated with the cytological and microsatellite results. Taken together, these characters allowed all 60 specimens included in our analyses to be assigned to the correct microsatellite cluster based on morphology alone. Thus, *B. lignifera* s. s. and *B. kelseyana* are diagnosable morphologically, in addition to satisfying various genetic species criteria (Mallet 1995). Based on these results, we recognize the latter as a new species.

Taxonomic Treatment

Boechera kelseyana Windham & Allphin, sp. nov.—TYPE: U. S. A. Arizona: Apache Co., SSE of Nazlini on bench of Red Mesa near Indian Route 27 ca. 2.35 road miles NW of its junction with Indian Route 26. (35.8752N 109.4433W; WGS84 Datum), 6,825 ft. elev., in sandy duff soil under *Juniperus* on gentle NW-facing slope with *Artemisia, Cercocarpus* & *Pinus* nearby, 21 Apr 2001, *M. D. Windham & D. Roth 2320* (holotype: DUKE 398927; isotypes: ARIZ, ASC, ASU, BRY, GH, MO, NMC 77565, NY, RM, RSA, SJNM, UC, UNM, UT).

Similar to *Boechera lignifera* but differing in being a sexual diploid (vs. apomictic diploid hybrid) with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen (vs. mostly ovoid-spheroid, asymmetrically multicolpate or malformed pollen); further distinguished by having fruiting pedicels that are usually ascending proximally (vs. divaricate or descending), seeds with a nearly continuous wing 0.1–0.2 mm wide (vs. mostly discontinuous and < 0.1 mm), and lower stem trichomes loosely appressed to spreading with a mixture of orientations and sizes (vs. appressed and more uniform), with the largest trichomes usually the least branched with 2–4 rays (vs. most branched with 5–7 rays).

Plants short-lived perennials, the caudices rarely woody and usually not raised above ground level, lacking crowded, persistent leaf bases; sexually reproducing, with mostly well formed, narrowly ellipsoid, symmetrically tricolpate pollen. Fertile stems arising laterally below a short, terminal sterile shoot, 1–5 per caudex branch per year, 1.2–4 dm long, lower parts with many, stalked, 2–7-rayed trichomes (0.1–)0.2–0.7 mm long, the trichomes loosely appressed to spreading, various orientations and sizes mixed, the largest trichomes spreading and usually the least branched (with 2–4 rays); upper parts of stems glabrous to sparsely pubescent. Leaves at stem bases narrowly oblanceolate to linear, 2–5(–7) mm wide, entire, often with a few, marginal simple or spurred trichomes to 1 mm long near petiole bases, the blade surfaces generally densely pubescent with short-stalked, (3–)4–7-rayed trichomes 0.1–0.3 mm long; leaves of the sterile shoot forming a dense, ascending tuft, the blades narrower and more densely pubescent than those of basal leaves; cauline leaves of fertile stems 4–13, usually ciliate proximally and pubescent distally, the uppermost with auricles 0.5–2 mm long. Inflorescences simple racemes or rarely sparingly branched, the main axis 5–15-flowered; lowermost fruiting pedicels (5–)8–21 mm long, usually ascending proximally, gently down-curved distally, sparsely pubescent with dendritic trichomes or nearly glabrous. Flowers: sepals 2.5–3.5 mm long, greenish to dull lavender with whitish margin ca. 0.1 mm wide, sparsely pubescent with trichomes similar to those of leaves and pedicels, petals 5–7 × 1–1.5 mm, whitish but often aging pale lavender, glabrous; ovules 44–58 per fruit. Fruits 3.6–5.6 cm × 1.4–2.0 mm at maturity, usually curved downward, widely pendent, not secund, glabrous; styles 0.1–0.2(–0.6) mm long. Seeds in one row in each locule or rarely somewhat irregular, 1.3–1.8 × 1–1.2 mm; pale marginal wing nearly continuous, 0.1–0.2 mm wide. Embryos resulting from fertilization of reduced (meiotically-derived) egg cells of monosporic haploid megagametophytes. Chromosome number $n = 7$ with normal pairing during meiosis I.

