

Cross-lineage hybridization in the genus *Arctostaphylos* (Ericaceae)

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Abstract

The evolutionary history of the genus *Arctostaphylos* is split between two deep lineages. Many *Arctostaphylos* species are hypothesized to result from hybridization, but cross-lineage hybrids are uncommon. I identified six species with nuclear ribosomal DNA sequences that contain nucleotide combinations from both lineages. In these cross-lineage hybrids, polymorphic nucleotides are common at the seven base positions that separate the two lineages. In cross-lineage hybrid species where individuals from multiple populations are available, nucleotide variation between populations is present at these seven positions. At least four of these cross-lineage hybrids are polyploids, suggesting that whole genome duplication enables hybridization between lineages. Sequence variation between populations suggests that they may result from multiple independent polyploidy. I searched for potential parental species for these cross-lineage hybrids by comparing their sequences with those of all other extant taxa and evaluating nucleotide additivity. The results offer insights into *Arctostaphylos* biogeography and paleoendemism as well as hybridization and polyploidy.

Introduction

The genus *Arctostaphylos* comprises 67 species (Kauffmann et al. 2015, FNA 2009). One species, *A. uva-ursi*, is globally distributed. The remainder are native to the western United States, northern Mexico, and southern British Columbia. Nearly three quarters of *Arctostaphylos* are local endemics; many of them are confined to “islands” of unusual and generally poor soil. Forty are of conservation concern. Eleven *Arctostaphylos* species are polyploids.

Manzanita Park in north Monterey County, California, contains more than 150ha of coastal chaparral on sandy soil. This shrubland habitat is dominated by three manzanita species: two narrow endemics, *Arctostaphylos pajaroensis* and *A. hookeri*, and a regional endemic, *A. crustacea*. The three taxa are easily distinguishable. *A. pajaroensis* is a tall, upright shrub with triangular leaves and gray shreddy bark, *A. hookeri* is a low, mat-forming shrub with shiny green leaves and smooth red bark, and *A. crustacea* is intermediate in stature and has a prominent basal burl. During more than a decade of frequent hikes at the park, I challenged myself to find individuals with these characters in unusual combinations. These apparent hybrids do occur, and they invariably combine the triangular leaves of *A. pajaroensis* with the burl of *A. crustacea*. I was motivated in this search by the knowledge that local nurseries sell a natural hybrid named *A. ‘Sunset’* that combines characters from *A. hookeri* with characters from *A. crustacea*. But I never found one in the wild.

It is not unusual to find two or three *Arctostaphylos* species growing together at the same site, occasionally more. Sometimes there are hybrids, sometimes there aren't. This study is an attempt to apply molecular data and the tools of phylogenetic analysis to answer the question: why?

Methods

I downloaded all *Arctostaphylos* nucleotide sequences from GenBank that include the ITS, 5.8S, or 26S regions of nuclear ribosomal DNA. These sequences result from several studies, particularly Markos et al. (1998), Boykin et al. (2005), and Wahlert et al. (2009). I also downloaded *Arctous rubra* sequences that include the same regions as an outgroup to polarize my resulting phylogeny. I chose *Arctous rubra*, an arctic/tundra shrub, based on its position as close sister to *Arctostaphylos* within the *Ericaceae* (Kron et al. 2002). I upgraded taxonomic nomenclature as necessary to agree with the current treatment (FNA 2009) and assigned a brief identifier (six-character acronym, optional single-character subspecies identifier, and differentiating number) to each sequence. Sequence identifiers are listed in Appendix 1 along with corresponding taxon name, GenBank accession number, ploidy, study author, and collection location and voucher identification if available. The ITS+5.8S dataset consists of sequences from all but four *Arctostaphylos* species (*A. bolensis*, *A. incognita*, *A. moranii*, and *A. nortensis*) and includes multiple sequences for many species (different subspecies and collection locations). The 26S dataset consists of a smaller sample (18 species).

I assembled and manually aligned the ITS1+5.8S+ITS2 and 26S sequences in separate nucleotide character matrices. Where ITS1 and ITS2 were separate accessions, I combined these before alignment. Manual alignment was straightforward due to a relatively small number of variable characters and indels. After alignment, I trimmed all sequences to uniform length (584 bp for ITS1+5.8S+ITS2 and 264 bp for 26S).

These aligned character matrices provided data necessary to infer the evolutionary history of *Arctostaphylos* through phylogenetic inference using the PAUP* software. In addition, they enabled direct observation of nucleotide variability and polymorphism within and between nucleotide sequences, providing a means for testing hypothesized diploid and polyploid hybridization between species.

Results

Phylogenetic analysis

Sequences from the ITS, 5.8S, and 26S regions in *Arctostaphylos* contain nucleotide substitutions and polymorphisms at relatively few base positions, so direct observation and recognition of between-species patterns (apomorphies) and species-specific patterns (autapomorphies) was relatively straightforward. I first removed all base positions with no phylogenetically informative characters (no state changes, or changes only within a single taxon); this dramatically reduced the size of the datasets. I further reduced dataset size by combining all identical sequences from a single species, including

bp	g139	g142	g156	g264	g164	g219	g260	g66	g82	g113	g115	g116	g231	g233	g240	g254
RUBRA_Z	A	T	G	G	T	T	C	G	C	G	C	T	C	C	C	C
RUDIS_Z	C	C	A	C	T	A	T	G	C	T	C	T	C	T	C	C
HOOKERZ	C	C	A	C	T	A	T	G	C	G	C	T	C	C	T	C
PATULAZ	C	C	A	C	T	A	T	G	C	G	C	T	C	C	C	C
SENSITZ	C	C	A	C	T	A	T	G	C	G	C	T	C	C	C	C
NUMMULmZ	C	C	A	C	T	A	T	G	C	G	C	T	C	C	C	C
STANFOZ	C	C	A	C	T	A	T	G	C	G/T	C	T	C	C	C	C
ANDERSZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
CANESCZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
CRUST1Z	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
GLAUCAZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
MONTANZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	G/A/T/C
NISSENZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
PRINGLZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
TOMEN1Z	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C
VISCIDZ	C	C	A	C	C	C	A	G	C	G	C	T	C	C	C	C

Figure 2. Reduced, rearranged, and colored 26S character matrix (nucleotides) with cross-lineage sequences removed.

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472
RUBRA_1	C	C	T	A	-	-	C	G	T	C	C	T	G	C	A	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
PUNGEN8_2	T	T	C/T	T	T	G	T	T	C	T	T	C/T	A/G	A/C	A/G	A/C	A/G	T	C	C	T	C	C	C	C	A	G	C	T	A	A	T
PUNGEN2	T	T	C	T	T	G	T	T	C	T	T	C	A/G	A	A/G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
UVAURS9_2	T	T	A/C	T	T	G	T	T	C	T	T	T	A	A	A	C	A	T	T	C	T	C	C	C	A	G	C	T	A	A	T	
MANZANI2	T	T	C/T	T	T	G	T	T	C	T	T	C	A	A/C	A	C	A	A	C/T	C	T	T	T	T	A	G	C	T	A	A	T	
MANZAN2	T	T	C	T	T	G	T	T	C	T	T	C	A/G	A/C	A/G	C	A	T	C	C	T	C/T	C	C	T	A	G	C	T	A	A	T
MANZANI1	T	T	C	T	T	G	T	T	C	T	T	C	A/G	A	A/G	C	A	A	C	C	C/T	C/T	C/T	C/T	T	A	G	C	T	A	A	T
MANZANr1	T	T	C	T	T	G	T	T	C	T	T	C	A/G	A	A/G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
PACIF1	T	T	C	T	T	G	T	T	C	T	T	C	G/T	A	G/T	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
TOMENT7_2	T	T	C	T	T	G	T	T	C	T	T	C	G	C	G	C	G	T	C	C	T	C	C	C	-	A	G	C	T	A	A	T
FRANC18	T	T	C	T	T	G	T	T	C	T	T	T	A/G	A/C	A/G	A	A	T	C	C	T	C	C	C	A	G	C	T	A	A	T	

