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A cytogeographic study, the relationship between chromosome number and geographical and ecological distribution, was made of the tetraploid and hexaploid chromosome forms of Achillea millefolium L. in western Oregon. Principal objectives of the investigation were three. First was a determination of the exact distributions of the two levels, as previous studies had indicated only the general distributional patterns. Second was a search for pentaploid hybrids at any contact zones between the two levels; and third was a determination of the factors enabling the interior tetraploid form to invade the coastal areas of Coos and Curry Counties, Oregon.

Distributional studies were carried out by chromosome counts of population samples from transects in western Oregon and northwestern California, the counts being from squashed mounts of microspores undergoing the first post-meiotic division. Internal pollen

diameters were utilized as indicators of ploidy level.

Results disclosed that the distributions of the two chromosome forms are not as simple as previously reported, but involve overlapping distributions, mixed populations, disjunct populations, and penetration of one form into areas occupied by the other. Pentaploid hybrids were found to occur in nature, morphologically resembling their tetraploid and hexaploid progenitors, and showing meiotic pairing of 18 II's and 9 I's with random distribution of the univalents. The maximum internal diameter of good binucleate pollen grains can be utilized as an indicator of ploidy level, although with some reservations. The distribution of the coastal tetraploid form in southwestern Oregon was correlated with the Klamath Mountains geological area. Plants of this area, especially those at its distributional limits, were observed to be producing a large number of tetraploid pollen grains that were apparently viable.

Several hypotheses are advanced as to the nature of the distributional pattern in western Oregon, the origins of the North American hexaploid chromosome form, and the cytogeographical patterns exhibited by the two chromosomal levels.

CYTOGEOGRAPHY OF ACHILLEA MILLEFOLIUM L. IN WESTERN OREGON

by

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CYTOGEOGRAPHY OF ACHILLEA MILLEFOLIUM L. IN WESTERN OREGON

I. INTRODUCTION

Previous research on the Achillea millefolium L.-complex in western North America has revealed that two chromosome forms exist, a tetraploid (n=18) and a hexaploid (n=27). Distributional studies by Ehrendorfer (1952b) indicate that the tetraploid occurs across the interior of North America, reaching the Pacific Coast in only three areas. One area is the coastal marshlands of Marin County, California, and portions of the adjacent counties of Napa, Solano, and Contra Costa. The second area is the coastal regions of Del Norte County, California, and Coos and Curry Counties, Oregon. The third is on the Olympic Peninsula in Jefferson and Clallam Counties, Washington, and Vancouver Island, British Columbia. The hexaploid is distributed along the Pacific Coast, being divided into three spatially isolated entities by the intrusion of the tetraploids in southwestern Oregon and on the Olympic Peninsula.

Distinguishing the two chromosome levels by external morphological criteria is extremely difficult due to population variability and individual plasticity, and the two levels have been delegated different taxonomic circumscriptions and ranks by various researchers. Although cytological investigations of Achillea by Clausen, Keck, and

Hiesey (1938), Turesson (1939), Lawrence (1947), Ehrendorfer (1952b), and Mulligan and Bassett (1959) indicate the general distribution of the two levels in Oregon, exact distributions are still unknown.

The objectives of the research reported here are three. First is a determination of the geographical distribution in western Oregon of tetraploid and hexaploid chromosome forms of Achillea millefolium L. Second is a search for hybrids at any contact zones between the two levels; and third is a determination of what environmental factors permit the tetraploid form to spread from the interior into the coastal areas of Coos and Curry Counties, Oregon. The present work is limited in its scope to the northern face of the tetraploid zone and does not examine the southern distributional limits in northwestern California.

Studies of plant distribution were carried out by chromosome counts of population samples on transects in western Oregon and northwestern California, these counts being primarily from squashed mounts of microspores undergoing the first post-meiotic division in anthers. Internal pollen diameters were also utilized as an indicator of ploidy level, as was done by Ehrendorfer (1952b) and Mulligan and Bassett (1959). From initial cytological studies, contact areas between the tetraploids and hexaploids were located, investigated in

the field, and verified for the presence of pentaploid hybrids. Changes in the chromosome number along the transects were examined for any possible correlations with environmental changes.

II. LITERATURE REVIEW

Taxonomic Studies

Achillea L. is a genus in Compositae-Anthemideae with approximately 75-100 species. It comprises plants whose breeding system is characteristically entomophilous, sexual, and outcrossing. With its greatest diversity in southeastern Europe and southwestern to central Asia, the genus is composed of diploids (n=9) and numerous polyploids at the tetraploid, hexaploid, and octoploid level. Hegi (1928) divides the genus, on morphological characteristics, into six sections: Ptarmica (Necker) Koch, Filipendulinae Boissier, Babounya (DC.) Hoffm., Santolinoidea DC., Arthrolepsis Boissier, and Millefolium (Adans.) Koch.

The Achillea millefolium L.-complex with circumpolar distribution in temperate zones of the northern hemisphere, is described by Ehrendorfer (1952b) as being composed of isolated diploid species with derived polyploid hybrids. In Eurasia there are four diploids (n=9): A. setacea W. & K., occurring on dry, sandy steppes; A. asplenifolia Vent., found in low moor vegetation; A. roseo-alba Ehrend., an introgressive hybrid of A. setacea and A. asplenifolia; and A. tomentosa L. whose relationship to the complex is questioned. Achillea collina Becker is a tetraploid (n=18) found in dry grasslands and open oak forests. Achillea pannonica Scheele is an octoploid

(n=36), distributed on dry slopes. Eurasian hexaploids (n=27) are classified as A. stricta Schleicher and A. millefolium L. sensu stricto. A. stricta, of open mountain forests, is believed to be more closely allied to the A. tanacetifolia All.-complex. This complex is not sharply differentiated from A. millefolium, the most widely distributed species and one which is found in areas with adequate amounts of summer water.

