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The Artemisia ludoviciana polyploid complex is a highly polymorphic assembledge of eight taxa in the Pacific Northwest, with a chromosome base number of x=9. A cytogenetic analysis of this complex was carried out to determine the sources of its variation, the types of polyploidy present, and the genetic processes active in its evolution.

Chromosome counts in the complex disclosed two diploid taxa, A. michauxiana and A. cavaticaulis, in addition to the previously counted diploids, A. suksdorfii and A. lindleyana.

Results of meiotic analysis and artificial hybridization experiments strongly suggested the autoploid origin of the polyploid members of the complex. These results included the constancy of multivalent formation among the polyploids, the maximum number of multivalents

formed in each taxon, the pairing relationships of the hexaploid and trisomic hexaploid A. douglasiana, the degree of homology between the included genomes of the artificial hybrids, and the presence of heteroploidy in at least five of the included taxa.

The attributes of the complex that might have contributed to the origin and success of autoploidy were also examined. Observations indicate that the diploids produce a low but constant number of unreduced pollen grains. Introgression from the diploid to the tetraploid level, through tetraploid progeny, provides a source of variation for the newly formed autoploids. The complex has a broad physiological base. Mechanisms are present in the meiotic apparatus that could allow for an increase in fertility of autoploid individuals.

The phylogenetic and taxonomic relationships among several of the taxa are discussed.

CYTOTAXONOMIC STUDIES IN THE ARTEMISIA LUDOVICIANA POLYPLOID COMPLEX OF THE PACIFIC NORTHWEST

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CYTOTAXONOMIC STUDIES IN THE ARTEMISIA LUDOVICIANA POLYPLOID COMPLEX OF THE PACIFIC NORTHWEST

I. INTRODUCTION

The Artemisia ludoviciana-complex is a polymorphic assemblage of closely related taxa in the Artemisia vulgaris-aggregation. The complex occurs throughout western North America, and the included variants form a polyploid series with three known chromosomal levels--diploid, tetraploid, and hexaploid. The more recent taxonomic treatments of the complex (Keck, 1946; Cronquist, 1955; Ward, 1960) recognize several species, defined as series of populations having correlated morphological characters, similar ecogeographic distribution, and a common chromosome number, if known. However, this taxonomic technique has proven to be difficult to apply in practice because of evident morphological continuity between the taxa.

The objective of this investigation is to apply cytogenetic methods to the taxonomic study of the members of this complex in northwestern North America, in order to determine the sources of its variation, the types of polyploidy present, and the genetic processes that have been active in the evolution of the complex. The results may lead to a better understanding of the systematic relationships of the taxa involved. The methods employed include

morphological and cytological observations of selected natural populations from the Pacific Northwest, transplant experiments with population samples, and cross-pollination of selected individuals, followed by analysis of the resulting progeny.

II. LITERATURE REVIEW

Classification of Polyploids

The degree of homology between the contributed genomes is the basis for the classification of polyploid individuals. There is a range from autopolyploids with completely homologous genomes, to allopolyploids with completely dissimilar genomes. Stebbins (1947) suggested the term "segmental allopolyploid" for those organisms intermediate between the two extremes, that is, similar in certain portions of the genome but dissimilar in others.

Pairing relationships of the chromosomes during the first meiotic prophase and metaphase are the criteria most easily utilized to establish the degree of homology between the genomes of a polyploid. Lack of multivalent formation, however, is not necessarily evidence against chromosomal homology. For example, Riley (1960) investigated the genetic control of diploid-like behavior in hexaploid wheat and found that the genes involved are localized in one arm of the B-5 chromosome. Hexaploid individuals that were monosomic for chromosome B-5 formed sexivalents and quadrivalents regularly during microsporogenesis. Genotypic control of pairing relationships has also been found in other organisms. Single recessive genes for desynapsis occur in Avena (Thomas and Rajhathy, 1966) and Oryza (Wang, et al., 1965) and affect the formation of bivalents.

Preferential pairing, due to small-scale structural differentation leads to bivalent rather than multivalent formation in plants with otherwise homologous chromosomes (Skirm, 1942). Cua (1952a) confirmed this observation in rice. John and Henderson (1962) observed that the frequency of multivalent formation is directly related to chromosome length and chiasma frequency in tetraploid spermatogonial cells of Schistocera. Polyploid groups, of which Achillea millifolium may be used as an example, exhibit variation (polymorphism) in their pairing relationships (Ehrendorfer, 1959b). From these results it is apparent that the chromosomal configurations of autoploids are under genetic control and, consequently, may be influenced by natural selection. Therefore, the classification of an individual organism or population as allopolyploid on the basis of its showing diploid-like chromosome behavior is ambiguous, and statements implying that there exist relatively more allopolyploids and segmental allopolyploids than autoploids (Stebbins, 1950, p. 308-335; Jones, 1961; Davis and Heywood, 1963, p. 215-225) must be reevaluated. As a consequence of his work on wheat, Riley (1960, p. 407) stated:

> ....an examination of many of the frequently quoted examples of allopolyploid behavior suggests that the direct origin of polyploids free from heterogenetic multivalents, with their depressing influence on fertility and stability, must be rare. Indeed, the cytologically diploid behavior which many natural polyploid species display must often have been acquired subsequent to the development of polyploidy.

By comparing the meiotic behavior of newly synthesized diploid hybrids between the putative parents of a tetraploid species, with that of a polyhaploid derived from the same polyploid, Riley concluded that four classical examples of allopolyploidy, Nicotiana tabacum, Poa annua, Brassica juncea, and Gossypium barbadense, are in reality autopolyploids.

Hybridization and Polyploidy

The important role of hybridity in the origin of successful polyploid races is widely accepted (Stebbins, 1950, p. 308, 316; Davis and Heywood, 1963, p. 216, 222). Even within autopolyploid complexes intervarietal polyploids are more prevalent than autoploids derived from the doubling of the chromosome number of non-hybrid individuals (Stebbins, 1950, p. 316). The advantage of "hybrid polyploids" over strict autoploids or their diploid progenitors is due to their greater adaptability; a wider ecological tolerance results from recombination of the parental genotypes which are either adapted to different habitats or which have different modes of adaptation to the same habitat (Stebbins, 1956). Polyploids originating in this manner are physiologically better equipped to colonize newly available sites (Reese, 1961). Reese suggested that because of this adaptability a higher proportion of polyploids than diploids occupied areas uncovered by the Pleistocene glacial recession, accounting for

the south to north increase in the relative frequency of polyploid species.

Stages in the Evolution of Polyploid Complexes

Although polyploidy may produce a more widely adapted genotype, according to Stebbins (1956), it also tends to decrease the potential rate of new morphological and physiological adaptations. Because of this slow rate of change, the evolution of a polyploid complex may pass through a series of stages. In discussing the role of polyploidy in the evolution of the grasses, Stebbins (1956) described four stages in this series and gave examples of each from the Gramineae. The four stages are as follows:

(1) The polyploid complex of recent origin. Complexes in this stage contain diploid taxa that are common and successful, and polyploids that may or may not exceed the distribution of the diploids. An example is the genus Aegilops.

(2) The polyploid complex with rapidly evolving polyploid members. Here the polyploids have largely replaced their parental populations and the remaining diploids tend to be narrow endemics. An example is the Dactylis glomerata-complex.

(3) The polyploid complex with secondary polyploid cycles. In this instance, most or all of the diploids are extinct, and a new series of polyploids has arisen. An example is Bromus section Bromopsis.

(4) The monotypic polyploid complex. This taxon usually has a very high chromosome number and is derived from an ancient polyploid complex. An example is Bromus section Ceratochloa.

Characteristics of Autopolyploids

Strict autopolyploidy may produce two virtually indistinguishable cytological races. These two races, diploid-tetraploid pairs (Davis and Heywood, 1963, p. 196), often can be separated, however, on characters that result from the doubling of the chromosome complement (Stebbins, 1950, p. 301-308) or from evolutionary processes that have altered one or both genotypes. Three examples will serve to illustrate the variational patterns frequently associated with diploid-tetraploid pairs.

1. The Epilobium angustifolium-complex contains three chromosomal races--diploid, tetraploid, and hexaploid. Hexaploids have been found only in one population in central Japan; however, the other races are circumboreal in distribution. The diploids and tetraploids are allopatric throughout most of their range, and they have different ecological preferences. A combination of four external characters showed some correlation with chromosome number, but abrupt discontinuities were not apparent. Mosquin (1966) recognized two subspecies in the complex, one diploid and one polyploid, because of the morphological continuity within the group.

2. Saxifraga hyperborea (2n=26)--Saxifraga rivularis (2n=52), as contrasted with Epilobium, above, may be unambiguously differentiated by the presence of stolons in the tetraploid and by a pinkish cast to the floral and vegetative portions of the diploid (Webb, 1964).

3. Intraspecific chromosomal races in Thelesperma simplicifolium (n=10, 11, and 22) do not seem to be correlated with morphological differences. However, they may be indentified by chromatographic examination of secondary biochemical constituents (Melchert, 1966).

Intervarietal autopolyploids range between the morphological extremes of the parents, morphologically uniting all three. Most autoploids of this type, and often intravarietal autoploids as well, are members of more involved complexes, such as in the Dactylis glomerata-complex. Although the total variation of this complex is within the limits of the ten or more distinct but interfertile diploid members, the characters are recombined in the tetraploids. The tetraploids more closely resemble the diploids that occur within the same geographic region, and in some instances the two races can be separated only by cytological examination. The tetraploids form a reticulate pattern of variation connecting all the diploids in a continuous morphological series. This pattern of variation arose during the Pleistocene ice age when various diploids came together and hybrids were produced. The polyploid derivatives swamped out the diploid

hybrids and colonized the ecological niches made available during the post-glacial periods. The tetraploids then hybridized with tetraploid populations advancing from other centers of diploid hybridization (Zohary and Nur, 1959).

Meiotic pairing in autopolyploids is highly variable; however, newly formed autotetraploids generally exhibit some degree of quadrivalent and trivalent-univalent formation (Riley, 1960). Table 1 is a compilation of the mean meiotic configurations per cell in both natural and induced autotetraploid grasses.

The frequency of various prophase configurations for four homologous chromosomes was found to be related to the chiasma frequency of the parental diploid in various grasses (Morrison and Rajhathy, 1960) and in the locust genus Schistocera (John and Henderson, 1962). Of the grasses examined in the former study only Hordeum bulbosum formed the maximum possible configuration of 8_{IV}, and then only rarely. In Schistocera full multivalent formation was not observed in any of the cells. John and Henderson were able to examine the frequency of multivalent formation of chromosomes of differing lengths in the complement of Schistocera, and from their results they concluded (p. 144): "Genes control the chiasma frequency per cell and hence, in polyploids, the multivalent frequency per cell. Relative size then determines chiasma and multivalent distribution throughout the complement." The conclusions were

Table 1. The reported frequency of quadrivalents in some autotetraploids (4x=28) in Gramineae.

Species	No. of cells	No. of quadrivalents per cell
	12	3.6
	23	3.8
Hordeum vulgare	82	3.0
	23	3.1
	--	1.9
	21	1.1
	69	2.4
Secale cereale	58	3.9
	100	3.9
	--	1.3
	248	4.6
	21	4.2
Hordeum bulbosum	--	3.0--4.0
	50	4.1
	120	4.7
	50	3.7
	1404	3.9
Dactylis glomerata	ca. 444	3.6
	--	3.0
	5433	3.5
Arrhenatherum elatius	176	3.8
Lolium perenne	--	4.0
	--	3.0
Agropyron cristatum	69	3.7
Agrostis canina	307	2.7

Modified from Morrison and Rajhathy, (1960, p. 306).

based on the following results (John and Henderson, 1962, p. 130-133): (1) The L-chromosomes formed multivalents 93.3 percent of the time, the M-chromosomes 73.6 percent, and the S-chromosomes only 17.3 percent. (2) Trivalents and univalents were rare and found only in the M- and S-chromosomes. (3) Univalents were produced without trivalents in three of twenty-five cells and only in the S-chromosomes. (4) Ring quadrivalent formation was proportional to the length of the chromosomes. (5) The chiasma frequency of the tetraploid cells was found to be more than twice that of the parental diploid cells, 44.44 to 19.33. (6) The number and complexity of the multivalents were a reflection of the chiasma frequencies of the homologous chromosomes in the diploids.

In autopolyploids, or segmental allopolyploids, all four chromosomes of a quadrivalent are homologous; therefore, the presence of quadrivalents at metaphase I should not decrease fertility, since either regular alternate or regular adjacent disjunction yields gametes with a complete genome. Induced autotetraploids, however, usually have a reduced pollen set (Stebbins, 1950, p. 305). Often this reduction results from the production of aneuploid pollen, and Myers (1943) cytologically demonstrated that aneuploid pollen production in Dactylis is correlated with the presence of univalents and not with the occurrence of quadrivalents. Randolph (1935), however, found low frequencies of univalents and laggards in corn; aneuploid

pollen, formed via unequal disjunction from quadrivalents, was common. In Sorghum and Secale, Garber (1955) and Hilpert (1957), respectively, found that parallel ring quadrivalents ([]) occasionally disjoined unequally while convergent ring quadrivalents (◇◇) always gave a 2:2 disjunction.

Even though aneuploid pollen may be scored as "good", the frequency of aneuploid individuals in polyploid populations is low in Holcus mollis (Jones, 1961).

In some organisms the causes of low fertility may be solely of a genetic rather than a cytologic nature (Stebbins, 1950, p. 305-307).

Evolutionary Trends in Autopolyploids

To be successful, populations that have undergone a significant reduction in fertility with the development of polyploidy must compensate for this reduction by asexual reproduction, the production of a higher number of seed, or selection for increased fertility through more regular meiosis. Because the cytological causes of low fertility vary, we might expect the pathways for the restoration of higher fertility to vary as well. Meiotic regularity may be achieved either in a one-step change or by the gradual accumulation of mutations and rearrangements. In an example of one-step restoration of meiotic stability, complete diploidization of the hexaploid bread wheats (Triticum) has been found to be under the control of a single

gene locus (Riley, 1960).

As a case of gradual restoration of meiotic stability, Gilles and Randolph (1951) found indications of a trend toward all bivalent pairing in tetraploid Zea mays. They selected for fertility and vigor in induced tetraploid corn over a period of ten generations. The mean quadrivalent formation per cell was then measured in the F_{10} progeny and in the remnants of the original population, and was found to have decreased from 8.47 to 7.46. In the low-fertility plants, 21 percent of the cells had a maximum of ten quadrivalents, while none of the cells in the high-fertility line had ten quadrivalents.

One means of reduction in the number of quadrivalents per cell is by selection for lower chiasma frequency. According to McCollum (1958) an extreme reduction in chiasma per cell would likely decrease fertility in the orchard grasses (Dactylis), because trivalent and univalent frequencies are negatively correlated with chiasma frequency. If the model for obligatory pairing for all chromosome ends as proposed by John and Henderson (1962, p. 142, Figure 61) is correct, however, the degree of trivalent and univalent formation is limited and would not increase with a reduction in chiasma frequency.

As Feldman (1965) explained for the tetraploid Aegilops, one genome may act as a "sterility-buffer" while the other is altered. Structural alteration of one genome of an autoploid could therefore cause diploidization by disrupting pairing between the two genomes.

Diploid-like behavior is not the only means of achieving stability, since regular disjunction of homologous quadrivalents also yields genomically complete pollen. Complete quadrivalent formation could even be of selective advantage over gradually attained complete bivalent formation, because, in the process of approaching the all-bivalent condition, univalents and trivalents could become more frequent and bring about aneuploid irregularities, as discussed above.

Because 3:1 disjunction, and consequent chromosomal instability, occurs in varying frequencies when parallel rings of four are formed, meiosis could be made more regular by being directed toward convergent ring formation. Several organisms that apparently have directed meiosis of this type are listed by McCollum (1958, p. 580).

Although polyploidy erects an immediate barrier to full gene exchange between the diploid and tetraploid races, evidence has been accumulating since 1937 that the barrier is far from complete. The sterility of the triploid barrier may be breached in one of two ways:

1. The production of viable gametes by "sterile" triploids. Satina and Blakeslee (1937) reported a high frequency of 2n and n gametes produced in triploid Datura. Then in 1954 Charlotte Avers described three triploid artificial hybrids in Aster which had about 90 percent good pollen. Cytologic observations indicated that unpaired univalents underwent division at both anaphase I and II,

resulting in pollen with a higher chromosome number than expected. Zohary and Nur (1959) found three triploid Dactylis individuals in nature that set seed in about 5 percent of the florets (compared to the usual 25-30 percent in 4x and 2x individuals). Of the nineteen seedlings obtained from the triploids that flowered, twelve were tetraploid and six were pentaploid.

2. The production of tetraploid hybrids from diploid-tetraploid crosses. These "exceptional offspring" are more difficult to detect than triploids; however, they have been reported in Zea, Galium, Campanula, Datura, Secale (Woodell and Valentine, 1961), Achillea (Ehrendorfer, 1959b), Solanum (Marks, 1966), Primula (Nagaharu and Hirotoshi, 1950), and Dactylis (Carroll and Borrill, 1965). The production of triploids is apparently rare in these crosses (Marks, 1966).

Considering the somewhat limited genetic heterogeneity of tetraploid races, however, the significance of this unidirectional introgression from the diploid to the tetraploid level can hardly be overestimated.

The Species Concept in Relation to the Autopolyploid Complex

The autopolyploid complex, with at least partial sterility barriers between the phenetically and phylogenetically most similar

types, but with free genetic interchange between the more widely separated forms at the same ploidy level, presents a most difficult problem for the taxonomist. There are two basic approaches to the classification of autopolyploid complexes, or combinations of the two, which the systematist may follow:

1. The biological species approach, based on interfertility, necessitates the division of the complex into morphologically parallel species, each at a different chromosomal level. Löve's (1960) original statement of this philosophy was based on the contention that the doubling of the chromosome complement erects a barrier to interbreeding, and that the two levels can always be identified. If this philosophy is applied, however, entities with the same lineage are split, and polyphyletic taxa are established. An example of this approach to the taxonomy of autopolyploids is the proposed treatment of the Biscutella laevigata-complex of Europe by Clausen, Keck, and Hiesey (1945, p. 139-140). Although the tetraploid members of the group inhabited the highland areas of Central Europe after the glacial recession, the diploids remained in the original dry, hot, lowland sites. The two chromosome levels are therefore goegraphically separated in the modern flora. Since all of the diploids interbreed, Clausen, et al. suggested that they are all subspecies of Biscutella coronopofolia. The tetraploids were then included in the parallel Biscutella laevigata. A similar treatment by Manton has proven to

be impractical for use in the field and herbarium (Guinea and Heywood, 1964, p. 326). Stebbins and Zohary (1959) modified this approach in their study of orchard grass. All the variants were included in one species, Dactylis glomerata, with two morphologically similar series of subspecies, one tetraploid and one diploid.

Recent workers have shown that gene flow does occasionally occur from diploid taxa to their sibling polyploids, and, in some instances, the two cytological levels cannot be satisfactorily separated. Thus Löve's original premise is certainly not universally applicable.

2. The second taxonomic philosophy is based on discontinuities in variation, and it divides autoploid groups across chromosome levels but between the modal peaks of variation. This results in species that are both morphologically and phylogenetically related. However, at higher levels of polyploidy interspecific hybridization obscures the valleys between the peaks. Heckard (1960), using this approach in the Phacelia magellanica-complex, recognized that the resulting species were not always distinct; however, because of the size of the complex, he felt that union of all of the variants into one species would prove too cumbersome. Heckard therefore defined the species in this complex as the morphological plateaus. They include more than one breeding system, and they hybridize with other species at the same level.

Origin of Autopolyploids

Autotetraploids may originate either by the doubling of the somatic chromosome complement or by the union of diploid gametes (Swanson, 1957). Somatic doubling presumably results from failure of the spindle apparatus and subsequent non-disjunction, or by the failure of cell wall formation followed by fusion of the two nuclei.

Diploid gametes are produced regularly by tetraploids, or they may also be produced at reduced rates by triploids (Zohary and Nur, 1959) and diploids (Satina and Blakeslee, 1935; Ehrendorfer, 1959b; Marks, 1966; Carroll and Borrill, 1965). In triploids the gametes arise from unequal disjunction, and approximately equal numbers of 1n and 2n pollen are produced. In the latter situation the mechanisms are not clearly understood, though in Solanum the second division spindles fuse (Marks, 1966) and in Datura dyad production is apparently under genetic control (Satina and Blakeslee, 1935).

Marks speculates that the occurrence of spontaneous autotetraploids in a population of two or more closely related diploids could serve as an intermediate for the production of allopolyploids[1] (Marks, 1966, Figure 1). Two other possibilities are also apparent for Marks' scheme: (1) autopolyploids could be derived in the same manner, and

[1] Number of allotetraploids possible = n - 1, where n = the number of diploid species present.

(2) the second diploid need not necessarily be a member of the original population to contribute to the variation; it may come in contact with the tetraploid race after the latter has expanded geographically.

Previous Taxonomic and Cytological Studies in Artemisia

The genus Artemisia is a widely distributed group of about 150 species of the Northern Hemisphere. The first comprehensive monograph of the genus was begun by Besser in the early 1800's. Besser died in 1842, however, and did not complete the taxonomic treatment; Hooker (1833) and DeCandolle (1837) presented portions of his unpublished work in Flora Boreali-Americana and Prodomus Systematis Naturalis, respectively. Besser (1829) established four sections in Artemisia--Dracunculus, Seriphidium, Abrotanum, and Absinthium. His division of the genus is largely followed today, although the sections are often elevated to the level of subgenus, and sections Absinthium and Artemisia (Besser's Abrotanum) may be united in the subgenus Artemisia (Poljakow, 1961).

Numerous chromosome counts and several outstanding morphologic treatments have been completed in Artemisia, and yet taxonomically the genus is not well understood. This is primarily because of the high incidence of hybridization-polyploidization cycles and the resulting reticulate evolutionary pattern (Suzuka, 1952; Ward,

1953; Ehrendorfer, 1964).

The basic chromosome number in Artemisia is x=9, and at least nine species of Artemisia have undergone dysploid reduction to a gametic number of n=8 (Ehrendorfer, 1964, p. 132).

The Subgenus Seriphidium

Besser distinguished this taxon (called a section in Besser's scheme) on the basis of homogamous heads and a glabrous receptacle. Both Ward (1953) and Beetle (1960) questioned Besser's exclusion from Seriphidium of A. biglovii, a species of section Artemisia which closely resembles members of Seriphidium, but has heads that are usually heterogamous and only occasionally lack ray florets. Although Ward maintained Besser's sections, Beetle included A. bigelovii in section Tridentatae Rydb.

This subgenus is most widespread in the xerophytic Great Basin and Columbia Plateau of the western United States. Its North American representatives comprise the Artemisia tridentata-complex, containing diploid, tetraploid, hexaploid, and octoploid races. The diploid races are widespread, and though they are ecologically differentiated from the derived polyploids, morphologically they are very similar (Ward, 1953). Because of this morphological pattern, Ward suggested that the complex, excluding A. rothrockii (4x, 6x, and 8x), was of autopolyploid origin. Extensive hybridization at the

tetraploid levels then produced allopolyploidy and increased the fertility of the populations. Another consequence of the hybridization, according to Ward, was the erection of a morphological continuum uniting all of the variants. Tetraploid hybrids from crosses between the diploid and tetraploid levels apparently exist in nature also (Ward, 1953, p. 202). Ward and Beetle both divided the complex on the basis of morphology; therefore, different chromosome levels were united at the species level.

Taylor, Marchand, and Crompton (1964) examined the cytology and ecology of A. tridentata itself at the northern limit of its range. In British Columbia the diploids and tetraploids were found to be morphologically distinguishable on the basis of number of florets per head, leaf width, and akene fluorescence in ultra-violet light. The two races are also well separated ecologically by different altitudinal and moisture requirements. Although in this region these two variants resemble the previously described diploid-tetraploid pairs, farther south the possibility for gene exchange with other taxa is increased, and this relationship is obscured. The authors used this information to substantiate Beetle's division of the species into A. tridentata ssp. tridentata (4x) and ssp. vaseyana (2x).

According to Stebbins' classification of the evolutionary stages in polyploid complexes, the Artemisia tridentata-complex is an example of Phase I (Ehrendorfer, 1964).

A variant of A. maritima from the upper Kurram has been studied rather extensively by Suzuka and his associates (Suzuka, Koriba, and Mitsuoka, 1955). This entity is the commercial source of santonine and was considered by Qazibash (1948) as a species (A. kurramensis) distinct from A. maritima. Autotetraploids of this group were artificially induced by colchicine treatment of seedlings grown from field collected akenes. Of the one hundred pollen mother cells (PMC's) examined from autotetraploid plants, 54 percent contained one to five quadrivalents per cell with a modal value of one quadrivalent per cell. Trivalents and univalents were also frequently observed.

Artemisia mendozana of the subgenus Seriphidium is octaploid, 2n=72 (Kawatani and Ohno, 1964).

The Subgenus Dracunculus

This small group with heterogamous heads, pistillate ray florets and pefect but sterile disc florets, has not been extensively studied cytologically. Polyploidy is apparently well developed, as three hexaploid and one octoploid race are known (Kawatani and Ohno, 1964). In North America only diploids (n=9 and 8) and tetraploids (n=18) have been counted, and the diploids are the most widespread (Cave, 1959-1965).

The Subgenus Artemisia

Hall and Clements (1923) considered this group of species to be the most primitive members of the genus, because the floral morphology is least specialized. The disc florets are perfect and fertile; the ray florets are pistillate and fertile. Besser originally separated plants of this group on the basis of one character--receptacle with long hairs in his section Absinthium and glabrous in Abrotanum (Artemisia). Asa Gray (1884) first united the two groups into the section Euartemisia. Hall and Clements acknowledged the soundness of Gray's treatment, but retained both sections for the sake of convenience.

Previously published chromosome numbers in the subgenus Artemisia include n=7 (Wiens and Richter, 1966), 8, 9, 16, 17, 26, 26+1, and 27 (Cave, 1959-1965). Suzuka (1950) suggested that hybridization followed by chromosome doubling gave rise to the n=17 individuals. Artemisia montana (n=26) of the Artemisia vulgaris-aggregation (Ehrendorfer, 1964), putatively arose from hybridization of parental types with n=17 (A. dubia or A. princeps of the same aggregation) and n=9 (Suzuka, 1950; Arano, 1962). Other n=26 and n=26+1 types were considered by Suzuka (1952) to have arisen by chromosome loss.

Two sympatric members of the subgenus are the morphologically

similar A. pattersonii (n=7) and A. scopulorum (n=9) (Wiens and Richter, 1966). Karyological examination of these supposedly related taxa by the authors did not yield evidence relating to the evolution of the n=7 form, although Wiens and Richter suggested two alternative hypothesis for the origin of the species.

Ehrendorfer (1964) cytologically compared the Eurasian groups Heterophyllae, Norvegicae, Pectinatae, and Rupestres and found diploids, tetraploids, and hexaploids. He noted few meiotic disturbances in the groups and concluded that the genomes were weakly differentiated. However, a limited number of restitution nuclei were found. According to Ehrendorfer the Heterophyllae are in the third phase in the evolution of polyploid complexes. That is, the diploid progenitors of the group are allopatric and of a very limited range while the polyploids are widely distributed. Artemisia oelandica and A. panicii, both hexaploid members of the Heterophyllae, are regressive relicts.