Figure 3. Reduced, rearranged, and colored ITS+5.8S cross-lineage sequences. All but one taxon are polyploid (red identifiers).

bp	g139	g142	g156	g264	g164	g219	g260	g66	g82	g113	g115	g116	g231	g233	g240	g254
RUBRA_Z	A	T	G	G	T	T	C	G	C	G	C	T	C	C	C	C
PUNGENZ	C	C	A	C	C/T	A/T	A/T	G	C	G/T	C	T	C	C	C	C
UVAUR2Z	C	C	A	C	G/A/T/C	C	A	G	C	G	C	T	C	C	C	C
UVAUR3Z	C	C	A	C	C	T	T	G/A/T/C	T	G	C	C	T	C	C	C
UVAUR1Z	C	C	A	C	C	C	T	G	C	G	C	T	C	C	C	C
MANZANZ	C	C	A	C	G/A/T/C	G/A/T/C	G/A/T/C	C	G	G	C	T	C	C	C	C

Figure 4. Reduced, rearranged, and colored 26S cross-lineage sequences. All taxa are polyploid.

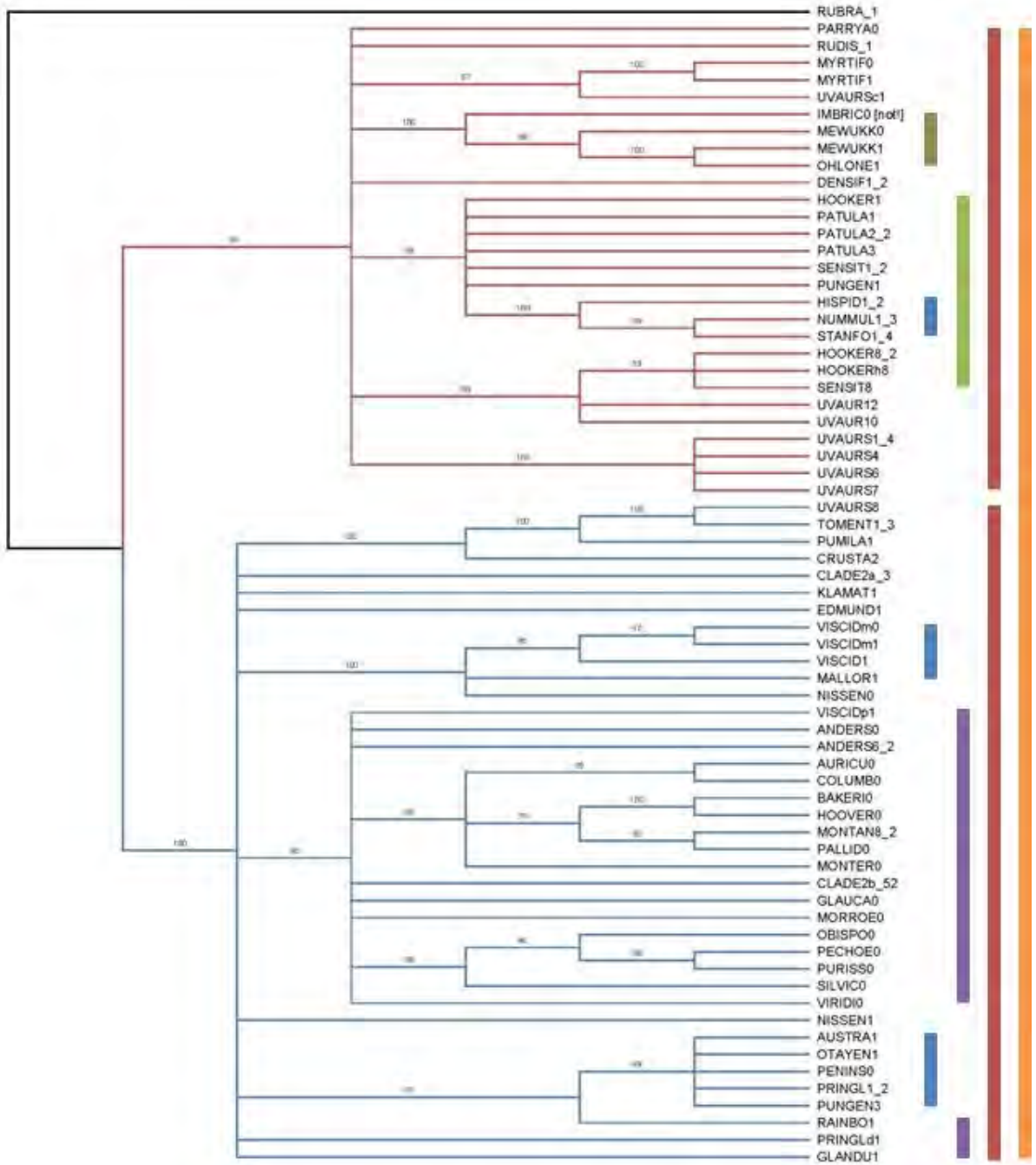


Figure 5. ITS+5.8S majority-rule consensus tree with cross-lineage sequences removed. Orange bar is the genus *Arctostaphylos*. Red bars are the patula and columbiana clades. Brown bar is the ohloneana clade.

subspecies, under a new common identifier with an appended number. Also, in two cases, I combined multiple taxa with identical sequences under a common identifier (CLADE2a_3 and CLADE2b_52). Membership of these combined sequence groups are listed in Appendix 2. All sequences with character differences, including polymorphisms, were retained. Finally, I rearranged the order of the characters (nucleotides) and colored groups of derived characters (apomorphies and autapomorphies) to aid visual identification of any patterns. Portions of the reduced, rearranged, and colored character matrices are shown in Figure 1 (ITS+5.8S) and Figure 2 (26S).

Several groups of derived characters are apparent within these nucleotide sequences. First, 15 character positions (orange)—seven in the ITS1 region, four in ITS2, and four in 26S—differ nearly uniformly from the ancestral state (the outgroup, RUBRA_1). These positions are obviously responsible for the synapomorphy that defines the genus *Arctostaphylos*. Also, seven character positions (red)—two in ITS1, two in ITS2, and three in 26S—assume complementary character states as a group. These positions are clearly responsible for a synapomorphy that splits the genus into two deep lineages. This two-clade phylogeny was first observed by Denford (1981) using flavonoids as markers. While investigating the monophyly of five *Arctostaphylos* subspecies, Markos et al. (1998) recovered these two clades from nuclear ribosomal DNA using ITS and 26S sequences and RFLP markers. Boykin et al. (2005) and Wahlert et al. (2009) demonstrated that the two-clade structure persisted as they increased the number of *Arctostaphylos* taxa sampled (Appendix 3). A few one and two-character apomorphies (blue and green) are also evident within the nucleotide sequences, as well as one longer derived group that encompasses seven character positions (brown). This seven-character apomorphy will be discussed below.