Species of the complex have radiated into many ecological niches with a greater differentiation of races and species in Europe than in North America, where diploids have not been found. Turesson (1939), however, commented that the complex is represented in North America by well marked ecotypes. In this region, the complex has been worked on taxonomically, and varying opinions have been expressed. Pollard (1899) noted that both A. ptarmica L. and A. millefolium occur in eastern North America and that Nuttall in 1834 described A. lanulosa as being distinct from the eastern A. millefolium. Bongard in 1832 described A. borealis, encompassing the achilleas of Alaska and northern British Columbia. Hooker (1834) in his Flora Boreali-Americana classified the plants of this region as A. millefolium.

About 1900, various western botanists such as Pollard, Heller, and Rydberg described several species in Achillea, but major taxonomic work began with that of Clausen, Keck, and Hiesey (1938,

1940, 1948) in their experimental studies on the nature of species. Utilizing chromosome counts and morphological observations these investigators (1940) developed a pattern of relationships in the western North American complex. The complex was divided by them into three ecospecies, retaining the names proposed by Nuttall and Bongard; two ecospecies were hexaploids and one was tetraploid. One hexaploid was A. millefolium L., the Eurasian species said to occur throughout the eastern portion of the United States. The second hexaploid was A. borealis Bong., occurring along the Pacific Coast from Alaska to Baja California, and comprised of three ecotypes which were recognized as subspecies. One of these was A. borealis ssp. arenicola (Heller) Keck, a maritime ecotype of the Oregon and California coastal dunes. A second was subspecies californica (Pollard) Keck, distributed in the Coast Range, Washington to Baja California; and the third was subspecies typica Keck, of the Arctic coasts of Alaska and British Columbia. The tetraploid ecospecies, Nuttall's A. lanulosa, was described as being highly polymorphic but divisible into two ecotypic subspecies. The first was subspecies typica Keck, widely distributed throughout the interior of North America, and the second was subspecies alpicola (Rydb.) Keck, of the alpine and subalpine Sierras, Cascades, and Rocky Mountains.

Between 1940 and 1960, experimental analyses by Clausen, Keck, Hiesey, and Nobs on the Achillea ecotypes revealed information

as to the nature of the complex. They state (1948, p. 1) "...the series of variations within the complex have no morphological breaks but consist of trends that seem to be indissolubly linked together by finely graded morphological steps." The problem of morphological gradations is also reported by Lawrence (1947) in his distributional studies of the genus. He noted that chromosome number provided the only dependable means of distinguishing between A. lanulosa and A. borealis because morphological characters were inadequate. Ehrendorfer (1952b) confirmed this, discovering that pollen grain size was the only definite character for separation of tetraploids and hexaploids as herbarium specimens. The taxonomic treatment of Clausen, Keck, and Hiesey, described above, was followed by several authors of floras encompassing the Pacific Northwest.

A second taxonomic opinion of the complex is that there is one species, A. millefolium L., extremely polymorphic but with recognizable ecotypes which may be designated as varieties. Nobs (1960) developed this concept, describing on morphological characters ten varieties, which he considers to be generally distinct but with intergrading intermediates. Thus the species and subspecies of Clausen, Keck, and Hiesey are reduced to varieties.

As the preceding discussion shows, the genus Achillea in North America has been subjected to considerable taxonomic revision at the specific and infraspecific level. This is due to its extreme

variability and morphological continuity. The taxonomist in attempting to develop a classification is confronted with the problem of the relationship between morphological and chromosomal discontinuities. Should a difference in chromosome number be emphasized in a formal taxonomy, or should it be considered equally with other characters? This problem is one aspect of the controversial subject of the nature of species.

Reproductive isolation is the major premise of the "biological species concept," a popular species definition being argued pro and con by taxonomists and geneticists. Löve (1964, p. 43) states:

> Biosystematists have shown that real species, or perhaps rather the biological species... is a natural and non-arbitrary unit of a genetically closed population system that has lost its ability to interbreed with other such systems. It usually coincides with the Linnaean species selected by aid of the reproductive gap. The genetical barrier has been found to be caused by cytological differences, and so it can be discovered and defined by aid of cytological methods.

Observations on various plant groups have revealed that there may be differences in chromosome number without accompanying morphological differences, and differences in morphology without differences in chromosome number. Illustrations of the former situation are found throughout the taxonomic literature. Work by Baldwin (1941) on the monotypic genus Galax L. disclosed the presence of a tetraploid chromosome form believed to be of autoploid origin. This tetraploid is distinguished only by slightly thicker leaves and more

robustness than its diploid progenitor. In the genus Claytonia L. there is present a vast array of polyploids and aneuploids, with chromosome numbers ranging from n=6 to n=36 and with few distinctive morphological differences (Rothwell, 1959; Rothwell and Kump, 1965). Mosquin (1963, 1966a, b) reports the existence of three chromosomal races, diploid, tetraploid, and hexaploid, within the single species Epilobium angustifolium L. stating (1966b, p. 205), "The name Epilobium angustifolium has remained stable, uncontroversial, biologically meaningful, and useful since the species was first described by Linnaeus." Similar situations have been detected in Sedum L. (Baldwin, 1942), Clarkia Pursh section Myxocarpa (Mosquin, 1966b), and Narcissus L. (Fernandes, 1951).

Mosquin (1966b) notes that morphologically distinct but reproductively compatible groups can also be found in nature, with barriers to reproduction being ecological rather than chromosomal. A classic example is the genus Platanus L., n=21, with two distinctive and spatially separated species, P. occidentalis L. and P. orientalis L. (Fernald, 1931). The experimentally produced hybrid, described as P. acerifolia Willd., is characterized by vigor and fertility with normal meiosis (Sax, 1933). The genus Catalpa Scop. (Smith, 1941) presents a second example of two spatially disjunct species capable of hybridizing to produce fully fertile progeny. Mosquin (1966b) comments that similar situations are found in Abies,

Salix, Delphinium, Aquilegia, and Cattleya.