The type species of the genus Artemisia is A. vulgaris. This species with its related forms comprises the A. vulgaris-aggregation. The aggregation is circumboreal in distribution (Figure 1), and the variants occupy a wide range of environments from the extremes of alpine or sub-alpine sites to exposed coastal bluffs. The majority of the members of this highly polymorphic complex, however, are plants of the xerophytic steppes of Europe, Asia, and America.

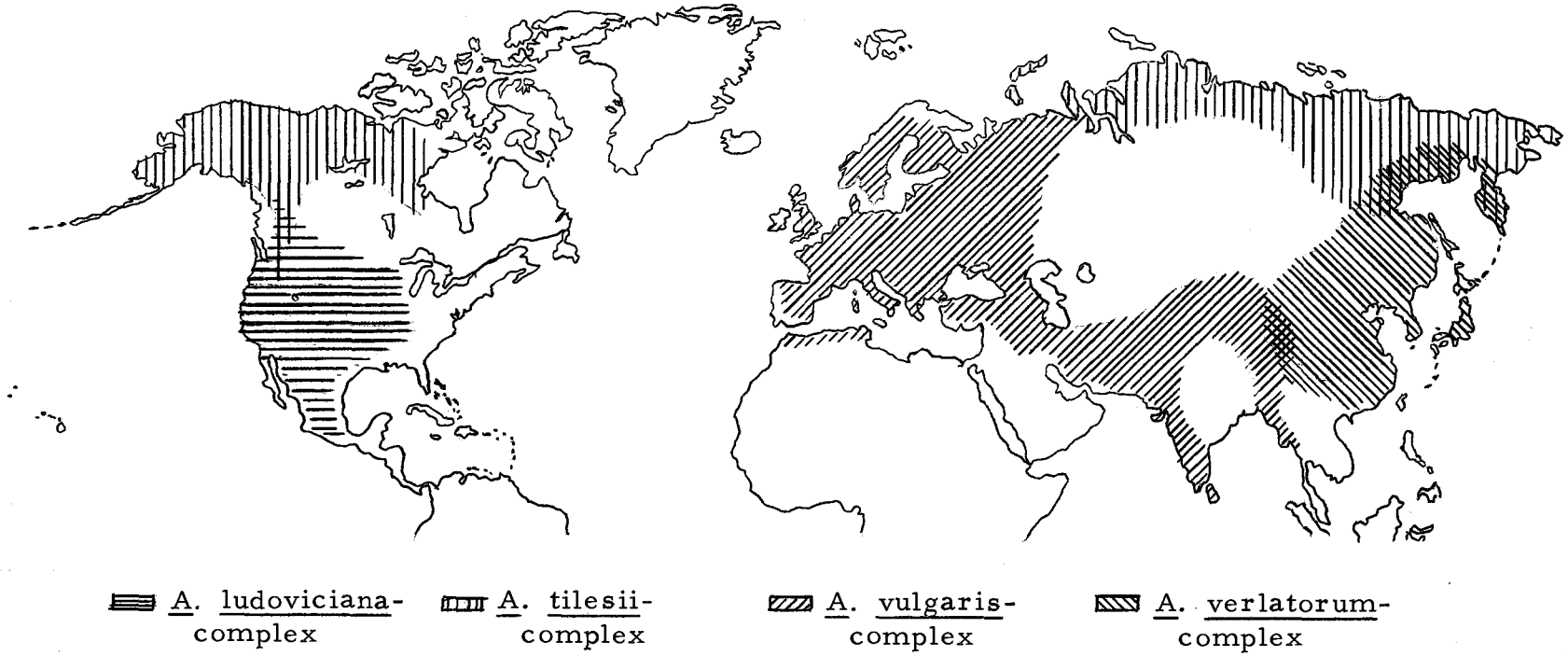

Figure 1. Distribution of Artemisia vulgaris, sensu lato. Boundaries are very approximate.

Artemisa vulgaris, sensu stricto, is diploid in Europe and North Africa with a haploid chromosome number of n=8 (Cave, 1959-1965). In India hexaploid, tetraploid, and diploid populations of A. vulgaris with a base number of x=9 have been reported by Khoshoo and Sobti (1958). They found a general increase in ploidy level with decreasing altitude and latitude, from the inner Himalayas (2x) to Burma, Ceylon, and Western India (6x). Koul (1964a and 1964b) examined the meiotic behavior of diploid and tetraploid A. vulgaris from the Ram Nath Chopra Garden of Medicinal Plants in Jammu, India. He discovered that both divisions of meiosis in the tetraploid were highly irregular, including: (1) poor spindle development, (2) absence of bivalent formation in some PMC's, (3) multivalent and univalent formation, and (4) unequal disjunction. The frequency of metaphase I configurations as determined by Koul (1964b, p. 409) is shown in Table 2. In addition only 14 percent of the meiotic divisions produced four microspores with regular nuclei.

Table 2. Total configurations in the tetraploid Artemisia vulgaris at metaphase I.

1. 8(IV) + 2(II)	2
2. 8(IV) + 1(II) + 2(I)	5
3. 8(IV) + 1(III) + 1(I)	3
4. 6(IV) + 2(II) + 8(I)	3
5. 6(IV) + 4(II) + 4(I)	9
6. 6(IV) + 2(III) + 6(I)	31
7. 4(IV) + 6(III) + 2(I)	17
8. 4(IV) + 4(III) + 8(I)	23
9. 2(IV) + 8(III) + 4(I)	27
10. 8(IV) + 3(II) + 6(I)	12
Total number of cells observed	132

Modified from Koul (1964b, p. 409).

The closely related Artemisia tilesii-complex of the Artemisia vulgaris-aggregation is centered in arctic Asia but occurs in northern North America as well (Hermann, 1956). Diploids (2n=18) and hexaploids (2n=54) have previously been described in this species (Cave, 1959-1965). The known hexaploid subspecies in America extends down the Cascade and Rocky Mountains to Oregon and Montana (Keck, 1946).

Other Eurasian taxa with known chromosome numbers are A. verlotorum (n=8 and 9) and A. mongolica (n=8) (Cave, 1959-1965).

P. A. Rydberg (1916) recognized numerous taxonomic entities within the aggregation in North America. Hall and Clements (1923) realized the complexity of the group, but they chose to emphasize the continuity of variation and united all Rydberg's taxa under the European species A. vulgaris, with fifteen subspecies. Continuing the study after Hall's death, Clausen, Keck, and Hiesey (1940) found three levels of polyploidy within Hall and Clements' conservatively treated species. On the basis of this additional evidence, Keck (1946) revised the North American members of this complex using geographic-ecologic criteria. The taxa recognized by Keck and utilized during the course of this study are listed below:

1. Artemisia vulgaris L. Apparently adventive in North America, occurring along the eastern seaboard and port cities in the western United States. The stems are extremely stout and may

be up to 2 m. tall. The primary leaves are green above and white-tomentose below, 5-12 cm. long and equally wide, with three to seven divisions that are 5-15 mm. broad and again lobed or toothed. n=8 in Europe and Canada (Keck, 1946; Cave, 1959-1965); n=9, 18, and 27 in India (Khoshoo and Sobti, 1958).

2. Artemisia michauxiana Bess. in Hook. Plants of talus slopes at alpine or sub-alpine elevations in the northern and central Rocky Mountains and the Cascade Mountains; local in other mountain areas in the Northwest. The leaves are green above and white-tomentose below, 2-5 cm. long and rather broad, bipinnately divided with irregular teeth on the salient lobes, the angle between the lobe and the central axis approaching 90°. Chromsome number unknown to Keck.

3. Artemisia longifolia Nutt. The leaves are linear or linear-lanceolate, usually entire, 3-12 cm. long and 2-5 mm. wide. Ranges throughout the northern prairie states into Canada. Not included in this study. n=18.

4. Artemisia serrata Nutt. The leaves are lanceolate to linear-lanceolate, serrate from tip to near base, green above and white-tomentose below, 8-12 cm. long and 12-20 mm. wide. The species occurs in the upper Mississippi Valley and intergrades with A. ludoviciana. It was included by Gray (1884) as a variant of the latter species. Not included in this study. n=18.

5. Artemisia carruthii Wood ex Carruth. The leaves are 1-3 cm. long, usually with both sides white-tomentose, divided into linear or filiform lobes. A common plant of the southern Rocky Mountains, it seems to intergrade with certain subspecies of A. ludoviciana. Not included in this study. n=9.

6. Artemisia ludoviciana Nutt. Keck divided this highly variable entity into seven subspecies, on the basis of leaf morphology, relative compactness of the panicle, and ecological distribution. Cronquist (1955), however, makes his separation mainly on the basis of panicle compactness, and he recognizes two subspecies, a northern group of three varieties with a tight panicle (including the type specimen), and a southern group of four varieties with a more open, leafy panicle. The subspecies or varieties that are within the scope of this study are:

a. Artemisia ludoviciana Nutt. ssp. ludoviciana. It occurs from the mid-Mississippi Valley westward to the Sierra-Cascade Mountains along lowland streams. The leaves are 3-10 cm. long and usually entire or with a few entire lobes, and are white-tomentose on both sides. The involucre is 2-3 mm. wide. This polymorphic group apparently intergrades with all of the taxa of the complex that are sympatric with it. n=18.

b. Artemisia ludoviciana ssp. candicans (Rydb.) Keck. It generally replaces the typical subspecies in the northwestern Great Basin and the Columbia Plateau at medium elevations in the foothills. The leaves are 5-10 cm. long and divided into narrow forward-projecting lobes, usually tomentose on both surfaces. The involucre is 3-7 mm. wide. Intergrades are found in eastern Washington linking this taxon with the interior valley Artemisia douglasiana and the montane A. ludoviciana ssp. incompta. Chromosome number unknown to Keck.

c. Artemisia ludoviciana ssp. incompta (Nutt.) Keck. It occurs at high elevations in the mountain ranges of the Northwest. The leaves are 2-8 cm. long, glabrate and green above and white-tomentose below, and divided into linear or lanceolate, forward projecting lobes. The involucre is 2.5-4 mm. wide. This subspecies was once included as a form of Artemisia michauxiana, and intergrades between the two frequently occur. n=18 and 27.

7. Artemisia lindleyana Bess. in Hook. The leaves are mostly less than 1 cm. wide, 2.5 cm. long, entire or serrate-dentate at the apex. Stems woody at the base, 2-4 cm. tall. Cronquist (1955) notes that this species only rarely spreads by rhizomes. It occurs along the banks of the Columbia River and its major tributaries, mostly

below the high water mark, and is closely related to A. ludoviciana ssp. ludoviciana and ssp. incompta. n=9 (Taylor and Brockman, 1966).

8. Artemisia suksdorfii Piper. The leaves are 1.5-3 cm. wide and 8-15 cm. long, broadly lanceolate or elliptic, and strongly discolored. The panicle is dense. The involucre is less than 2 mm. wide, yellow-green and shining. It occurs along the coastal bluffs or along rivers a short distance inland, from Sonoma County, California, to Vancouver Island. n=9.

9. Artemisia douglasiana Bess. in Hook. The leaves are lanceolate to elliptic, 1-5 cm. wide, and 7-15 cm. long, sparsely tomentose to glabrous above and grey-tomentose below, margin variable, entire to lobed. The involucre is 2-3 mm. wide. It is found in the interior valleys of Washington, Oregon, and California, west of the Cascade-Sierra mountain axis, and in Baja California, northern Idaho, the Yakima Valley, and the Lake Tahoe region. This hexaploid taxon was considered by Clausen, Keck, and Hiesey (1940) to be of amphidiploid origin, derived from the coastal A. suksdorfii (2x) and the interior A. ludoviciana (4x). It is intermediate geographically between the two putative parents and to some extent it is at a middle elevation as well. Stebbins (1965, p. 190) noted that A. douglasiana also occupies sites that are edaphically intermediate between the sandy bluffs along the Pacific Ocean and the poorly

drained mineral soils of the Northwestern steppes. Keck (1946) suggested that hybridization might have occurred in the Columbia River Gorge where the eastern and western entities might have been sympatric. Intermediates between A. douglasiana and A. ludoviciana ssp. candicans also occur east of the Cascade Mountains in Washington (Cronquist, 1955). n=27.

10. Artemisia prescottiana Bess. in Hook. The leaves are 2-5 cm. long, less than 1 mm. wide, green above with sparse arachnoid-pilose hairs below, and pinnatifid with 3-7 filiform entire lobes. The involucre is 4-5 mm. wide. It is known only from a single collection by David Douglas, "On the Quick Sand River, near the Grand Rapids of the Columbia."

Ward, in Abrams' Illustrated Flora of the Pacific States (1960, p. 408) stated that A. prescottiana is probably only a variety of A. lindleyana. Cronquist (1955), however, reduced this name to a synonym of A. lindleyana. Chromosome number unknown to Keck.

11. Artemisia tilesii Ledeb. spp. unalaskensis (Bess.) Hulten. The leaves are very variable but are usually deeply pinnately or even subpalmately lobed, and strongly discolored. The involucre is 4-8 mm. wide and often anthocyanous. It occurs from Hokkaido, Kamchatka, the Aleutian Islands, Alaska, and Yukon Territory, south to northern Oregon and Montana. This taxon is more closely related to the Eurasian members of the Artemisia vulgaris-aggregation; however,

intergrades with A. douglasiana are occasionally found in Oregon. n=27.

Keck considered the Vulgares-complex (Artemisia vulgaris-aggregation) to be a typical polyploid complex with the morphologically and ecologically diverse forms closely enough related for limited gene flow. This observation was based in part on the pairing relationships he observed in two F_1 hybrids of the cross, A. douglasiana X A. ludoviciana ssp. ludoviciana. These pentaploid hybrids seldom produced more than seven univalents and sometimes no more than three (Clausen, Keck, and Hiesey, 1940). The authors suggested that the extra pairs (i.e., the frequency of pairing obtained versus the pairing expected) indicated autosyndesis. However, they stated that all the taxa of "subsection Vulgares" possibly constitute one cenospecies.

Ehrendorfer (1964), utilizing the information in Keck's monograph, classified the North American members of the complex as an example of Phase II in the evolutionary series of polyploid groups. Ehrendorfer noted, in particular, the limited number of diploid representatives, the plastic and widely distributed tetraploids, the secondary polyploidy in A. douglasiana, and the presumed extinction of the diploid progenitors of some tetraploid lines (e.g., the progenitors of A. longifolia and A. serrata).

III. MATERIALS AND METHODS

Collections

Live specimens were collected by the author or his colleagues at Oregon State University from populations in Oregon, Washington, Idaho, northern California, Massachusetts, Oklahoma, and Montana. Rhizomes were dug from selected plants, wrapped in moist newsprint, and placed in plastic bags. They were propagated in sandy soil in plastic pots and grown in the Oregon State University greenhouse for the first season. In most instances the plants were then transferred to a common garden, of sandy, well drained Willamette River soil, that was cultivated and irrigated. The freshly dug plants from nature produced a more luxuriant growth if stored for about one to two weeks in the plastic bags at 5°C.; all of the later collections were handled in this manner. Other specimens were kindly sent by D. A. Wiens from Utah, I. G. Palmblad from central Washington, and G. D. Arnold from Oklahoma and New Mexico. All of the collections are listed by taxon, locality, and accession number in Appendix I. If the specimen was in flower at the time of collection, a pressed voucher was also prepared. Vouchers were taken from all of the material in the greenhouse and garden.

A special collection was made of the members of the Artemisia ludoviciana-complex at Mayer State Park, Rowena, Oregon. This site

includes A. lindleyana and A. prescottiana along the rocky-sandy Columbia River shore below the high water mark, and A. ludoviciana inland around two small river-fed lakes. One of the lakes is intermittant and provides a natural seed bed for possible hybrid forms. The purpose of this study was to determine if hybrid genotypes were produced at this point of close contact between the shore and inland forms. In order to sample the population's variation, plants were randomly sampled along eleven transects that were on 20 m. centers and perpendicular to the river shore. Sampling sites were placed at 20 m. intervals along each transect, and vouchers were collected from the nearest organism to the sampling site in each quadrant (modified from Cain and Castro, 1959, p. 134). If the sample plant was not in flower, it was tagged and the voucher collected at a later time. Notations as to time of flowering, associated species, type of substrate, and available moisture were recorded for each sample plant.

Representative genotypes from the site at Mayer State Park were also selected and divided into two clones; a portion of the rhizome was transplanted, by the procedures outlined above, in the Willamette Valley garden at Corvallis. Each parental clone in its native environment was marked, by a wooden stake driven into the soil, and observed throughout the growing season. Vouchers of these plants were taken from the surviving parental clones at the end of

the season. Because at the time of writing the transplanted clones have not yet completed one growth season in the garden, the results of this portion of the experiment are not included in this report.

Cytological Studies

Buds were collected from plants in the field, greenhouse, and garden, for the determination of chromosome number, meiotic analysis, and pollen fertility counts.

Meiotic Analysis

For meiotic preparations the involucre of the buds was first opened with a dissecting needle, and the buds were killed and fixed in a mixture of chloroform, 95% ethanol, and glacial acetic acid (6:3:1) for 24 hours. The material was washed and stored in 70% ethanol at -10°C. The buds were bulk stained at 55°- 60°C. in HCl-alcohol-carmine (Snow, 1963) for one day, washed free of excess stain in 70% ethanol, and dissected in 45% glacial acetic acid. Anthers containing the PMC's were then mounted in Hoyer's medium (Alexopoulis and Beneke, 1952) and squashed. Analyses of meiotic figures were conducted with Zeiss bright-field optics.

Chromosome number was determined at either diakinesis, prometaphase I, metaphase I, anaphase I, prophase II, or metaphase of the first mitotic division in microspores. The chromosome number

of only three specimens was determined from squashes of root-tip material. Pairing relationships were scored at prometaphase and metaphase I in the polyploids, and at diakinesis as well in the diploids, the features examined being the chromosomal associations and chiasma frequency. The regularity of chromosomal disjunction was determined at anaphase I and subsequent stages of division. The number and size of the nuclei resulting from meiotic division was also recorded at telophase II or while the sporads of microspores were still associated. The latter measurement was used as an indicator of meiotic stability.

Photomicrographs were taken at 1000X magnification with a Zeiss attachment camera and panatomic-X film.

Pollen Fertility

Pollen preparations from fresh material were fixed and stained in a manner similar to PMC preparations, except that the involucre was already open at collection time and artificial opening with a needle, for better penetration of the killing fluids, was unnecessary. The pollen was teased out of open florets, prior to anther dehiscence, and mounted in Hoyer's medium without squashing. Pollen with two sperm nuclei present at this stage of development were considered to be fertile (Dean, 1966); Artemisia pollen is normally trinucleate when shed. The florets open one to two hours prior to dehiscence; therefore,

to allow for variation in collection time, only pollen grains that had not yet begun nuclear division of the generative nucleus were considered to be non-fertile. A random sample of at least one hundred grains from several florets from different heads was used for the determinations.

Pollen Diameter

Pollen was also collected from selected herbarium specimens, to determine mean pollen diameters as a possible indicator of chromosome number in A. michauxiana and A. ludoviciana ssp. incompta. Anthers from open florets were selected and the pollen was released into an analine blue-lactophenol solution. The cover slip was placed on the liquid droplet without the application of pressure. External pollen diameter was measured from linear scale, camera lucida drawings at 240x magnification (750x bench level) of 10-20 randomly selected grains. Only fully developed grains that stained blue were measured.

Hybridization

The Artemisias are paniculate anemophiles with relatively small but numerous heads, and members of the subgenus Artemisia have perfect disc florets. These factors prevent mechanical emasculation and make artificial pollen transfer difficult. The following

method of hybridization was found to be the most practical of the various approaches attempted during the course of the study.

The entire inflorescence or sub-divisions of the panicle of the pistillate parent was bagged in parchment "ear protector" bags just prior to the appearance of the ray stigmas. After the ray florets had opened, the actively dehiscing, staminate parent was introduced to the bag at a slightly higher elevation. The bags were periodically shaken to cause the pollen to fall free. With this method it was impossible to determine which stigmas had been effectively pollinated; therefore, seed set could not be measured. Also, due to the extended flowering time, the bags were left in place for up to two or three weeks; hence, moisture and heat within the bag may have decreased the number of hybrid seed set. The akenes were harvested after they became loose within the involucral bracts, and they were propagated in individual plant bands by placing the akene on moist, screened soil and covering with screened sand and soil. The bands were sub-irrigated in the greenhouse and shaded with cheesecloth. After six to eight weeks the seedlings were transplanted to plastic pots in the greenhouse.

Akenes were also collected from selected garden individuals after open pollination, and they were grown in the same manner.

Leaf vouchers were pressed from each seedling, and buds for meiotic and pollen analysis were collected from selected F_1 plants.

Because many of the parental individuals were partially self-incompatible, the groups of seedlings were examined for probable hybrids on the basis of leaf morphology and growth habit.

IV. RESULTS

Cytology

Chromosome Counts

The chromosome numbers of 130 plants from 59 populations were determined. Appendix I includes a tabular report of the chromosome counts by taxon and accession. In general, the counts corresponded to previous reports by Keck (1946) and others (Cave, 1959-1965). Reports are given for three previously uncounted taxa:

Artemisia michauxiana	$2n=9_{II}$	$2n=18_{II}$
Artemisia prescottiana	$2n=18_{II}$	
Artemisia ludoviciana ssp. candicans	$2n=18_{II}$	$2n=27_{II}$

A previously undescribed diploid taxon, known only from one population, Clines Falls, in central Oregon, was also encountered. For a discussion of the morphological characteristics of this variant, referred to here as A. cavaticaulis, see the section entitled Discussion.

Three taxa were found to include chromosome levels different from those previously cited in the literature:

	Previous counts	Newly reported counts
Artemisia lindleyana	$2n=9_{II}$	$2n=18_{II}$
Artemisia douglasiana	$2n=27_{II}$	$2n=18_{II}$
Artemisia tilesii ssp. unalaskensis	$2n=27_{II}$	$2n=18_{II}$

The previous counts of A. douglasiana were confirmed from eight populations in the Willamette Valley.

Minor variations were observed in the following groups:

Artemisia douglasiana	$2n=27_{II}{}^{1}{}_{I}$	$2n=17_{II}{}^{1}{}_{I}$
Artemisia ludoviciana ssp. candicans	$2n=18_{II}{}^{2}{}_{IIB}$	

Heteroploidy was found to exist in six entities in addition to that already reported by Keck (1946) for A. ludoviciana ssp. incompta and Khoshoo and Sobti (1958) for A. vulgaris. These include:

Artemisia vulgaris	$2n=8_{II}$	$2n=18_{II}$	$2n=$ca. 27_{II} [2]
Artemisia douglasiana		$2n=18_{II}$	$2n=27_{II}$
Artemisia lindleyana	$2n=9_{II}$	$2n=18_{II}$	
Artemisia michauxiana	$2n=9_{II}$	$2n=18_{II}$	
Artemisia ludoviciana ssp. candicans		$2n=18_{II}$	$2n=27_{II}$
Artemisia tilesii ssp. unalaskensis		$2n=18_{II}$	$2n=27_{II}$

[2] Chromosome counts from the first mitotic division in microspores ranged from 22 to 30 with two counts of 27, in a sample of seven.

Pollen Diameter

The results of pollen diameter measurements for 35 plants are graphically illustrated in Figure 2. The data include measurements for five diploids and ten tetraploids whose chromosome numbers had previously been counted during microsporogenesis, and 20 plants whose numbers are not known. As demonstrated in Figure 2, the diploids and tetraploids cannot be definitely separated on the basis of pollen diameter. Likewise there is considerable overlap between the two taxa being tested, A. michauxiana and A. ludoviciana ssp. incompta. Nonetheless, the plants can be assigned to approximate ploidy levels, and these estimates were used in preparing the geographical plots on the distributional map in Figure 3. One individual of A. michauxiana from Steens Mountain (M-3) was judged to be highly sterile, on the basis of pollen stainability in analine blue-lactophenol.

Meiotic Analysis of the Diploids

The chromosomal configurations of nine diploids were tabulated at metaphase I and diakinesis of PMC's. The frequencies of bivalents and univalents per cell at both stages are shown in Table 3. Univalents were found in all of the taxa examined except A. carruthii, a Southwestern member of the complex. Whether the univalents arose via asynapsis or desynapsis could not be ascertained. However, four was

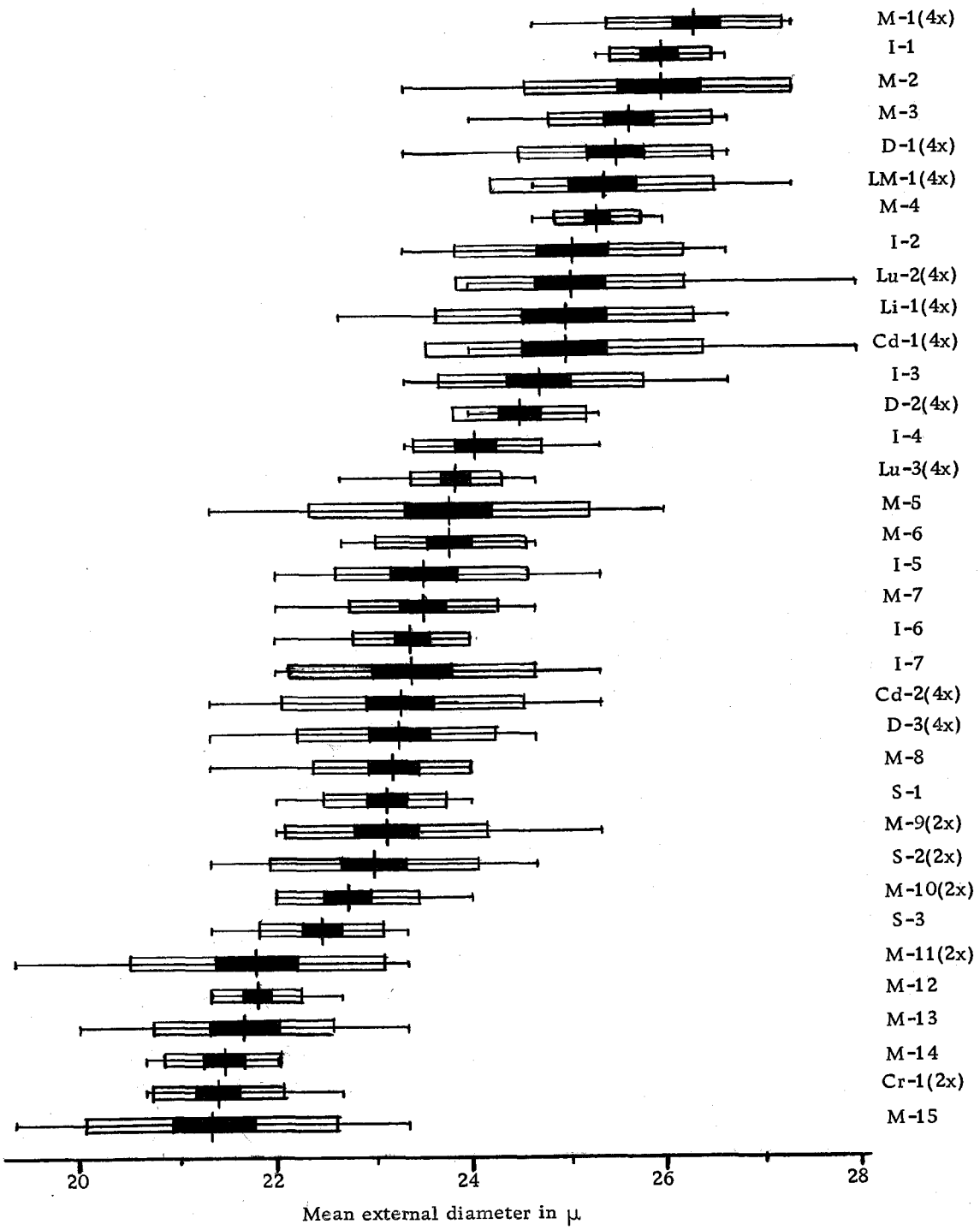

Figure 2. Comparative pollen grain sizes. For a tabulation of the accessions, see Appendix III. For a distributional map of A. michauxiana and A. ludoviciana ssp. incompta, see Figure 3.

