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Randomly amplified polymorphic DNA analysis (RAPD) of *Artemisia* subgenus *Tridentatae* species and hybrids

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Randomly amplified polymorphic DNA analysis (RAPD) of *Artemisia* subgenus *Tridentatae* species and hybrids

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This article is available in Great Basin Naturalist: [https://scholarsarchive.byu.edu/gbn/vol58/iss1/2](https://scholarsarchive.byu.edu/gbn/vol58/iss1/2)
RANDOMLY AMPLIFIED POLYMORPHIC DNA ANALYSIS (RAPD) OF ARTEMISIA SUBGENUS TRIDENTATAE SPECIES AND HYBRIDS

E. Durant McArthur1, Joann Mudge1,2,3, Renee Van Buren2,4, W. Ralph Andersen2, Stewart C. Sanderson1, and David G. Babbel1,2,5

ABSTRACT.—Species of Artemisia (subgenus Tridentatae) dominate much of western North America. The genetic variation that allows this broad ecological adaptation is facilitated by hybridization and polyploidization. Three separate studies were performed in this group using randomly amplified polymorphic DNA (RAPD). Fifty-seven 10-mer primers generated nearly 400 markers from genomic DNA obtained from leaf tissue. These studies were (1) a measure of the variability of plants within and between populations and between subspecies using 5 A. tridentata ssp. wyomingensis populations, 2 A. cana ssp. cana populations, and 1 A. cana ssp. vaseyana population; (2) an examination of the hypothesis that tetraploid (4x) Artemisia tridentata ssp. vaseyana derives de novo from diploid (2x) populations via autoploidy and (3) an examination of the validity of the status of putative hybrids that have been produced by controlled pollination. These latter hybrid combinations—A. tridentata ssp. tridentata × A. t. ssp. vaseyana, A. t. ssp. wyomingensis × A. tripartita, and A. cana ssp. cana × A. tridentata ssp. wyomingensis—were made to combine traits of parental taxa in unique combinations with possible management applications. RAPD marker data were subjected to similarity and UPGMA clustering analyses. RAPD markers were effective in measuring genetic diversity at different systematic levels. Individual plants within a population were approximately 55% to 80% similar to one another; populations within subspecies gave corresponding values of similarity, probably a result of the combined effects of large population sizes and wind pollination. The 2 subspecies of A. cana were approximately 45% similar. At least some 4x populations of A. tridentata ssp. vaseyana apparently derive de novo from 2x plants based on their being embedded in 2x phenogram groups, thus reinforcing evidence that autoploidy plays an important role in Tridentatae population biology. Two (A. tridentata ssp. tridentata × A. t. ssp. vaseyana and A. cana ssp. cana × A. tridentata ssp. wyomingensis) of the 3 putative hybrid combinations were confirmed to include hybrids. These hybrids may have potential in management applications. Additional use of RAPD technology combined with other techniques may be useful in delimiting genetic characteristics and in guiding artificial selection in the Tridentatae.

Key words: Artemisia, Tridentatae, RAPD, hybridization, diploid, tetraploid, polyploid, autoploidy.


**Materials and Methods**

**Plant Materials**

Plant populations sampled and used in the 3 studies together with their taxonomic affiliation are listed in Table 1. We obtained these plant materials from the following: natural populations, samples of natural populations that had been propagated from seed in our greenhouse, outplantings of progeny from natural populations in experimental plots and common gardens, and putative artificial hybrids grown in the greenhouse and at outplanting sites. The putative hybrid seed was obtained using methods described previously (McArthur et al. 1979, 1988, Noller and McArthur 1986), i.e., placing inflorescences with dehiscent anthers in doubled pollination (white bakery) bags just before anthesis of the maternal parent. We removed pollination bags 3 wk later and collected seed before the heads shattered. Representative voucher samples for all 3 studies are deposited in the Shrub Sciences Laboratory Herbarium (SSLP). Leaves for DNA extraction and analysis were collected from individual plants during the 1991-92 growing season. We extracted DNA either from fresh material immediately after collection (<3 h) or from material that had been immediately frozen in liquid nitrogen and stored at ultra-cold temperatures (~80°C) until the tissue was extracted. DNA analysis was performed on individual plants and on bulked population samples (5-8 plants). Chromosome counts were made to assist in hybrid confirmation using the techniques described by McArthur and Sanderson (in review).