In Achillea this problem of a cytological discontinuity and a morphological continuity was recognized by early researchers. As mentioned previously, Turesson (1939) commented on the slight morphological differences and ecotypic series at both levels, ranking them as ecospecies as did Clausen, Keck, and Hiesey (1938). In 1948 Clausen, Keck, and Hiesey again noted that chromosome numbers provided the only means of separation in areas of contact between the two forms. Lawrence (1947) and Ehrendorfer (1952b) concurred.

View set forth by Raven (1962, 1963) illustrate the complexity of the group and the importance of individual interpretation. In 1962, observing that polyploidy is a special type of reproductive isolation, he stated that chromosomal races without morphological characters of differentiation should not be recognized as species, but possibly only as subspecies. In 1963 he presented an opposing opinion, considering the tetraploid and hexaploid chromosome forms in Achillea to be distinct species with major isolation barriers, differences in morphology, and separate systems of populations. Emphasis was placed on differences rather than similarities.

Distributional Studies

Distributional studies of the chromosome forms of Achillea in western North America began in the late 1930's with work of Clausen, Keck, and Hiesey (1938, 1940). They reported that the genus was represented by three species: a hexaploid species occurring along the Pacific Coast from Alaska to Baja California; a second hexaploid being well established along the East Coast following introduction from Europe; and a tetraploid species occurring throughout the interior of the continent. Each species was observed to be composed of a series of ecotypes. The absence of diploids was also noted.

Turesson in 1939 made a transect along the Pacific Coast sampling 16 different populations. Counting the root tips of three plants per accession, he reported that the hexaploid form was restricted to the coast and the tetraploid form distributed in the interior thus confirming the work of Clausen, Keck, and Hiesey. However, he commented that tetraploid plants were found on coastal bluffs near Gold Beach, Oregon, and within a few miles of the coast at Coquille, Oregon; and that the hexaploid plants were found 20 miles inland at Longvale, California.

Lawrence (1947) continued cytological investigations in an attempt to determine definite geographic boundaries between the two chromosome levels. Basing his study on 108 counts, he made

several observations as to the distributions of the two forms. The hexaploids occupy a narrow strip along the Pacific Coast except for intrusions in northern and central California, where they are found approximately 120 miles inland. The tetraploids are distributed in the interior, but extend to the coast in southwestern Oregon and replace the hexaploid completely between Coos Bay, Oregon and Clam Beach, California. Diploids are not present, but a single octoploid plant was found in Squaw Valley, California. Lawrence noted that even though there appears to be a lack of environmental and/or geographical barriers separating the two levels, no distinct overlap between them was found.

Ehrendorfer (1952b), utilizing data from approximately 70 more chromosome counts and pollen diameter measurements from herbarium specimens as indicators of ploidy level, extended Lawrence's distributional pattern to encompass the entire Pacific Coast. He indicated that the tetraploid occurs across the interior of North America, reaching the Pacific Coast in only three areas. One area is the coastal marshlands of Marin County, California, and portions of the adjacent counties of Napa, Solano, and Contra Costa. The second area is the coastal regions of Del Norte County, California, and Coos and Curry Counties, Oregon. The third is on the Olympic Peninsula in Jefferson and Clallam Counties, Washington, and Vancouver Island, British Columbia. The hexaploid is distributed

along the Pacific Coast, being divided into three spatially isolated entities by the intrusion of the tetraploids in southwestern Oregon and on the Olympic Peninsula. This work defined the distributional limits more exactly, with Ehrendorfer stating (p. 127), "Various chromosomal races replace one another in different localities and are only very rarely found growing side by side."

Determining ploidy level by the measurement of pollen grain diameters from herbarium specimens, Mulligan and Bassett (1959) published data supporting the distributional patterns of the preceding researchers. The results of investigations on the distribution of the tetraploid and hexaploid chromosome forms of Achillea are pictorially summarized in Figure 1.

A second aspect of chromosome distributional patterns in Achillea, not directly related to this author's research, is the occurrence of hexaploids along the Atlantic Coast. As previously mentioned, Clausen, Keck, and Hiesey (1940) stated that a Eurasian species, A. millefolium, was introduced and well established in the eastern United States; however, subsequent investigations by Turesson (1939) and Lawrence (1947) revealed only the presence of tetraploids. Ehrle in 1958 reported root-tip counts from 26 accessions throughout Pennsylvania, all of which were tetraploid. He also noted that literature surveys failed to disclose cytological proof of a hexaploid A. millefolium L. sensu stricto in the eastern United States. Mulligan