Paratypes—U. S. A. Arizona: Apache Co., NNE of St. Johns on broad ridge N of Zuni River along U.S. 191 ca. 4.64 road miles S of its junction with State Route 61, *Windham 3344* [BRY; DUKE 398916; MO; NMC 77566; UT]; SSE of Wide Ruins on ridge above Bent Knee Wash near Navajo Route 9410 ca. 0.5 road miles N of its junction with U.S. Route 191, *Windham 3346* [ARIZ; BRY; DUKE 398911; MO; NMC 77578;

NY; UC; UT 124855]; Navajo Indian Reservation; W of Steamboat near the Navajo-Apache county line, *Heil et al. 5820* [SJNM 14588]. Navajo Co., Navajo Indian Reservation, Dilkon Community Site, *Fleming 531* [SJNM 9071]; 12 mi E of Keams Canyon, *Peebles & Smith 13438* [ARIZ 95593]. New Mexico: McKinley Co., NE of Navajo on slope overlooking Tohdildonih Wash along Navajo Route 31 ca. 1.25 road miles NE of its junction with Navajo Route 12, *Windham 3370* [MO; NMC 77569; UT 124857]. Sandoval Co., S of Lybrook on rim of mesa near head of Escrito Canyon ca. 3.00 km SSE of Escrito Spring, *Windham et al. 3950a* [DUKE 404122]; ridge with towers ca. 1 mi SSW of Lybrook, (36°12′22″N 107°33′41″), *O'Kane & Heil 4691* [SJNM 35092]; NW of Ojo Encino on Fruitland Shale knolls (36°05′31″N 107°30′15″), *O'Kane & Heil 4667* [SJNM 35050]. San Juan Co., Chaco Culture National Historical Park, top of Chacra Mesa across from Wijiji Ruins (T21N, R10W, S35, SW/NE), *Heil 7371* [SJNM 19777].

Distribution, Habitat and Phenology—*Boechera kelseyana* currently is known only from northeastern Arizona and northwestern New Mexico, on or adjacent to lands belonging to the Navajo Nation. It occupies sandy soils in evergreen woodland habitats, usually growing under pinyon pine (*Pinus edulis* Engelm.) or Utah juniper (*Juniperus osteosperma* (Torr.) Little) trees at elevations of 1700–2300 m. The species blooms from late April to early June and produces mature seeds in June and July.

Etymology—This species is named in honor of C. Ann Kelsey (1948–2013), long-time herbarium collections manager at the Utah Museum of Natural History, who played a crucial support role in our studies of *Boechera* and was a treasured friend who left us much too soon.

Acknowledgments. The authors thank the curators of the following herbaria for permission to remove pollen and small leaf fragments for molecular analyses: ARIZ, ASC, BRY, CS, DUKE, GH, NY, RM, SJNM, UT, and UTC; we are especially grateful to Ron Hartman for allowing us to sample the lectotype of *B. lignifera* preserved at RM. Collections from the type locality of *B. kelseyana* and populations near Wide Ruins, Arizona and Tohdildonih Wash, New Mexico (all on the Navajo Nation) were made under permit to Daniele Roth of the Navajo Natural Heritage Program. We acknowledge Becky Kowallis and Susan Nelson for assistance with the analyses of megagametogenesis. This research was funded by National Science Foundation awards DEB-0816560 (to M. Windham), DEB-0816789 (to L. Allphin), and DEB-0817003 (to C. D. Bailey), as well as support from the U.S. Department of Commerce, NIST, ATP cooperative agreement no. 70NANB4H3039 and the Utah Agricultural Experiment Station (to J. Carman).