Of course the datasets are not quite as uniform as Figures 1 and 2 imply. Within the group of seven character positions that divides *Arctostaphylos* into two lineages (four in ITS+5.8S and three in 26S), various assortments of individual nucleotides from both clades, and polymorphisms that combine nucleotides from both clades, appear in 15 sequences from six species (Figures 3 and 4).

Nevertheless, after removing those 15 cross-clade sequences from the ITS+5.8S matrix, maximum parsimony in PAUP* generated a majority-rule consensus tree for the genus *Arctostaphylos* that corresponds closely with the predicted phylogeny (compare the colored bars in Figure 5 with like-colored cells in Figure 1) and adds additional taxa to the phylogenies published by Markos et al. (1998), Boykin et al. (2005), and Wahlert et al. (2009).

Cross-lineage hybrid parentage

Cross-lineage sequences cause problems for phylogeny inference, but they provide valuable insights into hybridization between species. Hybridization in *Arctostaphylos* has been an active topic of research and conjecture for many decades. Many *Arctostaphylos* species are hypothesized to have arisen through diploid hybridization or polyploidy, but reports of cross-lineage hybridization are uncommon (Wahlert et al. 2006, Parker 2007).

I identified six hybrid species with nuclear ribosomal DNA sequences that contain nucleotide combinations from both lineages (Figures 3 and 4). In these cross-lineage hybrids, polymorphic nucleotides are common at the seven base positions that separate the two lineages. In cross-lineage hybrids where individuals from multiple sequences are available, nucleotide variation between populations is present at these seven positions. The prevalence of four-character-state ambiguity at the positions that split the genus in several 26S sequences suggests polyploidy in the origin of these species (Figure 4). Not surprisingly, the literature confirms that four of the cross-clade species are polyploids, and the ploidy of another is uncertain.

I tested whether the parents of known or hypothesized cross-lineage hybrids could be identified by performing pairwise sequence comparisons between all other extant taxa and evaluating nucleotide additivity in the target taxon. Similar attempts to evaluate possible hybridization and polyploidy with ITS sequence additivity have been reported (e.g. Whittall 2000, Hardig et al. 2002).

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472
PATULA2_2	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	G	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
MEWUKK1	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	A	A	T	A	C	T	T	T	T	A	G	C	T	A	A	T
VISCIDm1	T	T	C	T	T	G	T	T	C	T	T	T	G	A	G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	-	C	A

Figure 6. *A. patula* and *A. viscida* are not additive for *A. mewukka*; seven nucleotides (brown) are not available from either parent (ITS+5.8S).

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472
OHLONE1	T	T	T	T	T	G	T	T	C	T	T	C	A	C	A	C	A	A	T	A	C	T	T	T	T	A	G	C	T	A	A	T
MEWUKK1	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	A	A	T	A	C	T	T	T	T	A	G	C	T	A	A	T
[various]	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T

Figure 7. *A. ohloneana* is additive for *A. mewukka* in hybrids with any of various taxa from the same lineage (ITS+5.8S).

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472	
OHLONE1	T	T	T	T	T	G	T	T	C	T	T	C	A	C	A	C	A	A	T	A	C	T	T	T	T	A	G	C	T	A	A	T	
MANZAN2	T	T	C/T	T	T	G	T	T	C	T	T	C	A	A/C	A	C	A	A	C/T	C	T	T	T	T	T	A	G	C	T	A	A	T	
MANZAN2	T	T	C	T	T	G	T	T	C	T	T	C	A	A/G	A/C	A/G	C	A	T	C	C	T	C	C	T	A	G	C	T	A	A	T	
MANZAN1	T	T	C	T	T	G	T	T	C	T	T	C	A	A/G	A	A/G	C	A	A	C	C	C/T	C/T	C/T	C/T	T	A	G	C	T	A	A	T
MANZANr1	T	T	C	T	T	G	T	T	C	T	T	C	A	A/G	A	A/G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
[various]	T	T	C	T	T	G	T	T	C	T	T	T	G	A	G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T	

Figure 8. *A. ohloneana* is additive for all selections of *A. manzanita* in hybrids with any of various taxa from the opposite lineage (ITS+5.8S).

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472	
HOOKER8_2	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	G	T	C	C	T	C	C	C	C	A	G	C	T	A	A	T	
PUNGEN1	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	G	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T	
PUNGEN8_2	T	T	C/T	T	T	G	T	T	C	T	T	C/T	A/G	A/C	A/G	A/C	A/G	T	C	C	T	C	C	C	C	A	G	C	T	A	A	T	
PUNGEN2	T	T	C	T	T	G	T	T	C	T	T	C	A	A/G	A	A/G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T
CLADE2b_52	T	T	C	T	T	G	T	T	C	T	T	T	G	A	G	A	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T	

Figure 9. *A. hookeri* (and several other possible parents) is additive for all *A. pungens* sequences in hybrids with any of various taxa from the opposite lineage (ITS+5.8S).

Searching for potential hybrid parents of taxa with ITS+5.8S sequences that contain the seven-character apomorphy mentioned above (brown in Figures 1, 3, and 5) produced surprising results. *Arctostaphylos mewukka*, a species long hypothesized to be of hybrid origin, has been carefully studied (Epling 1947, Dobzhansky 1953, Roof 1967, Schmid 1968). *A. mewukka* was first described as a diploid hybrid between *Arctostaphylos patula* and *A. viscida*, two manzanitas with overlapping ranges (McMinn 1939). Schierenbeck et al. (1992) performed detailed morphological and cytological studies and declared the species and subspecies to be the result of multiple independent (polyphyletic) allopolyploidy with *A. patula* and *A. viscida* as parents. Yet pairwise sequence comparison shows that the combination of *A. patula* and *A. viscida* is not additive for *A. mewukka* (Figure 6). Only one extant species satisfies the additivity requirement: *A. ohloneana* (Figure 7). Furthermore, while *A. patula* and *A. viscida* are from different lineages, *A. mewukka* can result from hybridization between *A. ohloneana* and various taxa in the same lineage. *A. mewukka* is fairly widespread at middle elevations in California's Sierra Nevada. *A. ohloneana* is a rare, recently described species from just one coastal Santa Cruz County location—well over one hundred miles to the west (Kauffmann 2015).

There's more. *Arctostaphylos manzanita* and its subspecies are a group of morphologically and geographically diverse polyploid taxa. This variability is also evident in the variety of nucleotide combinations in its sequences. Three *A. manzanita* sequences contain some (but not all) of the same seven-character apomorphy, either as variable characters or polymorphisms (Figure 8). This variability suggests *A. manzanita*'s numerous subspecies and varieties may have originated through multiple independent polyploidy. Once again, pairwise comparison found *A. ohloneana* as the only possibility for one of *A. manzanita*'s hybrid parents (Figure 8). According to Vasey and Parker (2008), *A. ohloneana* superficially resembles *A. manzanita*. *A. manzanita* is found in a variety of locations and habitats, including a small site about 30 miles east of *A. ohloneana*'s coastal location (Kauffmann 2015).

Like *Arctostaphylos manzanita*, *Arctostaphylos pungens* has a variety of nucleotide combinations and polymorphisms in its sequences. As in *A. manzanita*, this variability suggests multiple independent polyploidy. Again I searched for potential hybrid parents for *A. pungens* by comparing sequences and evaluating nucleotide additivity. In this case, more than one species satisfies the additivity requirement; *A. Hookeri*, for example (Figure 9). *Arctostaphylos pungens* is generally considered diploid, but Roof (1976) writes: "With a single known exception, in southern Mexico, the *A. pungens* of Mexico and southern California has been determined to be a tetraploid, with a gamete number of 26". If Roof is correct, *A. pungens* is another cross-lineage polyploid. This helps explain why Keeley (1976) observed unexpected morphological characters emerge from hybridization between (tetraploid?) *A. pungens* and diploid *A. glauca*. Believing *A. pungens* to also be diploid, Keeley declared the population a hybrid swarm with backcrosses to both parents rather than variable offspring from a polyploid parent.