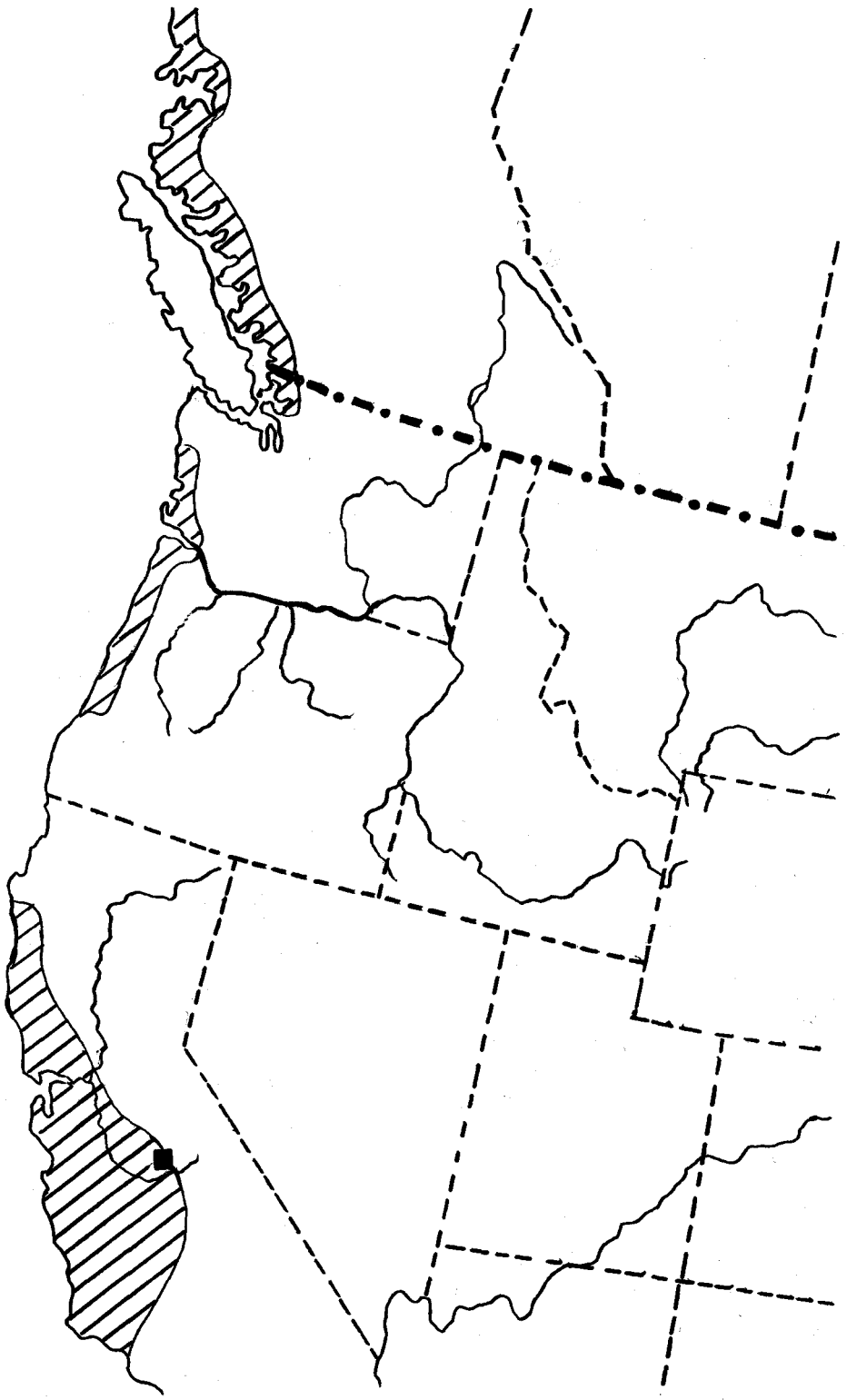

Figure 1. Distribution of tetraploid and hexaploid Achillea millefolium L. in western North America. Hexaploid, shaded area. Octoploid plant, square.

and Bassett (1959) confirmed this observation; but they reported hexaploids in Newfoundland and Nova Scotia, which upon closer examination were believed to be introduced. However, their pollen diameter studies indicated that the hexaploids of the Pacific Coast extended across northern Alaska and Canada. They state (p. 79): "...native A. borealis occurs not only along the Pacific Coast from southern California to the Aleutian Islands but also across the northern shorelines of Alaska, Yukon, Northwest Territories, Ontario, and Quebec."

Indicators of Ploidy Level

One aspect of the studies on the nature and the occurrence of polyploidy in higher plants has been the physiological and morphological effects resulting from an increase in chromosome number. Stebbins (1940, 1950) stated that the effects of polyploidy depend greatly upon the nature of the original genotype. Important phenotypic changes induced by polyploidy are: (1) An increase in cell size, particularly certain cells such as pollen grains or guard cells of stomata (Sax and Sax, 1937). (2) An increase in the water content of the cell, which is associated with changes in cell size. (3) A reduction in the growth rate with accompanying retardation and extension of flowering time. (4) Changes in various organs of the plant, predominantly determinate floral parts and seeds. Of these

changes the most common and notable are changes in cell size. Different investigators have employed this phenomenon in researches on various taxa. Babcock and Stebbins (1938) utilized stomate size as an indicator of ploidy level in their studies on apomixis in Crepis L. In general, the "larger" stomatal openings indicated a polyploid in contrast to the "smaller" stomates of the diploids. Noting that the sexual Crepis were normally diploid with small stomata and normal pollen and that the apomicts were polyploids with large stomata and sometimes abnormal pollen, they were easily able to determine distributions of the two types from herbarium specimens.

Measurements of pollen grain diameter has also been used in distinguishing various ploidy levels. In some cases the separation is definite while in others the correlation is somewhat indistinct. Bell (1954) in his work on the Sanicula crassicaulis DC.-complex employed such measurements in an attempt to separate diploids, tetraploids, hexaploids, and octoploids; but he discovered that intermediate sizes prevented distinct separations. Similar work has been done by Dean (1966) in Aster L., Gould (1957) in Andropogon L., and Heckard (1960) in Phacelia Juss.

In studies on Achillea several researchers have used the measurement of pollen diameters as a means of detecting the tetraploid and hexaploid forms in western North America. Ehrendorfer (1952b) reported a distinct correlation between pollen size and

chromosome number. Measuring the maximal inner width of stainable pollen grains, he reported the tetraploid pollen diameters to range between 20.5 and 23.5 microns and the hexaploid diameters to range between 24.0 and 28.0 microns. Diameters of measured European diploids were below 20.0 microns. Mulligan and Bassett (1959) similarly reported size differences for the two levels, utilizing measurements from spine to spine in polar view of ten well-formed pollen grains. Grains from herbarium specimens were measured and classified "tetraploid" or "hexaploid" depending upon whether the mean outside diameter was smaller or larger than 31.5 microns. In both papers there was no mention of overlap in the ranges of the mean diameters of the two levels.

Cytogenetic Studies

Beginning with the experimental studies on the nature of species by Clausen, Keck, and Hiesey in the early 1940's, data have been accumulating on the genetic structure of the ecological races of Achillea. Results of hybridization studies carried through several generations, between contrasting races at both the tetraploid and hexaploid level, have been combined with extensive cytological observations to develop a clear idea of the plant's cytogenetic system.