**STUDY 1.**—We examined DNA marker variability in populations at 2 different taxonomic levels (Table 1). First was the population level wherein we examined 5 different populations of *A. tridentata* ssp. *wyomingensis*. The 2nd level was between 2 subspecies of *A. cana* (2 populations of *A. cana* ssp. *cana* and 1 population of *A. c. ssp. *vaseyana*). We used both individual plants and bulked samples for DNA marker analysis.

**STUDY 2.**—We examined DNA markers from 7 *A. tridentata* ssp. *vaseyana* populations (Table 1, Fig. 1). Several locations where diploid (2x) and tetraploid (4x) populations of this taxon grow parapatrically or sympatrically have been discovered (McArthur and Sanderson in review). Some of those populations are included in this study (Table 1, Fig. 1). Initially, we thought the Hobble Creek location, which includes plants from both the mesic canyon bottom habitat and the more xeric south-facing slope habitat, included 2x and 4x populations. However, additional cytological study (McArthur and Sanderson in review, unpublished) has shown that the Hobble Creek location is essentially 2x with only an occasional 4x plant present. We kept the Hobble Creek population in the study for additional comparison of *A. tridentata* ssp. *vaseyana* DNA markers.

**STUDY 3.**—We examined DNA markers from 3 artificial hybridization experiments (Table 1). The 1st hybrid is *A. tridentata* ssp. *tridentata × A. t. ssp. *vaseyana*, including F1 and F2 hybrid generations that have been studied and confirmed as hybrids in morphological, chemical, and insect-host contexts (Noller and McArthur 1986, McArthur et al. 1988, 1992, Weber et al. 1994, Messina et al. 1996, Richards, Messina, and McArthur unpublished). This hybrid combination was made in an attempt to better understand hybridization dynamics and combine traits (palatability, nutrient content, growth rates) that might be favorable for large herbivore...
# Table 1. Location of sample collections, outplanting sites, and sample reference numbers.

| Taxon | Location and propagation information and sample reference number*
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STUDY 1</strong></td>
<td></td>
</tr>
</tbody>
</table>
| **Artemisia tridentata** Nutt. ssp. *wyomingensis* Beetle & Young | **Arco, Butte Co., ID, W s.n., October 1986; grown at Springville and Browns Park outplanting sites**  
3 km S of Dinosaur, Rio Blanco Co., CO, M&S 1438; grown at Springville and Browns Park outplanting sites  
Gordon Creek, 7 km W of Spring Glenn, Carbon Co., UT, W U-019; grown at Springville and Browns Park outplanting sites  
6 km N of Kemmerer, Lincoln Co., WY, M&J 1738 (U-028); grown at Springville and Browns Park outplanting sites  
1 km E of Warren, Carbon Co., MT, M&J 1743; grown at Springville and Browns Park outplanting sites  
  |
| **Artemisia cana** Pursh. ssp. *cana* | **Maybell, Moffat Co., CO, M&S 2120; grown in Shrub Sciences Laboratory Greenhouse**  
Sheridan, Sheridan Co., WY, W s.n., 1972, M&S 2128; grown at the Snow Field Station  
  |
| **Artemisia cana** Pursh. ssp. *viscidula* (Osterhout) Beetle | **Cart Creek, Daggett Co., UT, M&B 2204**  
  |
| **STUDY 2** |  |
| **Artemisia tridentata** Nutt. ssp. *caseyana* (Ryd.) Beetle | **Right Fork of Hobble Creek, Utah., UT, M&S 2164, 2185; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 2x  
4 km E of Salina Canyon Summit, Red Creek, Sevier Co., UT, M&S 2140; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 2x  
7 km E of Salina Canyon Summit, Red Creek, Sevier Co., UT, M&S 2149; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 4x  
Pine Valley, Washington Co., UT, M&S 2177; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 2x  
8 km N of St. George, E of Snows Canyon, Washington Co., UT, M&S 2189; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 4x  
Tabernacle Dome, Kolob Terrace, Zion National Park, Washington Co., UT, M&S 1821A; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 2x  
4 km N of Virgin, Washington Co., UT, M&S 2191; grown at Hobble Creek and Great Basin Experimental Range outplanting sites, 4x  
  |
| **STUDY 3** |  |
| **Artemisia tridentata** Nutt. ssp. *tridentata* | **12 km E of Dove Creek, Delores Co., CO, M&P U-076, V&Ba 22; grown at Snow Field Station and Hobble Creek outplanting sites**  
**Hobble Creek Canyon, Utah Co., UT, M&P U-001, M&S 1476, 2144, 2363, G, W, L, M, 21492, grown at Hobble Creek outplanting sites**  
**Sheridan, Sheridan Co., WY, M&S 2128; grown at the Snow Field Station**  
**West entrance, U.S. Sheep Station, Dubois, Clark Co., ID; M&S 2982**  
**Paddock 31A, U.S. Sheep Station, Dubois, Clark Co., ID; M&S s.n., August 1894**  
  |
| **Artemisia tridentata** Nutt. ssp. *wyomingensis* Beetle & Young | **Arco, Butte Co., ID, W s.n., October 1986; grown at Springville and Browns Park outplanting sites**  
6 km N of Kemmerer, Lincoln Co., WY, M&J 1738 (U-028); grown at Springville and Browns Park outplanting sites  
  |

