KARYOTYPES OF FOUR *ARTEMISIA* SPECIES: *A. CARRUTHII, A. FILIFOLIA, A. FRIGIDA,* AND *A. SPINESCENS*[1]

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ABSTRACT.— *Artemisia carruthii* and *A. frigida* of the subgenus Artemisia and *A. filifolia* and *A. spinescens* of the subgenus Dracunculus all have chromosome numbers based on $x = 9$. **Diploid** ($2n = 18$) karyotypes of each species are composed of large, medium, and small chromosomes that are mainly metacentric and submetacentric. The individual karyotypes are similar but distinctive. *Artemisia filifolia*'s karyotype and chemistry is quite similar to that of Section Tridentatae, but *A. filifolia* has significant morphological differences with respect to the Tridentatae. *Artemisia spinescens* includes a tetraploid ($2n = 36$) population as well as diploid populations. Karyotypic analysis of a tetraploid *A. spinescens* suggests that it is an autotetraploid, thus carrying out a common theme in *Artemisia* (autopolyploidy).

The genus *Artemisia* (Anthemideae, Compositae) is principally a temperate northern hemisphere plant group (Good 1974, Bailey Hortorium Staff 1976). A few of its 250 species, however, extend to South America and southern Africa. Most *Artemisia* phylogenists have suggested an origin for *Artemisia* in Eurasia because of the preponderance of diverse species growing there and because most of its Anthemideae relatives occur there (Stebbins 1974, Cronquist 1978, McArthur and Plummer 1978, McArthur 1979). Beetle (1979) recently suggested an American origin for the genus. Even disallowing Beetle's hypothesis, North America is without question a center of diversity for *Artemisia*. Several *Artemisia* species complexes (Clausen 1951: groups of closely related plants capable of intragroup gene exchange) appear to be evolving in North America (Hall and Clements 1923, Keck 1946, Ward 1953, Beetle 1960, Estes 1969, McArthur and Plummer 1978).

Artemisia has chromosome numbers based on $x = 6,7,8$, and 9 (Kawatani and Ohno 1964, Wiens and Richter 1966). By far the most common base number for *Artemisia* is $x = 9$, however, as it is for the whole of the Anthemideae (Persson 1974). Several *Artemisia* species and species complexes are composed of polyploid series. Euploid series based on $x = 9$ are most common (Table 1), but aneuploidy and amphiploidy based on other x's are also known (Suzuka 1950, 1952, Kawatani and Ohno 1964). The euploid complexes may be *auto*polyploid with one basic genome or *allo*polyploid with different genomes (Persson 1974) or somewhere in between—a segmental autopolyploid or allopolyploid (Stebbins 1971), in which case, genomes are partially differentiated.

Our work with *Artemisia* has been mainly with the *A. tridentata* Nutt. complex (= section Tridentatae) (Hanks et al. 1973, McArthur and Plummer 1978, McArthur et al. 1979, Welch and McArthur 1979). To better understand the Tridentatae, we have also looked at sympatric, non-Tridentatae, perennial *Artemisia*. This paper reports first publication of karyotypes of four non-Tridentatae species. Two species represent the subgenus Artemisia (*A. carruthii* Wood and *A. frigida* Willd.) and two represent the subgenus Dracunculus (*A. filifolia* Torr. and *A. spinescens* D. C. Eaton). The third subgenus in *Artemisia* is Seriphidium, which is principally Eurasian and North African. The section Tridentatae has been assigned to Seriphidium, but the Tridentatae are probably independent of and parallel to the Seriphidia (McArthur and Plummer 1978).

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MATERIALS AND METHODS

PLANT MATERIALS.— The plant materials studied were from the native collection sites and from transplanted wildings at the Snow Field Station in Ephraim, Utah. Each collection was assigned a culture number preceded by *U* to indicate its order of accession. Original locations of plant populations are given in Table 2. Voucher herbarium specimens for each accession have been deposited in the Shrub Sciences Laboratory Herbarium (SSLP).

KARYOTYPING.— Seed was collected from open-pollinated plants at the Snow Field Station for *A. carruthii*, *A. filifolia*, and *A. frigida*, and from the natural populations for *A. spinescens*. Root tips from seedlings germi-

TABLE 2.— *Artemisia* accessions studied.

Taxon	Culture	Utah collection site	Elevation (m)
A. carruthii	U4	Clear Creek Canyon, Sevier County	2,060
A. filifolia	U7	Kanab, Kane County	1,510
A. frigida	U9	Sunglow Park, Wayne County	2,070
A. spinescens	U3	Gunnison, Sanpete County	1,550
A. spinescens	U4	Ouray, Uintah County	1,420

TABLE 1.— Euploid patterns in $x = 9$ *Artemisia*.