Clausen, Keck, and Hiesey (1940) reported the complex to consist of three species, one tetraploid with 18 pairs of chromosomes

and two hexaploids with 27 pairs. Cytological observations were that the somatic chromosomes were approximately one micron in width and four to six microns in length. In hexaploid forms some meiotic irregularity, giving rise to aneuploid plants, was noted. Lawrence (1947) observed that the somatic chromosomes were extremely long in paraffin-embedded preparations of root tips, and Ehrle (1958) reported that the majority of the chromsomes at the tetraploid level were of equal length with median centromeres. Four chromosomes with subterminal centromeres were observed to bear satellites, which Ehrle believed to indicate that the tetraploid arose from satellite-bearing diploid precursors either by autoploidy or alloploidy.

Clausen, Keck, and Hiesey (1940) also reported the results of selfing and reciprocal hybridizations between hexaploid A. millefolium L. from Denmark and A. borealis ssp. californica from central California, stating that the plants were completely cross-incompatible, highly self-incompatible, and not apomictic (on the basis of failure to produce viable achenes).

A voluminous amount of data on the cytology of Achillea and its hybrids has been published by Ehrendorfer (1952a, b; 1959a, b, c, d, e, f; 1960; 1961; 1964). The meiotic behavior of the majority of tetraploid and hexaploid plants is reported as normal, with meiotic irregularities being one of four types: (1) derangements in synapsis;

(2) derangements in chromosome reproduction; (3) derangements in primary spindle function; and (4) derangements in cell wall formation. Usually two to five percent of the microsporocytes examined had univalents and multivalents at meiotic metaphase one. Other cytological observations, especially on interracial hybrids, revealed the presence of bridges, fragments, lagging chromosomes, and micronuclei; an occasional complete breakdown in meiotic behavior resulted in the formation of a restitution nucleus. The frequency of these aberrations varied from plant to plant and race to race, with anywhere from two to fifty percent of the tetrads affected. Ehrendorfer (1959a, p. 148), noting that, "A prerequisite to the successful establishment of sexual polyploids is the normalization of meiotic chromosome pairing," comments that cytological observations indicate: (1) the genomes of Achillea are essentially structurally homologous; (2) there is little multivalent formation and associated meiotic irregularities, even with a high frequency of chiasmata; (3) there is genetic regulation of the meiotic behavior of chromosomes; and (4) there are defects in spindle behavior and cell wall formations.

In studies on the western North America tetraploids (1959e), he made an analysis of 51 individual plants, observing that seventy-five percent of the plants had no meiotic abnormalities and the remaining individuals had some univalents, trivalents, and higher multivalents. He found in one plant a meiotic bridge at first anaphase

persisting into metaphase II. Defective cell wall formations were also found, in which the walls formed successively rather than simultaneously as is characteristic of Achillea. However, Ehrendorfer indicated that meiotic disturbances are not as pronounced in North American tetraploids as in Eurasian ones.

At the hexaploid level, he examined 21 individuals from western North America finding no defects in meiotic processes or spindle apparatus except for an occasional univalent, multivalent, or unpaired homologue. Some aneuploids were found, which were believed due to irregular chromosome distributions in both anaphase I and II.

As evidence that natural hybridization between the two ploidy levels occurs, a partially fertile pentaploid hybrid ($2n=45$) and various aneuploid segregates were reported (Schneider, 1958; Ehrendorfer, 1959a) from Europe and North America.

III. MATERIALS AND METHODS

In order to determine the distributions of the two chromosome forms of Achillea, a series of collections was made along transects in western Oregon and northwestern California during the summer months of 1966. These transects are presented in Figure 2. Initial collections were made at ten-mile intervals. They involved first a random sampling of material, followed by collections from specific plants noted to have unusual morphological characteristics. A population sample normally consisted of material from two or three separate plants. At each collection site, heads in various stages of flowering were collected and placed in vials containing a modified Carnoy's killing-fixing solution (chloroform, 95% ethanol, glacial acetic acid; 6:3:1). Each locality was assigned an accession number and located by range, township, and section. Observations as to habitat and environment were also recorded. Due to the clonal habit of Achillea, it was possible to collect from each clump a pressed voucher specimen and rhizomes for transplanting to the greenhouse at Oregon State University. Collected buds were kept in the killing-fixing solution for a minimum of 24 hours, then washed and stored in 70% ethanol at 4°C until prepared for cytological observations.