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Appendix 1. Voucher information for DNA samples used in this study. Locality and collector(s) data followed by [herbarium acronym and accession # (unique alphanumeric assigned to DNA extraction; # of microsatellite loci resolved; # of these loci heterozygous; pollen morphologies in order of frequency >5%: S = sexual, A = apomictic, M = malformed, dash = no data)].

Boechera kelseyana Windham & Allphin (18 samples): Arizona: Apache Co., SSE of Nazlini on bench of Red Mesa near Indian Route 27 ca. 2.35 road miles NW of its junction with Indian Route 26, *Windham & Roth 2320* holotype: [DUKE 398927 (JB129; 15; 0; S)]; isotype: [RM (JB1409; 15; 0; S)]; NNE of St. Johns on broad ridge N of Zuni River along U.S. 191 ca. 4.64 road miles S of its junction with State Route 61, *Windham 3344* [DUKE 398916 (JB237; 15; 0; S)]; [BRY (JB1410; 15; 0; S)]; [UT 124846 (FW1450; 15; 0; S)]; SSE of Wide Ruins on ridge above Bent Knee Wash near Navajo Route 9410 ca. 0.5 road miles N of its junction with U.S. Route 191, *Windham 3346* [DUKE 398911 (JB238; 15; 0; S)]; [UT 124855 (JB1408; 15; 0; S)]; [ARIZ (FW1474; 15; 0; S)]; Round Top Mesa, 1 mi from Ganado, *Anderson & Young 137* [ASC 24169 (JB917; 15; 0; –)]; Navajo Indian Reservation; W of Steamboat near the Navajo-Apache county line, *Heil et al. 5820* [SJNM 14588 (JB913; 15; 0; –)]. Navajo Co., Navajo Indian Reservation; Dilkon Community Site, *Fleming 531* [SJNM 9071 (JB918; 15; 0; –)]; 12 mi E of Keams Canyon, *Peebles & Smith 13438* [ARIZ 95593 (JB507; 15; 0; –)]. New Mexico: McKinley Co., NE of Navajo on slope overlooking Tohdildonih Wash along Navajo Route 31 ca. 1.25 road miles NE of its junction with Navajo Route 12, *Windham 3370* [NMC 77569 (PJA213; 15; 0; S)]; [UT 124857 (JB321; 15; 0; S)]. Sandoval Co., S of Lybrook on rim of mesa near head of Escrito Canyon ca. 3.00 km SSE of Escrito Spring, *Windham et al. 3950a* [DUKE 404122 (FW228; 15; 0; S)]; ridge with towers ca. 1 mi SSW of Lybrook (36°12′22″ N 107°33′41″), *O'Kane & Heil 4691* [SJNM 35092 (JB916; 15; 0; –)]; NW of Ojo Encino on Fruitland Shale knolls (36°05′31″N 107°30′15″), *O'Kane & Heil 4667* [SJNM 35050 (JB915; 15; 0; –)]. San Juan Co., Chaco Culture National Historical Park; top of Chacra Mesa across from Wijiji Ruins (T21N, R10W, S35, SW/NE), *Heil 7371* [SJNM 19777 (JB920; 15; 0; M, S)].

Boechera lignifera (A. Nels.) W.A. Weber (42 samples): Colorado: Garfield Co., N of Loma on slope below Book Cliffs near CO Route 139 ca. 3.5 road miles N of Mesa County line, *Windham 3871* [DUKE 401966 (JB1265; 15; 11; A)]. La Plata Co., SW of Durango on slope overlooking La Plata River near State Route 140 ca. 1.5 road miles N of the New Mexico state line, *Windham et al. 4194* [DUKE 404774 (FW1561; 15; 11; –)]. Mesa Co., Watson Creek, 2.5 mi due SE of Palisade (T1S, R2E, S13, NE), *Neese & Chatterley 15567* [RM 359853 (JB660; 15; 11; A)]. Moffat Co., NNW of Dinosaur on ridge overlooking Trail Creek near County Road 16S (Miners Draw Rd) ca. 0.07 km E of Utah state line, *Windham 3874* [DUKE 401963 (JB1268; 11; 9; M, A)]; W of Vermillion Creek on hill overlooking Irish Canyon road ca. 1.93 km N of the summit (6939) of