	Number of species[2] with 2*n* chromosomes											
Subgenus[1]	18	27	36	54	72	18–36	18–45	18–54	18–72	18–90	36–54	References[2]
Artemisia	49	—	19	6	—	8	—	2	—	—	1	Keck (1946) Suzaka (1950, 1952) Arano (1962, 1963, 1968) Ehrendorfer (1964) Kawatani and Ohno (1964) Estes (1969) Korobkov (1972)
Dracunculus	9	—	13	1	—	2	—	—	—	1	—	Kawatani and Ohno (1964) Rousi (1968) Filatova (1971) Korobkov (1972) McArthur and Pope (1977)
Seriphidium	15	1	13	—	1	3	1	1	—	—	1	Suzuka (1952) Kawatani and Ohno (1964) Filatova (1974a and 1974b) Persson (1974)
Tridentatae[1]	—	—	—	—	—	8	—	1	1	—	—	Ward (1953) Taylor et al. (1964) McArthur and Plummer (1978) McArthur (unpublished)
	73	1	45	7	1	21	1	4	1	1	2	

[1]See McArthur and Plummer (1978) and McArthur (1979) for historical development. The section Tridentatae has not been formally proposed as a subgenus, but it is independent of and more or less parallel to the three recognized subgenera.

[2]The references and hence the number of species are not exhaustive, but are representative.

nated in petri dishes were pretreated with colchicine, fixed in 1:3 acetic alcohol, stained in acetocarmine, and squashed on a microscope slide in Hoyer's solution (McArthur and Plummer 1978). Slides are stored at the Shrub Sciences Laboratory.

Several seedlings of each accession were checked for chromosome number (McArthur and Pope 1977), but karyotypes were prepared from one slide per accession—a slide with flat photogenic cells. Five randomly selected metaphase plates from each slide were photomicrographed (Fig. 1). Prints at a magnification of 3120X were used to prepare the karyotypes in a manner slightly modified from that outlined in our earlier paper