The collected heads were stained in bulk, using Snow's

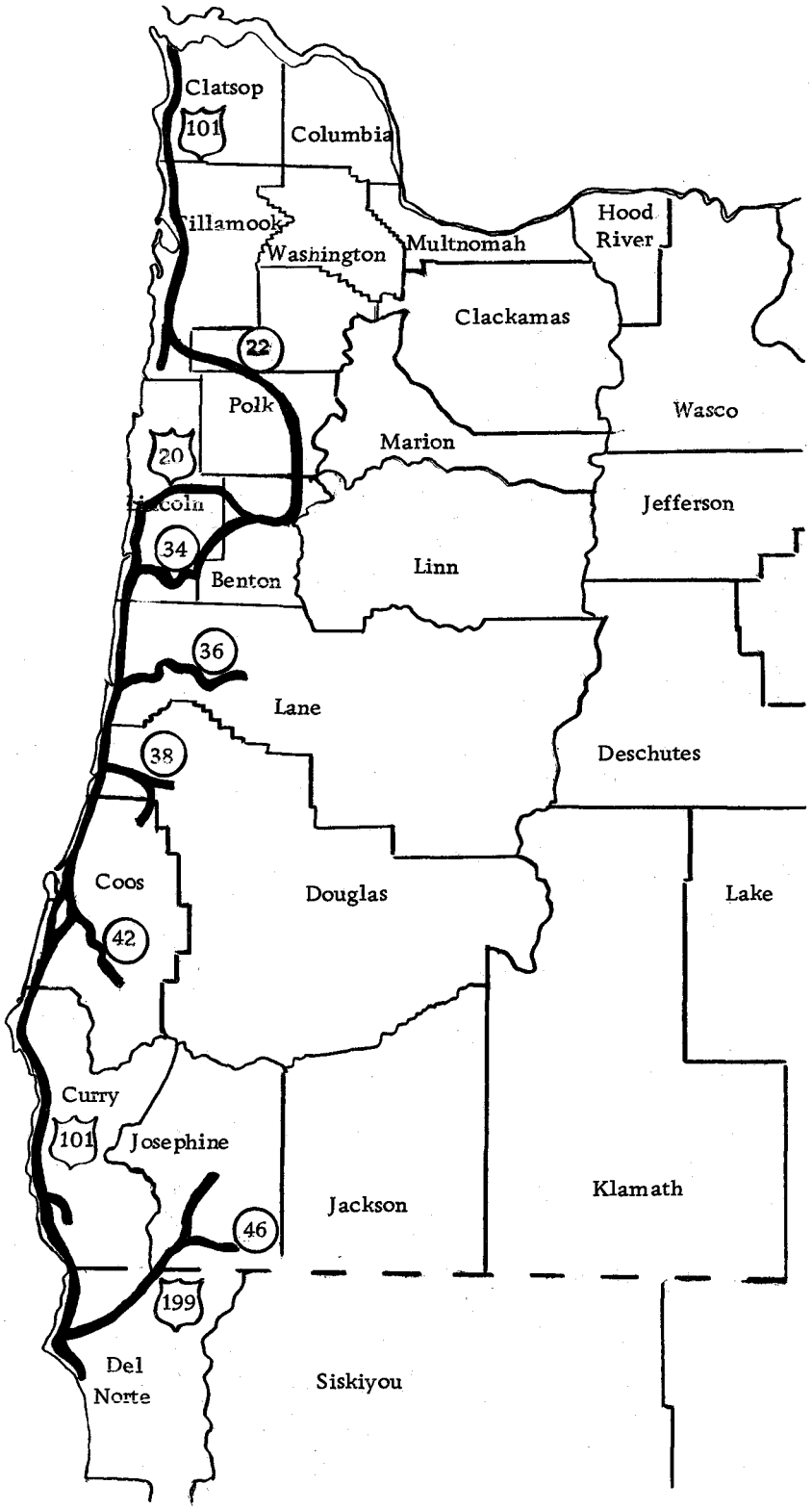

Figure 2. Collection transects for Achillea accessions. Collections were primarily from highway right of way. Transects in bold line.

carmine stain (Snow, 1963) for 24 hours at 60°C and were then washed completely and stored in 70% ethanol. It was determined that heads with ligulate flowers emerging from the bracts provided anthers most suitable for cytological observations. Intact anthers were dissected from a tubular flower in a drop of 45% acetic acid. Extranous material was drawn off and a drop of Hoyer's mounting medium (Alexopoulos and Beneke, 1952) added, followed by a coverslip. Pressure was then applied to rupture the anther sacs and release the microspores. Prior to observation, stain differentiation was accomplished by heating the slide over steam for approximately ten seconds.

Microscopic examinations were made utilizing a Leitz Wetzlar bright-field microscope with Kohler illumination and yellow-green and daylight blue filters. The chromosomes could be readily distinguished at metaphase in the first post-meiotic division of the microspores, and counts were made primarily on cells at this stage. Microsporocyte meiosis was examined only when unusual conditions were noted in the microspore mitosis. Counts were obtained from three or four microspores of each plant.

Following the chromosome number determination, the internal diameter of the pollen grains (microgametophytes) was measured using a camera lucida apparatus with a bench level magnification of X 750. The mean diameter was based on the measurement of ten

good binucleate pollen grains immediately following mitosis. The presence of unreduced grains, high frequency of microgametophyte abortion, or other unusual properties was then determined.

IV. RESULTS

Chromosome Studies

Morphological observations in the field were of little use in distinguishing ploidy level. Each population tended to be distinctive, and within populations there was sometimes extreme variation. Most notable was the contrast between plants growing in direct sunlight and those in deep shade, or between plants of a protected forest habitat and an exposed coastal bluff habitat. Characters varying within a population were head size, herbage color, floret number, bract margin coloration, pubescence, and leaf dissection. Observations made on greenhouse transplants, after several months had elapsed, revealed a relative uniformity in general appearance with respect to color, habit, and leaf dissection between and within different populations; however, coastal ecotypes retained their thick succulent herbage.

Collection data, determinations of chromosome number, and measurements of pollen grain diameters are summarized in appendices I and II. Other than the observations on chromosome numbers, extensive karyotypic studies were not carried out. Two chromosomes, in both the tetraploid and hexaploid plants, had satellites. The chromosome complement, as seen at metaphase of the first post-meiotic division, consisted of 18 or 27 chromosomes of

approximately equal length, the majority being metacentric or submetacentric. Aneuploidy occurred frequently at both chromosome levels, the usual variation being microspores with gametic numbers of 17 and 26. The hyperploid condition (19 and 28 chromosomes) was seldom observed. Investigations of meiosis in some plants at both levels sometimes revealed a lagging chromosome at the spindle plate. In plant 117-1, a micronucleus was observed. The aneuploid condition appeared to be present to varying degrees in pollen of all plants examined, but no correlation between aneuploidy and degree of microspore abortion was attempted. Abortion of the majority of microspores within one anther sac was occasionally observed, but examination of earlier stages in the same individual revealed an apparently regular meiosis and first post-meiotic division.

Photomicrographs of the tetraploid and hexaploid chromosome complements at metaphase of the first post-meiotic division are presented in Figure 3.