Discussion

Two lineages (*patula* and *columbiana* clades)

Several authors have proposed biogeographical explanations for the rapid post-glacial radiation of *Arctostaphylos*. Raven and Axelrod (1978) describe the Xerothermic Period, a time of warming and

drying climate, when boundaries between northern forest-dominated vegetation (the Arcto-Tertiary Geoflora) and southern scrub-dominated vegetation (Madro-Tertiary Geoflora) were changing rapidly. They attribute much of the explosive speciation in species like *Arctostaphylos* to these changes. The two deep lineages within the *Arctostaphylos* phylogeny likely originated with these two vegetation types.

Because *Arctostaphylos* diversity and taxonomy are heavily influenced by the split between two lineages, these lineages deserve more imaginative names than “Group One/Group Two” (Markos et al. 1998) or “Clade 1/Clade 2” (Boykin et al. 2005, Wahlert et al. 2009). I recommend that the most representative or widespread diploid species in each lineage should lend its name, and provisionally suggest “*patula* clade” for those species with Great Basin/Rocky Mountain (Madro-Tertiary) affinities, and “*columbiana* clade” for those species with northern (Arcto-Tertiary) affinities.

The *ohloneana* clade

The small clade that shares a distinctive seven-nucleotide apomorphy is also deserving of a name. Its rare, recently-described diploid member, *Arctostaphylos ohloneana*, may be the paleoendemic remnant from a formerly wide-ranging species (Vasey and Parker 2008) that participated in multiple independent polyploidy to produce an assortment of *A. manzanita* and *A. mewukka* subspecies. I suggest “*ohloneana* clade” for this group that is providing valuable insights into diversification and biogeography in *Arctostaphylos*.

Too many manzanitas?

Two very different patterns of diversification are evident in *Arctostaphylos*. Same-lineage species conform rigidly to a nucleotide “signature”, the handful of apomorphies, each involving just a few base positions, that collectively defines its place in the phylogeny (colored cells in Figures 1 and 2 and colored bars in Figure 5). Ninety percent of *Arctostaphylos* taxa exhibit this consistency. One signature is common to 34 species, half the genus. In contrast, each cross-lineage population acquires its own unique arrangement of nucleotides and polymorphisms, especially at the seven base positions that separate the lineages, rather than rigidly conforming to a common, species-wide signature (figures 3 and 4). Their phylogeny is a reticulated network rather than a simple tree. In every case but one, these hybrid species are polyploids, suggesting that whole genome duplication enables hybridization between lineages (Schierenbeck et al. 1992).

Arctostaphylos taxonomists have been enthusiastic “splitters”—at least from the perspective of nuclear ribosomal DNA. Raven (1969) characterized the situation:

A variety of workers have continued to present new combinations and new taxa without ever approaching the overall view of the group necessary to achieve taxonomic synthesis. A useful taxonomic system for a complex group such as *Arctostaphylos* will never be built up on such blocks, and indeed, the overall pattern of variation tends to become more and more obscure as the new taxa are proliferated.

Roof (1976), following Raven’s lead, took a “fresh approach” to the genus and attempted a complete revision based on just six basic species. All the rest he considered hybrids to be reclassified as subspecies and varieties (inconsistently applied terms for regional or local “coherent evolutionary

subsets”, Hamilton and Reichard 1992). For same-lineage species, one nucleotide signature is typically shared by a variety of populations, whether species, subspecies, or same-lineage hybrids that have undergone concerted evolution. For these taxa, Roof’s radical “lumping” approach seems appropriate, notwithstanding conservation concerns (will endangered populations continue to receive protection if they’re no longer called species?). But not so for cross-lineage polyploids and hybrids. The best documented example of cross-lineage diversification in this study is *Arctostaphylos manzanita*, with individuals from nine populations. Roof describes “combinations too numerous and devious to recite” in *A. manzanita* and worries that “there is danger of attaching to it more subspecies than it can possibly bear”. Such taxa demand a rethinking of the species concept. What is a species if every population has its own unique arrangement of nucleotides? Should every population constitute a separate species? If so, does every species deserve endangered species protection?

bp	i27	i31	i36	i47	i60	i62	i72	i428	i496	i568	i577	i37	i435	i207	i468	i33	i114	i106	i187	i416	i417	i469	i494	i506	g284	i32	i79	i226	i535	i182	i231	i472	
HOOKER8_2	T	T	C	T	T	G	T	T	C	T	T	C	A	C	A	C	G	T	C	C	T	C	C	C	C	C	A	G	C	T	A	A	T
PAJARO1_2	T	T	C	T	T	G	T	T	C	T	T	T	G	A	G	A	A	T	C	C	T	C	C	C	C	T	A	G	C	T	A	A	T
CRUSTAL_2	T	T	C	T	T	G	T	T	C	T	T	T	G	A	G	C	A	T	C	C	T	C	C	C	T	A	G	C	T	A	A	T	

Figure 10. The three *Arctostaphylos* at Manzanita Park (ITS+5.8S). A diploid in the patula clade, a diploid in the columbiana clade, and a polyploid. What would a polyploid *A. ‘Sunset’* look like?

Arctostaphylos ‘Sunset’

Hybridization has been reported to be relatively frequent between some pairs of *Arctostaphylos* species. For other pairs, hybrids appear infrequently, if ever. Reproductive barriers are presumed to be stronger between the two lineages than within (Parker 2007). In studies where hybridization was frequent, the parents were typically members of the same lineage; where hybridization was uncommon, they were from opposite lineages (Vasey and Parker 2014). A similar situation exists in the genus *Ceanothus*: two deep lineages are separated by strong reproductive barriers, which are believed to be geographical and edaphic rather than intrinsic (Hardig et al. 2002).

Manzanitas from the two lineages are frequently found growing together in the wild. Often they are accompanied by a polyploid. This is the situation at Manzanita Park. *Arctostaphylos hookeri* is a diploid in the *patula* clade, *A.pajaroensis* is a diploid in the *columbiana* clade, and *A. crustacea* is polyploid.

Arctostaphylos ‘Sunset’ is a cross-lineage hybrid. It would be interesting to learn if it is polyploid.

Needs

The existence of two deep lineages in the inferred *Arctostaphylos* phylogeny, the *patula* and *columbiana* clades, is only moderately supported by bootstrap resampling (Markos et al. 1998, Boykin et al. 2005, Wahlert et al. 2009). With the exception of the *ohloneana* clade, clades deeper in the phylogeny receive even lower support. The most obvious path to increasing statistical support, and for determining

whether the conclusions reported in this study are robust, is to acquire sequence data beyond the nuclear ribosomal repeats—including low-copy coding regions and plastid genomes.

The assumption has been that, because of concerted evolution, only a single individual from a single population is necessary to obtain representative sequence data for *Arctostaphylos* (Boykin et al. 2005). For same-lineage species, this is likely the case. But for cross-lineage hybrids, the few available sequences from different populations in a single species (*Arctostaphylos manzanita*, *A. pungens*, and *A. uva-ursi*) clearly contradict the assumption. Therefore a greater breadth of sampling among the six cross-lineage species is also needed. These sequences should capture the geographic range of each species, with emphasis on unusual edaphics (e.g. serpentine) and regions that overlap with other *Arctostaphylos* taxa.