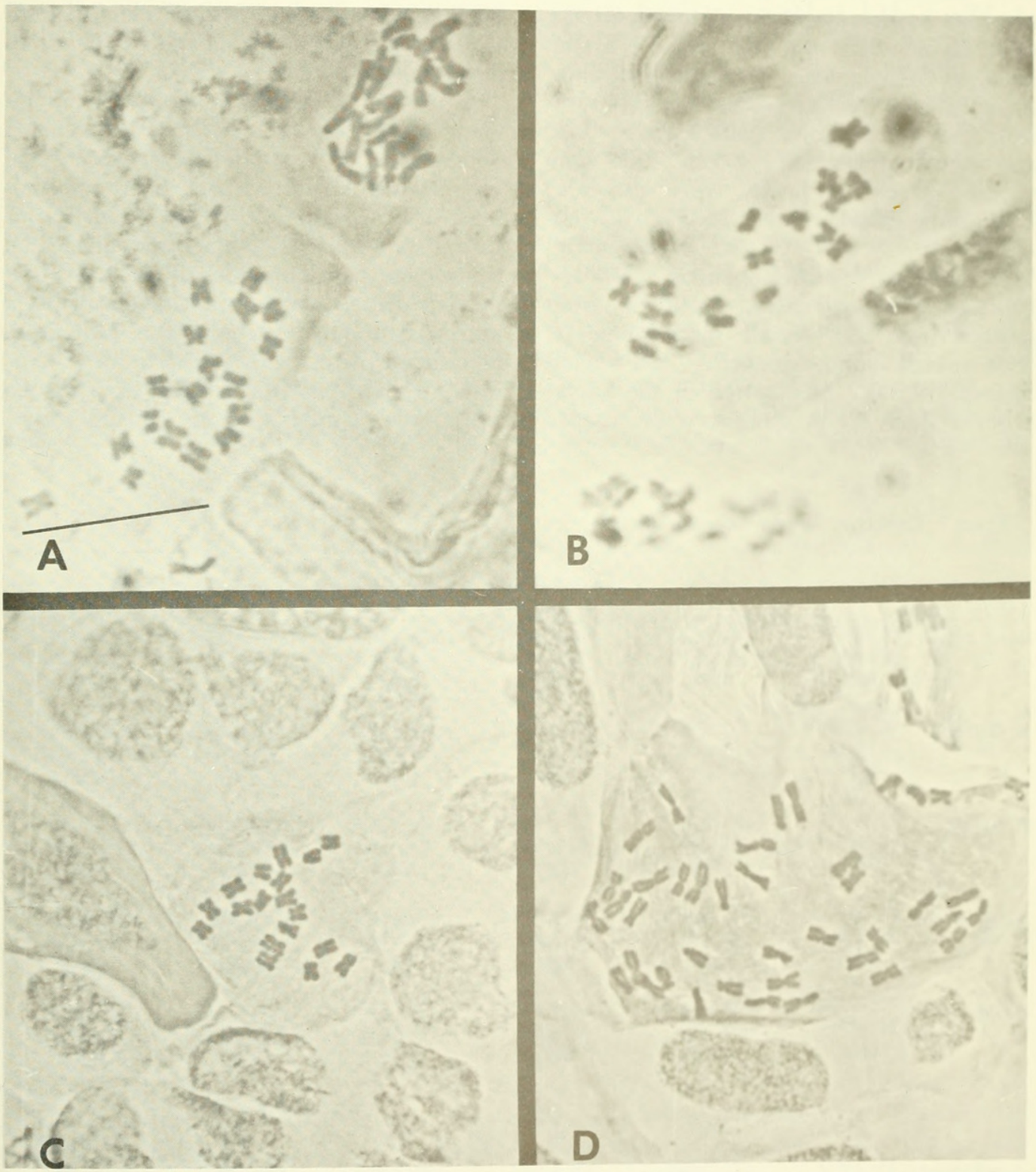

Fig. 1. *Artemisia* spp.: Photomicrographs of colchicine arrested metaphase plates of root tips (1400 X). A, *filifolia*, culture U7, $2n = 18$. B, *frigida*, culture U9, $2n = 18$. C, *spinescens*, culture U3, $2n = 18$. D, *spinescens*, culture U4, $2n = 36$. The line in Fig. 1A defines the end of a cell.

(McArthur and Plummer 1978). Each chromosome complement was assigned 100 arbitrary length units so that the actual measurement per chromosome was proportionalized. For diploid accessions, large chromosomes (L) were defined as those >12.6 units, medium (M) as 9.6–12.6, and small (S) <9.6. For the tetraploid accession, these values were halved. The centromere position was recorded as the proportional length of the short chromosome arm with respect to the length of the long arm. Metacentric chromosomes (M) were defined as those with a ratio >0.75, submetacentric (SM) as 0.50–0.75, and subteliocentric (ST) <0.50. Thus nine classes of chromosomes were possible: LST = large subteliocentric, LSM = large submetacentric, LM = large metacentric, MST = medium subteliocentric, MSM = medium submetacentric, MM = medium metacentric, SST = small subteliocentric, SSM = small submetacentric, SM = small metacentric. For preparation of the karyotypes of Table 3, chromosomes were grouped into the nine classes and paired by relative length and centromere position within each class. Interclass pairing was occasionally required so that all chromosomes could be paired. In such cases, length was given priority over centromere position. To confirm fit of pairing choices, each chromosome pair was visually inspected on the photomicrographs. The five samples for each accession were then averaged ($\bar{x}$) for each chromosome pair and a standard error of the mean (se) computed.

A means of measuring karyotype asymmetry (Wiens and Richter 1966), F percent is obtained for a karyotype by averaging the proportional length of each short chromosome arm in respect to its long arm. In our case, F percent was obtained by halving the centromere position values of Table 3. As F percent decreases from a maximum of 50, a more asymmetric karyotype is indicated. Metaphase plates were available for *A. carruthii* (Keck 1946) and *A. frigida* (Knaben 1968). We compared our F percent results

TABLE 3. Karyotypic data for *Artemisia* species.

Taxon	Accession	Chromosome characteristics[1]	1	2	3
A. carruthii	U4	L	12.75 ± .07	13.63 ± .24	12.18 ± .10
		C	.75 ± .05	.90 ± .02	.93 ± .03
		CC	LSM	LM	MM
A. filifolia	U7	L	14.12 ± .28	13.75 ± .33	12.63 ± .14
		C	.71 ± .02	.92 ± .03	.92 ± .03
		CC	LSM	LM	LM
A. frigida	U9	L	13.65 ± .33	10.59 ± .19	12.34 ± .22
		C	.92 ± .05	.57 ± .07	.96 ± .01
		CC	LM	MSM	MM
A. spinescens	U3	L	13.40 ± .16	12.11 ± .06	11.15 ± .20
		C	.78 ± .06	.71 ± .05	.64 ± .03
		CC	LM	MSM	MSM
A. spinescens	U4	L	6.71 ± .11	6.30 ± .04	6.91 ± .11
		C	.81 ± .02	.82 ± .01	.63 ± .02
		CC	LM	LM	LSM
			10	11	12
A. spinescens	U4	L	5.74 ± .03	5.62 ± .04	5.46 ± .06
		C	.89 ± .03	.85 ± .03	.84 ± .03
		CC	MM	MM	MM

[1]Relative chromosome length (L), centromere position (C), and chromosome class (CC)

with values obtained from these previously published metaphase plates.