Data obtained from measurements of the internal diameter of binucleate pollen grains could be successfully used as an indicator of ploidy level, although with some reservations. As the data presented in Table I and Figure 4 reveal, there was not a distinct discontinuity between the diameters of the grains of the tetraploids and the hexaploids; however, the degree of overlap was relatively small. Experimentation was not attempted as to the effect on pollen grain diameter

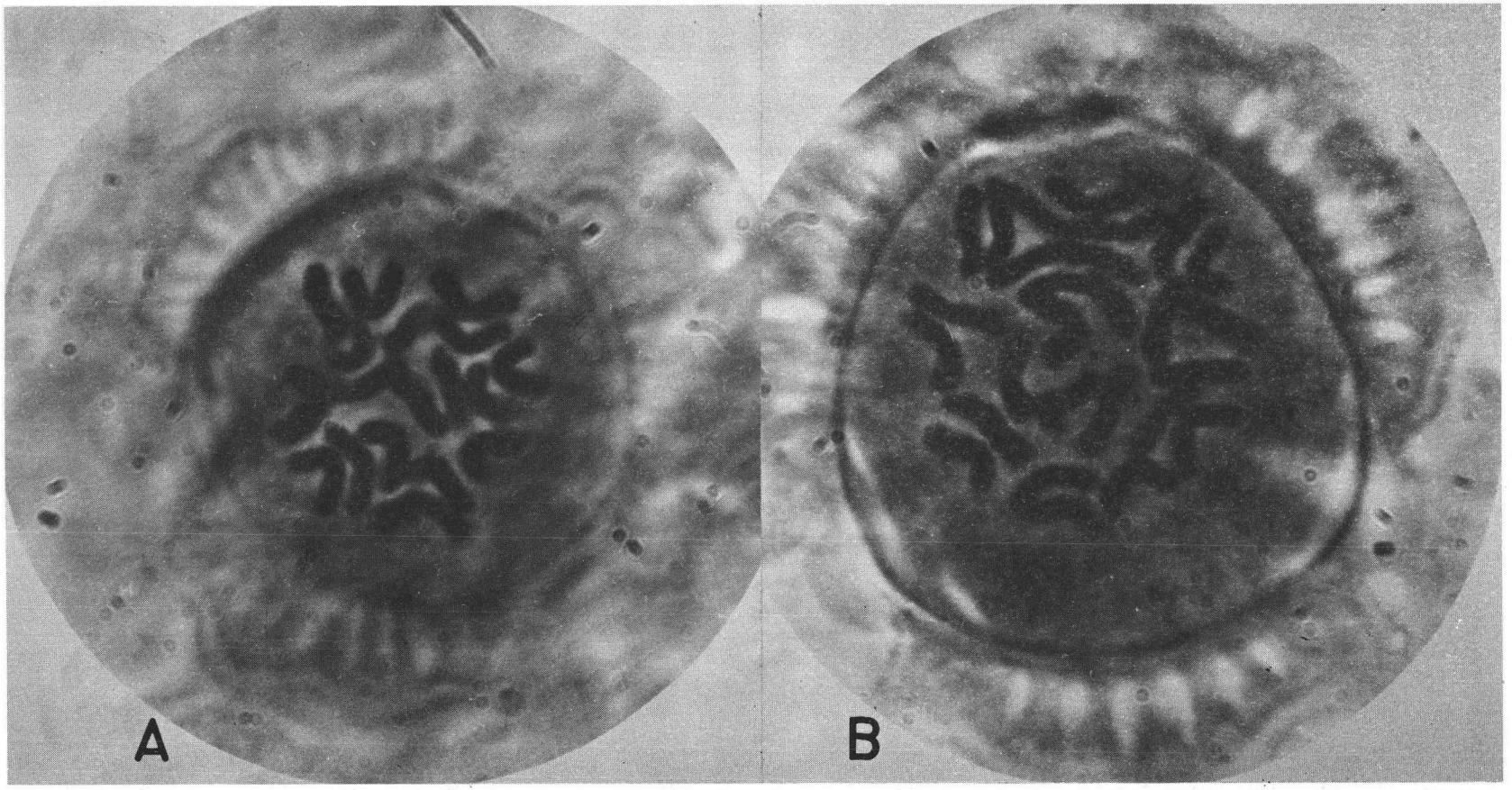

Figure 3. Photomicrographs of chromosome complements in Achillea.
A. Tetraploid complement; accession 124-2 X 4000
B. Hexaploid complement; accession 82-1 X 4000

Table I. Comparison of the means of the tetraploid and hexaploid pollen measurements (size in microns).

Tetraploid		Hexaploid	
Mean	Number of plants	Mean	Number of plants
17.2	1	18.4	1
17.3	1	20.4	2
17.6	1	20.5	3
17.7	1	20.7	1
17.9	1	20.8	3
18.1	2	20.9	1
18.3	1	21.1	1
18.7	1	21.2	2
18.8	3	21.3	5
18.9	5	21.5	1
19.1	2	21.7	3
19.2	7	21.8	2
19.3	4	21.9	3
19.5	2	22.0	3
19.7	3	22.1	2
19.8	4	22.3	6
19.9	4	22.4	1
20.0	5	22.5	2
20.1	7	23.2	3
20.3	2	23.3	1
20.4	1	23.5	1
20.5	3	23.8	1
20.7	1	23.9	1
20.8	3	24.0	1
20.9	1	24.1	1
21.1	1	24.5	1
21.2	2	24.7	1
21.5	1	25.1	1
21.7	2	25.2	1
21.8	3		
22.0	1		
22.4	1		
22.5	1		
24.1	1		
24.4	1		