*Initials for collections are B = David G. Babbed, BS = Jerry B. Barker, G = Sheri Goodrich, J = Gary L. Jorgensen, L = Nest E. Lewis, M = E. Dustin McArthur, MO = Stephen B. Morrison, P = A. Perry Plumlee, S = Stewart C. Sanderson, V = Gordon A. Van Egge, W = Bruce L. Welch, and Wi = Alma H. Winward. The U collection numbers refer to seed and plant culture accession numbers maintained at the Great Basin Experimental Range, Ephraim, UT. For locations of the outplanting sites see Figure 1.
consumption (McArthur et al. 1988, 1992, Weber et al. 1994). The 2nd putative hybrid combination is A. t. ssp. wyomingensis × A. tripartita (4 different combinations involving the Warren, Montana, and Gordon Creek, Utah, populations of A. tridentata ssp. wyomingensis as female parents and 2 A. tripartita populations from the entry snow fence and paddock 31A of the U.S. Sheep Station, Dubois, Idaho, as pollen donors). The 3rd combination is A. cana ssp. cana × A. tridentata ssp. wyomingensis (3 combinations with A. c. ssp. cana from Sheridan, Wyoming, as the female parent and A. t. ssp. wyomingensis from Arco, Idaho, and Kemmerer, Wyoming, as pollen donors). These latter 2 putative hybrid combinations were
designed to attempt to combine the drought tolerance of *A. t.* ssp. *wyomingensis* with the root-sprouting, fire-tolerant adaptation of *A. c.* ssp. *cana* and *A. tripartita* (McArthur et al. 1992, McArthur 1994). *Artemisia tridentata* ssp. *wyomingensis* is not fire tolerant, and much of its range has been usurped by alien fire-adapted weed species such as *Bromus tectorum* (McArthur et al. 1990, Monsen and Kitchen 1994).

**DNA Extraction and Amplification**

We extracted DNA from individual plants or bulked samples using a method adapted from Delaporta et al. (1983). After the tissue was ground to a powder in liquid nitrogen using a mortar and pestle, about 1–1.5 ml extraction buffer (10 mM EDTA, pH 8.00; 50 mM NaCl, 100 µL 20% SDS and 5 µL 2 β-mercaptoethanol) per gram of plant tissue was added and the mixture ground further. One ml portions of the homogenate were transferred to 1.5-ml centrifuge tubes and incubated for 20 min at 68°C in a water bath. Following incubation, 500 µL of 1 M potassium acetate was added to each tube and the tubes mixed thoroughly. We then incubated the samples at 4°C for 20 min. Following incubation, the samples were centrifuged at 14,000 rpm for 5 min. The supernatant was transferred through microcloth to a clean microcentrifuge tube containing 500 µL of isopropanol. The samples were mixed by gently inverting and the samples incubated overnight at 4°C to help precipitate the DNA (Fairbanks et al. 1993).

Because of the high amount of carbohydrate obtained after the above extraction, we performed a carbohydrate wash (Sederhof; North Carolina State University, Chapel Hill, personal communication). All tubes containing DNA from a particular plant or bulked sample were combined to form single pellets. Each pellet was resuspended in 700 µL of 1 M NaCl and vortexed gently (DNA dissolves in the salt but carbohydrates do not). The samples were incubated at 4°C for 20 min and then centrifuged at 14,000 rpm for ca 5 min. The resulting supernatant was added 1:1 to isopropanol and incubated overnight at 4°C to aid DNA precipitation. The DNA was pelleted and washed with 70% ethanol, dissolved in TE (10 mM Tris, pH 8.00; 1 mM EDTA, pH 8.0), and stored at −20°C until use.