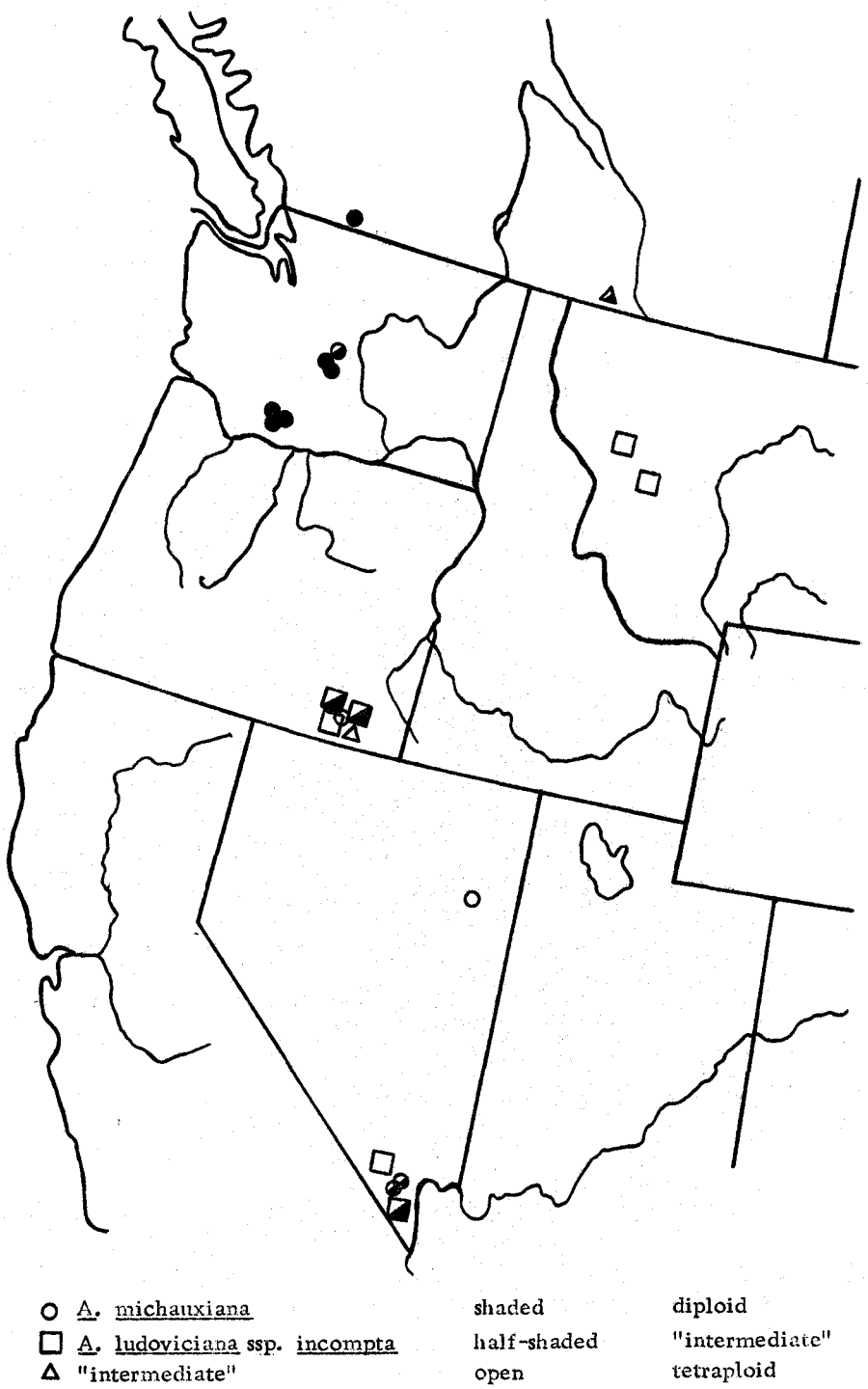

Figure 3. Distribution of Artemisia michauxiana and Artemisia ludoviciana ssp. incompta. For a tabulation of the accessions, see Appendix III. The chromosome level was determined from mean external pollen diameter comparison (Figure 2).

Table 3. Frequencies of diakinesis and metaphase I configurations in the diploids.

	Cells		Bivalents/Cell		Univalents/Cell	
	Diakinesis	Metaphase I	Diakinesis	Metaphase I	Diakinesis	Metaphase I
Artemisia cavaticaulis						
8A	73	83	8.90	8.95	0.20	0.10
8B	2	104	8.50	8.85	1.00	0.30
8C	29	32	8.69	8.75	0.62	0.50
	104	219	8.84	8.87	0.21	0.27
Artemisia suksdorfii						
4A	--	6	----	9.00	----	----
4B	41	95	8.95	8.98	0.10	0.04
19	88	148	8.90	8.95	0.20	0.10
27	45	135	8.91	8.97	0.18	0.06
	174	384	8.91	8.97	0.18	0.07
Artemisia carruthii						
52	90	97	8.91	9.00	0.18	----
Artemisia vulgaris						
85E	21	55	7.90	7.82	0.20	0.36

the maximum number of univalents found in any one cell (Table 4, plants with seven bivalents). The diploid race of A. michauxiana was not analyzed at either metaphase or diakinesis, for lack of flowering material.

The number of chiasmata per cell and chiasmata per chromosome pair[3] were computed for each of the taxa at metaphase I (Table 5). The frequencies fail to approach 2.0 chiasmata per pair; correspondingly the ratio of rod to ring bivalents is only 1.23:1. The one A. vulgaris plant investigated demonstrated an extremely low number of chiasmata per chromosome pair, only 1.03. Koul (1964b), in comparison, reported that only 11 percent of the bivalents had single chiasma in Indian A. vulgaris; however, Koul's Figure 1, page 408, his only photomicrograph of meiosis from the diploid race, shows all rod bivalents at late diplotene.

Two means of estimating meiotic stability were employed, pollen viability (Table 6) and sporad analysis (Table 7). Appreciable numbers of triads and dyads were observed in the analysis of sporads of microspores; they were distinguished from earlier stages of microsporogenesis by the presence of the cell plate, which does not form until late telophase II (Figure 4a). In addition, they were associated with the "normal" tetrads of microspores. The nuclei of the dyads,

[3] Chiasmata per chromosome pair = the chiasma frequency per cell/ the gametic number of the organism.

Table 4. Frequency distribution of numbers of bivalents per cell in the diploids.

	9	8	7
Artemisia cavaticaulis			
8A	79	4	-
8B	90	12	2
8C	25	5	2
Artemisia suksdorfii			
4A	6	-	-
4B	93	2	-
19	141	6	1
27	131	4	-
Artemisia carruthii			
52	97	-	-
Artemisia vulgaris			
85E	47	6	2

Table 5. Chiasma frequency at metaphase I in the diploids.

	Cells	Chiasmata/ Cell	Chiasmata/ Chromosome pair
Artemisia cavaticaulis			
8A	63	11.7	1.30
8B	84	13.9	1.54
8C	23	12.4	1.38
	170	12.8	1.42
Artemisia suksdorfii			
4A	2	16.5	1.83
4B	50	13.4	1.49
19	104	17.0	1.89
27	86	12.4	1.38
	242	14.6	1.62
Artemisia carruthii			
52	89	16.8	1.87
Artemisia vulgaris			
85E	57	8.2	1.03

Table 6. Pollen viabilities of the different taxa and ploidy levels.

	Total grains	Percent viability
Artemisia cavaticaulis (2x)		
8C	238	95.0
Artemisia suksdorfii (2x)		
4B	148	95.2
19	375	91.8
27	294	94.9
	817	93.5
Artemisia michauxiana (2x)		
106C	152	100.0
Artemisia ludoviciana (4x) ssp. *ludoviciana*		
29	347	52.4
98C	251	89.6
	598	68.1
ssp. *candicans*		
9B	267	78.7
9C	367	80.7
41B	243	07.0
	877	59.7
(excluding 41B)	634	79.8
ssp. *incompta*		
79B	286	94.4
108C	238	86.1
108F	253	67.6
115D	226	99.1
	1003	86.7
Artemisia prescottiana (4x)		
42-559	401	83.8
Artemisia michauxiana (4x)		
119A	328	93.9

Table 6. (continued)

	Total grains	Percent viability
Artemisia douglasiana (4x)		
11A	226	98.7
11B*	417	92.6
11C	198	99.5
11F	210	99.0
11H	313	97.8
11I	415	29.9
11J	242	97.5
82D	247	97.8
	2268	84.7
(excluding 11I)	1853	97.0
Artemisia lindleyana (4x)		
1A	229	97.8
1B	284	98.9
1F	144	92.4
1V	340	80.3
1X	181	100.0
36A	375	96.5
36D	234	93.6
36E	553	81.4
103A	340	89.1
103G	307	94.5
103H	443	95.9
	3430	91.6
Artemisia douglasiana (6x)		
48+	453	62.2
80A+	117	98.3
	570	69.6
Artemisia ludoviciana ssp. candicans (6x)		
28D	230	96.5
Artemisia vulgaris (6x)		
2	193	96.9

*monosomic tetraploid

+trisomic hexaploid

Table 7. Frequencies of the types of sporads of microspores.

	Tetrads	Triads	Dyads	Monads	Others
Artemisia cavaticaulis (2x)					
8B	98.4	----	1.6	---	---
8C	86.3	10.1	3.7	---	---
Artemisia suksdorfii (2x)					
4B	95.0	1.8	1.2	1.9	---
27	85.5	7.6	7.0	---	---
Artemisia ludoviciana (4x)					
ssp. ludoviciana					
98C	98.6	1.3	----	---	---
ssp. candicans					
44F	100.0	----	----	---	---
79J	98.8	1.2	----	---	---
113A	100.0	----	----	---	---
113X	100.0	----	----	---	---
ssp. incompta					
79B	98.8	1.2	----	---	---
108C	97.4	2.6	----	---	---
108F	100.0	----	----	---	---
115D	95.4	4.5	----	---	---

Table 7. (continued)	Tetrads	Triads	Dyads	Monads	Others
Artemisia lindleyana (4x)					
1A	100.0	----	----	---	---
1D	93.6	3.5	----	---	2.8
1E	100.0	----	----	---	---
1V	78.7	----	----	---	21.3
33B	81.1	6.0	9.8	2.9	---
33C	100.0	----	----	---	---
36A	85.9	14.1	----	---	---
36B	100.0	----	----	---	---
103A	95.9	2.2	0.4	0.8	0.4
103C	99.5	0.5	----	---	---
115I	93.7	3.8	2.4	---	---
Artemisia douglasiana (4x)					
11A	100.0	----	----	---	---
11C	100.0	----	----	---	---
11H	100.0	----	----	---	---
11I	13.5	8.1	74.9	3.1	0.3
11J	99.1	0.9	----	---	---
82A	98.8	1.2	----	---	---
82C	100.0	----	----	---	---
82D	100.0	----	----	---	---
Artemisia ludoviciana (6x) ssp. candicans					
28D	97.9	1.4	----	---	0.8
Artemisia douglasiana (6x)					
48	69.0	16.6	11.8	4.1	1.3
Artemisia vulgaris (6x)					
2	87.6	12.4	----	---	---

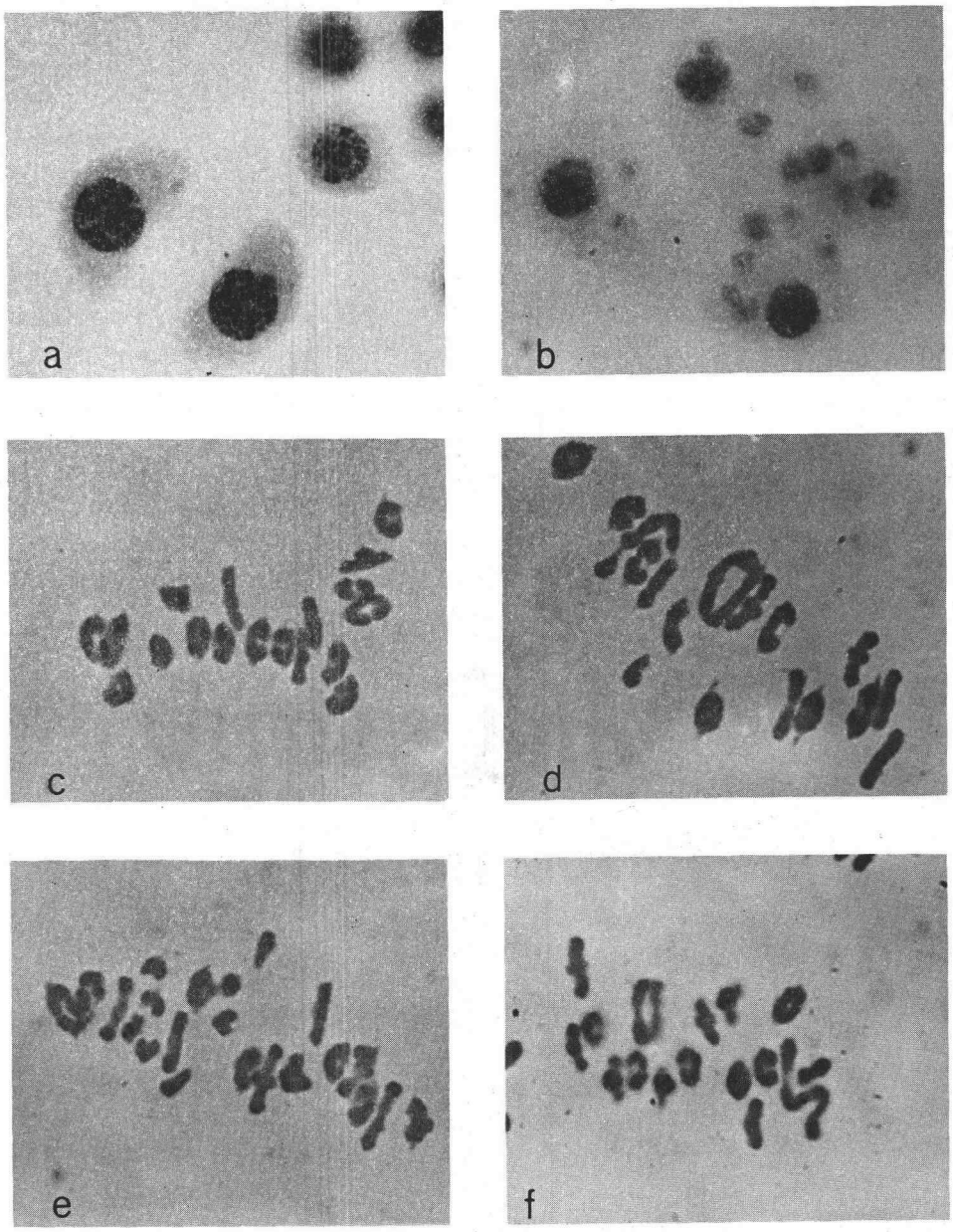

Figure 4. Meiosis in the Artemisia ludoviciana-complex. (a) Dyad of microspores. (b) Tetrad of microspores with 21 micronuclei. (c) Metaphase I, $2^{\infty}{}_{IV}14_{II}$. (d) Metaphase I, $1^{O}{}_{IV}\,1^{\sqcap}{}_{IV}\,14_{II}$. (e) Metaphase I, $1^{\infty}{}_{IV}\,16_{II}$. (f) Metaphase I, $1^{\sim}{}_{IV}16_{II}$. (a) A. suksdorfii, (b) - (f) A. lindleyana.

and one of the three nuclei of triads, were observed to be larger in diameter than the nuclei of tetrads and the remaining two nuclei of the triads. The cells within dyads and triads that contained the larger nuclei were considered to be unreduced microspores. There were three monads observed in one of the A. suksdorfii plants from the Yeon State Park population. In this same individual at least one restitution nucleus was observed at an earlier stage. Various numbers of micronuclei were found in all of the diploids; these consisted of from one to several chromosomes (Figure 4b). In spite of these irregularities, however, pollen set was over 90 percent normal in all the diploid plants.

Meiotic Analysis of the Tetraploids

The majority of the tetraploids examined formed multivalent associations in PMC's with the median of the averages being 0.66 multivalents per cell (Table 8). The configuration patterns of multivalents at metaphase were of three primary types (Figure 4c, d, e, and f), and they were distributed according to the data given in Table 9. Interestingly, only 7.2 percent of the multivalents appeared as trivalents. The maximum number of chromosomes involved in multivalents in any cell was 24, or 2/3 of the genome (Table 10). Chiasma frequency was also computed for the tetraploid individuals (Table 11), and the average chiasma frequency was found to be less

Table 8. Frequencies of metaphase I configurations in the tetraploids.

	Cells	Quadrivalents/* Cell	Bivalents/ Cell	Univalents/ Cell
Artemisia ludoviciana				
ssp. ludoviciana				
9B	27	0.67	16.66	----
9C	20	1.40	15.00	0.40
14B	20	0.25	17.50	----
25	71	0.61	16.78	----
29	4	----	18.00	----
46B	13	0.23	17.38	0.30
98C	43	0.61	16.79	----
	198	0.66	16.72	0.00
ssp. candicans				
41B	11	1.00	16.00	----
79J	18	0.56	16.83	0.12
113X	16	0.63	16.75	----
	45	0.70	16.60	0.00
ssp. incompta				
115D	21	0.19	17.23	0.76
Artemisia lindleyana				
1A	21	1.09	15.76	0.10
1B	92	0.85	16.28	0.04
1E	84	0.86	16.22	0.12
1F	12	0.42	17.08	0.16

Table 8. (continued)

	Cells	Quadrivalents/* Cell	Bivalents/ Cell	Univalents/ Cell
Artemisia lindleyana (continued)				
1V	55	0.74	16.49	0.04
33B	2	----	18.00	----
33C	37	1.24	15.46	0.10
34C	41	0.81	16.36	0.04
34E	56	1.39	15.19	0.04
36B	37	0.70	16.54	0.10
36E	10	0.30	17.40	----
37A	10	0.40	17.20	----
42-203	49	0.43	17.14	----
65A	26	0.62	16.76	----
103A	35	0.66	16.46	0.46
103C	27	0.59	16.74	0.14
103H	18	0.94	16.11	----
115I	22	0.64	16.63	0.18
	634	0.81	16.33	0.09
Artemisia prescottiana				
42-559	153	0.55	16.89	0.00+
Artemisia michauxiana				
119A	21	1.29	15.29	0.28

Table 8. (continued)

	Cells	Quadrivalents/* Cell	Trivalents/ Cell (0.27)	Bivalents/ Cell	Univalents/ Cell
Artemisia douglasiana					
11A	10	0.70		16.60	----
11B+	33	0.78		15.08	0.97
11C	18	1.00		15.94	0.10
11F	29	0.69		16.62	----
11H	10	0.60		16.80	----
11I	40	----		0.43	35.12
11J	33	0.85		16.30	----
82A#	65	0.24		17.52	----
82D	48	1.50		14.88	0.20
	286	0.72		14.02	5.06
Excluding 11I and 11B	213	0.78		16.40	0.06

* including trivalents plus univalents

\+ monosomic tetraploid

\# tetraploid with two pairs of accessory chromosomes

Table 9. Frequencies of metaphase I multivalent configurations in the tetraploids.

	Total	 IV	IV	 IV	 IV	III
Artemisia ludoviciana						
ssp. ludoviciana						
9B	18	0.22	0.61	0.17	----	----
9C	24	0.37	0.37	0.16	----	0.10
14B	5	0.40	0.40	0.20	----	----
25	43	0.30	0.22	0.35	----	0.13
46B	3	----	----	1.00	----	----
98C	26	0.61	0.23	0.15	----	----
	119	0.38	0.32	0.26		0.07
ssp. candicans						
41B	11	0.18	0.64	----	----	0.18
79J	10	0.40	----	0.50	----	0.10
113A	10	0.50	0.20	0.30	----	----
113X	5	0.60	----	0.40	----	----
	36	0.39	0.25	0.28		0.08
Artemisia lindleyana						
1E	65	0.26	0.29	0.32	0.02	0.11
1F	6	0.50	0.17	0.33	----	----
1V	42	0.17	0.21	0.48	0.02	0.11
34A	2	----	----	0.50	----	0.50
34C	35	0.54	0.17	0.26	----	0.02
36B	26	0.54	0.12	0.35	----	----
36D	18	0.22	0.33	0.39	----	0.06
36E	3	0.67	----	0.33	----	----
37A	4	----	1.00	----	----	----

Table 9. (continued)

	Total	◇◇IV	□IV	⟋⟍IV	⊣IV	III
Artemisia lindleyana, (continued)						
42-203	20	0.70	0.10	0.20	----	----
65A	16	0.06	0.56	0.31	----	0.06
103A	23	0.35	0.04	0.48	0.04	0.08
103C	16	0.56	0.13	0.19	----	0.13
103H	17	0.59	0.06	0.24	----	0.11
115I	14	0.57	0.43	----	----	----
	307	0.38	0.22	0.32	0.01	0.07
Artemisia prescottiana						
42-559	81	0.22	0.32	0.46	----	----
Artemisia michauxiana						
119A	22	0.18	0.41	0.36	----	0.06
Artemisia douglasiana						
11A	7	0.29	0.43	0.29	----	----
11B*	35	0.26	0.37	0.11	----	0.26
11C	17	0.46	0.24	0.24	----	0.06
11F	22	0.18	0.50	0.32	----	----
11H	6	0.50	0.33	0.17	----	----
11J	25	0.28	0.40	0.32	----	----
82A+	14	0.36	0.22	0.43	----	----
82D	69	0.54	0.17	0.17	----	0.12
	195	0.39	0.30	0.22		0.09
Excluding 11B	160	0.41	0.28	0.25		0.06

* $2n=17_{II}{}^{1}I$
\+ $2n=18_{II}+2_{IIB}$

Table 10. Maximum number of multivalents in the polyploids.

Artemisia ludoviciana ssp. ludoviciana	$^{4}_{IV}{}^{9}_{II}{}^{2}_{I}$
ssp. candicans	$^{4}_{IV}{}^{10}_{II}$
ssp. incompta	$^{2}_{IV}{}^{14}_{II}$
Artemisia lindleyana	$^{4}_{IV}{}^{1}_{III}{}^{8}_{II}{}^{1}_{I}$
Artemisia prescottiana	$^{5}_{IV}{}^{8}_{II}$
Artemisia michauxiana	$^{4}_{IV}{}^{10}_{II}$
Artemisia douglasiana (4x)	$^{6}_{IV}{}^{6}_{II}$
Artemisia douglasiana (6x and 6x+1)	$^{1}_{VII}{}^{1}_{VI}{}^{4}_{IV}{}^{13}_{II}$ or $^{3}_{VI}{}^{3}_{IV}{}^{11}_{II}{}^{2}_{I}$

Table 11. Chiasma frequency at metaphase I in the tetraploids.

	Cells	Chiasmata/ Cell	Chiasmata/ * Chromosome pair	Chiasmata/ # Bivalent
Artemisia ludoviciana				
ssp. ludoviciana				
9B	22	21.59	1.20	1.30
9C	15	24.13	1.37	1.64
14B	19	22.11	1.23	1.26
25	68	20.34	1.13	1.21
46B	11	22.27	1.24	1.28
98C	38	22.92	1.27	1.37
	173	21.71	1.21	1.30
ssp. candicans				
41B	3	25.33	1.41	1.58
79J	18	20.78	1.15	1.23
113A	13	21.69	1.21	1.30
	34	21.53	1.20	1.29
ssp. incompta				
115D	16	20.88	1.16	1.21
Artemisia lindleyana				
1A	19	25.00	1.39	1.59
1E	50	21.48	1.19	1.32
1V	30	22.67	1.26	1.37
33C	37	23.54	1.31	1.52
34E	48	29.38	1.63	1.93
36B	36	22.81	1.27	1.38
36E	9	22.67	1.26	1.30

Table 11. (continued)

	Cells	Chiasmata/ Cell	Chiasmata/* Chromosome pair	Chiasmata/# Bivalent
Artemisia lindleyana, (continued)				
37A	5	23.80	1.32	1.38
42-203	39	21.82	1.21	1.27
65A	25	23.12	1.28	1.38
103A	33	21.91	1.22	1.33
103C	26	22.19	1.23	1.33
103H	16	22.94	1.27	1.42
115I	18	21.89	1.22	1.32
	391	23.39	1.29	1.44
Artemisia prescottiana				
42-559	137	23.60	1.31	1.40
Artemisia michauxiana				
119A	16	22.25	1.24	1.46
Artemisia douglasiana				
11A	17	23.24	1.29	1.40
11C	15	24.13	1.34	1.51
11F	28	21.86	1.21	1.32
11J	31	24.87	1.38	1.53
82A	65	20.09	1.11	1.15
82D	48	24.04	1.34	1.62
	204	22.55	1.25	1.39

* Total chiasmata/gametic chromosome number

\# Total chiasmata in bivalents/total bivalents

than twice that of the diploid populations. The ratio of rod to ring bivalents was 4.06:1.

Of the three tetraploids that did not exhibit multivalent formation at metaphase I (Table 8), two had too small a sample size to be significant. The third, a tetraploid A. douglasiana, plant number 111 from the Satus Creek population, was highly unusual, producing 0.43 bivalents and 35.12 univalents per cell. Observations of pairing behavior at zygotene and pachytene (Figure 5a) demonstrated the desynaptic nature of this plant. Total desynapsis had occurred in most of the cells by diakinesis with the "pairs" in close proximity or lying parallel to each other (Figure 5b). A true metaphase plate did not form unless a few bivalents remained (Figure 5c). Disjunction, therefore, was entirely random, and the ratios of chromosomes distributed to the anaphase I poles ranges from 18:18 to 4:32 (Table 12). Occasional cells at anaphase I had laggard chromosomes that did not migrate to either pole; therefore, the ratios did not always equal 36 total chromosomes. The cells in which all the chromosomes were included in the resulting nuclei are within squares in Table 12.