Finally, more definitive chromosome counts are needed for a few cross-lineage species. Is there a range of ploidy in *Arctostaphylos mewukka* and *A. pungens*? Chromosomal variations likely influence the trajectory of species evolution and hybridization.

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Appendices

Sequence	Taxon	GenBank1	GenBank2	Ploidy	Author	Location	Voucher
ANDERS0	andersonii	AF297780	AF297825	2n = 26	boykin	Santa Cruz	MV 0089
ANDERS1	andersonii	GQ280910		2n = 26	wahlert	San Mateo Co	V.T.Parker & M.C.Vasey 764
ANDERS6	andersonii	AH006955		2n = 26	hileman		V 0086
RUBRA_0	arctous rubra	AF297792	AF297837		boykin		
RUBRA_1	arctous rubra	AF091944			hileman		P 0860
AURICU0	auriculata	AF297779	AF297824	2n = 26	boykin	Contra Costa	MV 0170
AURICU1	auriculata	GQ280911		2n = 26	wahlert	Contra Costa Co	M.C.Vasey 840
AUSTRA1	australis	GQ280912		2n = 26	wahlert	Baja Norte	M.C.Vasey 799
BAKERI0	bakeri	AF297774	AF297819	2n = 52	boykin	Sonoma	VTP & MV 0547
BAKERI1	bakeri	GQ280913		2n = 52	wahlert	Sonoma Co	M.C.Vasey 71
BAKERIs1	bakeri sublaevis	GQ280914			wahlert	Sonoma Co	V.T.Parker & M.C.Vasey 548
CANESCO	canescens	AF297781	AF297826	2n = 26	boykin	Santa Cruz	MV 0179
CANESC1	canescens	GQ280915		2n = 26	wahlert	Marin Co	V.T.Parker & M.C.Vasey 443
CANESCs1	canescens sonomensis	GQ280916		2n = 26	wahlert	Lake Co	M.C.Vasey 282
CATALI0	catalinae	AF297787	AF297832	2n = 26	boykin	Santa Catalina Island	VTP & MV 155
CATALI1	catalinae	GQ280917		2n = 26	wahlert	Santa Barbara Co	V.T.Parker & M.C.Vasey 155
COLUMB0	columbiana	AF297765	AF297810	2n = 26	boykin	Mendocino	VTP 0299
COLUMB1	columbiana	GQ280918		2n = 26	wahlert	Humboldt Co	M.C.Vasey 1030
CONFER1	confertiflora	GQ280919		2n = 26	wahlert	Santa Barbara Co	V.T.Parker & M.C.Vasey 900
CRUSTA1	crustacea 1	GQ280987		2n = 52	wahlert	Monterey Co	V.T.Parker 584
CRUSTA2	crustacea 2	GQ280988		2n = 52	wahlert	Santa Cruz Co	M.C.Vasey 786
CRUSTAi1	crustacea insulicola	GQ280990		2n = 52	wahlert	Santa Barbara Co	V.T.Parker & M.C.Vasey 903
CRUSTAr1	crustacea rosei	GQ280991		2n = 52	wahlert	Monterey Co	M.C.Vasey 822
CRUSTAs1	crustacea subcordata	GQ280992		2n = 52	wahlert	Santa Barbara Co	V.T.Parker & M.C.Vasey 901
CRUZEN0	cruzensis	AF297788	AF297833	2n = 26	boykin	San Luis Obispo	VTP & Schierenbeck 0012
CRUZEN1	cruzensis	GQ280920		2n = 26	wahlert	San Luis Obispo Co	V.T.Parker et al. 12
DENSIF0	densiflora	AF297753	AF297798	2n = 26	boykin	Sonoma	MV 0069
DENSIF1	densiflora	GQ280921		2n = 26	wahlert	Sonoma Co	M.C.Vasey 69
EDMUND1	edmundsii	GQ280922		2n = 26	wahlert	Monterey Co	M.C.Vasey 819
FRANCI1	franciscana	GQ280931		2n = 26	wahlert	Strybing Arboretum	strybing
FRANCI8	franciscana [hookeri ssp.]	AF106816		2n = 26	markos	[San Francisco]	tilden
GABILA1	gabilanensis	GQ280923		2n = 26	wahlert	San Benito Co	V.T.Parker & M.C.Vasey 1086
GLANDU0	glandulosa	AF297775	AF297820	2n = 52	boykin	Santa Barbara	VTP & MV 0157
GLANDU1	glandulosa 1	GQ280925		2n = 52	wahlert	San Diego Co	M.C.Vasey 724
GLANDU2	glandulosa 2	GQ280926		2n = 52	wahlert	San Benito Co	V.T.Parker & M.C.Vasey 456
GLANDUc1	glandulosa cushingiana	GQ280924		2n = 52	wahlert	Santa Barbara Co	M.C.Vasey 1002
GLANDUm1	glandulosa mollis [glaucomolli	GQ280927			wahlert	Los Angeles Co	M.C.Vasey 1000
GLAUCA0	glauca	AF297778	AF297823	2n = 26	boykin	San Luis Obispo	VTP 0235
GLAUCA1	glauca	GQ280928		2n = 26	wahlert	Los Angeles Co	M.C.Vasey 809
GLUTIN1	glutinosa	GQ280929		2n = 26	wahlert	Santa Cruz Co	M.C.Vasey 401
HISPID0	hispidula	AF297752	AF297797	2n = 26	boykin	Del Norte	MV 0360
HISPID1	hispidula	GQ280930		2n = 26	wahlert	Del Norte Co	M.C.Vasey 360
HOOKER0	hookeri	AF297756	AF297801	2n = 26	boykin		Strybing Arboretum
HOOKER1	hookeri	GQ280932		2n = 26	wahlert	Santa Cruz Co	V.T.Parker & M.C.Vasey 55
HOOKER8	hookeri [ssp. hookeri]	AF106818		2n = 26	markos	Monterey Co	SM,VTP,&MV 0607
HOOKERh8	hookeri hearstiorum	AF106817		2n = 26	markos	San Luis Obispo Co	SM,VTP&MV 0603/strybing
HOOVER0	hooveri	AF297773	AF297818	2n = 26	boykin	Monterey	MV 0667
HOOVER1	hooveri	GQ280934		2n = 26	wahlert	Monterey Co	M.C.Vasey 1050
IMBRIC0	imbricata?	AF297758	AF297803	2n = 26	boykin		[wrong taxon?]
IMBRIC1	imbricata	GQ280935		2n = 26	wahlert	San Mateo Co	M.C.Vasey 18
INSULA1	insularis	GQ280936		2n = 26	wahlert	Santa Barbara Co	Anonymous s.n.

Appendix 1: ITS+5.8S sequence identifiers, taxon names, GenBank accession numbers, ploidy, study authors, collection locations, and voucher identification.