DNA markers were amplified with either AmpliTaq DNA Polymerase or AmpliTaq DNA Polymerase Stoffel Fragment (Perkin Elmer-Cetus, Norwalk, CT). Both enzymes were used to obtain different DNA bands with the same primer because AmpliTaq tended to amplify higher molecular weight markers than did Stoffel Fragment (Subral and Honeycutt 1993). Amplifications were performed according to Williams et al. (1990) as modified by Mudge et al. (1996). Reagents for RAPD amplification were obtained from Perkin Elmer-Cetus and Promega Corp. (Madison, WI), primers from Operon Technologies, Inc. (Alameda, CA) and the University of British Columbia Biotechnology Laboratory (Vancouver, BC). The reaction preparation was automated by means of a Biomek 1000 work station (Beckman Instruments Inc., Fullerton, CA). Each sample for amplification had a total volume of 15 µL. Amplifications were carried out on 3 MJ Research PTC-100 96-well thermocyclers (MJ Research Inc., Watertown, MA) with different programs for AmpliTaq and Stoffel Fragment. The AmpliTaq program consisted of an initial denaturation step at 92°C for 3 min, followed by 48 cycles of denaturation step at 86°C for 1 min, 36°C for 1 min 45 sec, and 72°C for 2 min. Once the 48 cycles were complete, samples were held at 72°C for 7 min and then stored at 4°C until electrophoresis. Minimum ramp times were used between each step. The Stoffel Fragment program consisted of an initial denaturation step at 94°C for 3 min followed by 40 cycles of 96°C for 1 sec, a 0.5°C s⁻¹ ramp to 35°C which was held for 1 sec, a 0.3°C s⁻¹ ramp to 72°C which was held for 1 sec, and a 0.2°C s⁻¹ ramp to 96°C. Once the 40 cycles were complete, samples were held at 72°C for 7 min and then stored at 4°C until electrophoresis.

DNA amplification products were separated by electrophoresis in 20 × 25- or 20 × 40-cm 20 g L⁻¹ 1:4 Low (2%) EEO agarose: FMC Metaphor (FMC Bioproducts, Rockland, ME) gels with up to 28 lanes. The entire 15-µL sample plus 2–3 µL of bromophenol blue dye in glycerol was added to each lane. DNA size markers (pUC-19 207, Biosynthesis, Inc., Lewisville, TX) were added to at least 2 lanes in each gel for reference and ease in scoring gels. Samples were electrophoresed at 150 V for 3 to 4 h at room temperature.
Gels were stained with 0.5 μg ethidium bromide per ml in both gel and gel buffer. They were not destained. Gels were visualized on a UV transilluminator and photographed with a camera system, or they were viewed on a video imaging system that had greater resolution and storage capabilities than photographic methods have (Mudge et al. 1996). Amplified bands were scored and recorded as presence or absence of bands of the same molecular weight (Fig. 2). Bands of the same mobility were presumed to be homologous.

Analysis of Amplified DNA Products

The NTSYS-pc statistical software package was used to analyze coded DNA markers (Rohlf 1993). Presence or absence of specific DNA bands (markers) was analyzed for estimating percent similarity with Jaccard’s coefficient of similarity (Jaccard 1912) using NTSYS-pc, version 1.80. UPGMA clustering analysis (NTSYS-pc, SAHN) and a phenetic tree (NTSYS-pc, TREE) were generated to graphically show the percent similarity among appropriate samples. Phenetic trees were constructed from individual plant data except for the Artemisia tridentata ssp. wyomingensis population study. In that study both individual plant and bulked data were used. The bulked data were not weighted; i.e., they were treated analogously to a single plant. Bulked samples included 5–8 individual plants.

RESULTS

Fifty-seven primers (3–24 per study) produced nearly 400 (6–216 per study) scorable markers that were used to construct similarity phenograms (Tables 2, 3, Figs. 3–6). The primer and marker totals of Table 2 are not additive because 21 of the primers and as many as 150 of the DNA markers were shared between or among the separate studies.