In order to compare the diploid ($2n = 18$) and tetraploid ($2n = 36$) chromosome complements of *A. spinescens*, we used a paired t-test (Woolf 1968). The assumption was that the relative lengths of the doubled pairs (1 with 2, 3 with 4, . . .17 with 18 of Table 3) would be significantly different ($P < 0.05$) from the diploid pairs (1,2,...9) if the diploid chromosome complement had not doubled to form the tetraploids. We point out that because size was the first pairing criterion, the possible doubled pairs' relative length of tetraploids are systematically biased (e.g., $1 > 2$, $3 > 4$, ...$17 > 18$). For centromere position, the average of tetraploid pairs was compared to the diploids (1 and 2 with 1, 3 and 4 with 2, ...17 and 18 with 9).

Results and Discussion

The four diploid accessions have different, but not radically different, karyotypic patterns (Table 3, Fig. 1). Persson (1974:168) reported that the whole genus *Artemisia* has a relatively similar karyotype. We believe this tendency for a relatively similar karyotype is present in the genus, but some *Artemisia* taxa have quite different karyotypes (Filatova 1971, 1974a, 1974b), as well as probable aneuploid chromosome number reductions (Wiens and Richter 1966).

Artemisia carruthii.— The accession of *A. carruthii* that we examined has chromosome pairs as follows: 1 LSM, 1 LM, 5 MM, 1 SSM, and 1 SM (Table 3). *Artemisia carruthii* is a member of the *A. ludoviciana* Nutt. species complex of the subgenus Artemisia. It occurs in inland western North America and is known only as a diploid ($2n = 18$). Keck's (1946) metaphase plate has an F percent of 37 as compared to our 44. Despite the apparent difference, the chromosomes are probably similar. Estes's (1969) evidence (hybridization along with meiotic chromosome pairing) supported autopolyploidy in the *A. ludoviciana* complex.

Artemisia frigida.— Like *A. carruthii*, *A. frigida* is also a member of the subgenus Artemisia. *Artemisia frigida*, however, forms its

Chromosome pair						
4	5	6	7	8	9	F %
11.45 ± .15 .90 ± .03 MM	10.96 ± .14 .99 ± .01 MM	10.65 ± .15 1.00 ± .00 MM	10.43 ± .20 .79 ± .05 MM	9.53 ± .29 .67 ± .06 SSM	8.39 ± .16 .96 ± .02 SM	44
9.99 ± .31 .50 ± .04 MSM	11.89 ± .28 .90 ± .03 MM	10.95 ± .32 .84 ± .04 MM	10.10 ± .20 .97 ± .01 MM	8.50 ± .38 .38 ± .02 SST	9.05 ± .30 .86 ± .03 SM	39
11.82 ± .16 .82 ± .04 MM	11.32 ± .21 .98 ± .01 MM	10.78 ± .18 .91 ± .08 MM	10.53 ± .17 .96 ± .02 MM	9.58 ± .13 .49 ± .02 SST	9.36 ± .16 .85 ± .04 SM	41
10.62 ± .21 .73 ± .08 MSM	11.92 ± .19 .91 ± .05 MM	11.34 ± .19 .87 ± .03 MM	10.40 ± .13 .94 ± .05 MM	9.78 ± .20 .85 ± .09 MM	9.31 ± .14 .62 ± .07 SSM	39
6.36 ± .08 .62 ± .02 LSM	5.86 ± .12 .50 ± .05 MSM	5.58 ± .13 .50 ± .05 MSM	5.41 ± .09 .60 ± .04 MSM	4.95 ± .06 .53 ± .03 MSM	6.03 ± .04 .91 ± .01 MM	–

Chromosome pair						
13	14	15	16	17	18	
5.27 ± .10 .87 ± .03 MM	5.22 ± .10 .87 ± .03 MM	4.78 ± .06 .85 ± .05 SM	4.60 ± .03 .87 ± .02 SM	4.62 ± .10 .54 ± .06 SSM	4.58 ± .11 .41 ± .02 SST	36

own species complex. It ranges from Mexico through Alaska to Siberia (McArthur et al. 1979). *Artemisia frigida* is known only as a diploid ($2n = 18$) (Löve and Löve 1964, Knaben 1968, Kovanda 1972, Mulligan and Cody 1972, Hartman 1977, McArthur and Pope 1977). Its karyotype consists of 1 pair LM, 1 pair MSM, 5 pair MM, 1 pair SST, and a pair of SM chromosomes with an F percent of 41 (Table 3, Fig. 1A). Knaben's (1968) cell had an F percent of 43.