Sequence	Taxon	GenBank1	GenBank2	Ploidy	Author	Location	Voucher
KLAMAT1	klamathensis	GQ280937		2n = 26	wahlert	Siskiyou Co	M.C.Vasey 756
LUCIAN1	luciana	GQ280938		2n = 26	wahlert	San Luis Obispo Co	M.C.Vasey 1046
MALLOR1	malloryi	GQ280939		2n = 26	wahlert	Shasta Co	V.T.Parker & M.C.Vasey 834
MANZAN1	manzanita 1	GQ280944		2n = 52	wahlert	Mendocino Co	V.T.Parker & M.C.Vasey 369
MANZAN2	manzanita 2	GQ280945		2n = 52	wahlert	Humboldt Co	M.C.Vasey 851
MANZAN3	manzanita 3	GQ280946		2n = 52	wahlert	Amador Co	M.C.Vasey 767
MANZAN4	manzanita 4	GQ280947		2n = 52	wahlert	Sonoma Co	M.C.Vasey 424
MANZANe1	manzanita elegans	GQ280941		2n = 52	wahlert	Glenn Co	M.C.Vasey 216
MANZANg1	manzanita glaucescens	GQ280942			wahlert	Sonoma Co	V.T.Parker & M.C.Vasey 508
MANZANI1	manzanita laevigata 1	GQ280943			wahlert	Contra Costa Co	M.C.Vasey 894
MANZANI2	manzanita laevigata 2 [bowern	GQ280940			wahlert	Contra Costa Co	M.C.Vasey 169
MANZANr1	manzanita roofii	GQ280948		2n = 52	wahlert	Glenn Co	M.C.Vasey 883
MEWUKK0	mewukka	AF297759	AF297804	2n=26/52	boykin	Butte	MV 0029
MEWUKK1	mewukka	GQ280952		2n=26/52	wahlert	Tuolumne Co	M.C.Vasey 575
MONTAN1	montana	GQ280933		2n = 52	wahlert	Marin Co	S. Markos 613
MONTAN8	montana [hookeri]	AF091951		2n = 52	hileman	Marin Co	SM,VTP&MV 0601 / M 0610
MONTANr8	montana raveni [hookeri ssp.]	AF106819		2n = 52	markos	[San Francisco Co]	tilden
MONTAR1	montaraensis	GQ280953		2n = 26	wahlert	San Mateo Co	M.C.Vasey 743
MONTER0	montereyensis	AF297770	AF297815	2n = 26	boykin	Monterey	VTP 0581
MONTER1	montereyensis	GQ280954		2n = 26	wahlert	Monterey Co	M.C.Vasey 656
MORROE0	morroensis	AF297763	AF297808	2n = 26	boykin	San Luis Obispo	VTP & MV 0149
MORROE1	morroensis	GQ280955		2n = 26	wahlert	San Luis Obispo Co	K. Bode 823
MYRTIF0	myrtifolia	AF297760	AF297805	2n = 26	boykin	Amador	VTP & MV 0497
MYRTIF1	myrtifolia	GQ280956		2n = 26	wahlert	Amador Co	M.C.Vasey 733
NEVADA1	nevadensis	GQ280957		2n=26/52	wahlert	Siskiyou Co	M.C.Vasey 757
NISSEN0	nissenana	AF297782	AF297827	2n = 26	boykin	El Dorado	VTP & MV 0490
NISSEN1	nissenana	GQ280958		2n = 26	wahlert	Placer Co	M.C.Vasey 892
NUMMUL1	nummularia	GQ280959		2n = 26	wahlert	Mendocino Co	V.T.Parker & M.C.Vasey 516
NUMMULm1	nummularia mendocinoensis	GQ280951			wahlert	Mendocino Co	G.Wahlert s.n.
NUMMULm0	nummularia mendocinoensis	AF297750	AF297795		boykin	Mendocino	McCabe & Shierenbeck 0037
OBISPO0	obispoensis	AF297764	AF297809	2n = 26	boykin	San Luis Obispo	VTP & MV 0236
OBISPO1	obispoensis	GQ280960		2n = 26	wahlert	Monterey Co	M.C.Vasey 1052
OHLONE1	ohloneana	GQ280961		2n = 26	wahlert	Santa Cruz Co	V.T.Parker & M.C.Vasey 111
OSOENS1	osoensis	GQ280962		2n = 26	wahlert	San Luis Obispo Co	K. Bode 780
OTAYEN1	otayensis	GQ280963		2n = 26	wahlert	San Diego Co	M.C.Vasey 748
PACIFI1	pacifica	GQ280964		2n = 52	wahlert	San Mateo Co	M.C.Vasey 20
PAJARO0	pajaroensis	AF297772	AF297817	2n = 26	boykin	Monterey	VTP & MV 0459
PAJARO1	pajaroensis	GQ280965		2n = 26	wahlert	Monterey Co	V.T.Parker & M.C.Vasey 459
PALLID0	pallida	AF297771	AF297816	2n = 26	boykin	Contra Costa	VTP & MV 0565
PALLID1	pallida	GQ280966		2n = 26	wahlert	Alameda Co	M.C.Vasey 436
PARRYA0	parryana	AF297757	AF297802	2n = 52	boykin	San Bernardino	J Keeley 22,291
PATULA0	patula	AF297754	AF297799	2n = 26	boykin	Sierra	VTP 0313
PATULA1	patula 1	GQ280967		2n = 26	wahlert	White Pine Co NV	M.C.Vasey 626
PATULA2	patula 2	GQ280968		2n = 26	wahlert	Plumas Co	M. Wood 316
PATULA3	patula 3	GQ280969		2n = 26	wahlert	Calaveras Co	V.T.Parker & M.C.Vasey 770
PECHOE0	pechoensis	AF297767	AF297812	2n = 26	boykin	San Luis Obispo	Markos 0264
PENINS0	peninsularis	AF297785	AF297830		boykin	Baja California	MV 0804
PILOSU0	pilosula	AF297766	AF297811	2n = 26	boykin	San Luis Obispo	VTP 0233
PILOSU1	pilosula	GQ280970		2n = 26	wahlert	San Luis Obispo Co	M.C.Vasey 233
PILOSU2	pilosula [wellsii]	GQ281005		2n = 26	wahlert	San Luis Obispo Co	M.C.Vasey 1041
PRINGL0	pringlei	AF297784	AF297829	2n = 26	boykin	Pima AZ	MV 0232