STUDY 1.—Figures 3 and 4 show individual plant similarity within populations of A. tridentata ssp. wyomingensis, A. cana ssp. cana, and A. c. ssp. viscidula; among-population similarity within each taxon; and similarity between subspecies of A. cana. These results are within expected ranges at those systematic levels (Van Buren et al. 1994, Gang and Weber 1995, McArthur et al. 1998). Individual plants within each population are generally but not always more similar to other plants in their own population than plants of the same taxon in other populations.

The Gordon Creek, Utah, and Warren, Montana, populations of A. tridentata ssp. wyomingensis are less homogeneous than the other 3 populations of A. t. ssp. wyomingensis (Arco, Idaho; Kemmerer, Wyoming; Dinosaur, Colorado). Bulked samples show that the 3 geographically clustered populations (Dinosaur, Colorado; Gordon Creek, Utah; and Kemmerer, Wyoming) are slightly more similar to each other than to the more geographically isolated populations, Arco, Idaho, and especially Warren, Montana (Figs. 1, 3). Under the conditions of our study, individual plants within populations were approximately 55% to >80% similar in DNA markers. All populations are at least 50% similar to each other except for 1 outlying plant from Warren, Montana (Fig. 3).

The 2 populations of A. cana ssp. cana were about 54% similar to each other, whereas those populations were only 45% similar to the population of A. cana ssp. viscidula included in the study (Fig. 4). The Sheridan, Wyoming, population of A. c. ssp. cana and the Cart Creek, Utah, population of A. c. ssp. viscidula are more homogeneous than is the Maybell, Colorado, population of A. c. ssp. cana. The similarity between A. cana subspecies is supportive of their conspecific affinity and placement within the subgenus Tridentatae (McArthur et al. 1998).

STUDY 2.—Comparisons of the 2x and 4x populations of A. tridentata ssp. caseyana are presented in the Figure 4 phenogram as individual plants. In general, as in study 1, individual plants for each population clustered on the same stem. All plants and populations for this subspecies were >50% similar as was the case for plants and populations within subspecies in study 1. Results reveal 4 groups with >55% similarity (Fig. 5): the top one comprises the 11 Salina Canyon (Utah) 2x plants, 16 of the 21 Hobble Creek (Utah) 2x plants, and 1 of the 11 Kolob Terrace (Utah) 4x plants; the 2nd group is composed of all 8 Pine Valley (Utah) 2x plants, all 12 Pine Valley 4x plants, and 11 of the 12 Kolob Terrace 2x plants; the 3rd one is composed of the 9 Salina Canyon 4x plants, 5 of the 21 Hobble Creek 2x plants, and 1 Kolob Terrace 4x plant; the bottom group comprises 9 of the 11 Kolob Terrace 4x plants and a single Kolob Terrace 2x plant. These
results are consistent with geographic separation (Fig. 1) except for 2 Kolob Terrace 4x plants that are in the 1st and third groups, respectively, rather than the 4th group (Fig. 5). The 1st and 2nd groups, 55% similar, are 2x with the exception of the 4x Pine Valley plants and a single 4x Kolob Terrace plant, whereas the 3rd and 4th groups, 54% similar, are primarily
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TABLE 2. Number of primers and DNA marker bands used in separate studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of primers</th>
<th>No. of bands</th>
<th>Primer sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>STUDY 1</td>
<td>Artemisia tridentata ssp. wyomingensis populations</td>
<td>22</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Artemisia cana subspecies</td>
<td>24</td>
<td>216</td>
</tr>
<tr>
<td>STUDY 2</td>
<td>Artemisia tridentata ssp. vaseyana, 2x and 4x</td>
<td>14</td>
<td>133</td>
</tr>
<tr>
<td>STUDY 3</td>
<td>Artemisia tridentata ssp. tridentata × A. t. ssp. vaseyana</td>
<td>11</td>
<td>19</td>
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<tr>
<td></td>
<td>Artemisia tridentata ssp. wyomingensis × A. tripartita</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Artemisia cana ssp. cana × A. tridentata ssp. wyomingensis</td>
<td>36</td>
<td>6</td>
</tr>
</tbody>
</table>

*Operon = Operon Technologies, Inc., UBC = University of British Columbia Biotechnology Laboratory.