Blue Hill, *Windham 99-156* [DUKE 400941 (JB727; 15; 13; A)]. Montrose Co., SW of Montrose on lower slopes of the Uncompahgre Plateau along State Route 90 ca. 4.5 road miles SW of its intersection with Shavano Valley Road, *Windham & Allphin 4219* [DUKE 405160 (FW1580; 15; 11; –)]. Rio Blanco Co., 2.2 mi S of White River along E Fork of Quinn Draw (T2N, R100W, S19, NW, NW, SE); on ledge of the Uinta Formation, *Baker et al. 82-32* [CS 67162 (JB926; 15; 11; A)]; SSE of Rangely near CO 139 at mouth of East Fourmile Draw ca. 0.39 km ENE of its confluence with Douglas Creek, *Windham 3873* [DUKE 401962 (JB1267; 15; 12; A)]. Idaho: Custer Co., near mouth of Elbow Canyon ca. 12 mi E of Mackay, *Rollins et al. 83265* [GH (FW700; 12; 9; –)]. Nevada: Eureka Co., NW of Eureka near Devils Gate along U.S. Route 50 ca. 4.9 road miles WNW of its junction with State Route 278, *Windham 3914* [DUKE 401959 (JB1303; 15; 13; M)]. Utah: Beaver Co., WNW of Milford along drainage separating The Needles from the Mountain Home Range ca. 1.06 air miles NW of the summit (7542) of The Toad, *Windham & Allphin* 4262 [DUKE 405158 (FW1612; 15; 12; –)]; W of Milford along road N of Vance Spring near low divide ca. 1.70 air miles NW of the summit (7273) of Sawtooth Peak, *Windham & Allphin 4265* [DUKE 405157 (FW1615; 15; 12; –). Box Elder Co., on slope above Grouse Creek Valley along road to Lynn ca. 5.1 road miles N of the town of Grouse Creek, *Windham 99-094* [DUKE 398921 (JB239; 15; 12; M, A)]. Daggett Co., road to Browns Park ca. 1 mi from Wyo. State Line; clay hills with scattered juniper, *Heil & Porter 7444* [SJNM 18853 (JB620; 15; 11; A)]; near Flaming Gorge, 15 mi SE of Manila, *Rollins 2274* [UTC 42530 (CR1317; 15; 12; M)]. Emery Co., WNW of Castle Dale on N slope of Straight Canyon along State Route 29 ca. 3.92 road miles ESE of Joes Valley Dam, *Windham* 4247 [DUKE 405159 (FW1598, FW1599; 15; 13; –)]. Rich Co., S end of Crawford Mts. ca. 4 mi NE of Woodruff; NE-facing slope (41°32.79′N by 111°06.40′W), *Moon & Moon 255* [BRY 479020 (JB1591; 15; 12; A)]. San Pete Co., Elk Ridge ranch; mountains on W side of valley from Indianola, 4 mi from Hwy. (T12S, R3E, S34), *Collins 693* [NY 281259 (FW647; 15; 13; –)]. Summit Co., NNW of Coalville on slope overlooking Echo Reservoir ca. 3.06 km S of Beckwith Spring, *Windham 99-076* [UT 121315 (JB728; 15; 12; A)]. Tooele Co., NE of Ibapah on slope above Pony Express Wash along Lower Gold Hill Road ca. 2.8 road miles NE of its junction with BIA Hwy. 1, *Windham 3916* [DUKE 401960 (JB1305; 15; 12; M)]. Uintah Co., NNW of Red Fleet Reservoir on lower slopes of the Uinta Mts. ca. 2.07 km NW of the confluence of Constantine and Pothole Canyons, *Windham 99-060* [DUKE 400944 (JB834; 15; 12; A)]; ESE of Jensen at base of Snake John Reef along Miners Draw Road ca. 0.5 road miles ENE of