The tetraploids appeared to be only slightly less fertile than the diploids according to the pollen fertility measurements, Table 6. The desynaptic individual described above, however, was highly infertile. As indicated by Table 7, unreduced microspores were produced at relatively high rates by plants of the A. lindleyana group

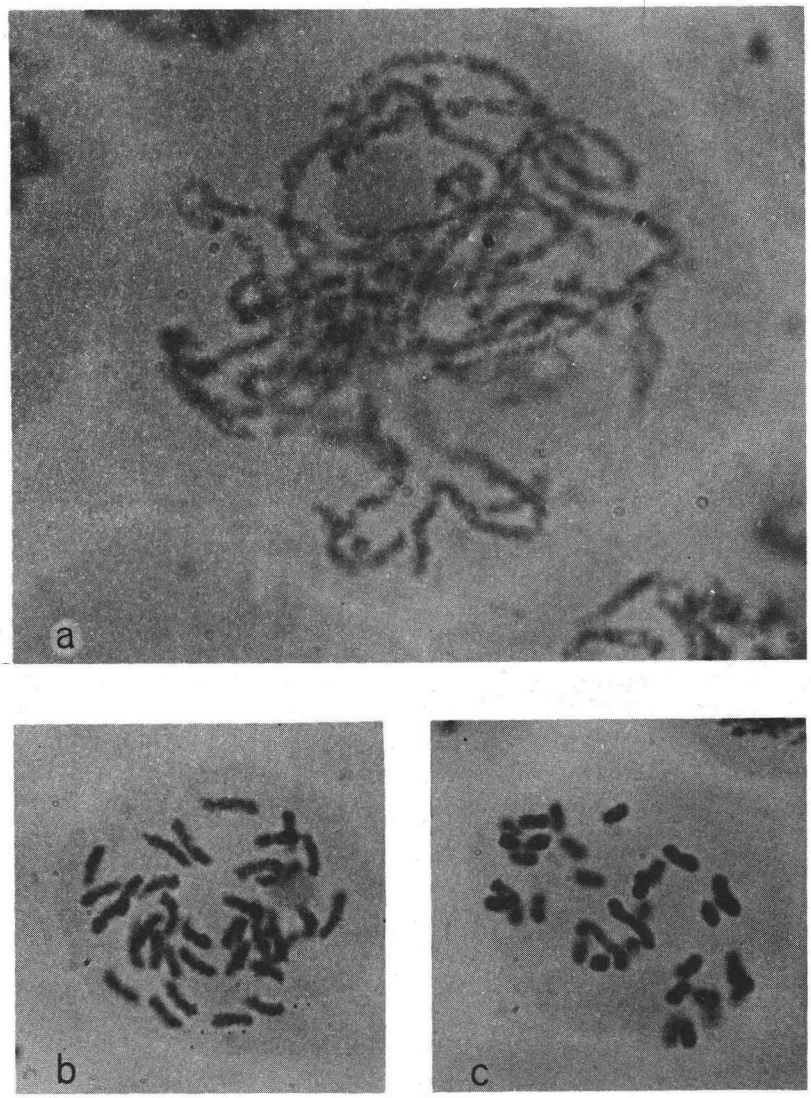

Figure 5. First meiotic division of the desynaptic, tetraploid Artemisia douglasiana. (a) Pachytene, synapsed chromosome arms. (b) Diakinesis, 36_I with the homologs lying parallel. (c) Metaphase I, $2_{II}\ 32_I$ with random and irregular chromosome migration.

Table 12. Disjunctional ratios at anaphase I in a desynaptic tetraploid of Artemisia douglasiana.

Number of Chromosomes at the Minor Pole	Number of Chromosomes at the Major Pole																			
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
4																	1					[1]
5																						
6																				[1]		
7																			[1]			
8										1								[1]				
9	1													1			[2]					
10															1	[1]						
11			1												[1]							
12					1		1						1	[1]								
13						2	1						[3]									
14												[5]										
15					1				2		[4]											
16										[8]												
17									[2]													
18								[2]														

and by the desynaptic plant of A. douglasiana from Satus Creek, as well as by the diploids. All of the polyploid individuals examined exhibited micronuclei during the sporad stage; of 4863 total sporads examined in the tetraploids (excluding plant 11I), 570 (11.7 percent) contained one or more micronuclei per sporad.

Meiotic Analysis of the Hexaploids

Two of the three Artemisia douglasiana hexaploids selected as parental stock for the hybridization experiments and later as subjects for meiotic analysis were trisomic hexaploids. All possible types of metaphase I configurations were found in the three individuals, with quadrivalents and bivalents most frequent (Table 13). However, the maximum number of multivalent associations found per cell was only six (Table 10); one ring bivalent was counted for every 1.96 rod bivalents. The frequency of configuration types is shown in Table 14, and a photomicrograph of a metaphase I configuration with an explanatory camera lucida diagram is shown in Figure 6. Fertility in the hexaploids, including A. douglasiana and A. vulgaris, was rather high considering the degree of meiotic disturbances (Tables 6 and 7).

Hybrids

Akenes were produced in 64 of the artificial hybridization experiments. One half of the akenes from each cross, up to a maximum

Table 13. Frequencies of metaphase I configurations in hexaploid Artemisia douglasiana.

Accession	Cells	VII/ Cell	VI/ Cell	V/ Cell	VI/ Cell	III/ Cell	II/ Cell	I/ Cell
48*	29	0.03	0.48	0.17	2.07	0.52	20.34	0.59
80*	21	0.05	0.24	0.05	0.90	0.62	23.33	0.86
124	11	----	0.09	0.09	1.18	0.27	23.27	0.91
	61	0.04	0.33	0.11	1.50	0.51	21.90	0.74

Table 14. Frequencies of metaphase I multivalent configurations in hexaploid Artemisia douglasiana.

Accession	Total	VII	⋀⋀VI	◇◇◇VI	⬚VI	V	◇◇IV	⋀IV	⬚IV	III
48*	92	0.01	----	0.04	0.10	0.05	0.14	0.26	0.22	0.17
80*	31	0.03	0.06	0.06	0.03	0.03	0.10	0.23	0.23	0.23
124	17	----	0.06	----	----	0.06	0.18	0.47	0.06	0.18
	140	0.01	0.02	0.04	0.07	0.05	0.14	0.28	0.20	0.18

* trisomic hexaploid

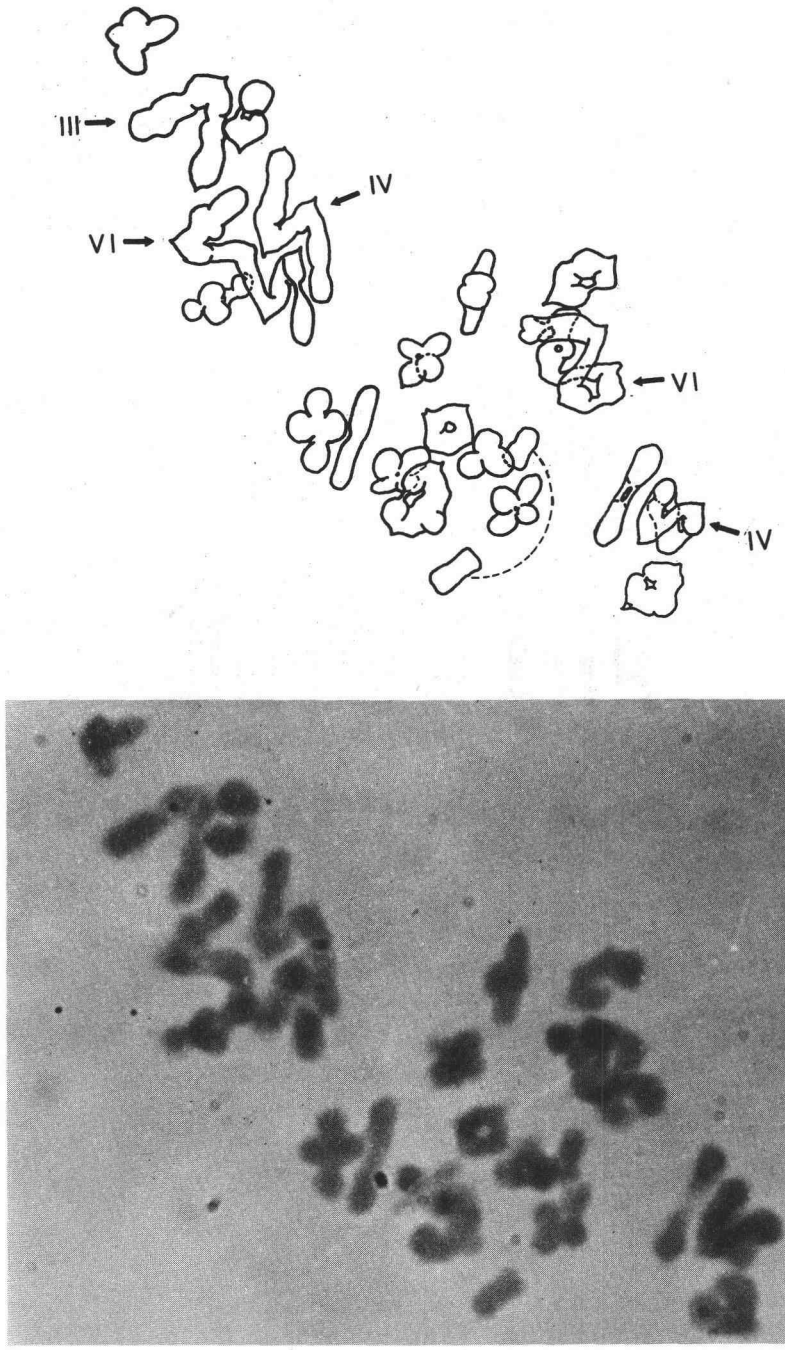

Figure 6. Metaphase I configuration in the trisomic hexaploid Artemisia douglasiana. $2_{VI}\ 2_{IV}\ 1_{III}\ 16_{II}$.

of 25, were randomly selected and planted during the summer of 1966. Germination occurred within a period of four to fourteen days, and the seedlings became established quickly. In only 34 of the total number of crosses were seedlings produced that were morphologically intermediate between the parent plants, and only these were considered to be hybrids or probable hybrids. These 34 hybrid experiments are listed in Table 15. Illustrations of the leaf shapes of selected hybrids and their parents are presented in Appendix II; the selected crosses are designated by a "+" in Table 15. The number planted data shown in Table 15 was computed as equal to the total number of akenes planted minus the number of selfed progeny that germinated. Likewise, the number of germinations, surviving seedlings, and seedlings flowering the first season include only hybrid individuals. In cases where the parents were morphologically similar, the progeny were difficult to screen for hybridity, but with two notable exceptions they were considered to be hybrids. The exceptions were progeny of attempted intraspecific crosses in A. cavaticaulis and in A. douglasiana. These two taxa were highly self-fertile; for example, 32 of 33 seedlings produced in intertaxon crosses with A. douglasiana as the pistillate parent were classed as non-hybrid, and 59 seedlings out of 73 total in A. cavaticaulis were also the product of self-fertilization. Self-fertilization may also be prevalent in A. suksdorfii; however, the number of akenes

Table 15. Artificial hybrids.

	Akenes Planted	Embryos Germinated	Seedlings Survived	Seedlings Flowered First Season
Artemisia cavaticaulis X Artemisia suksdorfii				
+ 8B X 27	6	6	6	0
X Artemisia douglasiana (4x)				
*8B X 82C	17	11	2	0
Artemisia suksdorfii X Artemisia suksdorfii				
4B X 27	25	6	4	0
X Artemisia cavaticaulis				
*27 X 8B	25	22	22	0
X Artemisia ludoviciana ssp. candicans				
*27 X 113X	25	8	8	0
Artemisia ludoviciana X Artemisia lindleyana ssp. ludoviciana				
17 X 36E	5	5	5	3

Table 15. (continued)

	Akenes Planted	Embryos Germinated	Seedlings Survived	Seedlings Flowered First Season
Artemisia ludoviciana X *Artemisia cavaticaulis* ssp. *candicans* (4x)				
+ 113A X 8A	8	8	8	3
X *Artemisia ludoviciana* ssp. *incompta*				
41B X 115C	2	2	2	1
Artemisia ludoviciana ssp. *incompta* X *Artemisia ludoviciana* ssp. *incompta*				
115J X 79B	14	6	6	6
115C X 115H	21	15	11	10
115D X 115F	25	3	3	3
X *Artemisia lindleyana*				
+ 108F X 103H	26	19	19	19
115J X 1D	14	9	5	5

Table 15. (continued)

	Akenes Planted	Embryos Germinated	Seedlings Survived	Seedlings Flowered First Season
Artemisia lindleyana X *Artemisia lindleyana*				
1V X 1E	5	3	3	3
33A X 1X	4	4	4	4
33A X 1D	2	2	1	1
X *Artemisia cavaticaulis*				
103A X 8B	1	1	1	1
+ 103C X 8A	12	2	1	1
+ 103G X 8B	26	8	8	3
X *Artemisia ludoviciana* ssp. *ludoviciana*				
34A X 29	3	3	2 (1 died after flowering)	3
X *Artemisia ludoviciana* ssp. *candicans* (4x)				
+ 1V X 44F	4	3	3	3
X *Artemisia douglasiana* (4x)				
34A X 82C	25	24	24	24
* 36C X 11I	1	1	1	1

Table 15. (continued)

	Akenes Planted	Embryos Germinated	Seedlings Survived	Seedlings Flowered First Season
Artemisia michauxiana (4x) X Artemisia ludoviciana ssp. incompta				
+ 119A X 115J	21	17	17	13
X Artemisia lindleyana				
+*119A X 42-203	24	10	9	1
Artemisia douglasiana (4x) X Artemisia douglasiana (4x)				
11A X 82A	6	6	6	4
X Artemisia cavaticaulis				
* 82C X 8B	6	3	3	0
X Artemisia suksdorfii				
* 82A X 4B	11	4	4	0
X Artemisia ludoviciana ssp. candicans (4x)				
+ 82D X 79J	21	11	10	9
Artemisia douglasiana (4x) X Artemisia lindleyana				
11A X 103H	1	0	-	-
+ 11C X 36A	3	2	2	0

Table 15. (continued)

	Planted	Germinated	Seedlings Survived	Seedlings Flowered First Season
Artemisia prescottiana X Artemisia cavaticaulis				
+ 42-559 X 8B	25	20 (slug damage)	11	11
Artemisia ludoviciana X Artemisia suksdorfii ssp. candicans (6x)				
28D X 19	1	0	-	-
Artemisia douglasiana (6x) X Artemisia ludoviciana ssp. incompta				
+ 80 X 108C	6	2	1	0

* hybrid nature not clearly established by morphological analysis

+ leaf shape illustrated in Appendix II

produced in crosses of this taxon was not sufficient to provide adequate data. In the above three instances the selfed offspring were uniform in morphology and closely resembled the parental plant, suggesting homozygosis. Selfed progeny were also identified in three of the hybrid crosses--8B X 27, 8B X 82C, and 82A X 4B.

The progeny generally were extremely vigorous; however, two anomalous growth forms were noted in the hybrids. (1) The "dwarf" characteristic (Figure 7a) was manifested in the young seedlings by extremely short internodes, weak stems, malformed, small, and sometimes chlorotic leaves, and a depauperate root system. Less severe forms, termed "semi-dwarfs" (Figure 7b) first showed the above characteristics, but then developed strong lateral shoots or rhizomes and quickly overcame the physical abnormalities. Two of the "dwarf" individuals elongated slightly after eight months, and the upper leaves were normal in appearance though still dwarfed; none flowered within the first year, however. Table 16 lists the "dwarf" genotypes by hybrid number; however, all are believed to be products of self-fertilization. In all instances the putative parent was a diploid, and only two genotypes were involved--Clines Falls and Gold Beach. (2) The "hooded" phenotype (Figure 8a and b) is characterized by a malformation of the involucral phyllaries. The phyllaries are leaf-like and curl loosely around the semi-exposed receptacle and florets. This character was present at a low frequency and only in crosses

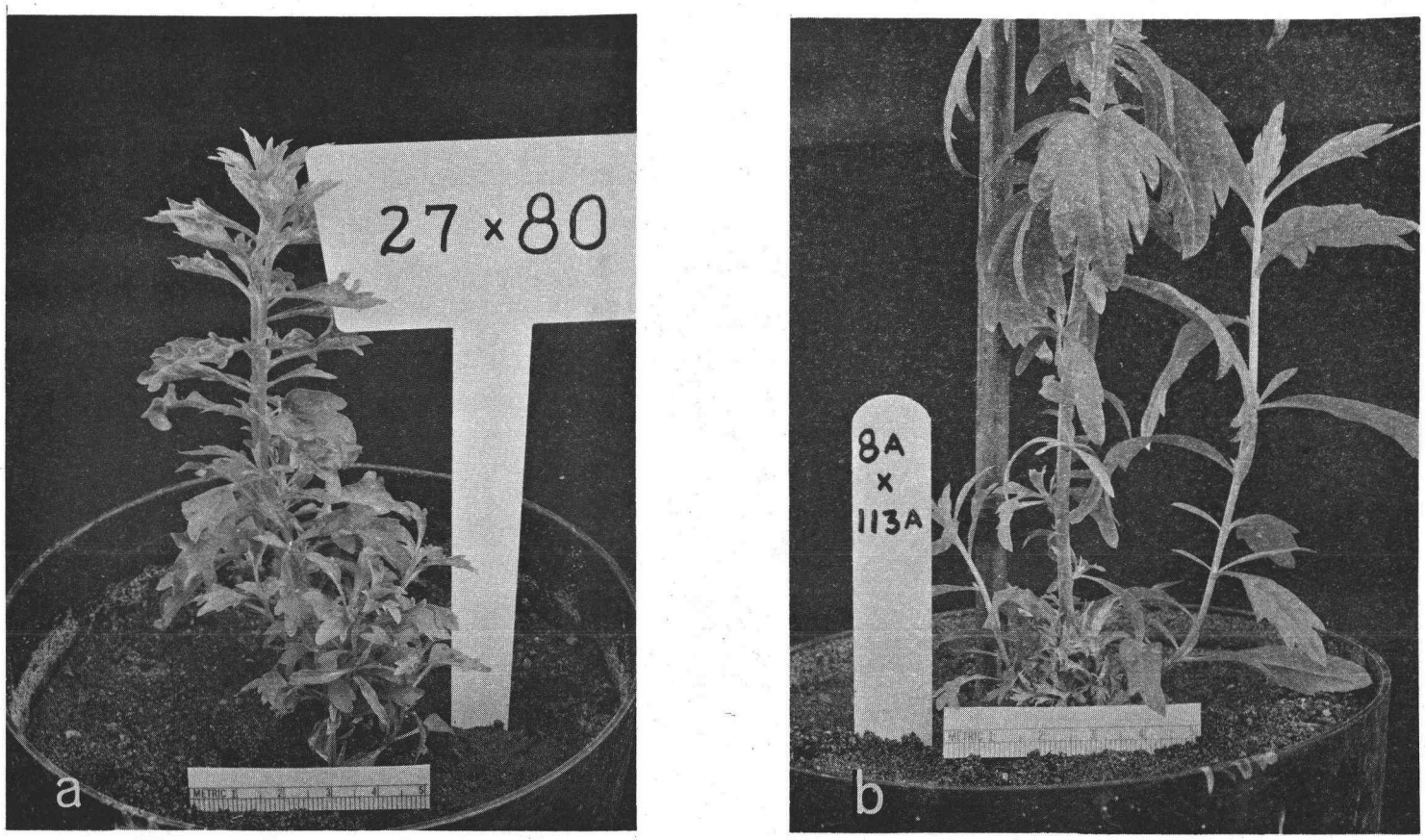

Figure 7. Abnormal hybrid seedlings. (a) "Dwarf" form. (b) "Semi-dwarf" form.

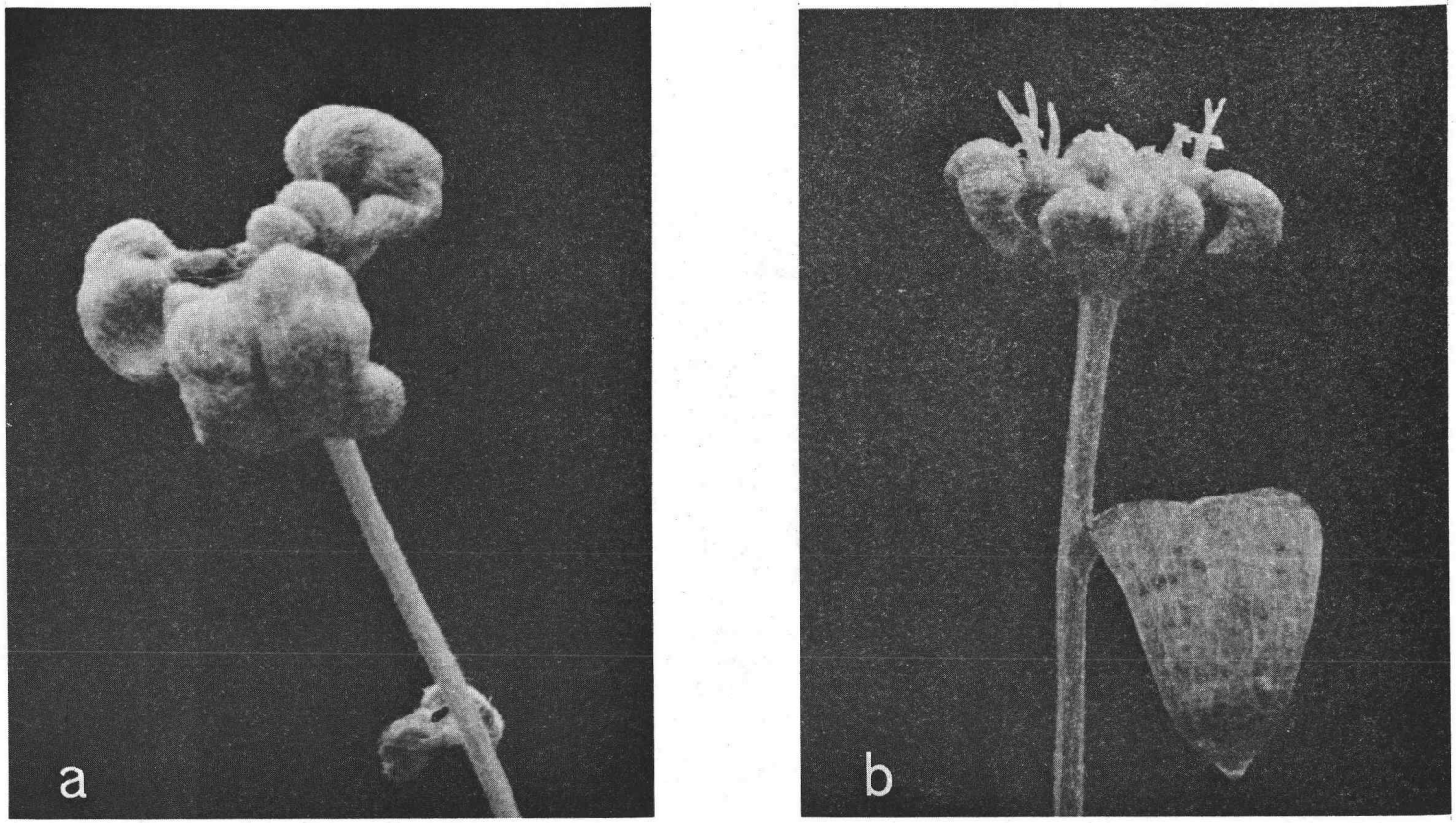

Figure 8. Hooded condition in the S_1 generation of Artemisia douglasiana (4x and 6x). (a) Extreme condition with florets undeveloped. (b) Less extreme condition. 6x

involving A. douglasiana (4x and 6x). Florets did not develop on two of the plants.

Table 16. Numbers of dwarf and semi-dwarf progeny.

	Total progeny	Dwarf	Semi-dwarf
8A X 28D	20	4 (2)*	2
8A X 82A	27	2 (2)*	4
8A X 8B	16	2	2
8A⊗	8	1	2
8A X 45A	12		4
8A X 113A	18	7	2
	101	16 (4)*	16
27 X 80	7	4 (1)*	-
27 X 103B	7	2	-
	14	6 (1)*	

* Died in early seedling stage.

In addition to plants showing the above two traits, four other selfed progeny of A. douglasiana produced small floral buds, but development was inhibited and they did not mature.

The morphology of one hybrid combination is worthy of special note; the others will be discussed where pertinent in the following sections. This cross involved A. ludoviciana ssp. candicans X A. cavaticaulis, 113A X 8A. The progeny were triploid and intermediate in morphology. They bore a strong resemblance to A. douglasiana

and in all instances could only be classed as members of this taxon, though some had a slightly different pattern of leaf lobing (Appendix II, p. 147 and 148).

Cytology of the Hybrids

Chromosomal associations and pollen fertility were determined for the hybrids that had flowered up to the time of this report. This information is recorded in Table 17.

Triploid offspring were observed in two heteroploid crosses, A. ludoviciana ssp. candicans (113A) X A. cavaticaulis (8A), and A. lindleyana (103C) X A. cavaticaulis (8A). The leaf morphology of the parents and progeny of both crosses are illustrated in Appendix II, p. 147 and 148 for the former cross, and p. 143 for the latter. In the cross 113A X 8A, eight hybrids were grown, but only two have flowered to date. These have been counted; #4 was 2n=26 and #8 was 2n=25. The maximum possible meiotic configuration, $8_{III} + 1_{II}$, was found in one of thirteen cells in plant #4. In the cross 103C X 8A, four of eight hybrids have been examined cytologically. Three of the progeny examined were strict triploids, the other was 2n=26. The other four individuals were screened for triploidy by observing the amount of pollen produced, and the test was positive for all four. In plant #1 (2n=26), three cells out of thirty displayed six trivalents. In the remaining three plants, taken together, two out of thirty-one

Table 17. Cytology and pollen fertility of selected F_1 hybrids.