TABLE 3. Primer name, sequence, and number of markers generated from each primer used for amplification of sample DNA for study 2 (2x and 4x Artemisia tridentata ssp. vaseyana populations).

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5'→3')</th>
<th>Number of markers</th>
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<tbody>
<tr>
<td>UBC-157</td>
<td>CCTGGGCGAGG</td>
<td>11</td>
</tr>
<tr>
<td>UBC-180</td>
<td>CCGGACACGCT</td>
<td>12</td>
</tr>
<tr>
<td>UBC-199</td>
<td>CTTTCCGGCAC</td>
<td>12</td>
</tr>
<tr>
<td>UBC-457</td>
<td>CCACGCGGTTC</td>
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<td>UBC-459</td>
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<td>UBC-515</td>
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<td>UBC-598</td>
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<tr>
<td>UBC-601</td>
<td>CGCCCGAATCG</td>
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</tr>
<tr>
<td>UBC-615</td>
<td>CCTCGACGGC</td>
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</table>

4x with the exception of 5 Hobble Creek and 1 Kolob Terrace 2x plants.

STUDY 3.—DNA marker similarities among the putative hybrid plants and their parents are illustrated in Figure 6. The 1st hybrid combination (A. tridentata ssp. tridentata × A. t. ssp. vaseyana), which had been confirmed by previous studies (McArthur et al. 1988, Weber et al. 1994, Messina et al. 1996), yielded 3 major groups plus several outliers (Fig. 6). The major groups are the A. tridentata ssp. vaseyana parent (top), the F1 and F2 hybrids (center), and the A. t. ssp. tridentata parent (near bottom). The outliers are some A. t. ssp. tridentata parent plants and especially F2 plants (near bottom of top group and near bottom). These results of a parent through F2 hybrid generations give evidence of hybridization. The parent plants are well separated in the phenogram (Fig. 6). The F1 and F2 plants are closer to the maternal parent than expected. F2 hybrids do, however, show additional segregation over the F1 plants as expected (Fig. 6). Similarity values of Figure 6 are less than others presented herein and in McArthur et al. (1998) because we worked with taxon-specific markers to the extent that we could find them; therefore the values should be considered relative and not absolute. The other 2 putative hybrid combinations (A. tridentata ssp. wyomingensis × A. tripartita and A. cana ssp. cana × A. tridentata ssp. wyomingensis) gave contrasting results (data not shown). The A. tridentata ssp. wyomingensis × A. tripartita combination was not successful. All female parent plants (N = 10), progeny of self-pollinated control plants (N = 7), and putative hybrid plants (N = 15) clustered in 1 stem above 50% similarity, whereas pollen parents (2 different A. tripartita populations, each N = 6) clustered in a separate group above 40% similarity. The 2 groups are only about 22%
similar. The *A. cana* ssp. *cana* × *A. tridentata* ssp. *wyomingensis* combination, on the other hand, yielded results that had several putative hybrid plants intermediate in similarity to the 2 parental stocks. These results are corroborated, in part, by cytological studies. The *A. cana* ssp. *cana* × *A. tridentata* ssp. *wyomingensis* combination yielded 6x plants, which would be expected in an 8x (A. *cana* ssp. *cana*) × 4x (A. *tridentata* ssp. *wyomingensis*) combination. Because both *A. tridentata* ssp. *wyomingensis* and *A. tripartita* are 4x, cytological results are not instructive in that combination. Meiotic figures of this combination display numerous multivalents, especially quadrivalents, as did *A. tridentata* ssp. *wyomingensis* plants and other polyploid taxa in an earlier cytological study (McArthur et al. 1981).

**DISCUSSION**

**USE OF RAPD MARKERS.**—The set of studies reported herein adds weight to the evidence that RAPD markers are useful in systematic problems at various hierarchical levels from individual plants to genera (e.g., Levi et al. 1993, Santos et al. 1994, Van Buren et al. 1994, Gang and Weber 1995, McArthur et al. 1998) and in hybridization problems including hybrid zones and natural and artificial hybridizations (e.g., Huen and Helentjaris 1993, Bradshaw et al. 1994, Kennard et al. 1994, Dean and Arnold 1996, Lin and Ritland 1996, Mudge et al. 1996).