Artemisia filifolia.— This species is assigned to the subgenus Dracunculus, but has no close relatives (Hall and Clements 1923). Beetle (1979) recently suggested a possible affinity between *A. filifolia* and the Tridentatae. Our analyses show it to have pairs of chromosomes as follows: 1 LSM, 2 LM, 1 MSM, 3 MM, 1 SST, 1 SM with an F percent of 39 (Table 3, Fig. 1B). This karyotype is quite similar to the Tridentatae—differing by two chromosome pairs. The mean Tridentatae F percent is 38 (calculated from McArthur and Plummer 1978). When compared to the Tridentatae karyotype, *A. filifolia* has an extra LM pair in place of an MSM (McArthur and Plummer 1978). Kelsey and Shafizadeh (1979) point out that the sesquiterpene lactone colartin is shared by *A. filifolia* and the Tridentatae. Our chromatographic data (Hanks et al. 1973 and unpublished data stored at the Shrub Sciences Laboratory) of phenolic compounds also show some similarities between the two taxa. Before any close relationship can be inferred, however, more definitive chemotaxonomic and systematic study is required. The taxa differ widely in floral characteristics and wood anatomy (Moss 1940, McArthur 1979) and apparently do not hybridize despite areas of sympatric distribution.

Artemisia spinescens.— This species is also currently assigned to the subgenus Dracunculus (McArthur 1979). It differs from other species of *Artemisia* because it is both spring flowering and deciduous. Only four populations have had chromosome numbers determined (Powell et al. 1974, McArthur and Pope 1977). Of these, three were diploid ($2n = 18$) and one was tetraploid ($2n = 36$). In the present study, the diploid karyotype has 1 LM, 3 MSM, 4 MM, and 1 SSM chromosome pairs with an F percent of 39, whereas the tetraploid has a karyotype of 2 LSM, 2 LM, 4 MSM, 6 MM, 1 SST, 1 SSM, and 2 SM chromosome pairs with an F percent of 36. The tetraploid karyotype appears to be an approximate doubling of the diploid one. Our paired t-tests for relative length and centromere position showed tetraploid chromosomes to be about what would be expected in doubling the diploid chromosomes. The approximate doubling was especially true for relative length ($P < 0.50$). The centromere positions were not significantly different from doubling ($0.10 > P > 0.05$), but did not indicate an exact doubling. Another measure of centromere position, F percent, was about the same for the diploid and tetraploid accessions (Table 3).

Of particular interest were two pairs of LSM and SM and the single pairs of SST and SSM in the tetraploid. The LSM and SM could have been derived from the largest MSM and the smallest MM pairs (Pairs 3 and 8: Table 3) of the diploids by translocation. The SST and SSM pairs of the tetraploid could, with repatterning (e.g., pericentric inversions), have been derived from the diploid SSM pair 9. Persson (1974) illustrated the analogous nonsimilar $4x$ and $6x$ polyploid grouping that occurs in the *A. maritima* L. complex.

It is hard for us to visualize the tetraploid *A. spinescens* as anything other than autotetraploid. *Artemisia spinescens* has no close relatives. Although the diploid genome may have differentiated in various populations so that the tetraploid(s) may have arisen from hybrids between slightly different parents, the hypothetical differentiated diploids must surely have had a common source in the recent past. Further support for the autotetraploid nature of tetraploid *A. spinescens* is the apparent tendency for autopolyploidy in the genus *Artemisia*. Table 1 certainly suggests such a tendency. Furthermore both the *A. ludoviciana* (Estes 1969) and the *A. tridentata* (Ward 1953, McArthur and Plummer 1978) complexes are riddled with autopolyploidy.

CONCLUSIONS

The four species examined in this report mirror much of the genus *Artemisia*'s chromosomal picture. Their karyotypes are,

in general, quite similar although there are distinctive differences. The four species are all $x=9$, as is most of *Artemisia*. *Artemisia spinescens* shows apparent autopolyploidy, a phenomenon quite common in *Artemisia*. *Artemisia filifolia* has a karyotype quite similar to that found in the *A. tridentata* complex, but morphological and anatomical differences do not support a close relationship between these taxa.

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