Accession	2n	Cells	IV/Cell	III/Cell	II/Cell	I/Cell	Maximum configuration	Pollen set	Total grains
Artemisia ludoviciana ssp. *candicans* (4x) X *Artemisia cavaticaulis*									
113A X 8A									
-4	26	13	----	3.47	5.47	4.68	$8_{III}{}^{1}{}_{II}$	0.00+	1000+
-8	25	--	----	----	-----	----	----	----	----
Artemisia ludoviciana ssp. *incompta* X *Artemisia ludoviciana* ssp. *incompta*									
115J X 79B									
-1	36	27	0.56	----	16.88	----	$3_{IV}{}^{12}{}_{II}$	93.0	100
-14	36	15	1.27	----	15.44	----	$2_{IV}{}^{14}{}_{II}$	98.0	100
Artemisia ludoviciana ssp. *incompta* X *Artemisia lindleyana*									
108F X 103H									
-11	36	2	1.33	----	15.34	----	$2_{IV}{}^{14}{}_{II}$	95.0	100
-18	36	28	1.11	0.04	16.20	0.04	$2_{IV}{}^{1}{}_{III}{}^{12}{}_{II}{}^{1}{}_{I}$	----	----

Table 17. (continued)

Accession	2n	Cells	IV/Cell	III/Cell	II/Cell	I/Cell	Maximum configuration	Pollen set	Total grains
				Artemisia lindleyana X Artemisia lindleyana					
1V X 1E									
-1	36	51	0.96	----	16.08	----	$4_{IV}10_{II}$	100.0	100
33A X 1D		(V/Cell)							
-3	37	15(0.07)	1.13	0.07	15.53	0.87	$3_{IV}12_{II}1_{I}$	----	---
33A X 1X									
-1	36	47	1.09	0.38	14.56	0.38	$5_{IV}8_{II}$	----	---
				Artemisia lindleyana X Artemisia cavaticaulis					
103C X 8A									
-1	26	30	----	3.13	5.83	4.93	$6_{III}3_{II}2_{I}$	0.04	310
-3	27	3	----	6.67	2.00	3.00	$8_{III}1_{II}1_{I}$	0.05	375
-4	27	18	----	3.50	5.72	5.06	$8_{III}1_{II}1_{I}$	0.05	423
-6	27	11	----	3.55	5.45	5.45	$6_{III}3_{II}3_{I}$	----	---
103G X 8B									
-20	36	27	1.37	----	15.26	----	$4_{IV}10_{II}$	----	----

Table 17. (continued)

Accession	2n	Cells	IV/Cell	III/Cell	II/Cell	I/Cell	Maximum configuration	Pollen set	Total grains
	Artemisia lindleyana X Artemisia ludoviciana ssp. candicans (4x)								
1V X 44F									
-1	--	--	----	----	-----	----	----	75.0	113
-3	--	--	----	----	-----	----	----	88.6	123
-4	--	--	----	----	-----	----	----	94.1	134
36C X 11I									
-1	72	--	----	----	-----	----	----	----	---
	Artemisia prescottiana X Artemisia cavaticaulis								
42-559 X 8B									
-1	36	55	0.40	----	17.13	0.14	$3_{IV}12_{II}$	96.0	100
-2	36	55	0.13	----	17.62	0.25	$1_{IV}1_{III}14_{II}1_{I}$	----	---
-4	36	56	0.46	----	16.98	0.21	$2_{IV}14_{II}$	97.0	100
-7	36	26	0.95	----	16.60	----	$5_{IV}8_{II}$	95.0	100
-21	37	85	0.69	0.11	16.47	0.96	$3_{IV}12_{II}1_{I}$	----	---

Table 17. (continued)

Accession	2n	Cells	IV/Cell	III/Cell	II/Cell	I/Cell	Maximum configuration	Pollen set	Total grains
Artemisia michauxiana (4x) X *Artemisia lindleyana*									
119A X 42-203									
-6	36	40	0.70	----	16.58	0.05	$2_{IV}14_{II}$	94.0	100
Artemisia michauxiana (4x) X *Artemisia ludoviciana* ssp. *incompta*									
119A X 115J									
-3	36	33	0.76	----	16.48	----	$3_{IV}12_{II}$	77.0	100
-19	36	30	0.86	----	16.28	----	$3_{IV}12_{II}$	96.0	100
Artemisia douglasiana (4x) X *Artemisia ludoviciana* ssp. *candicans* (4x)									
82D X 79J									
-5	35	41	1.00	0.10	15.35	----	$5_{IV}1_{III}6_{II}$	----	---
-6	36	29	1.90	----	14.20	----	$5_{IV}8_{II}$	----	---
-12	--	--	----	----	-----	----	----	94.0	100
-19	36	38	0.61	0.03	16.76	0.03	$3_{IV}12_{II}$	98.0	100
Artemisia douglasiana (4x) X *Artemisia lindleyana*									
11C X 36A									
-3	--	--	----	----	-----	----	----	99.0	100
Artemisia douglasiana (4x) (open pollinated)									
11I									
-1	40	--	----	----	-----	----	----	----	----
-11	39	17	0.41	0.35	17.63	1.06	$2_{IV}15_{II}1_{I}$	----	----

cells displayed trivalents. It is notable that all of the triploids in both crosses produced some viable pollen.

Progeny of the cross A. prescottiana (42-559) X A. cavaticaulis (8B) were intermediate between both parents and were expected to be triploid as well (Appendix II, p. 145); however, chromosome counts of five plants indicated that they were 2n=36 or 2n=37. Their siblings all produced copious amounts of pollen and were considered to be tetraploid as well.

Another example of a diploid-tetraploid cross that produced 4x progeny was Artemisia lindleyana (103G) X Artemisia cavaticaulis (8B) (Appendix II, p. 144). In this instance, the morphology was not as unequivocal as in the first example, and all of the offspring were not intermediate. A sister cross 103A X 8B also produced one hybrid; however, it has not been examined cytologically.

Progeny observed from crosses between normally synaptic tetraploids produced some multivalents in all instances, and with three exceptions, pollen fertilities were greater than 90 percent.

The cross 36C X 11I produced only one akene; the seedling showed poor root development, and the plant developed slowly. Morphologically, the plant resembled the pistillate parent; however, there were tendencies toward the pollen plant in the leaves, which were entire and wider. The chromosome number was n=36, octoploid! The staminate parent was the desynaptic A. douglasiana (4x)

which produced a high proportion of non-reduced pollen (ca. 85 percent). Although the production of non-reduced pollen in plant 36C was not measured, in general the A. lindleyana group demonstrated the ability to produce 2n pollen as well. Therefore, the derivation of this octoploid individual is in doubt, and an origin from self-fertilization cannot be excluded.

Thirty-two seedlings germinated from 108 akenes sown from the unbagged inflorescence of plant 11I in the garden; however, the rate may be higher, because germination is continuing at the time this is written, 22 weeks after sowing. The offspring were highly variable (their leaves are illustrated in Appendix II, p. 151 and 152), and all that survived a period of poor seed-bed maintenance were vigorous. The only plants counted to date had chromosome numbers of 2n=39 and 2n=40. Akenes were also collected from the two A. douglasiana (4x) individuals (11C and 11J) situated adjacent to 11I in the experimental garden. No peculiarities were noted in the progeny.

Other akenes produced from open pollination in the garden were collected from adjacent A. suksdorfii (19) and A. cavaticaulis (8A) plants. All of the progeny from 8A appeared to be the product of selfing or intrataxon crosses. Four of eighteen seedlings from plant 19, however, were intermediate between A. suksdorfii and A. cavaticaulis and appeared to be hybrid genotypes (Appendix II, p. 140).

V. DISCUSSION

Autopolyploidy within the Complex

Indicators of Autopolyploidy

A significant conclusion from this study is that autopolyploidy has been a prime evolutionary force within the Artemisia ludoviciana-complex of the Northwest. The results of cytological observations include the following five indicators of autoploidy:

1. The formation of multivalents, according to the sampled individuals, is a common feature of the polyploid races. This observation, first made during the chromosomal survey of natural populations and later confirmed in meiotic analyses of selected organisms, is indicative of a high degree of chromosomal homology between the parental genomes. Complete homology, if it could be shown, would be definitive of strict autopolyploidy. The full extent of this cytological trait in the taxa studied is unknown, although indications are that it may be more widespread in the Vulgares-aggregation. For instance, Koul (1964b) reported meiotic disturbances in a tetraploid A. vulgaris in India; he concluded that autoploidy was the likely source of the meiotic instability. Also Figure 7 of Keck's 1946 taxonomic revision (p. 425) shows a ring of four chromosomes at metaphase I in a plant of A. ludoviciana ssp. ludoviciana

from Manitou, Colorado.

2. The majority of the tetraploid cells examined in this study were either "normal" with 18_{II} or they contained one multivalent. However, as Table 10 demonstrates, the maximum number of multivalents observed in cells of each tetraploid taxon is from four to six with the sole exception of A. ludoviciana ssp. incompta in which relatively few cells had been counted. Therefore, 44-67 percent of the total complement of chromosomes may be involved in quadrivalent formation. As Morrison and Rajhathey (1960) indicated, the maximum possible number of multivalents is very seldom attained even in artificially induced autotetraploids. In the genus Artemisia, Arano, Koriba, and Mitsuoka's (1955) observations on the cytology of artificially induced autotetraploids of A. kurramensis demonstrated that 54 percent of the PMC's formed only one to five quadrivalents, and that only one quadrivalent was the most frequently observed multivalent configuration. Trivalents were present in 35 percent of the cells and univalents were common as well. Meiotic associations did not approach the maximum multivalent configurations even though the chromosomes were homologous.

3. Evidence of intergenomic homology was found among all three of the genomes contributing to the A. douglasiana genotype. Of the examined individuals all produced PMC's with configurations that included the highest number of chromosomes possible (i.e.,

septivalents in the trisomic hexaploids and sexivalents in the hexaploids). About 41 percent of the meiotic divisions of A. douglasiana from the Siskiyou Mountains (population number 48) contained associations of five or more chromosomes, and one of the cells included three sexivalents and three quadrivalents. The other two examined individuals had 33 percent and 18 percent of the cells with quinquivalent associations or larger.

4. The results of the cytological observations of the artificial hybrids demonstrate that the same degree of homology is present between the genomes of different taxa as within one taxon. That is, the diploid progenitors of the northwestern polyploid races were members of the same genetic unit, an ecospecies.

The cytology of the triploid progeny quite clearly illustrates that the A. cavaticaulis genome is homologous to both genomes of A. lindleyana (103) and A. ludoviciana ssp. candicans (113), and that both of the genomes included in each of these taxa are also homologous. Trivalents were not only as prevalent as bivalents (Table 17), but maximum configurations were found as well.

Hybrids were also obtained from three other interracial crosses. Floral buds of one of these, A. douglasiana (80) X A. ludoviciana ssp. incompta (108), putatively a pentaploid, were just beginning to develop at the time of writing. In the two remaining heteroploid hybridizations A. cavaticaulis served as the staminate parent. The

pistillate parents were A. prescottiana (42-559) and A. lindleyana (103). The latter hybridization was accomplished with both plant 103A and plant 103G. Chromosome counts of the progeny of two of these three crosses made to date have revealed only tetraploid progeny; the progeny of the cross 103A X 8B had not been counted at the time of writing. All of the offspring of the 42-559 X 8B cross were morphologically intermediate between both parental types and were considered to be F_1 hybrids. Eight of thirteen seedlings of 103A X 8B, and one of eight of 103G X 8B progeny, were scored as hybrids by the same criterion. The origin of these "exceptional offspring" will be discussed below; the point under consideration here is that the individuals that have been examined cytologically behave like the natural tetraploids.

Multivalents have been found in every hybrid examined in crosses within each polyploid level; and intertaxon homologies are of the same order as those of the natural tetraploids, as well. A summary of the determined genomic homologies is presented in Figure 9.

Clausen, Keck, and Hiesey (1940) obtained two pentaploid A. ludoviciana ssp. ludoviciana X A. douglasiana hybrids. These authors maintained that their data demonstrated that both A. ludoviciana ssp. ludoviciana genomes are homologous to two of the three A. douglasiana genomes. However, the remaining genome of the

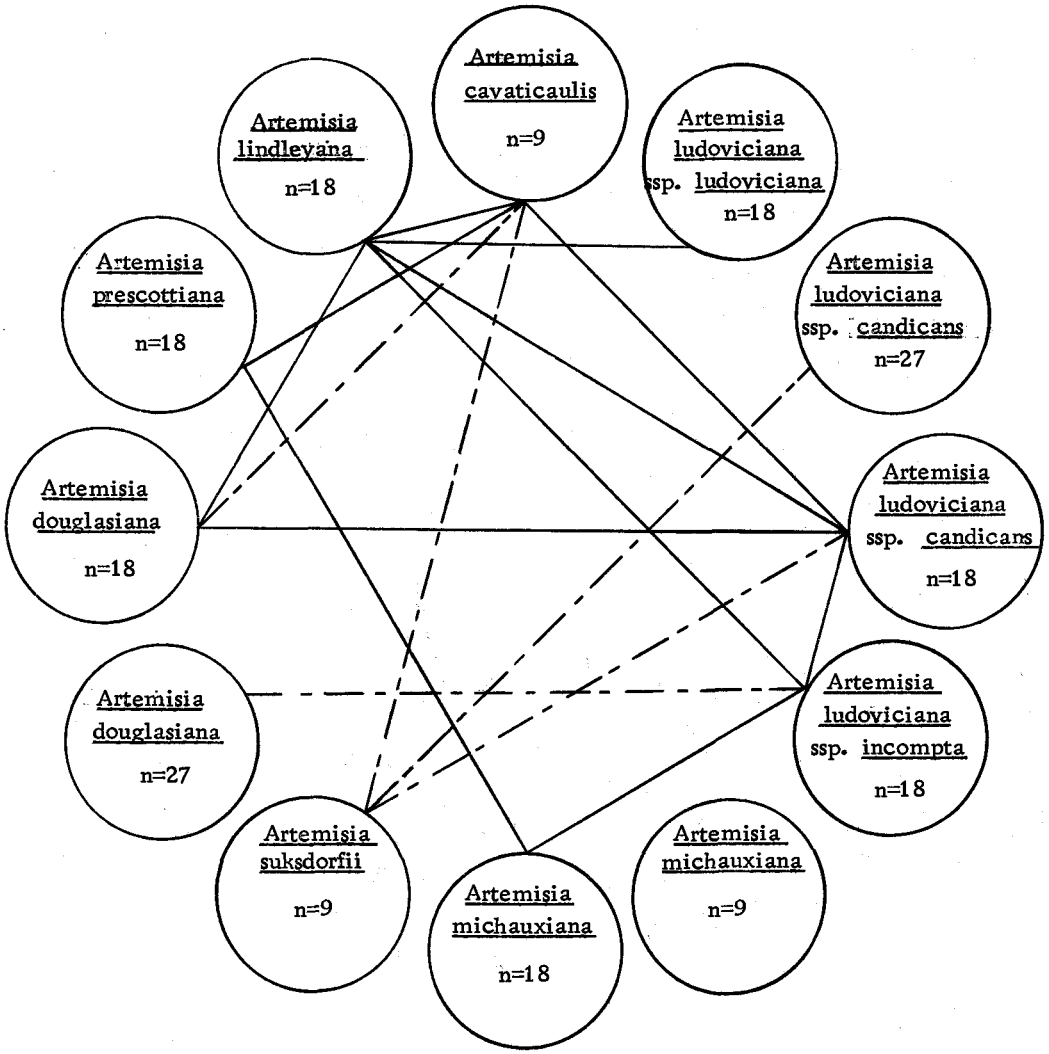

Figure 9. Genomic relationships in the Artemisia ludoviciana-complex.

hexaploid was seldom found as 9_I. In light of the pairing relationships now discovered in natural A. douglasiana populations, it seems likely that the tendency for pairing of these chromosomes is due to their homology with the paired genomes, rather than to autosyndesis as suggested by the above authors.

5. Heteroploidy was found to exist in at least five of the morphological entities within the complex (see also Clausen, Keck, and Hiesey, 1940, and Taylor and Brockman, 1966). The five heteroploid taxa are: (1) Artemisia michauxiana, a taxon which may prove to be an example of a diploid-tetraploid pair relationship, that is, with the polyploid having arisen directly from the diploid race. However, the exact ecologic and geographic distributions are not yet known. (2) A. lindleyana, in which the diploid falls within the morphological range of the tetraploids, but the variation is so complicated at the higher level that this diploid cannot be considered the sole progenitor. (3) A. douglasiana (4x and 6x), in which the hexaploid race appears to be widespread throughout the Puget-Willamette Trough, the Central Valley of California, and the limiting foothills. The tetraploids have been found only in the Yakima Valley of central Washington. The tetraploid race cannot be distinguished from the northern hexaploids on the basis of external morphology; however, Keck (1946) reports a more deeply lobed form from the San Francisco Bay region. (4) A. ludoviciana ssp. candicans, in which the

hexaploid is known from only one location, Tombstone Prairie, a moist montane meadow on the western slope of the central Oregon Cascades. Although this is outside the previously described range of A. ludoviciana ssp. candicans and approaches the range of A. douglasiana, the presence of a large involucre, and leaves that are divided, pubescent on both surfaces, and less than 1.5 cm. wide, led to the plants being classified as A. ludoviciana ssp. candicans. (5) A. ludoviciana ssp. incompta, for which Clausen, Keck, and Hiesey (1940) suggest that the hexaploid is an isolated individual in an otherwise tetraploid population (as opposed to the instance of A. ludoviciana ssp. candicans, where apparently the entire population was hexaploid). However, these authors reported chromosome counts from only two plants in the population, one tetraploid and one hexaploid. Clausen, Keck, and Hiesey also proposed that the hexaploid was formed by the union of a normal haploid gamete and an unreduced gamete. The latter putatively arose in response to the low montane temperatures.

Examples of heteroploid or multilevel taxa are common throughout the entire genus Artemisia. Keck (1946) suggested that A. carruthii of the Artemisia ludoviciana-complex might include chromosomal races. Three levels have been reported for the closely related A. vulgaris, and Koul's drawing (1964a) indicates that morphologically the three levels are one taxonomic unit. Ward (1953) used the

presence of heteroploidy to imply autoploid relationships among the western sagebrush species, though Ehrendorfer (1964) disagreed with his conclusion. Also the tetraploid A. tilesii ssp. unalaskensis, reported here (Appendix I), is indicative that the Artemisia tilesii-complex may have followed evolutionary pathways similar to the Artemisia ludoviciana-complex.

The portion of the Artemisia ludoviciana-complex included in this study displays attributes that might have played a significant role in the development of autopolyploidy. Four such characteristics are: (1) the capacity to produce unreduced pollen in the diploid races; (2) hybridization as a means of introducing variation in the more slowly evolving polyploids; (3) the presence of a wide series of ecotypes, to favor increased adaptability of the polyploids; and (4) genetic variation in meiotic mechanisms that could possibly allow for the improvement of autoploid fertility, if necessary.

The Formation of Unreduced Pollen

The production of unreduced microspores, and hence gametes, at a low but constant rate provides a means of formation of polyploid individuals within a population. The frequency of such individuals is dependent upon the frequency of unreduced gametes, the degree of pollen tube competition, and the viability of the polyploid embryos.

The production of unreduced pollen in the northwestern members

of the complex was inferred from the presence of dyads and triads of microspores; this observation was confirmed by the production of "exceptional progeny" from heteroploid crosses, described above. Megasporogenesis was not examined; however, the likelihood of unreduced egg production is favored by the octoploid F_1 hybrid derived from the 36C X 11I mating.

Although direct evidence for the origin of the 2n pollen was not obtained, the most probable cause is meiotic spindle malfunction and failure of anaphase migration. The observations implying this are: (1) the discovery of at least one restitution nucleus in a plant of A. suksdorfii (Yeon); (2) the occurrence of occasional monads, which probably contain restitution nuclei; (3) the failure to observe any metaphase I or diakinesis cells with twice the "diploid" chromosome number, i.e., cell fusion or somatic doubling did not precede meiosis; (4) the occurrence of triads, which could result only from a defect at meiosis II; and (5) the fact that post-meiotic doubling cannot account for the production of dyads and triads. That failure of spindle formation, or spindle malfunction, does occur within the Vulgares-aggregation was shown by Koul (1964b). For a comparative example from another plant group, non-reduction in Solanum results from spindle fusion in the second division (Marks, 1966).

The Introduction of Variation

The production of and subsequent hybridization among numerous polyploids, from an initially diploid population, may provide for considerable variability within a newly formed polyploid population. In addition, as Marks (1966) pointed out, interlevel introgression may occur from the diploids to the tetraploids, if the capacity to produce unreduced gametes is present in the diploid race. Two instances of artificial introgression on this pattern are found in the hybridization experiments reported above--one in A. prescottiana and one in A. lindleyana X A. cavaticaulis. The production of "exceptional 4x progeny" has been discovered only with a diploid serving as pollen parent. However, crosses with the diploid as the seed parent most often resulted in self-fertilization, and none of the offspring have flowered to date. Both the selfs and hybrids of these crosses must be screened for triploidy and tetraploidy.

The introduction of other diploid taxa may initiate allopolyploid series from previously autoploid complexes (Marks, 1966), or if the diploids are members of the same ecospecies, then it may simply introduce variation into the autoploid race.

Although the production of tetraploids from heteroploid crosses has been previously reported, according to Carroll and Borrill (1965) the cytological cause in other species has not been ascribed either to

digamy or non-reduction. Mark's (1966) observations, however, definitely suggest non-reduction as the origin of autotetraploids in Solanum. The occurrence in nature of introgression between chromosomal races resulting from unreduced gametes has been suspected by Stebbins (1956) in Dactylis and Ward (1963) in Artemisia section Seriphidium.

Another possible source of variation is hybridization between triploids and their progenitors, as has been recorded in nature by Zohary and Nur (1959) in Dactylis. The Artemisia triploids obtained during the hybridization experiments all produce some good pollen, and five akenes were developed from uncontrolled pollinations on ten plants.

Ecotypic Variation

As Stebbins (1956) and Jones (1961) have pointed out, the ecological tolerance of polyploids formed from differing physiological races may be greater than the sum of the tolerances of the two contributing parents. That is, sibling races may be able to compete with the parental races in the original niche, to occupy sites that are intermediate between the two parents, or to inhabit completely different sites. This adaptability may be due to the inclusion of genes from separate adaptive systems, and interaction of the various enzyme and hormonal systems resulting from this.

The known diploids of the Artemisia ludoviciana-complex appear to be as widely separated physiologically as they are morphologically. For instance, A. suksdorfii's more usual habitat is along the open, sandy, coastal bluffs, which are well drained and have a low mineral content (Stebbins, 1965). The climate is mild during both summer and winter; the annual precipitation is about 80-100 inches. Artemisia carruthii, on the other hand, occurs at elevations from 2000-3000 m. along canyon walls and meadows in the Upper Sonoran and Transition Zones of the southern Rocky Mountain States and northern Mexico, and extends eastward into the Great Plains along river banks. Artemisia cavaticaulis has been collected only from the moist river shore of the Deschutes River in a well protected canyon at Clines Falls State Park in central Oregon. The park is located within the low rainfall Juniper-belt of the western Great Basin. Apparently, the diploid races of A. michauxiana and A. lindleyana are not ecologically separated from the tetraploid races. Artemisia michauxiana occurs on loose talus slopes above 3600 m. in the northern Rocky and Cascade Mountains, and sporadically throughout other mountainous areas south to Nevada and Colorado; however, the diploid has been definitely identified only from central Washington near Ellensburg and Wenatchee (Appendix I, accessions 105 and 106). Diploid A. lindleyana is known only from a single collection, Calder 37036 (DAO), in southern British

Columbia, along a sandy lake shore of the Columbia River system. It is also probable that other diploid taxa, either now extinct or as yet undiscovered, contributed to the variation of the group, because the total morphological range of the polyploids far exceeds that of the known diploids. It is obvious, however, that the polyploids have descended from a broad physiological base.

At this point in the study, direct evidence that the diploids are members of one ecospecies has not been obtained; however, it appears highly probable that the contributing diploids had similar genomes at the time of formation of the polyploid races.

Mechanisms for Fertility Improvement

Meiotic instability is an important factor in the origin of most or all autopolyploid groups, for even if pollen fertility and seed set are not appreciably reduced by the production of unbalanced gametes, aneuploid offspring resulting from gametes with deviating chromosome numbers may suffer a higher differential mortality rate in nature (Jones, 1961). The desynaptic plant (11I) from the Satus Creek population that produced large numbers of aneuploid microspores, had little effect on the homogeneity of the population's chromosome numbers. Morphological examination of the progeny from open pollination of the desynaptic plant and two adjacent sister plants in the garden demonstrated a high degree of variation among

the offspring of 11I (Appendix II, p. 151 and 152), while the progeny from the other two plants were within the morphological limits of the parental population. The natural population was morphologically uniform also.

Because of the reduced fertility resulting from meiotic instability, genetic factors that would tend to increase stability would be favored in nature. Evidence suggests that such an increase in stability has occurred within the Artemisia ludoviciana-complex. First, as previously discussed, the complex is considered to be of autopolyploid derivation; yet the frequency of multivalent formation is considerably less than that of recently induced autotetraploids (compare Tables 1, 2, and 8). There is, of course, the possibility that the factors were already present within the genotype of the individuals; A. kurramensis may be an example of this phenomenon. Second, however, the artificial triploids produced during this series of experiments formed trivalents at a much higher rate than the tetraploids--both F_1 hybrids and natural--formed quadrivalents. This relationship was present even when the triploid and tetraploid hybrids were derived from sister crosses (Table 17, A. cavaticaulis X A. lindleyana). In other words, genetic control operating within the tetraploids to limit multivalent formation is not operating in the newly formed triploids. Another example of this phenomenon is in an endemic Colorado crucifer, Physaria vitulifera, studied by

Mulligan (1966). The diploid (n=4) and tetraploid (n=8) races form a simple diploid-tetraploid pair; however, both regularly form only bivalents at prophase I. Cytological proof of the autoploid origin of the tetraploid from the diploid was obtained by Mulligan from an artificial triploid that formed up to 4_{III}'s during meiosis. Further investigation demonstrated that the diploid race averages 2.0+ chiasmata per bivalent while the tetraploid uniformly has one localized chiasma per bivalent. Whereas a reduction in chiasmata frequency appears to have been the only genetic factor involved in the diploidization of Physaria vitulifera (4x), two factors may have been significant within the Artemisia ludoviciana-complex:

1. A comparison of the chiasmata frequency of the tetraploid Artemisias versus the diploids demonstrates that the tetraploids have a significantly lower frequency of chiasmata per bivalent than do the diploids. This is in spite of the evidence presented by John and Henderson (1962) that the chiasmata frequency of newly derived tetraploid cells is higher than in their diploid progenitors.

Assuming a relatively static chiasmata frequency in the diploids, the tetraploids have undergone a considerable reduction in chiasmata frequency. The basis for this assumption is the comparative rod- to ring-bivalent ratios; the ratio for the tetraploids is 4.06:1 and for the diploids 1.23:1. Though the diploids are somewhat below the expected range of 2-3:1 (Ehrendorfer, 1959a), the

tetraploids exceed the normal.

Since quadrivalent formation requires a minimum of three chiasmata per two bivalents, a one-step reduction in the chiasmata frequency to 1.0 per bivalent would lead to immediate diploidization of an organism. A gradual reduction of chiasmata frequency, however, must traverse a zone where trivalent and univalent frequency increases with decreasing chiasmata frequency. Because trivalents and univalents upset regular disjunction more severely than quadrivalents, we might expect the establishment of an equilibrium in which quadrivalents are produced at a low frequency and trivalents are rare (Table 8).

2. Another possible factor in the stabilization of meiosis is the establishment of regular disjunction via directed meiosis. According to McCollum (1958) deviations from a 1:1 ratio of parallel ring (⊏⊐) to convergent ring (◇◇) configurations of four chromosomes in favor of the convergent configuration is indicative of just such a directed meiosis, since parallel rings have been shown to yield unequal anaphase I disjunctions more frequently. As shown by the data recorded in Table 9, the number of convergent rings of four are more frequent than parallel rings of four. Also, it may be that the chains of four in the convergent configuration (⋀⋁) are more frequent than those in parallel (⊓⊔) or indifferent (⊣⊢) configurations, although data on this point were not recorded. A random spot check

of various slides suggested that they do indeed form the vast majority of the chains of four. If this check was accurate, then the metaphase I arrangement is highly directed. The development of this characteristic could occur concurrently with the chiasmata reduction, and therefore play a role in the formation of an equilibrium point, or it could serve to further stabilize disjunction after a bivalent-multivalent equilibrium has been formed.