**GENETIC DIFFERENTIATION AT VARIOUS SYSTEMATIC LEVELS.**—The amount of genetic differentiation among individual plants within
Fig. 4. Phenogram produced using UPGMA clustering analysis (NTSYS-pc; Rohlf 1983) for *Artemisia cana* subspecies and populations. Individual plants are as follows: *A. cana* ssp. *nina*, Sheridan (individual plants 1–5), Maybell (individual plants 1–5); *A. cana* ssp. *viscidula*, Cart Creek (individual plants 1–5).

Populations as revealed by genomic DNA markers in this set of studies (Figs. 3–6) is similar to that of populations determined by bulked samples (Fig. 3; Van Buren et al. 1994, Gang and Weber 1995, McArthur et al. in review). Similarity between individual plants within populations is usually in the range of 50–85%. An exception is in the *A. tridentata* ssp. *tridentata* × *A. t. ssp. vaseyana* hybridization study (Fig. 6), where fewer subspecies contrasting markers were used and similarity in hybrid and parental plants ranges from 20% to 100% Most plants within populations of that study are above 50% in similarity, but several outliers are only about 20–25% similar to other plants in their populations.

We suspect that individual plants are as different from one another within closely spaced populations as populations within taxa are from one another because of the wind-pollinated nature of the *Tridentatae* (McArthur et al. 1979, 1988, McArthur 1989). In a wind-pollination system, pollen of landscape-dominant plants is dispersed not only within populations but also between populations (Grant 1975, Franklin 1981, McArthur 1989). Further evidence of the spread of *Tridentatae* pollen is that during its fall pollination season, *Tridentatae* (= sagebrush) pollen counts are given out by weather reporters to the public in areas removed from stands of plants for the benefit of those allergic to *Tridentatae* pollen.

Subspecies range from 25% (*A. tridentata*; see Fig. 6) to 45% (*A. cana*; see Fig. 4) similarity among populations, which is generally more similar than the between-species similarities reported in the genera *Ranunculus*, *Artemisia*, and *Sphaeromeria* except for closely related (usually within same subgenus) species (Van Buren et al. 1994, McArthur et al. 1998). The 25% value may be low because DNA markers were selected to contrast the subspecies. Between genera similarity values in a companion study (McArthur et al. 1998) were also lower, 7–18%.

**De novo origin of 4x A. tridentata ssp. vaseyana.**—Cytological evidence (karyotypic structure and high multivalent frequencies in polyploids) suggests that the *Tridentatae* include a high frequency of autoploidy (McArthur et al. 1981). DNA marker data (Fig. 5) are useful in addressing the hypothesis that 4x populations and plants that are adjacent or intermixed with 2x populations may be of *de novo*, *in situ* origin. Data suggest that the hypothesis is at least partially correct. The 4x plants from near Pine Valley fall within the same grouping as the nearby 2x plants from Pine Valley and Kolob Terrace (Fig. 5). We suggest that these 4x plants are of *de novo* origin from the local 2x population(s). Cytological evidence gives additional credence to this hypothesis as we have located 3 populations in the Pine Valley area that are indistinguishable morphologically and chemically (coumarin compound content) but contain individual 2x and 4x plants (McArthur and Sanderson in review). Since the other 4x populations (Salina Canyon and Kolob Terrace) did not cluster tightly with adjacent 2x populations, they may not be recent autoploids. However, our studies show that all sampled *A. tridentata* ssp. *vaseyana* plants are quite similar (above 50%; see Fig. 5), suggesting earlier or more distant autoploidy as the source of 4x populations. These 4x populations have apparently dispersed, given the evident intertwining DNA marker (Fig. 5) and geographic patterns (Fig. 1). Recently,
autoopolyploidy has been recognized as playing a more important role in evolution in a wide array of plant species than has been traditionally recognized, e.g., Small 1985, Bayer 1987, Ness et al. 1989, Lumaret et al. 1989, Van Dijk et al. 1992, Soltis and Soltis 1993, Bretagnolle and Thompson 1996, Laushman et al. 1996. We believe the data we present here give further credence to the importance of auto­polyploidy in Tridentatae.