Appendix 1 (cont.): ITS+5.8S

Sequence	Taxon	GenBank1	GenBank2	Ploidy	Author	Location	Voucher
PRINGL1	pringlei	GQ280972		2n = 26	wahlert	Pima Co AZ	M.C.Vasey 232
PRINGLd1	pringlei drupacea	GQ280971			wahlert	Riverside Co	M.C.Vasey 993
PUMILA1	pumila	GQ280973		2n = 26	wahlert	Monterey Co	V.T.Parker & M.C.Vasey 677
PUNGEN0	pungens	AF297762	AF297807	2n=26/52	boykin		
PUNGEN8	pungens	AF106820		2n=26/52	markos	[San Diego Co]	tilden
PUNGEN1	pungens 1	GQ280974		2n=26/52	wahlert	San Bernardino Co	M.C.Vasey 194
PUNGEN2	pungens 2	GQ280975		2n=26/52	wahlert	San Diego Co	M.C.Vasey 696
PUNGEN3	pungens 3	GQ280976		2n=26/52	wahlert	Pima Co AZ	M.C.Vasey 228
PURISS0	purissima	AF297769	AF297814	2n = 26	boykin	Santa Barbara	VTP 0238
PURISS1	purissima	GQ280977		2n = 26	wahlert	Santa Barbara Co	V.T.Parker et al. 2
RAINBO1	rainbowensis	GQ280978		2n = 26	wahlert	San Diego Co	K. Bode 775
REFUGI0	refugioensis	AF297776	AF297821	2n = 26	boykin	Santa Barbara	MV 0156
REGISM1	regismontana	GQ280979		2n = 26	wahlert	San Mateo Co	M.C.Vasey 557
RUDIS_1	rudis	GQ280981		2n = 26	wahlert	San Luis Obispo Co	V.T.Parker & M.C.Vasey 151
SENSIT1	sensitiva	GQ280982		2n = 26	wahlert	Marin Co	V.T.Parker 306
SENSIT0	sensitiva [nummularia]	AF297755	AF297800	2n = 26	boykin	Marin	Dunne 0040
SENSIT8	sensitiva [nummularia]	AF091949		2n = 26	hileman	Santa Cruz Co	V 0040 / MV&VTP 0398
SILVIC0	silvicola	AF297768	AF297813	2n = 26	boykin	Santa Cruz	MV 0082
SILVIC1	silvicola	GQ280983		2n = 26	wahlert	Santa Cruz Co	V.T.Parker & M.C.Vasey 865
STANFO0	stanfordiana	AF297751	AF297796	2n = 26	boykin	Sonoma	MV 0468
STANFO1	stanfordiana	GQ280986		2n = 26	wahlert	Napa Co	M.C.Vasey 392
STANFOd1	stanfordiana decumbens	GQ280984			wahlert	Sonoma Co	M.C.Vasey 422
STANFOr1	stanfordiana raichei	GQ280985			wahlert	Mendocino Co	M.C.Vasey 277
TOMENT1	tomentosa [ssp tomentosa]	GQ280993		2n = 52	wahlert	Monterey Co	M.C.Vasey 243
TOMENT0	tomentosa [ssp. tomentosay]	AF297786	AF297831	2n = 52	boykin	Monterey	MV 0243
TOMENT8	tomentosa [ssp. tomentosa]	AF106823		2n = 52	markos	Monterey	MV&VTP 0243
TOMENT7	tomentosa	AH006954		2n = 52	hileman		V 0243
TOMENTd1	tomentosa daciticola	GQ280989			wahlert	San Luis Obispo Co	M.C.Vasey 1008
UVAUR12	uva-ursi	FJ378570		2n = 26/52	liu		KUN:D.E.Boufford 40494
UVAURS0	uva-ursi	AF297761	AF297806	2n = 26/52	boykin	San Mateo	MV 0019
UVAURS7	uva-ursi	KR005087		2n = 26/52	van ee		
UVAURS8	uva-ursi	AF106821		2n = 26/52	markos	Monterey Co	VTP&MV 0046
UVAURS10	uva-ursi	AF091950		2n = 26/52	hileman	Ocean Co NJ	VTP 0440 / T 0440
UVAURS9	uva-ursi	AF106822		2n = 26/52	markos	San Mateo Co	MV 0019
UVAURS1	uva-ursi 1	GQ280994		2n = 26/52	wahlert	Ocean Co NJ	V.T.Parker 437
UVAURS2	uva-ursi 2	GQ280995		2n = 26/52	wahlert	Sleeping Bear National	McEaghan 933
UVAURS3	uva-ursi 3	GQ280996		2n = 26/52	wahlert	Yellowstone National	V.T.Parker & M.C.Vasey 942
UVAURS4	uva-ursi 4	GQ280997		2n = 26/52	wahlert	Gunnison Co CO	M.C.Vasey 643
UVAURS5	uva-ursi 5	GQ280998		2n = 26/52	wahlert	Alaska	J. Bishop 684
UVAURS6	uva-ursi 6	GQ280999		2n = 26/52	wahlert	Clallam Co WA	M.C.Vasey 340
UVAURSc1	uva-ursi cratericola	GQ281000			wahlert	U.C. Botanical Garden	92- 0545
VIRGAT1	virgata	GQ281001		2n = 26	wahlert	Marin Co	M.C.Vasey 783
VIRIDIO	viridissima	AF297777	AF297822	2n = 26	boykin	Santa Barbara	MV 0875
VISCID1	viscida	GQ281004		2n = 26	wahlert	Eldorado Co	V.T.Parker & M.C.Vasey 849
VISCIDm0	viscida mariposa	AF297783	AF297828	2n = 26	boykin	Tuolumne	VTP & MV 0569
VISCIDm1	viscida mariposa	GQ281002		2n = 26	wahlert	Tuolumne Co	M.C.Vasey 499
VISCIDp1	viscida pulchella	GQ281003			wahlert	Sonoma Co	M.C.Vasey 470
XMANST1	x manzanita x stanfordiana	GQ280949			wahlert	Sonoma Co	M.C.Vasey 425
XMEDIA1	x media	GQ280950			wahlert	Mendocino Co	M.C.Vasey 253
XREPEN1	x repens	GQ280980		2n = 26	wahlert	Marin Co	V.T.Parker & M.C.Vasey 276

Appendix 1 (cont.): ITS+5.8S

Sequence	Taxon	GenBank	Ploidy	Author	Location	Voucher
ANDERSZ	andersonii	AF091976	2n = 26	hileman	Santa Cruz Co	V 0086 / VTP&MV 0089
RUBRA_Z	arctous rubra	AF091968		hileman		P 0860
CANESZ	canescens	AF106813	2n = 26	markos	Santa Cruz Co	MV&VTP 0179
CRUST1Z	crustacea [rosei?, tomentosa]	AF106814	2n = 52	markos	San Francisco Co	MV&VTP 0095
GLAUCAZ	glauca	AF106805	2n = 26	markos	San Luis Obispo Co	VTP&MV 0235
HOOKERZ	hookeri [ssp. hookeri]	AF106806	2n = 26	markos	Monterey Co	SM,VTP,&MV 0607
MANZANZ	manzanita	AF351934	2n = 52	bidartondo		
MONTANZ	montana [hookeri]	AF091977	2n = 52	hileman	Marin Co	SM,VTP&MV 0601 / M 0610
NISSEZ	nissenana	AF106804	2n = 26	markos	Eldorado Co	MV&VTP 0490
NUMMULMZ	nummularia mendocinoensis	AF106803		markos	Mendocino Co	VTP&MV 0037
PATULAZ	patula	AF106807	2n = 26	markos	Glenn Co	VTP&MV 0102
PRINGLZ	pringlei	AF106815	2n = 26	markos	Puma Co AZ	MV&VTP 0232
PUNGENZ	pungens	AF106808	2n = 26/52	markos	[San Diego Co]	tilden
RUDIS_Z	rudis	AF106809	2n = 26	markos	Santa Barbara Co	VTP&MV 0237
SENSITZ	sensitiva [nummularia]	AF091974	2n = 26	hileman	Santa Cruz Co/Marin C	V 0040 / MV&VTP 0040
STANFOZ	stanfordiana	AF106810	2n = 26	markos	Sonoma Co	MV&VTP 0468
TOMEN1Z	tomentosa	AF091972	2n = 52	hileman		V 0243
UVAUR3Z	uva-ursi	AY596455	2n = 26/52	neyland		
UVAUR2Z	uva-ursi	AF106811	2n = 26/52	markos	Monterey Co	VTP&MV 0046
UVAUR1Z	uva-ursi	AF091975	2n = 26/52	hileman	Ocean Co NJ	T 0440
VISCIDZ	viscida	AF106812	2n = 26	markos	Plumas Co	VTP&MV 0314

Appendix 1: 26S sequence identifiers, taxon names, GenBank accession numbers, ploidy, study authors, collection locations, and voucher identification.