Taxonomic Relationships within the Complex

The polyploid members of the Artemisia ludoviciana-complex apparently form a morphological continuum across western North America, with A. ludoviciana itself forming the connecting link between all the variants. However, the Northwestern representatives of this morphological cline more closely resemble the diploids of the same area than they resemble diploids or polyploids from other regions. The logical supposition based on this, and on the presence of heteroploidy in two of the diploid taxa, is that some or all of the diploids gave rise directly to autopolyploid derivatives. Hybridization with the other autoploids from the Northwest and/or gene migration through introgression from the diploids then initiated genetic recombination. Hybridization with other groups of polyploids radiating out from other geographic centers of distribution would account for the distinctive morphological cline of A. ludoviciana. This type

of reticulate evolutionary pattern has been described for other complexes within the genus by Ehrendorfer (1964), though they were cited by him as examples of alloploidy. The alternative to this scheme would require hybridization between most of the diploids followed by chromosome doubling. Due to the current very different habitat preferences of the diploids, and the occurrence of A. suksdorfii and A. carruthii in areas that have been stable since mid-Tertiary or earlier (Keck, 1946), this alternative seems less likely though it is by no means excluded.

Hybridization is still a factor in the variation pattern of the complex; numerous populations were examined that were either intermediate between two taxa or included one or more taxa with intermediates. These were most prevalent between the various subspecies of A. ludoviciana. Individual examples will be discussed below. Generally few external barriers to hybridization exist among the polyploids. All taxa flower profusely and for extended periods of time in the garden, and observations in nature indicate an indeterminate flowering period. For example, A. lindleyana's flowering period is dependent upon the time and period of the annual spring flood of the Columbia River. Also, the collection of A. ludoviciana ssp. candicans (6x) from Tombstone Prairie, Oregon, flowerd in the early spring in the Willamette Valley garden while the natural population was still snow covered. The plants in nature then flowered later

in the season. Ecological isolation of the polyploid members of the complex is also incomplete, and the included taxa are either sympatric or they come in close contact with the remaining races.

Hybridization data obtained during the course of this study indicate that internal barriers are also lacking among the polyploids. Thus, there does not seem to be a deterrent to free gene exchange at the higher chromosome levels.

Intertaxon Relationships

1. The origin of *Artemisia douglasiana*. Clausen, Keck, and Hiesey (1940) considered *A. douglasiana* to be an amphidiploid originating from *A. ludoviciana* (4x) and *A. suksdorfii* (2x). The authors noted, as the bases for their conclusion, the geographic intermediacy of the hexaploid, the resemblance of its herbage to that of *A. suksdorfii*, and the similarities between the inflorescence of the hexaploid and that of *A. ludoviciana*.

Evidence was gathered in this study, in addition to the pairing relationships already described, that casts doubt on Clausen, Keck, and Hiesey's explanation of the origin of *A. douglasiana*: (a) The discovery of another diploid, *A. cavaticaulis*, that is closely related to *A. suksdorfii* but that varies from it in the direction of *A. douglasiana*. (b) The occurrence of tetraploid populations of *A. douglasiana* in the Yakima Valley of eastern Washington, in sites similar to those

occupied elsewhere by the hexaploid. (c) The artificial production of triploid hybrids that resemble A. douglasiana, from A. ludoviciana ssp. candicans X A. cavaticaulis (Appendix II, p. 147 and 148).

The conclusion favored here is that A. douglasiana originated from organisms with strongly homologous genomes, and that at least two of these genomes were contributed by the tetraploid race of A. douglasiana. The third set of chromosomes is either the result of autoploidy arising through the union of a non-reduced and a reduced gamete from the tetraploid population or by hybridization of the tetraploid and a similar diploid genotype, such as A. cavaticaulis. Possibly, however, the hexaploid arose from more than one pathway, as the lobed forms in the San Francisco Bay region might suggest. This form, which apparently varies from the "typical" form only in this one character (Keck, 1946, p. 459), likely has one of the lobed A. ludoviciana subspecies in its ancestral line. Artemisia ludoviciana ssp. incompta and an entire-leaved A. douglasiana (6x) formed a highly vigorous hybrid whose leaves were deeply lobed, but it resembled the A. douglasiana parent in all other characteristics (Appendix II, p. 153). We may also conclude that the A. douglasiana of the Yakima Valley is a relict of a larger population that was displaced by the hexaploid race. The origin of the tetraploid is unknown. Hybrids with A. cavaticaulis and A. suksdorfii, two diploids that may have played a part in its evolutionary history, were weak with

shriveled leaves and consequently furnished little taxonomic information.

In all probability the tetraploid A. douglasiana hybridizes with A. ludoviciana ssp. candicans in eastern Washington, thereby contributing to the taxonomic confusion between these two taxa in that region (Keck, 1946; Cronquist, 1950). Hybrids between these two tetraploid genotypes were artificially constituted (Appendix II, p. 149). They are vigorous and fertile, and form a series between the two parental types. Up to six multivalents have been observed at meiosis in the hybrids.

2. The taxonomic position of Artemisia prescottiana. Neither Cronquist (1955) nor Ward (1960) considered A. prescottiana to be deserving of specific rank. The former author relegated it to synonomy under A. lindleyana without comment. Ward, however, stated, "Plants with characters intermediate between this (note: A. prescottiana) and A. lindleyana are also common in this area, and it is probably at best only a variety of that species." At least one population (Mayer State Park, Oregon) was found during this study to include both morphological types, and intermediates were also present. Progeny tests of the offspring from open pollination of 42-559, an A. prescottiana form, were conducted, and all progeny were A. lindleyana-like, though very variable. This study, therefore, substantiates Cronquist and Ward's conclusions concerning this name,

and all future reference here to A. lindleyana will be in this broader sense, to include A. prescottiana.

3. The relationship between Artemisia ludoviciana and Artemisia lindleyana. Keck (1946) recognized A. lindleyana as a chamaephyte of the Columbia River and its tributaries, closely related to A. ludoviciana ssp. ludoviciana and ssp. incompta. He, and later Cronquist (1955), separated A. lindleyana from the latter two subspecies on the basis of its having a more reduced panicle, a woody base, leaves apically toothed and less than 1.0 cm. in width, and plants taprooted and generally without spreading rhizomes.

Artemisia lindleyana occupies what are perhaps the most specialized and precarious habitats in the complex. The plants are semiannually innundated by swiftly flowing currents that often sweep away the sand from the rootstock or cover the plant with silt and debris. The stalks are usually broken back, but they resprout after the water recedes. Few other plant species occur within this rocky-sandy zone below the high water mark; yet A. lindleyana is capable of producing rather large populations and of flowering consistently, at least during the three seasons that it has been observed.

Evidence obtained from hybridization studies and field and garden observations indicates that A. lindleyana, rather than being completely distinct, is an expression of ecotypic variation to this harsh environment in the same sense that the subspecies of

A. ludoviciana are examples of ecotypic variation. This evidence may be summarized as follows: (a) The "species" characters are not absolutely correlated; thus, plants of the large Wanapum population (103) have highly divided leaves reminescent of A. ludoviciana ssp. incompta, while other populations are like A. ludoviciana in containing some rhizomatous members (Mayer State Park and Hayden Island). (b) When maintained in an experimental garden free of spring innundation, the plants grow to heights equal to A. ludoviciana, and the inflorescense is slightly more spreading. However, the leaves remain characteristically narrow and the growth habit remains clump-like. (c) Hybrids produced between the two species are vigorous, like most of the intralevel crosses. (d) The panicle of A. lindleyana remains narrow and non-leafy, even in the garden. It therefore falls within the general limits set by Cronquist (1955) for the Pacific Northwest members of A. ludoviciana--all members of one subspecies, but differentiated at the varietal level in his scheme.

It seems highly probable, therefore, that the taproot system and woody base of A. lindleyana are adaptations to the river-shore habitat. In fact, in the instances examined by this author of the same traits in A. ludoviciana, the plants were growing in rocky soil in a stream bed (John Day, Pateros, Kettle Falls, and Frenchglen populations). Other characters such as small leaves, reduced height, and narrow panicle may be adaptations related to rapid growth rate

during the short post-flooding period available for resprouting and flowering.

Although A. lindleyana contains two chromosomal levels, it is most certainly not a simple diploid-tetraploid pair. That is to say, the diploid could not have contributed to the entire variation of the polymorphic tetraploid race. A comparison of the cytological races is presented in Table 18.

Table 18. A morphological comparison of the diploid and tetraploid races of Artemisia lindleyana.

	Diploid	Tetraploid
Inflorescence	Narrow panicle.	Narrow panicle.
Leaf length	4.0 cm.	2.5-5.5 cm.
Leaf width	0.5 cm.	0.3-0.4 (rarely 0.8 cm.).
Leaf vesture	Upper loosely villous above and densely tomentose below; lower glabrous above and densely villous below.	Loosely villous to glabrous above and densely tomentose below.
Leaf shape	Upper entire; lower irregularly apically toothed, lanceolate, revolute.	Entire, apically toothed to deeply lobed (lobes up to 2 cm. long and lanceolate), usually entire in the inflorescence, linear-lanceolate to lanceolate, revolute.
Heads	4.0-4.5 mm. high, 4.0-5.0 mm. wide, all heads as wide or wider than high, campanulate.	3.0-4.5 mm. high, 3.0-5.0 mm. wide, occasionally higher than wide, campanulate.
Stem	4.5 dm. tall, woody at base, appears to be somewhat rhizomatous.	2.0-6.5 dm. tall, woody at the base, usually not rhizomatous.

The role of the diploid A. lindleyana in the evolution of the complex is still unknown. Probably, however, it contributed both the genomes of the tetraploid race. If this situation is correct, then introgression with A. ludoviciana ssp. incompta is the most likely source of the lobed leaf patterns present in central Washington. An artificial hybridization of this nature was attempted between an apically toothed form of the tetraploid A. lindleyana (103H) and A. ludoviciana ssp. incompta (108). The A. ludoviciana ssp. incompta parent is from a lower elevation site immediately above the Columbia River at Pateros, Washington. The hybrids as expected are lobed in the same manner as the lobed-form of A. lindleyana (Appendix II, p. 146).

The diploid race may also have contributed to the subspecies of Artemisia ludoviciana through hybridization with A. michauxiana and/or other diploid races in the Northwest, although there is not yet any evidence to either support or refute this supposition.

4. Artemisia michauxiana, a diploid-tetraploid pair, and Artemisia ludoviciana ssp. incompta. The distribution of pollen size classes within A. michauxiana and A. ludoviciana ssp. incompta (Figures 2 and 3) indicates that the latter taxon is largely tetraploid in the far Western States, but that A. michauxiana includes both levels along its north-south range. That intermediates occur between these two taxa was first reported by Keck (1946) and verified by

herbarium studies in this report. One of the intermediates from Steens Mountain included in the pollen study was found to be almost completely sterile. Its pollen diameter was well within the 4x class; however, the sample cannot be considered random because certain larger pollen sizes may be preferentially fertile. This evidence suggests that this individual is a sterile triploid, although diploid A. michauxiana has yet to be identified in that geographic region. The diploid-tetraploid nature of A. michauxiana, the morphological similarity between the two taxa (recall that they once were considered subspecies of the same species), and the presence of intermediates suggests that A. ludoviciana ssp. incompta either arose via hybridization of A. michauxiana and another taxon of the complex, or that A. michauxiana is currently affecting the variation pattern of A. ludoviciana ssp. incompta by introgression.

Hybridization between the two taxa was accomplished during this study, and the 4x hybrids have the same cytologic characteristics as the others that have been described. Morphologically, the offspring are within the limits of A. ludoviciana ssp. incompta.

5. Artemisia suksdorfii and Artemisia cavaticaulis. Though these two taxa are distinct they bear the greatest morphological similarities among the diploids. The two are compared in Table 19.

Artemisia suksdorfii apparently has a very constant morphology as the plants undergo little modification when transplanted to either

Table 19. A morphological comparison of the diploids, Artemisia suksdorfii and Artemisia cavaticaulis.

	A. suksdorfii	A. cavaticaulis
Inflorescence	Extremely dense, wide and leafy panicle.	Narrow panicle.
Leaf length	8-15 cm.	7-9 cm.
Leaf width	1.5-3.0 cm.	0.9-1.4 cm.
Leaf shape	Broadly lanceolate, oblanceolate, or elliptic with a few irregular and coarse teeth or lobes, occasionally entire, lobes up to 2.0 cm. long; plane or seldom narrowly revolute.	Linear-lanceolate, lanceolate, or oblanceolate with 2-4 lobes, lobes up to 3.0 cm. long and slightly incurved or more commonly lanceolate, the lobes often irregularly toothed; narrowly revolute.
Leaf vesture and surface	Strongly discolored, bright green and glabrous or sparingly villous above with a rugose surface and yellow-green veins, silvery lanate below.	Villous and non-rugose above, lanate below.
Heads	3.0-4.5 mm. high, less than 2 mm. wide, terete or narrowly ovoid, glabrous to villous and yellow-green, seldom with anthocyanous margins, 5-10 ray florets, 2-10 disc florets.	2.8-3.7 mm. high, 2.5-3.5 mm. wide, campanulate to globose, villous and green to gray-green, margins not anthocyanous, 8-12 florets, 20-33 disc florets.
Stem	7.5-12.5 dm. tall, simple and clustered from a rhizomatous base, often suffrutescent, striated and red to brown, sparingly villous; pith present.	6.5-9.0 dm. tall, branched, from a woody caudex, striate and green, densely villous; pith lost early in development; overwinters above the soil line as short scale-covered shoots that elongate in the spring.

the experimental garden or the greenhouse. In addition, the adventitious roadside populations of the west slope of the Cascade Mountains at an elevation of about 1000 m. (Snoqualmie Pass, Washington) did not demonstrate any observable deviation from the coastal populations. On the other hand, A. cavaticaulis exhibits considerable phenotypic plasticity. The description in Table 19 includes only measurements taken from plants in their natural habitat. In comparison, the four plants in the Willamette Valley experimental garden attained a plant height of only 3.0-4.5 dm. Their leaf size was also reduced to ca. 4.5 cm. in length and a maximum of 1.0 cm. in width. The degree of lobing did not vary from the natural condition; however, few teeth on the lobes appeared in the transplanted individuals. In addition, the upper leaf showed a decrease in the amount of surface hairs so that the leaf appeared discolored, though the color was not a bright green as in A. suksdorfii. Other than a corresponding reduction in the length of the inflorescence there was no change in the floral parts.

No change in height was noted in the greenhouse plants; however, their leaf size increased to 8.5-10.0 cm. in length and 2.0-3.0 cm. in width. The leaves differ from those of A. suksdorfii primarily by four traits--the upper surface is not rugose, the veins are the same coloration as the dark green surface, the upper surface is not shiny nor the under surface silvery, and the lobing pattern is more regular.

The self-fertilized progeny of A. cavaticaulis, however, developed to a height of 20-25 dm. without branching in one growing season.

Hybridization between the two taxa proved successful in one reciprocal cross (8B X 27 and 27 X 8B, see Tables 15 and 17 and Appendix II, p. 140). In all instances but one, the hybrids were robust; however, none have flowered as yet. In addition, putative hybrids between the two taxa were obtained from open pollination in the garden (Tables 15 and 17 and Appendix II, p. 141).

All of the offspring of the cross-fertilization are essentially A. suksdorfii-like; the stems are red, sparingly-villous with a pith and the leaves are irregularly toothed and glabrous above. The lower surface of the leaf is not as silvery as in A. suksdorfii, and the rhizomes are intermediate (Figure 10). The hybrids appear to be highly susceptible to leaf rust, and this is also characteristic of A. cavaticaulis but not of A. suksdorfii.

A determination of the genetic and taxonomic affinities of these two species must await further hybridization experiments and/or the production of floral buds on the current hybrids. However, it is quite possible that the two diploid taxa, A. suksdorfii and A. cavaticaulis, are relicts of a once larger, continuous population in the Northwest.

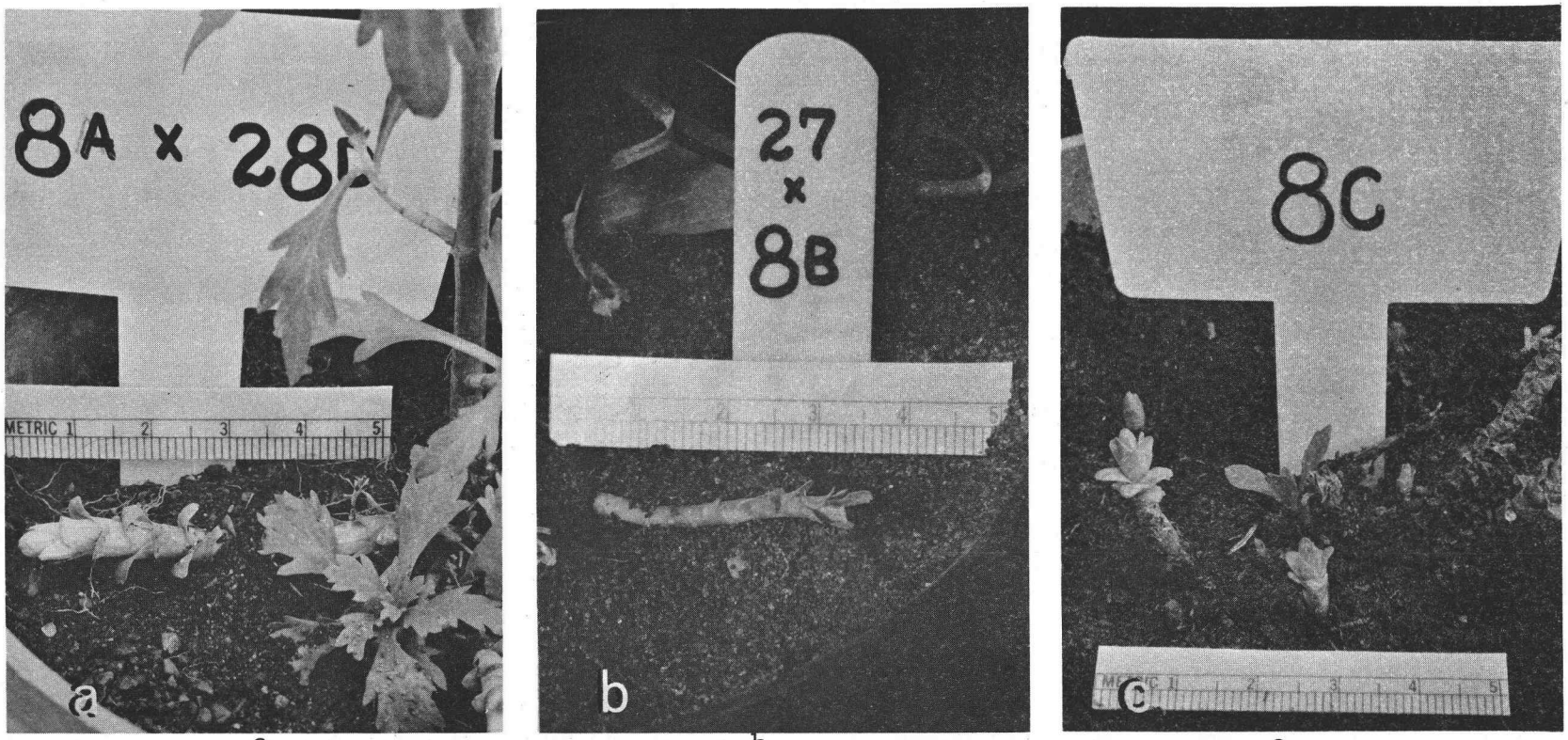

Figure 10. Rhizomes of Artemisia cavaticaulis and Artemisia suksdorfii X Artemisia cavaticaulis F_1 hybrids. (a) Emerging rhizome of A. cavaticaulis. (b) Emerging rhizome of hybrid. (c) Rhizome of A. cavaticaulis resprouting after dormancy.

Taxonomy of the *Artemisia ludoviciana*-Complex

Hybridization experiments and studies of pairing relationships are of little taxonomic value within a group having the composition of the *Artemisia ludoviciana*-complex. If one wishes to produce a phylogenetic taxonomic scheme, he must disregard as a criterion the ability to produce fertile F_1 hybrids between two types, for inherent in a complex of autoploid origin is the capacity to cross all possible types within chromosomal levels. Instead, the taxonomist must concentrate on evidence of genetic lineage.

Keck's classification which separated the forms along cytological lines is not phylogenetic, and it is difficult to utilize his system in field studies of natural populations. The latter fault will be a part of any system of classification devised for this group, however, because of the intensive hybridization in nature. Partly because of this, but also because of the extremely long list of synonomy within the complex, the author suggests that a detailed revision of the taxonomy not be undertaken until (1) the entire complex and its connections with the Eurasian complexes has been thoroughly examined, and (2) the genetic and ecological relationships between the extant diploids are better known. However, Cronquist's (1955) treatment of *Artemisia prescottiana* as a synonym of *Artemisia lindleyana* should be followed.

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APPENDICES

APPENDIX I. SITE DATA AND CHROMOSOME NUMBERS FOR *ARTEMISIA* POPULATIONS.