RAPD CONTRIBUTION TO HYBRIDIZATION STUDIES IN TRIDENTATAE.—The Tridentatae are thought to have evolved through a pattern of geographic migration, introgression, and hybridization (Ward 1953, Beetle 1960, Hanks et al. 1973, McArthur and Plummer 1978, McArthur et al. 1981, 1985, Thompson 1991). Therefore, studies that contribute to the understanding of hybridization processes in the group are needed to better understand the group’s dynamic population biology and evolution. Our DNA marker data are from 3 different hybrid combinations. These data confirm the hybrid nature of the A. tridentata ssp. tridentata × A. t. ssp. vaseyana progeny studied previously by other techniques (McArthur et al. 1988, 1992, Weber et al. 1994, Messina et al. 1996). The segregation of RAPD markers in F1 and F2 generations is a pattern that can be explained as the consequence of hybrid segregation (Fig. 6). Our data also support a successful hybridization of the A. cana ssp. cana × A. tridentata ssp. wyomingensis combination. Seven of the 13 putative hybrid plants examined for RAPD markers are intermediate in marker patterns in respect to their parents, whereas 6 are similar to the maternal parent. These results are consistent with our previous results on hybridization wherein substantive fractions of the progeny of successful hybrid combinations are indeed of hybrid origin and other substantive fractions are the result of self-pollinations (McArthur et al. 1988). The other hybrid combination, A. tridentata ssp. wyomingensis × A. tripartita, was not success­ful. All putative hybrid progeny are similar to their maternal parents.

These hybrid combinations, aside from helping us better understand Tridentatae breeding systems, were made for specific purposes (McArthur et al. 1985, 1988, 1992, McArthur 1988). The A. tridentata ssp. tridentata × A. t. ssp. vaseyana combination has been extended to the F3 generation with the goal of maintaining the growth and woody biomass characteristics of the paternal parent and the leafiness and palatability (= essential oil profile) to large ungulates of the maternal parent. We are currently evaluating those characteristics as well as the adaptation of the hybrids with respect to parental stock (Noller and McArthur 1986, McArthur et al. 1988, Weber et al. 1994, Messina et al. 1996, McArthur unpublished). The other 2 hybrids were made to combine the drought tolerance and widespread adaptability of the landscape-dominant A. tridentata ssp. wyomingensis with the root-sprouting, fire­tolerance capabilities of A. cana ssp. cana or A. tripartita (Beetle 1960, McArthur 1994). Much of the natural range of A. tridentata ssp. wyomingensis has been lost to cheatgrass and other alien fire-tolerant annual weeds (McArthur et al. 1990, Monsen and Kitchen 1994). The successful F1 hybrids have been outplanted and are apparently fertile (we recently collected filled seed). Additional evaluation of both successful hybrid lines is necessary before they can be considered for wide-scale planting. Moreover, the use of such material should be critically evaluated by land managers and others with interest in the well-being of our landscapes.

We have discussed some traits in Tridentatae species and hybrids that might be desirable to combine. The location of such traits on a genetic map would be useful information. RAPD, in concert with other molecular genetic tools and additional hybrid stock, could be used to document chromosomal locations as has been done with other plants, e.g., Penner et al. 1993, Bradshaw et al. 1994, Kennard et al. 1994, Santos et al. 1994, 1995, Mudge et al. 1996. Such information would also be useful in the ongoing work of understanding the dynamics of hybrid zones between the subspecies of A. tridentata (McArthur et al. 1988, Freeman et al. 1991, 1995, Weber et al. 1994, Graham et al. 1995, Messina et al. 1996, Wang et al. 1997).

Fig. 5 (see facing page). Phenogram produced using UPGMA clustering analysis (NTSYS-pc, Rohlf 1993) for Artemisia tridentata ssp. vaseyana 2x and 4x populations. Individual plants are keyed as follows: 2x = diploid, 4x = tetraploid, HC = Hobble Creek, KT = Kolo­b Terrace, PV = Pine Valley, SC = Salina Canyon. See Figure 1 and Table 1 for more detailed location information. Circled numbers, e.g., 6, are the major groups discussed in the text.
Fig. 6 (see facing page). Phenogram produced using UPGMA clustering analysis (NTSYS-pc, Rohlf 1993) for Artemisia tridentata ssp. tridentata × A. t. ssp. vaseyana including parental and F₁ and F₂ plant populations. Individual plants are keyed as follows: DC = A. t. ssp. tridentata parent plant (Dove Creek), HC = A. t. ssp. vaseyana parent plant (Hobble Creek), F₁ = 1st generation hybrid plant, F₂ = 2nd generation hybrid plant. Circled numbers, e.g. 0, are the major groups discussed in the text.

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