its junction with U.S. Route 40, *Windham 3876* [DUKE 401957; JB1270; 15; 12; A)]; N of Vernal on slope above Steinaker Reservoir near U.S. Route 191 ca. 4.3 road miles NNE of its junction with U.S. Route 40, *Windham 3877* [DUKE 401964 (JB1271; 15; 12; A)]; ENE of Vernal near mouth of Stuntz Draw along Echo Park Road ca. 0.2 road miles SW of the Colorado state line, *Allphin & Snyder 13-067* [BRY (LA121; 15; 12; –); (LA122; 15; 12; –); (LA123; 12; 9; –)]. Wyoming: Carbon Co., on ridge between Muddy, Cow, and Wild Cow Creeks and County Road 608 ca. 40 air mi SW of Rawlins (T15N, R91W, S8), *Nelson & Ward 37845* [RM 789444 (JB1008; 15; 13; A)]. Fremont Co., SE of Lander just below crest of Beaver Divide at a point ca. 1.83 km direct distance SSW of Bain Spring, *Windham 3799* [DUKE 400196 (JB130; 15; 11; A)]. Lincoln Co., Green River Basin ca. 5.3 air mi W of Fontenelle Dam (T24N, R112W, S31), *Cramer 5461* [RM 638980 (JB1009; 15; 13; A)]. Natrona Co., Laramie Range: ridge on W side of Corral Creek ca. 3 air mi E of WY Hwy. 487 (T31N, R79W, S31), *Fertig 13735* [RM 601719 (JB1010; 15; 12; –)]; 2 mi NE of Alcova near Wyo. Hwy. 220; juniper area, *Rollins & Rollins 7917* [BRY 235438.1 (CR1127; 15; 12; M)]. Sublette Co., Green River Basin: North Alkali Draw ca. 15 air mi E of Big Piney (T30N, R109W, S33), *Cramer & Hartman 6987* [RM 638672 (JB1011; 15; 11; A)]. Sweetwater Co., Green River; draws along the Green River Cliffs where it seeks the protection of sage-brush, *Nelson 4711* lectotype: [RM 12591.1 (CR1128; 15; 13; M, A)]; NW of the town of Green River on bluffs overlooking the river ca. 3.65 km WNW of the highest point on Tollgate Rock, *Windham 3033* [DUKE 395472 (JB426; 15; 13; A)]; NW of the town of Green River in draws of cliffs overlooking the river ca. 3.65 km WNW of the highest point on Tollgate Rock, *Windham* 3805 [DUKE 400244 (FW1028; 15; 13; A)]; bluffs overlooking Green River (41°33′06″N 109°31′ 31″W, *Carman BB 4.59* [UTC (CR1358; 15; 13; –); *Carman BB 3.98* = seed progeny of *BB 4.59* (CR1359; 15; 13; –)]; SSW of Rock Springs along tributary of Little Firehole Canyon near Little Firehole Road ca. 6.0 road miles SSW of its junction with US Route 191, *Windham 3809* [DUKE 400201 (JB131; 15; 13; A)]; just E of Richards Gap at S edge of Red Creek Basin ca. 1.70 km SSW of the confluence of Red Creek and Daniels Creek, *Windham 00-081* [DUKE 395471 (FW53; 15; 13; M, A)]. Uinta Co., stony hillside near Lyman; among sagebrush, *Rollins 2308* [GH (CR1024; 15; 12; –)]; E of Mountain View near rim of Cottonwood Bench ca. 6.08 km S of the summit of Turtle Hill, *Windham 99-168* [DUKE 400942 (JB835; 15; 12; A)]. Washakie Co., Bighorn Basin; ca. 12 air mi SE of Ten Sleep (T45N, R87W, S34); sandy areas on sandstone mesa with great deal of caprock, *Dueholm & Hartman 9262* [NY (FW649; 15; 12; –)].