Accession	Chromosome number	Locality
Artemisia carruthii		
52 (Boulder Lake)	$2n=9_{II}$	Oklahoma: Comanche County. Shore of Boulder Lake in the Wichita Mountains Wildlife Refuge. *Arnold s. n.*
67 (Cimmaron Canyon)		New Mexico: Colfax County. *Arnold s. n.*
Artemisia cavaticaulis		
8 (Clines Falls)	$2n=9_{II}$	Oregon: Deschutes County. Grassy river bank of the Deschutes River in a sheltered canyon at Clines Falls State Park. Associated with *Spiraea* and *Salix*. 900 m.
Artemisia michauxiana		
92 (Little Blitzen Creek)	$2n=18_{II}$	Oregon: Harney County. Moist flood-plain of the headwaters of Little Blitzen Creek and in the rocky stream bed. Steens Mountain. Associated with *Mimulus* and native grasses. 2300 m.
93 (Little Blitzen Gorge)		Oregon: Harney County. Basaltic crevices of the rim of the Little Blitzen Gorge. Steens Mountain. Associated with *Achillea*. 2600 m.
98 (Blitzen River)		Oregon: Harney County. Head of a gravel bar in the Blitzen River. Possibly washed down river. 1800 m.
105 (Mt. Mission)	$2n=9_{II}$	Washington: Kittitas County. Loose talus slope in Douglas fir zone. Roots in the moist underlying sand layer. Along Mission Trail. 1250 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia michauxiana (continued)		
106 (Mt. Lillian)	$2n=9_{II}$	Washington: Kittitas County. Talus bank of mountain stream above the Liberty-Beehive Trail, below the summit of Mt. Lillian. 1800 m.
119 (Indian Creek)	$2n=18_{II}$	Oregon: Harney County. Basaltic outcropping of canyon rim above Indian Creek. Steens Mountain. Associated with *Achillea*. 2600 m.
Artemisia suksdorfii		
4 (Yeon)	$2n=9_{II}$	Oregon: Multnomah County. Rocky soil at side of lower McCord Falls Trail in the open at Yeon State Park. 25 m.
19 (Multnomah Falls)	$2n=9_{II}$	Oregon: Multnomah County. Rocky river shore of the Columbia River on Interstate 80N causeway fill below Multnomah Falls. 25 m.
24 (Eagle Creek)		Oregon: Hood River County, Abundant in sandy soil on hillsides along the roadway to the overnight camping area in Eagle Creek Recreation Area, Mt. Hood National Forest. 25 m.
27 (Gold Beach)	$2n=9_{II}$	Oregon: Curry County. Exposed coastal bluff overlooking the Pacific Ocean, one mile north of the mouth of the Rogue River. *Chambers s. n.*
69 (Taft)	$2n=18^{*}$	Oregon: Lincoln County. Open bluff exposed to the Pacific Ocean and the Siletz River.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia suksdorfii (continued)		
83 (Snoqualmie Pass)		Washington: King County. Adventive populations occupying road cuts along US 10 west of the Cascade Summit. The soil was gravelly and the plants were spreading by rhizomes. Up to 1000 m.
126 (Mendocino)		California: Mendocino County. Coastal bluffs just north of Rockport. *Chambers s. n.*
Artemisia vulgaris		
2 (Rooster Rock)	$2n=ca.54^{+}$	Oregon: Multnomah County. Adventive on sandy backwater of the Columbia River at Rooster Rock State Park. Associated with *Salix*. 20 m.
68 (Glencoe)	$2n=36^{*}$	Oregon: Multnomah County. Adventive in weedy area in Portland Metropolitan Area. 25 m.
85 (Cambridge)	$2n=8_{II}$	Massachusetts: Middlesex County. In a weedy vacant lot. *Gottleib s. n.*
Artemisia douglasiana		
11 (Satus Creek)	$2n=18_{II}$; $2n=17_{II}{}^{1}I$	Washington: Yakima County. Sand and gravel floodplain and bank of Satus Creek along US 97. Associated with *Penstemon* and *Aster*. 300 m.
23 (Ruthton Point)		Oregon: Hood River County. Highly disturbed site near an old dwelling above the Columbia River. May have been under cultivation. 30 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia douglasiana (continued)		
48 (Siskiyou)	$2n=27_{II}{}^{1}{}_{I}$	Oregon: Jackson County. Sheep Springs Camp. East flank of Dutchman's Peak. 2100 m. Robinson s. n.
53 (Sauvies Island)	$2n=27_{II}$	Oregon: Multnomah County. Common roadside plant on Sauvies Island. Associated with Rubus.
54 (Independence)	$2n=27_{II}$	Oregon: Polk County. Abundant in the clearings between the wooded shore line of the Willamette River and the neighboring fields. 100 m.
55 (Skyline Road)	$2n=27_{II}$	Oregon: Polk County. Roadside weed at the base of South Salem Heights along Oregon 22. 100 m.
56 (Buena Vista)	$2n=27_{II}$	Oregon: Polk County. Sandy river shore at the Willamette River ferry crossing. 100 m.
72 (Roche Road)	$2n=27_{II}$	Oregon: Linn County. Roadside weed along Roche Road above the Willamette River in sandy soil. 70 m.
80 (Alturas)	$2n=27_{II}{}^{1}{}_{I}$	California: Modoc County. Sandy soil on roadside, just south of junction US 395 and California 299, north of Alturas. 1200 m. Chambers and Tyrl s. n.
82 (Thrall)	$2n=18_{II}$	Washington: Kittitas County. On the Yakima River near Thrall. Chambers s. n.
100 (Yakima)		Washington: Yakima County. Scattered clumps along the flood-plain of Satus Creek in rocky, sandy soil. Associated with sagebrush. 300 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia douglasiana (continued)		
101 (Yakima River)		Washington: Kittitas County. On a sandy-rocky shelf above the Yakima River. Associated with *Rhus* in a Ponderosa pine area. 400 m.
112 (Sherman Creek)§		Washington: Ferry County. Small population along the roadside in a disturbed site. Associated with *Populus*, *Spiraea lucida*, and roadside grasses. 600 m. Intermediate between *A. douglasiana* and *A. ludoviciana* ssp. *candicans*.
124 (Rogue River)	$2n=27_{II}$	Oregon: Curry County. Sandy bank of the Rogue River. *Johnston s. n.*
125 (Moro Bay)		California: San Luis Obispo County. Rocky canyon opening out on the ocean, 1 1/2 miles south of Moro Bay. *Chambers s. n.*
Artemisia lindleyana		
1 (Hayden Island)§	$2n=18_{II}$	Oregon: Multnomah County. Grassy shore of the Columbia River on Hayden Island, in moist sand below the high water mark of the river. Some plants in this population approach *A. ludoviciana* in their rhizomatous habit, width of leaves, and pubescence. 20 m.
22 (Stephen's Beach)		Oregon: Hood River County. Sandy beach along the Columbia River, below the summer water line and *Salix*. 25 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia lindleyana (continued)		
33 (Umatilla)	$2n=18_{II}$	Oregon: Umatilla County. Gravel-sand bank of the Columbia River slough, below the high water mark. Associated with young *Salix*. 90 m.
34 (Boardman) A-B, D-	$2n=18_{II}$	Oregon: Morrow County. Plants of sand-gravel banks of the Columbia River, just down river from the Boardman City Dump. Associated with young *Populus*. *A. ludoviciana* ssp. *candicans* above water line. 70 m.
36 (Blalock)	$2n=18_{II}$	Oregon: Gilliam County. In crevices of basaltic outcroppings along the Columbia River shore-line, below the high water mark; roots secure in rocks but in meagre soil. 70 m.
37 (Willow Creek)	$2n=18_{II}$	Oregon: Gilliam County. In sand and rocks at the mouth of Willow Creek where it empties into the Columbia River, below the high water mark. *Lupinus* immediately above the high water mark. 70m.
40 (Arlington)	$2n=18_{II}$	Oregon: Gilliam County. In sand among basaltic outcroppings below the high water mark of the Columbia River. 70 m.
42 (Mayer State Park)[§]	$2n=18_{II}$	Oregon: Wasco County. Rocky-sandy shore of the Columbia River below the high water line; *Gaillardia* and *Coreopsis* just at the high water mark. "*Prescottiana*" forms abundant. Closely associated with an inland population of *A. ludoviciana*. 50 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia lindleyana (continued)		
65 (Snake River)	$2n=18_{II}$	Washington: Whitman County. Rocky to sandy soil below the high water mark of the Snake River on a large alluvial fan. 550 m. *Wakefield s. n.*
103 (Wanapum)	$2n=18_{II}$	Washington: Grant County. East shore of the Columbia River in a large gravel and sand area below Wanapum Dam; the slope is gentle and the population extends farther inland than other populations of *A. lindleyana*. The population density decreases sharply as the amount of grass cover increases. Associated with *A. campestris* and scattered *Lupinus*. A single *A. ludoviciana* ssp. *candicans* (103E) was located about 100 m. above the high water line in sandy soil. 175 m.
115 (Waiwai)[§] I-M	$2n=18_{II}$	Washington: Whitman County. Rocky shore of the Snake River in the outwash of a small rocky stream. The stream bed and adjacent grass land was fenced and apparently grazed by cattle. *A. ludoviciana* ssp. *incompta* occurs in this rocky situation and a few intermediates occur between the two populations. 525 m.
Artemisia ludoviciana ssp. *ludoviciana*		
13 (Buck Creek)	$2n=18_{II}$	Oregon: Lake County. Bank of an irrigation canal draining into Buck Creek above Summer Lake.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *ludoviciana* (continued)		
15 (Chandler's Wayside)		Oregon: Lake County. Sand and gravel of Crooked Creek bed, below an outwash from Drakes Peak. Associated with *Achillea*, *Rosa*, and *A. tridentata* above the creek's banks.
25 (Jantzen Beach)		Oregon: Multnomah County. Appears to be adventive in the sandy soil along the roadside on Hayden Island near the Jantzen Beach Amusement Park. 20 m.
29 (Prineville)	$2n=18_{II}$	Oregon: Crook County. In a grassy waste-area atop the sandy bank of the Crooked River at the Prineville city limits. 1000 m.
31 (Crooked River)	$2n=18_{II}$	Oregon: Crook County. Gravel bar in the Crooked River below high water. Associated with *Salix*. 1000 m.
32 (John Day River)		Oregon: Wheeler County. Gravel bar of the John Day River bed below the high water mark. Very abundant. 600 m.
35 (Deschutes River)		Oregon: Wasco County. Base of basaltic slide area above a small pond created by highway fill. Isolated population, but *A. tridentata* is the dominant plant of the area. 50 m.
38 (Heppner Junction)		Oregon: Gilliam County. Sandy open area along Willow Creek about 100 m. above the mouth. 70 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *ludoviciana* (continued)		
39 (North Richland)		Washington: Benton County. Sandy, disturbed site above the Columbia River at a public boat launching ramp. 100 m.
57 (Rabbit Creek)		Oklahoma: Cotton County. Moist meadow along Rabbit Creek with scattered *Prosopsis*. *Baber s. n.*
97 (Harney)	$2n=18_{II}$	Oregon: Harney County. In a rocky outwash below a 2400 m. peak in a juniper-sage area. 2000 m.
120 (Frenchglen)§	$2n=18_{II}$	Oregon: Harney County. Abundant in the bed of a rocky water course, 5 miles east of Frenchglen on the Fish Lake Road. *Juniperus*, *Lupinus*, *Sarcobutus*, and *A. tridentata* above the stream and *A. arbuscula* in the "intermediate" zone. Associated with *A. ludoviciana* ssp. *incompta* in the stream and intermediates are common. 1300 m.
Artemisia ludoviciana ssp. *candicans*		
9 (Modoc Billy Creek)	$2n=18_{II}$	Oregon: Klamath County. Open meadow on the edge of pine forest. Associated with *A. tridentata* and *Chrysothamnus*. West of Beatty where Oregon 66 crosses Modoc Billy Creek. *Pembrook s. n.*
10 (Wasco)§		Oregon: Wasco County. Sandy, open alluvial fan at the mouth of a hillside ravine, above the Columbia River. Associated with *Chrysothamnus*. Approaches *A. douglasiana*. 80 m.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *candicans* (continued)		
14 (Summer Lake)	$2n=18_{II}$	Oregon: Lake County. Sandy, rocky soil at the mouth of an outwash on Oregon 31 above Summer Lake. Associated with *Achillea*, *Purshia*, and *A. tridentata*. 1250 m.
17 (Quartz Mountain)	$2n=18_{II}$	Oregon: Lake County. A series of roadside weeds in rocky dry soil. 1600 m.
18 (Sprague River)	$2n=18_{II}$	Oregon: Klamath County. On the sandy bank of the Sprague River, between *Spiraea* and *Rosa* and the water line. 1250 m.
28 (Tombstone Prairie)	$2n=27_{II}$	Oregon: Linn County. Abundant in Douglas-fir clearing in moist rocky soil. 1250 m.
30 (Crooked River Bridge)		Oregon: Crook County. Sandy banks of the Crook River where US 26 crosses the river. 1000 m.
34 (Boardman)[§] C	$2n=18_{II}$	Oregon: Morrow County. Immediately above the high water mark in Boardman. See *A. lindleyana* 34.
41 (Grant)	$2n=18_{II}$	Oregon: Sherman County. Sand-rock shore of an artificial pond between the railroad embankment and US 30. 50 m.
42 (Mayer State Park)[§]	$2n=18_{II}$	Oregon: Wasco County. In moist sandy or rocky soil in moist meadows, intermittant lake bottoms, and riverfed lake shores. Tendency toward a clumped

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *candicans* (continued)		
42 (Mayer State Park) (continued)		habit in the lake bottom. Approaches *A. douglasiana*. 50 m. See *A. lindleyana* 42.
44 (Jacks Creek)§	$2n=18_{II}$	Idaho: Owyhee County. Stream bank in a deep rocky canyon, growing in moist silt and gravel. Associated with *Equisetum*, *Solidago*, *Toxicodendron* and *Melilotus*. Population mixed with *A. ludoviciana* ssp. *incompta*. 900 m. Johnson and Beckman s. n.
46 (Priest Lake)	$2n=18_{II}$	Idaho: Bonner County. Along the Priest River just above the water line in sandy-gravelly wet soil. Associated with *Carex*, *Juncus*, *Agrostis*, and *Aster*. Dean s. n.
76 (Big Falls of the Deschutes)§	$2n=18_{II}$	Oregon: Deschutes County. Basaltic rock-river sand shore of the Deschutes River immediately above the Big Falls in a deep sheltered canyon. Associated with *Salix*, *Populus*, and *Spiraea*. Approaches *A. douglasiana*, but leaves more narrow. 1000 m.
79 (Cedar) J	$2n=18_{II}$	California: Modoc County. Few clumps on road bank near stream in a region with scattered *Abies concolor* and *Juniperus* on sagebrush-rabbitbrush slopes. 1950 m. Chambers and Tyrl s. n.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *candicans* (continued)		
90 (Wallowa Lake)	$2n=18_{II}$	Oregon: Wallowa County. Abundant along the east lateral moraine at Wallowa Lake. Associated with *Clarkia*, *Achillea*, *Lupinus*, and *Balsamorhiza*. 1250 m.
113 (Kettle Falls)	$2n=18_{II}$	Washington: Ferry County. Abundant in sand or rock situations along the shore of the Kettle River. Associated with *Salix* and *Populus*. Plants in the rocks tending to a woody root system and those in the sand to rhizomes. 500 m.
114 (Rosalia)§		Washington: Whitman County. Waste area between creek and railroad. Associated with orchard grass and *Rosa*. Tending to *A. douglasiana*. 700 m.
Artemisia ludoviciana ssp. *incompta*		
12 (Satus Pass)	$2n=18_{II}$	Washington: Klickitat County. Roadside at the Satus Pass summit on US 97 in sandy rocky soil. 1000 m.
44 (Jacks Creek)§	$2n=18_{II}$	Idaho: Owyhee County. See *A. ludoviciana* ssp. *candicans* 44.
51 (Iron Peak)		Washington: Kittitas County. West slope of Iron Peak; 3/4 mile from the origin of Beverly Creek-Turnpike Trail and about 200 m. above the trail; in a deep cut along a small stream. Wenatchee National Forest. Associated with *Senecio pauperculus*, *Salix*, *Alnus*, and *Cirsium edule*. Palmblad s. n.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *incompta* (continued)		
70 (Stansbury Mountains)		Utah: Tooele County. One mile below the guard station at South Willow Creek. *Wiens s. n.*
71 (North Bench)		Utah: Salt Lake County. Above the university at Salt Lake City. *Wiens s. n.*
79 (Cedar) A-I	$2n=18_{II}$	California: Modoc County. Abundant in sand and among rocks in streamway, and on roadside bank. Associated with *Populus*, *Prunus*, *Cercocarpus*, *Symphoricarpus*, and *Rosa*. Variable in color of the foliage. 1000 m. *Chambers and Tyrl s. n.*
86 (Aneroid Trail)§ C-N		Oregon: Wallowa County. Extending along the trail and flaring out into small populations in the rocky clearings in the Douglas-fir forest. Moisture seemingly abundant from springs. Associated with *Penstemon* on dryer slopes and *Mimulus* on the more moist areas. Occasional *A. ludoviciana* ssp. *candicans* types at the lower elevations. From 1700 m. to 2100 m.
88 (Hurricane Trail)§		Oregon: Wallowa County. In a moist rocky meadow along Hurricane Creek. Associated with *Achillea*, *Spiraea*, and *Thalictrum*. 1700 m. Population includes both *A. ludoviciana* ssp. *incompta* and *candicans* with abundant intermediate types.

APPENDIX I (continued)

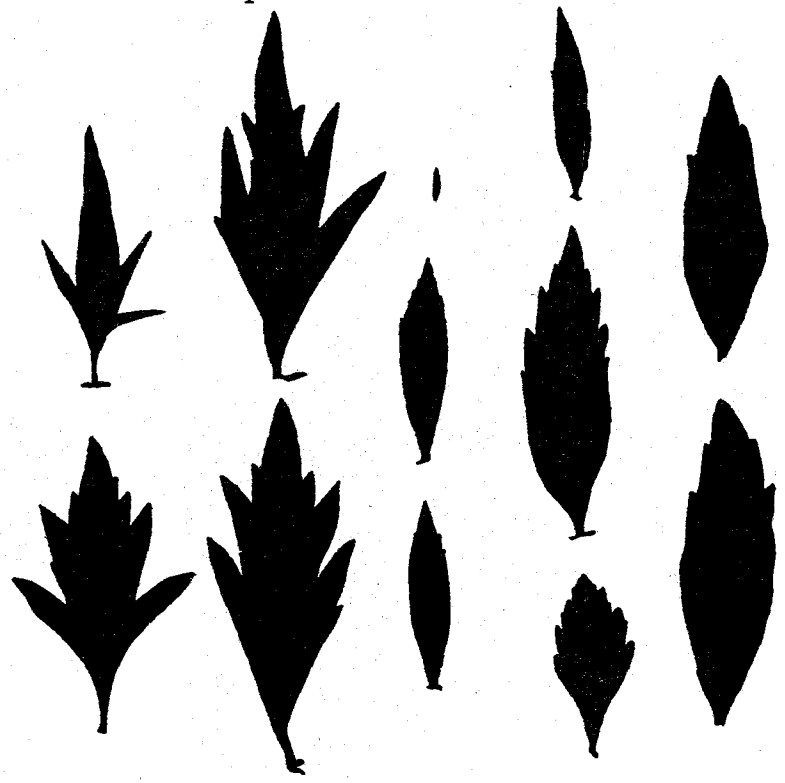
Accession	Chromosome number	Locality
Artemisia ludoviciana ssp. *incompta* (continued)		
99 (Little Fish Creek)		Oregon: Harney County. Head-waters of Little Fish Creek in moist, sandy soil along a running rivulet. Steens Mountain. Associated with *Mimulus*. 2400 m.
108 (Pateros)	$2n=18_{II}$	Washington: Okanogan County. Abundant on a gravel bar at the mouth of the Methow River, with scattered *Salix*. 400 m.
115 (Waiwai)[§] A-H	$2n=18_{II}$	Washington: Whitman County. See *A. lindleyana* 115.
116 (Anatone)		Washington: Asotin County. In crevices of dry granite slope with some seepage from a nearby stream. In a *Pinus ponderosa* zone. 900 m.
120 (Frenchglen)[§]	$2n=18_{II}$	Oregon: Harney County. See *A. ludoviciana* ssp. *ludoviciana*.
123 (Blitzen River)		Oregon: Harney County. In the basin of the Little Blitzen River under the partial shade of aspen along a dry stream bed. Associated with *Balsamorhiza*. 2000 m.
Artemisia ludoviciana ssp. *mexicana*		
81 (Wichita Mountains)[§]		Oklahoma: Comanche County. On the shore of Boulder Lake, Wichita Mountains Wildlife Refuge. *Arnold s. n.* See *A. carruthii* 52.

APPENDIX I (continued)

Accession	Chromosome number	Locality
Artemisia tilesii ssp. *unalaskensis*		
3 (Warrendale)	$2n=18_{II}$	Oregon: Multnomah County. Shaded by hardwood trees along a small rocky draw, beside Walker Road. 40 m.
6 (Starvation Creek)	$2n=18_{II}$	Oregon: Multnomah County. Roadside plant just above Starvation Creek State Park. 50 m.
45 (Teanaway River)	$2n=18_{II}$	Washington: Kittitas County. East bank of the Teanaway River at its influx with the Yakima River. In moist sand among boulders where the water has receded. Rare in the area. Associated with *Populus*, *Salix* and *Gilia*. *Palmblad* 4-1052.

§ Populations include either two or more taxa or intermedite genotypes.
* Determined from root-tip mitoses.
+ Determined from microspore mitoses.

APPENDIX II. LEAF OUTLINES OF SELECTED F_1 HYBRIDS AND THEIR PARENTS.

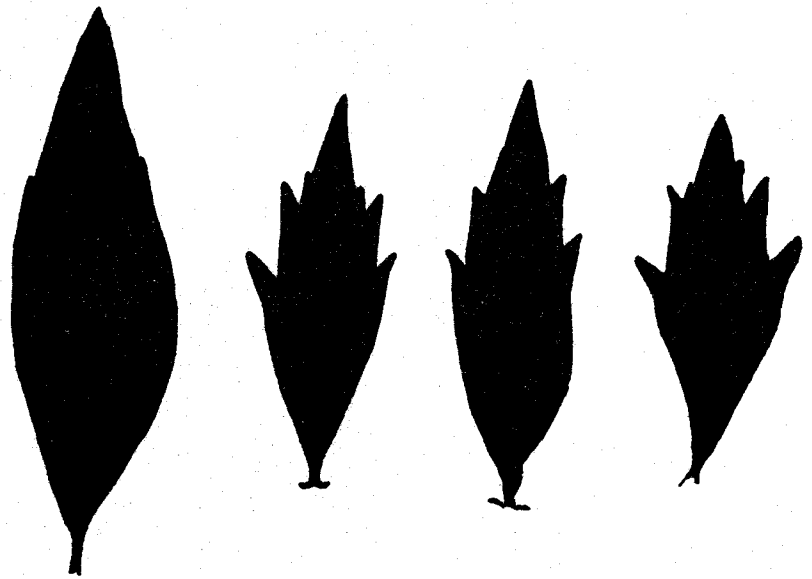


A B C D E

Artemisia cavaticaulis X Artemisia suksdorfii

(A) A. cavaticaulis (Clines Falls); (B) S_1; (C-D) hybrids; (E) A. suksdorfii (Gold Beach). 9/20 x

APPENDIX II. (continued)

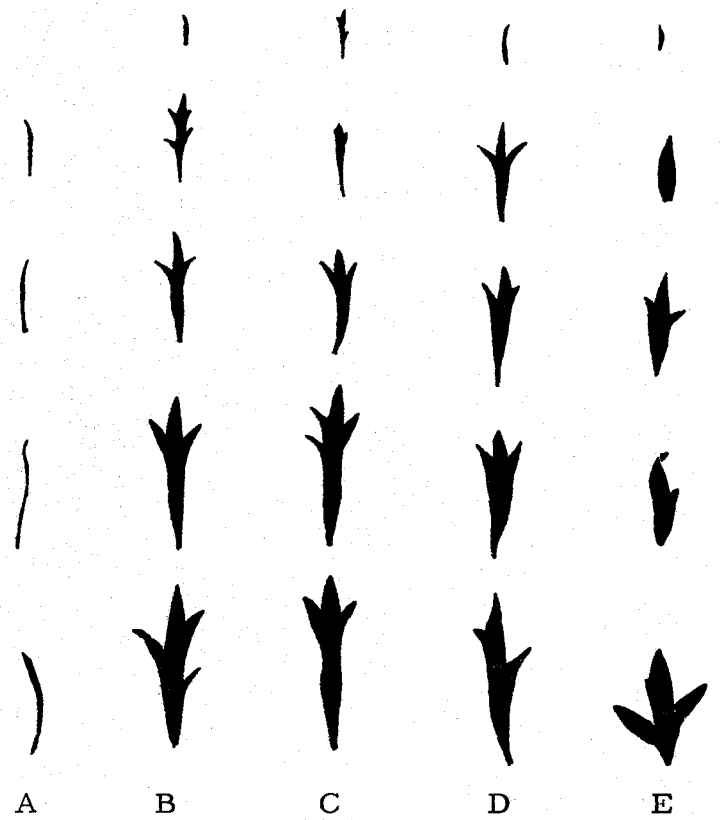


A B C D

Artemisia suksdorfii (open pollinated)

(A) A. suksdorfii (Multnomah Falls); (B-C) hybrids; (D) A. cavaticaulis (Clines Falls), putative staminate parent. 1/2 x

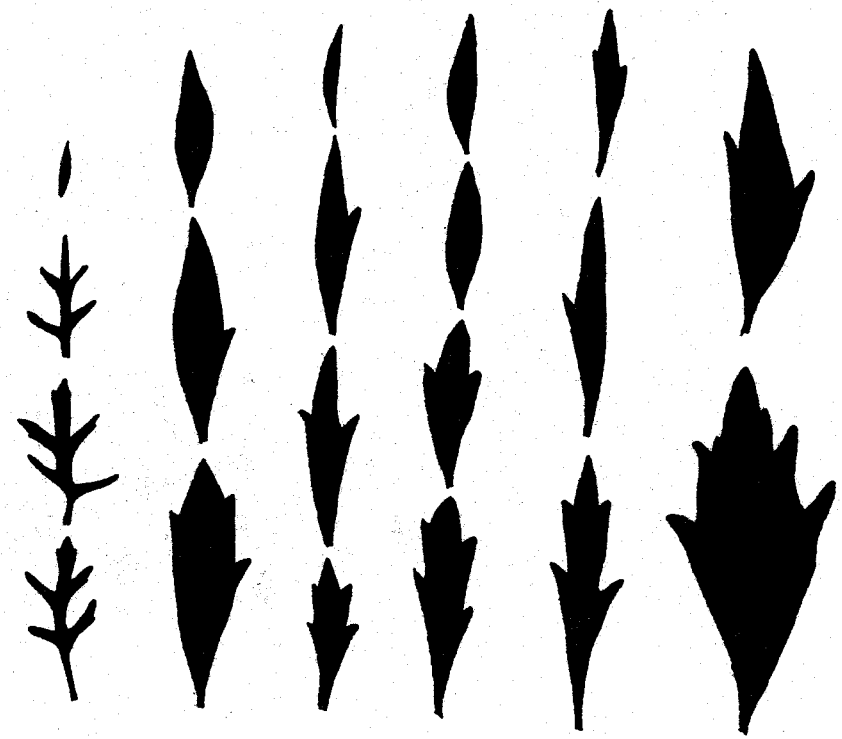
APPENDIX II. (continued)



Artemisia lindleyana (4x) X Artemisia ludoviciana ssp. candicans (4x)

(A) A. lindleyana (Hayden Island); (B-D) hybrids;
(E) A. ludoviciana ssp. candicans (Jacks Creek). 1/2 x

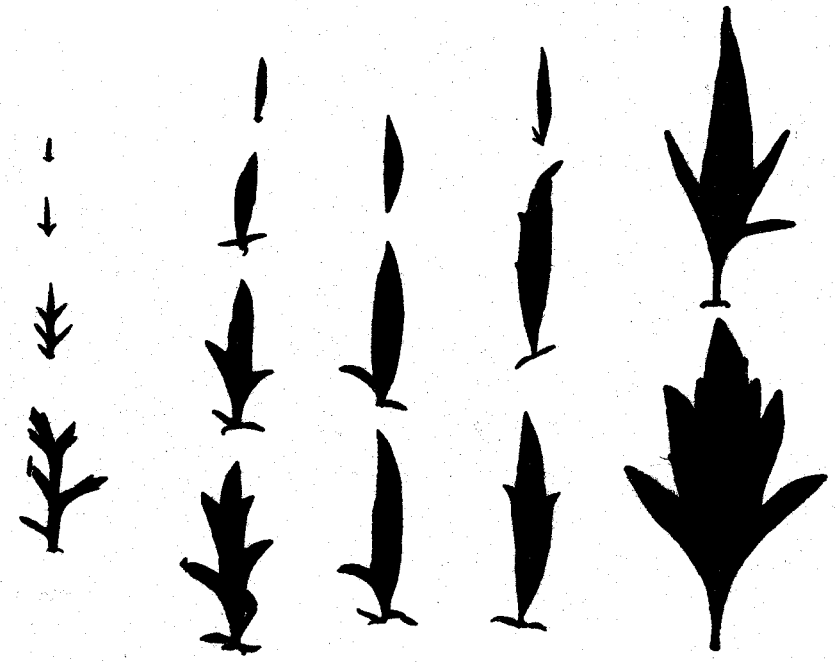
APPENDIX II. (continued)



Artemisia lindleyana (4x) X Artemisia cavaticaulis

(A) A. lindleyana (Wanapum); (B-E) hybrids;
(F) A. cavaticaulis (Clines Falls). 1/2 x

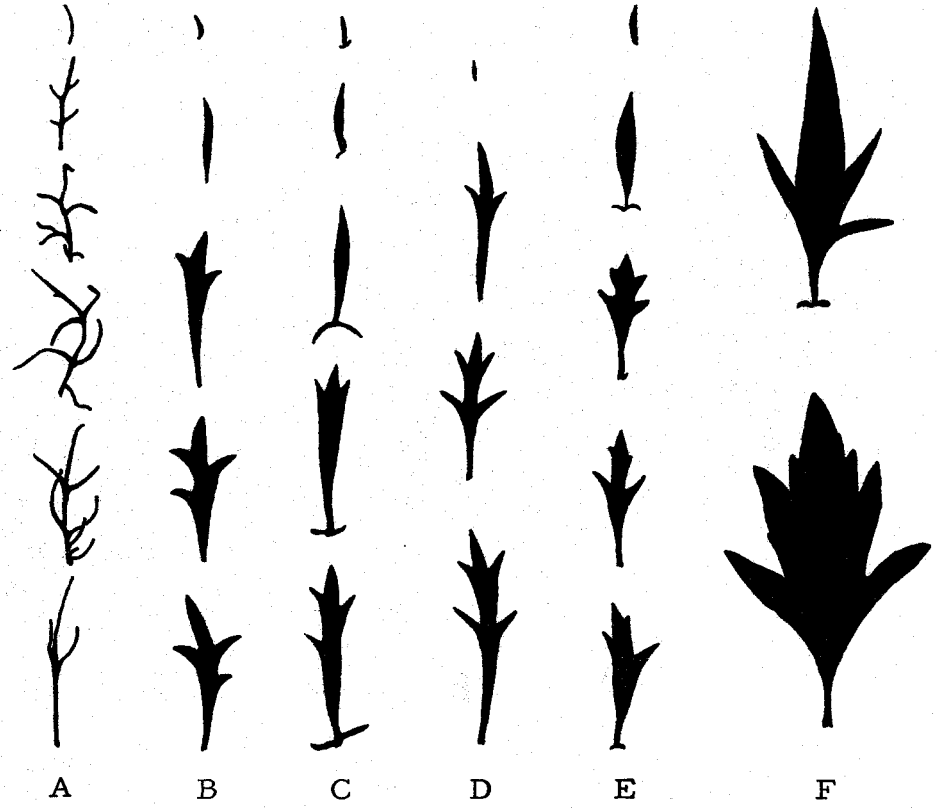
APPENDIX II. (continued)



Artemisia lindleyana (4x) X Artemisia cavaticaulis

(A) A. lindleyana (Wanapum); (B-D) hybrids;
(E) A. cavaticaulis (Clines Falls). 1/2 x