CLADE2a_3:

NEVADA1, CRUSTA1, CRUSTAs1, GLANDU2

CLADE2b_52:

MANZAN1, MANZAN3, MANZAN4, MANZANe1, MANZANG1, TOMENTd1, CRUSTAi1, CRUSTAr1, ANDERS1, AURICU1, BAKERI1, BAKERIs1, CANESCO, CANESC1, CANESCs1, CATALI0, CATALI1, COLUMB1, CONFER1, CRUZEN0, CRUZEN1, FRANCI1, GABILA1, GLANDU0, GLANDUc1, GLANDUm1, GLAUCA1, GLUTIN1, HOOVER1, IMBRIC1, INSULA1, LUCIAN1, MONTAN1, MONTAR1, MONTER1, MORROE1, OBISPO1, OSOENS1, PAJARO0, PAJARO1, PALLID1, PILOSU0, PILOSU1, PILOSU2, PURISS1, REFUGIO, REGISM1, SILVIC1, VIRGAT1, XMANST1, XMEDIA1, XREPEN1

Appendix 2: Identical sequences combined under a common identifier (CLADE2a_3 and CLADE2b_52).

Sequence	Species	Denford 1981	Markos 1998 (ITS)	Markos 1998 (26S)	Markos 1998 (RFLP)	Boykin 2005	Wahlert 2009	Van Dyke 2017
DENSIF	densiflora				patula	patula	patula	patula
HISPID	hispidula				patula	patula		patula
HOOKERh	hookeri hearstiorum		patula		patula		patula	patula
HOOKER	hookeri	patula	patula	patula	patula	patula	patula	patula
MEWUKK	mewukka				patula			patula
	mewukka truei					patula		
MYRTIF	myrtifolia	columbiana				patula		patula
NUMMULm	nummularia mendocinoensis			patula	patula	patula	patula	patula
NUMMUL	nummularia		patula	patula	patula	patula	patula	patula
OHLONE	ohloneana						patula	patula
PARRYA	parryana				patula	patula		patula
PATULA	patula	patula		patula	patula	patula	patula	patula
RUDIS_	rudis			patula	patula		patula	patula
SENSIT	sensitiva						patula	patula
STANFOd	stanfordiana decumbens						patula	patula
STANFOR	stanfordiana raichei						patula	patula
STANFO	stanfordiana	patula		patula	patula	patula	patula	patula
FRANCI	franciscana		columbiana		columbiana		columbiana	cross-lineage
MANZANe	manzanita elegans	patula					columbiana	cross-lineage
MANZANG	manzanita glaucescens						columbiana	cross-lineage
MANZANI	manzanita laevigata	patula						cross-lineage
MANZAN	manzanita	patula			columbiana			cross-lineage
MANZANr	manzanita roofii							cross-lineage
PACIFI	pacifica	patula						cross-lineage
PUNGEN	pungens	patula	columbiana	patula	patula	columbiana	patula	cross-lineage
TOMENTd	tomentosa dacticola						columbiana	cross-lineage
TOMENT	tomentosa		columbiana	columbiana	columbiana	columbiana	columbiana	cross-lineage
UVAURS	uva-ursi		columbiana	columbiana	columbiana	columbiana	patula	cross-lineage
ANDERS	andersonii	columbiana		columbiana	columbiana	columbiana	columbiana	columbiana
AURICU	auriculata					columbiana	columbiana	columbiana
AUSTRA	australis						columbiana	columbiana
BAKERI	bakeri				columbiana		columbiana	columbiana
BAKERIS	bakeri sublaevis					columbiana	columbiana	columbiana
CANESC	canescens	columbiana		columbiana	columbiana	columbiana	columbiana	columbiana
CANESCs	canescens sonomensis						columbiana	columbiana
CATALI	catalinae				columbiana	columbiana	columbiana	columbiana
COLUMB	columbiana				columbiana	columbiana	columbiana	columbiana
CONFER	confertiflora						columbiana	columbiana
CRUSTA	crustacea						columbiana	columbiana
CRUSTAi	crustacea insulicola						columbiana	columbiana
CRUSTAr	crustacea rosei	columbiana					columbiana	columbiana
CRUSTAs	crustacea subcordata						columbiana	columbiana
CRUZEN	cruzensis					columbiana	columbiana	columbiana
EDMUND	edmundsii	columbiana			columbiana			columbiana
GABILA	gabilanensis						columbiana	columbiana
	glandulosa crassifolia	columbiana						
GLANDUc	glandulosa cushingiana						columbiana	columbiana
	glandulosa gabrielensis				columbiana			
GLANDU	glandulosa	columbiana			columbiana	columbiana	columbiana	columbiana
GLANDUm	glandulosa mollis						columbiana	columbiana
GLAUCA	glauca			columbiana	columbiana	columbiana	columbiana	columbiana
GLUTIN	glutinosa						columbiana	columbiana
HOOVER	hooveri					columbiana	columbiana	columbiana
IMBRIC	imbricata						columbiana	columbiana
INSULA	insularis						columbiana	columbiana
KLAMAT	klamathensis				columbiana		columbiana	columbiana
LUCIAN	luciana						columbiana	columbiana
MALLOR	malloryi						columbiana	columbiana

Appendix 3: Assignment of taxa to patula and columbiana clades by Denford (1981, flavonoids), Markos (1998; RFLP, ITS, and 26S), Boykin (2005, ITS), Wahlert (2009, ITS), and this study (ITS). Note the confusion when attempting to classify cross-lineage taxa.

Sequence	Species	Denford 1981	Markos 1998 (ITS)	Markos 1998 (26S)	Markos 1998 (RFLP)	Boykin 2005	Wahlert 2009	Van Dyke 2017
MONTAN	montana montana	patula	columbiana		columbiana		columbiana	columbiana
MONTANr	montana ravenii		columbiana	columbiana	columbiana		columbiana	columbiana
MONTAR	montaraensis						columbiana	columbiana
MONTER	montereyensis					columbiana	columbiana	columbiana
MORROE	morroensis					columbiana	columbiana	columbiana
NEVADA	nevadensis	patula			columbiana		columbiana	columbiana
NISSEN	nissenana			columbiana	columbiana	columbiana		columbiana
OBISPO	obispoensis	columbiana				columbiana	columbiana	columbiana
OSOENS	osoensis						columbiana	columbiana
OTAYEN	otayensis							columbiana
PAJARO	pajaroensis					columbiana	columbiana	columbiana
PALLID	pallida	columbiana				columbiana	columbiana	columbiana
PECHOE	pechoensis					columbiana		columbiana
PENINS	peninsularis					columbiana		columbiana
PILOSU	pilosula					columbiana	columbiana	columbiana
PRINGLd	pringlei drupacea					columbiana		columbiana
PRINGL	pringlei			columbiana	columbiana		columbiana	columbiana
PUMILA	pumila				columbiana		columbiana	columbiana
PURISS	purissima					columbiana	columbiana	columbiana
RAINBO	rainbowensis						columbiana	columbiana
REFUGI	refugioensis				columbiana	columbiana		columbiana
REGISM	regismontana						columbiana	columbiana
SILVIC	silvicola					columbiana	columbiana	columbiana
VIRGAT	virgata						columbiana	columbiana
VIRIDI	viridissima					columbiana		columbiana
VISCIDm	viscida mariposa					columbiana	columbiana	columbiana
VISCIDp	viscida pulchella						columbiana	columbiana
VISCID	viscida	columbiana		columbiana	columbiana			columbiana

Appendix 3 (cont.): Assignment of taxa to patula and columbiana clades.