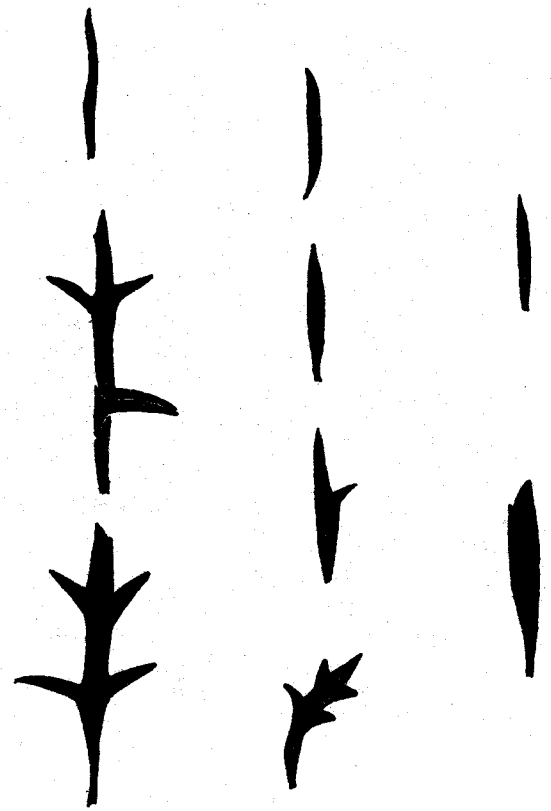
APPENDIX II. (continued)



Artemisia prescottiana X Artemisia cavaticaulis

(A) A. prescottiana (Mayer State Park); (B-E) hybrids;
(F) A. cavaticaulis (Clines Falls). 1/2 x

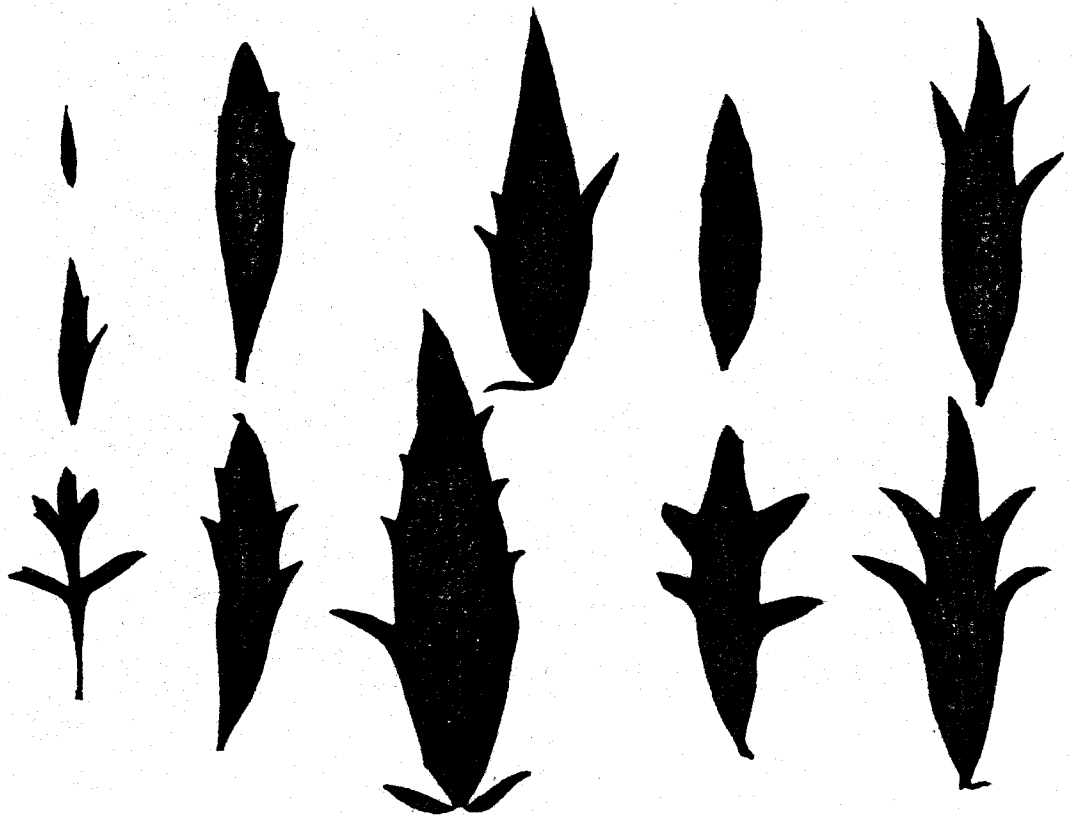
APPENDIX II. (continued)



Artemisia ludoviciana ssp. incompta (4x) X Artemisia lindleyana (4x)

(A) A. ludoviciana ssp. incompta (Pateros);
(B) hybrid; (C) A. lindleyana (Wanapum). 1/2x

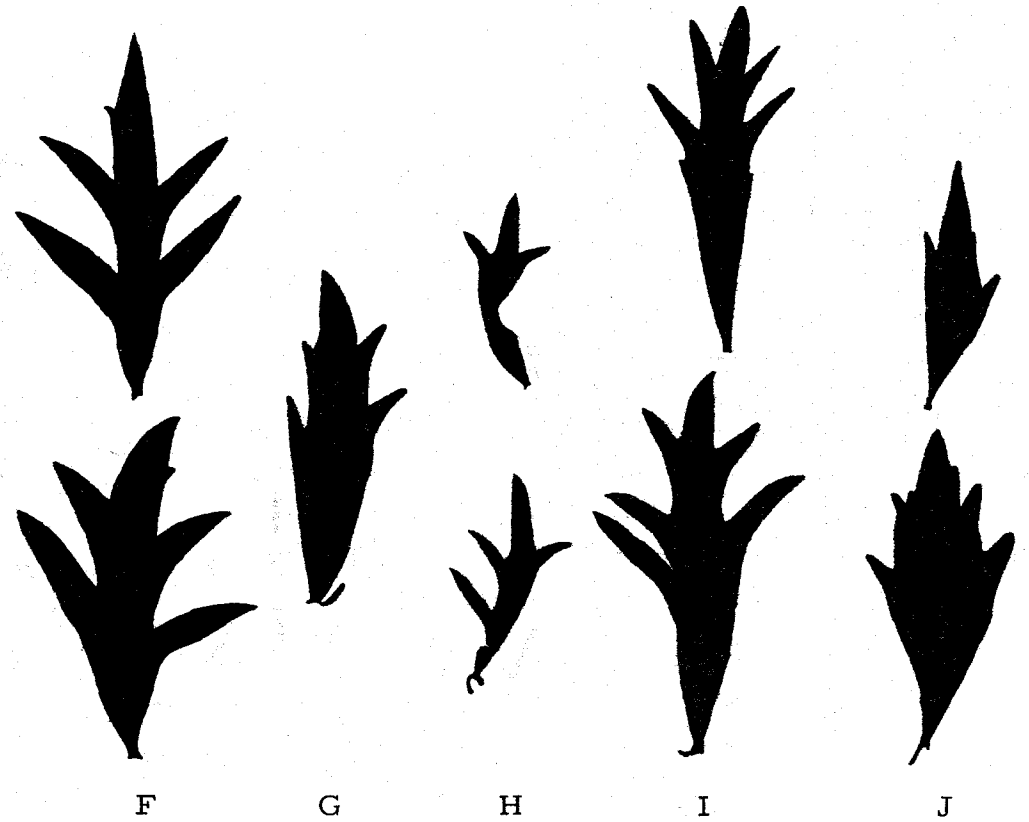
APPENDIX II. (continued)



Artemisia ludoviciana ssp. candicans (4x) X Artemisia cavaticaulis

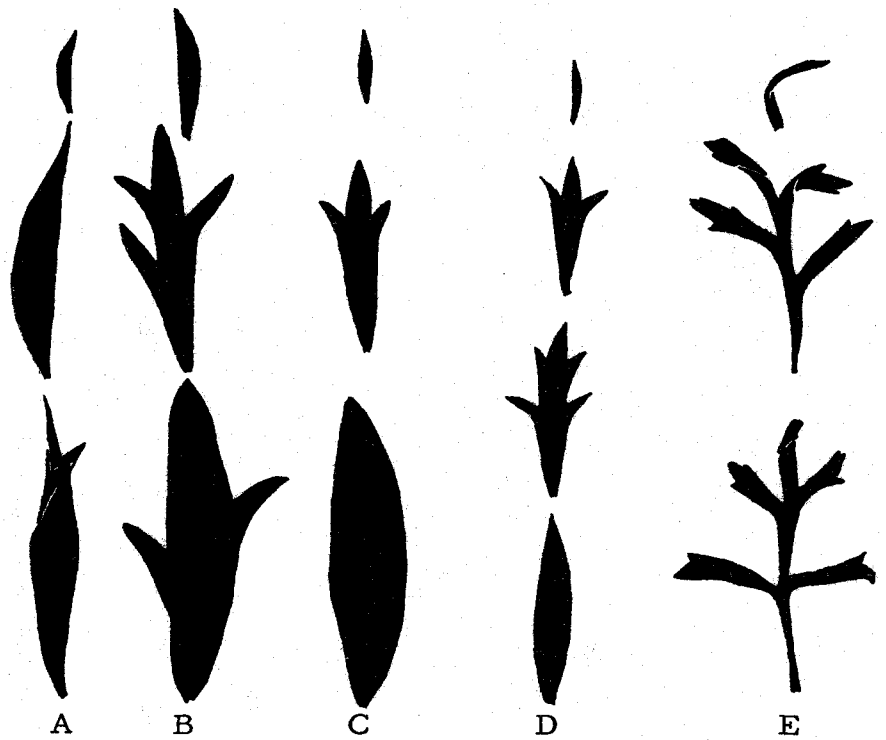
(A) A. ludoviciana ssp. candicans (Kettle Falls); (B-E) hybrids. 9/20 x

APPENDIX II. A. ludoviciana ssp. candicans X A. cavaticaulis, (continued)



(F-I) hybrids; (J) A. cavaticaulis (Clines Falls). 9/20 x

APPENDIX II. (continued)



Artemisia douglasiana (4x) X Artemisia ludoviciana ssp. candicans (4x)

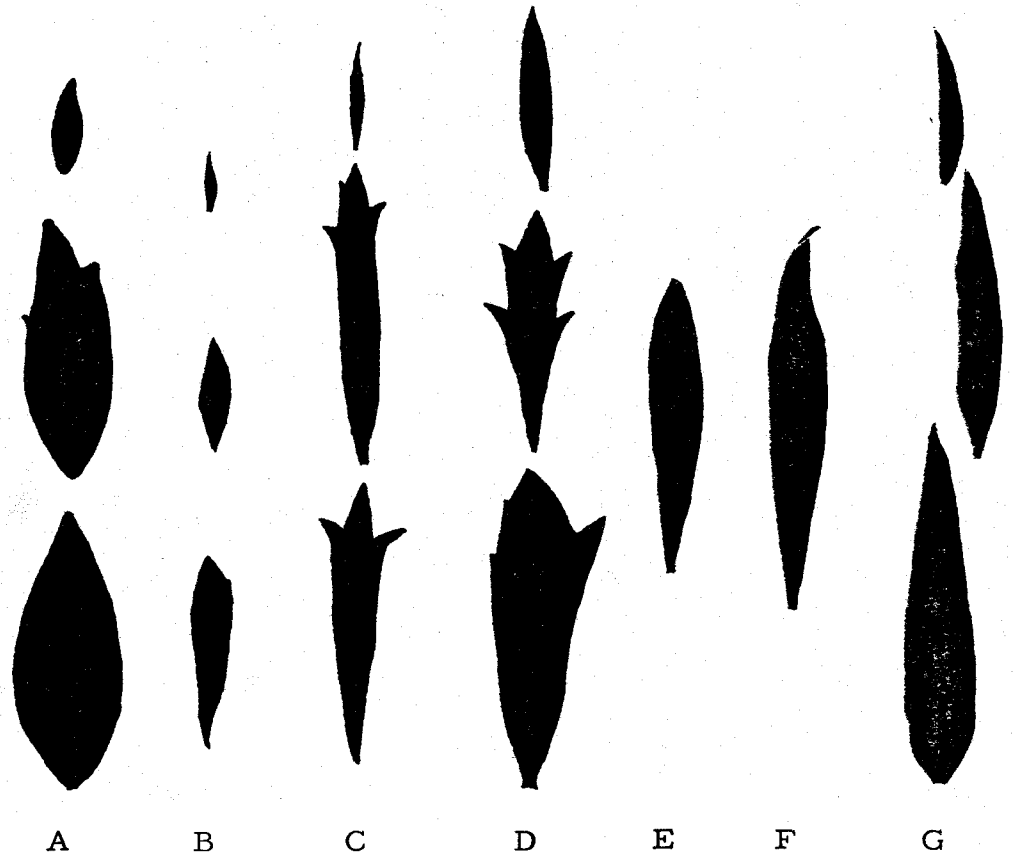
(A) A. douglasiana (Thrall); (B-D) hybrids;
(E) A. ludoviciana ssp. candicans (Cedar). 1/2 x

APPENDIX II. (continued)

Artemisia douglasiana (4x) X Artemisia lindleyana (4x)

(A) A. douglasiana (Satus Creek); (B-C) hybrids;
(D) A. lindleyana (Blalock). 1/2 x

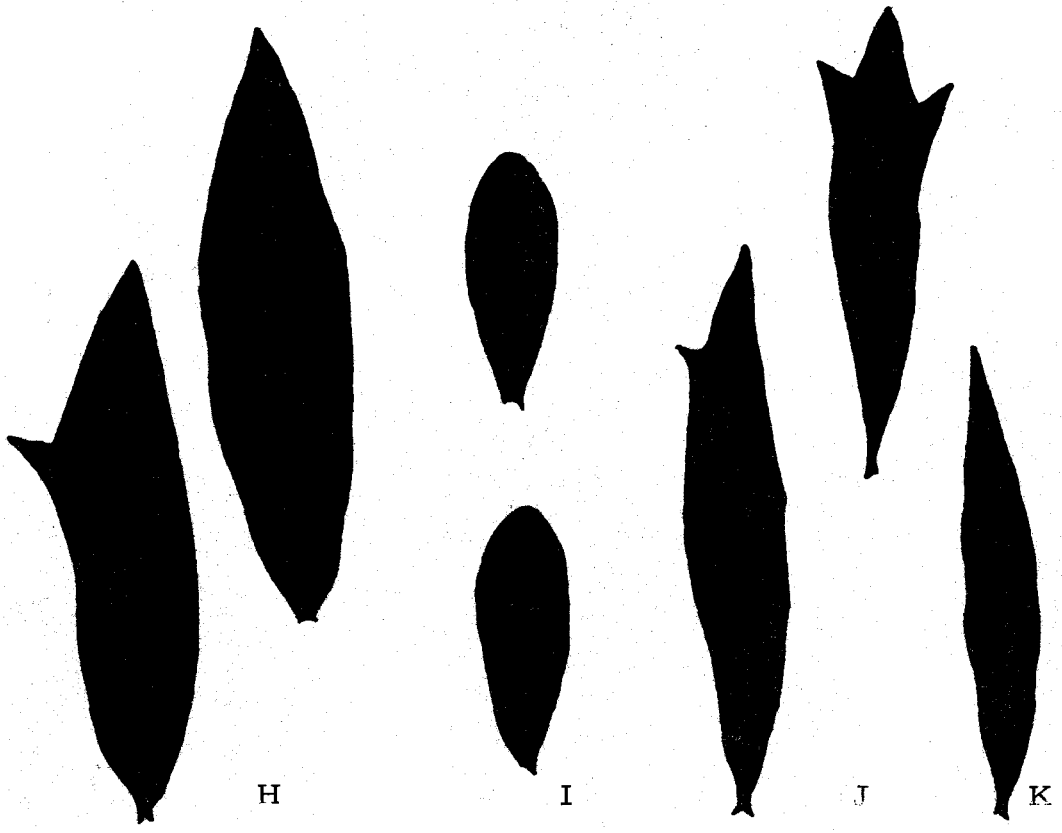
APPENDIX II. (continued)



Artemisia douglasiana (4x) open pollinated

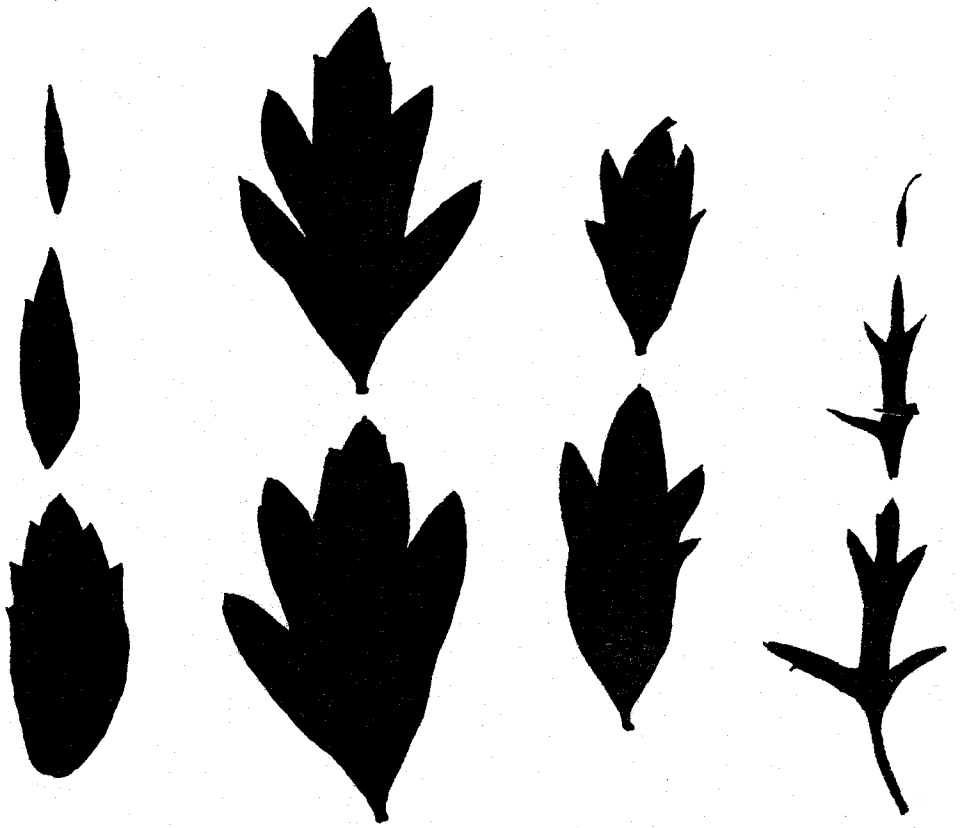
(A) A. douglasiana (Satus Creek); (B-G) hybrids. 9/20 x

APPENDIX II. A.douglasiana open pollinated, (continued)



(H-K) hybrids. 9/20 x

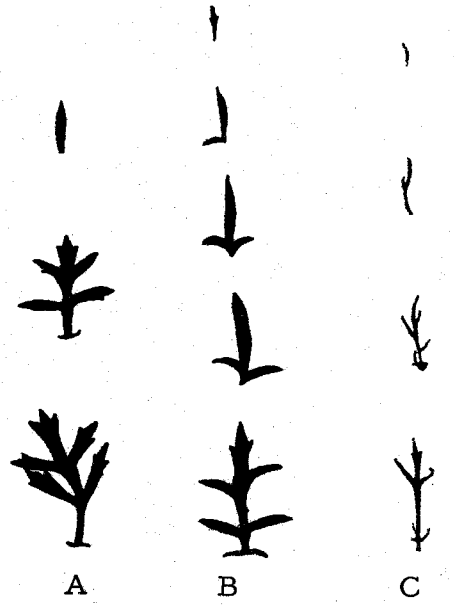
APPENDIX II. (continued)



Artemisia douglasiana (6x) X Artemisia ludoviciana ssp. incompta (4x)

(A) A. douglasiana (Alturas); (B-C) hybrids;
(D) A. ludoviciana ssp. incompta (Pateros). 9/20 x

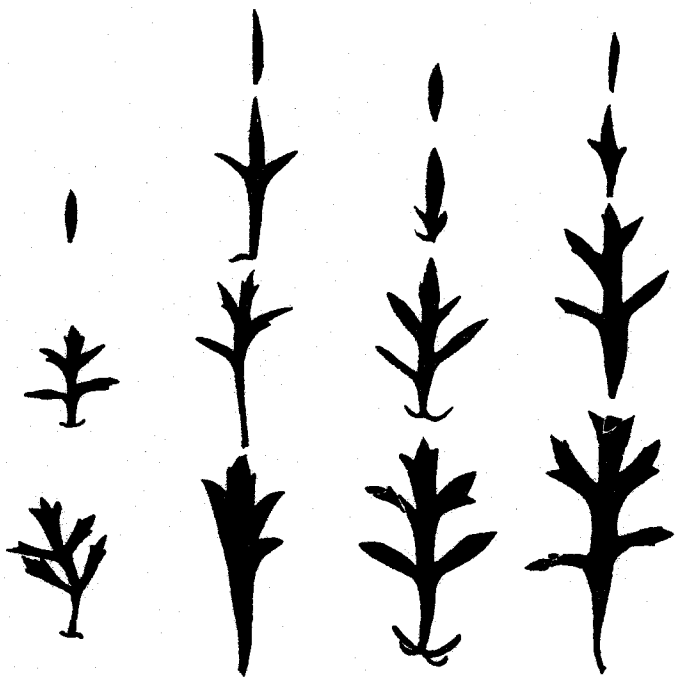
APPENDIX II. (continued)



Artemisia michauxiana (4x) X Artemisia lindleyana (4x)

(A) A. michauxiana (Steens Mountain); (B) hybrid;
(C) A. lindleyana (Mayer State Park). 1/2 x

APPENDIX II.(continued)



Artemisia michauxiana (4x) X Artemisia ludoviciana ssp. incompta (4x)

(A) A. michauxiana (Steens Mountain); (B-C) hybrids;
(D) A. ludoviciana ssp. incompta (Waiwai). 1/2 x

APPENDIX III. RELEVANT DATA FOR SPECIMENS USED IN POLLEN STUDIES.

Accession	Mean pollen diameter (μ)	Chromosome number	Locality
Artemisia michauxiana			
M-1	26.4±.2	n=18	Oregon: Harney County. Basaltic outcropping of canyon rim above Indian Creek, Steens Mountain. 2600 m. *Estes 119A.*
M-2	26.0±.4		Oregon: Harney County. Scattered in grass-sedge zone; dry loamy soil; rocky hillside; open; Steens Mountain. 2600 m. *Chas. G. Hansen 573* (OSC).
M-3	25.6±.2	(Partially sterile)	Oregon: Harney County. Dry canyon rimrocks; alpine; Dino Summit, Steens Mountain. 2750 m. *Percy Train n. s.* (OSC).
M-4	25.3±.2		Nevada: White Pine County. South side Sherman Ridge, south end of Ruby Range. 3050 m. *C. L. Hitchcock and J. S. Martin 5651* (OSC).
M-5	23.8±.4		Washington: Kittitas County. At Mt. Lilian, Table Rock Area, just below summit, 2 mi. off Liberty-Wenatchee Road, about 1700 m., loose talus of conglomerate and sandstone. *A. R. Kruckeberg 3046* (OSC).
M-6	23.8±.2		Nevada: Clark County. Kyle Canyon. Rainbow Falls. Hillside. Charleston Mountains. 2650 m. *I. W. Clokey 8568* (OSC).

APPENDIX III. (continued)

Accession	Mean pollen diameter (μ)	Chromosome number	Locality
Artemisia michauxiana (continued)			
M-7	23.5±.2		British Columbia: Kootenay County. Talus; Sage Creek, Flathead. 1600 m. J. Davidson Bell n. s. (BC).
M-8	23.2±.2		Nevada: Clark County. See M-6. I. W. Clokey 178526 (US).
M-9	23.2±.3	n=9	Washington: Kittitas County. Along talus shore of mountain stream above Liberty-Beehive Trail. 1800 m. Estes 106A.
M-10	23.0±.2	n=9	Washington: Kittitas County. See M-9. Estes 106H.
M-11	21.8±.5	n=9	Washington: Kittitas County. See M-9. Estes 106C.
M-12	21.8±.1		British Columbia: Yale County. Open space in heavy timber; Thunder Lake, Manning Park. 1100 m. K. I. Beamish, S. Stone, and F. Vrugtman 7809 (BC).
M-13	21.7±.4		Washington: Yakima County. Along spring, moist places under rim of canyon, Ridge of Wonders. Mt. Adams, James Langdon 168 (OSC).
M-14	21.5±.2		Washington: Yakima County. Alpine slopes of a canyon. East Mt. Adams. L. F. Henderson 1771 (OSC).

APPENDIX III. (continued)

Accession	Mean pollen diameter (μ)	Chromosome number	Locality
Artemisia michauxiana (continued)			
M-15	21.4±.5		Washington: Yakima County. See M-13. James Langdon 168 (OSC).
Artemisia ludoviciana ssp. *incompta*			
I-1	26.0±.2		Montana: Powell County. North Fork about 15 mi. Ovando. J. E. Kirkwood 1348 (ORE).
I-2	25.1±.4		Montana: Lake County. St. Mary's Lake. St. Ignatius, Mission Mountains. 1200 m. J. E. Kirkwood 1347 (ORE).
I-3	24.7±.3		Nevada: Clark County. Kyle Canyon. Low Hills, with *Pinus scopulorum* and *Cerocarpus ledifolius*, Charleston Mountains. 2200 m. I. W. Clokey 8567 (ORE).
I-4	24.1±.1		Oregon: Harney County. In aspen zone, with sagebrush in open meadow; dry loamy soil; hillside, west exposure. Steens Mountain. 2150 m. Chas. G. Hansen 592 (OSC).
I-5	23.5±.3		Oregon: Harney County. Common on dry sandy-rocky streambank in juniper zone; east slope of canyon; open. Steens Mountain. 1900 m. Chas. G. Hansen 1003 (OSC).

APPENDIX III. (continued)

Accession	Mean pollen diameter (μ)	Chromosome number	Locality
Artemisia ludoviciana ssp. *incompta* (continued)			
I-6	23.4±.2		Nevada: Clark County. Yellow pine belt; Charleston Park. Charleston Mountains. 2300 m. I. W. Clokey 7387 (US).
Artemisia ludoviciana ssp. *ludoviciana*			
Lu-1	25.4±.4	n=18	Oregon: Crook County. Gravel bar in the Crooked River bed, with Salix. 1000 m. Estes 31B.
Lu-2	25.0±.4	n=18	Oregon: Lake County. Sandy, rocky soil at the mouth of an outwash above Summer Lake with Achillea and Chrysothamnus. 1300 m. Estes 14D.
Lu-3	24.9±.1	n=18	Oregon: Lake County. See Lu-2. Estes 14A.
Artemisia ludoviciana ssp. *candicans*			
Cd-1	25.0±.4	n=18	Oregon: Klamath County. Open meadow on edge of pine forest, with A. tridentata and Chrysothamnus. West of Beatty where Oregon 66 crosses Modoc Billy Creek. Estes 9B.
Cd-2	23.3±.3	n=18	Oregon: Morrow County. Sand and gravel shore of the Columbia River in Boardman. Mixed population of A. lindleyana and A. ludoviciana ssp. candicans. 70 m. Estes 34C.