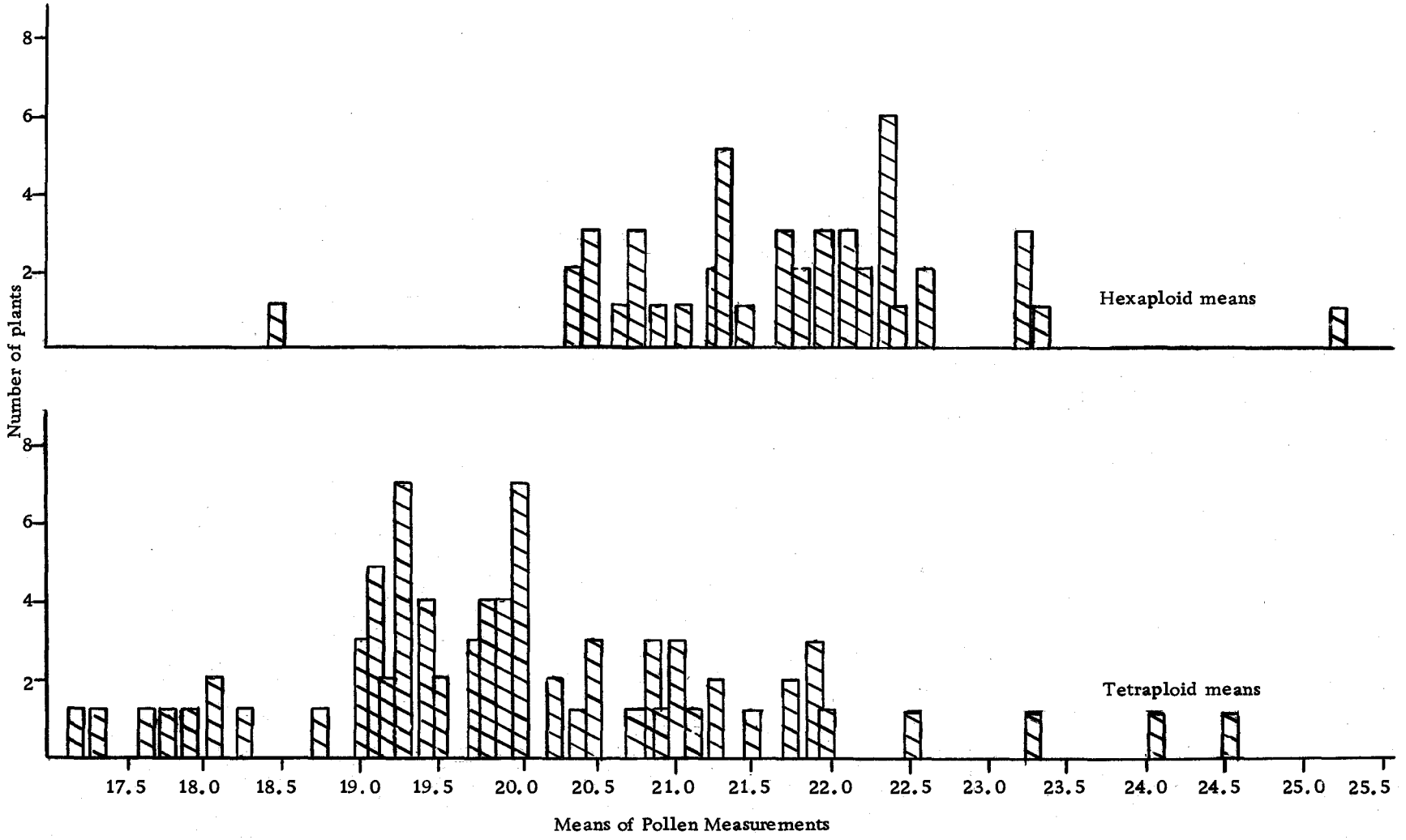

Figure 4. Comparison of the means of the tetraploid and hexaploid pollen measurements (size in microns).

of varying the proportions of chemicals in the killing-fixing solution.

Microscopic examination of accession number 85-4 revealed the plant to be a pentaploid (2n=45), with 18 bivalents and 9 univalents at first meiotic metaphase. Chromosome numbers in the microspores at the first post-meiotic division ranged from 19 to 26, and pollen diameters varied from the size range of tetraploids to that of hexaploids. The plant was found in a tetraploid population and was not morphologically distinctive, having been collected in the course of a normal random sampling.

Tetraploid plants from various populations were found to have a number of unreduced microspores, which could easily be distinguished by their large diameters (in the range of 32.7μ to 48.7μ). In microspore mitosis, 36 chromosomes were counted. Some of the grains appeared to be aborted, lacking cytoplasm. The majority of the grains were observed to be clustered in the basal portion of the anther sac, but sometimes two or three were found near the apex among normally reduced grains (n=18). The number of tetraploid grains varied from two to twenty-five per anther.

Populations exhibiting this phenomenon were centered primarily in coastal southwestern Oregon. Tetraploid plants from central and northern Oregon were rarely noted to have unreduced grains. An exception was accession number 56, a mixed population in which a single hexaploid plant was found among tetraploid plants some of which

were producing unreduced grains. The plants that produced these grains were morphologically indistinguishable from other tetraploids.

Population Distributions

The chromosome level and locality are pictorially related in Figures 5-9. The results indicate that the distributions of the two chromosome levels overlap in some areas and are spatially distinct in others. The observed situation in the lower Alsea River valley illustrates this overlapping distribution (Figure 8). An isolated clonal clump, accession 60-1, growing in the graveled margin of Oregon Highway 34 was found to be a tetraploid while adjacent collections (59-1,2; 61-1,2; 155-1; 156-1) were hexaploid. A similar situation was observed along a coastal transect on U. S. Highway 101 between Bandon and Florence (Figures 6 and 9). Tetraploid plants extended northward from Bandon to within approximately six miles of Coos Bay, and were noted to be producing a large number of unreduced pollen grains. Hexaploids were found along both sides of the bay and extended northward along the coast. In the area between Coos Bay and Florence, five populations of tetraploids (100, 101, 112, 150, 151) were found.

Disjunct distributions were also found in the lower portion of the Umpqua River valley, with accession 113, a hexaploid population, being found 15 miles inland from the coast, well past intervening

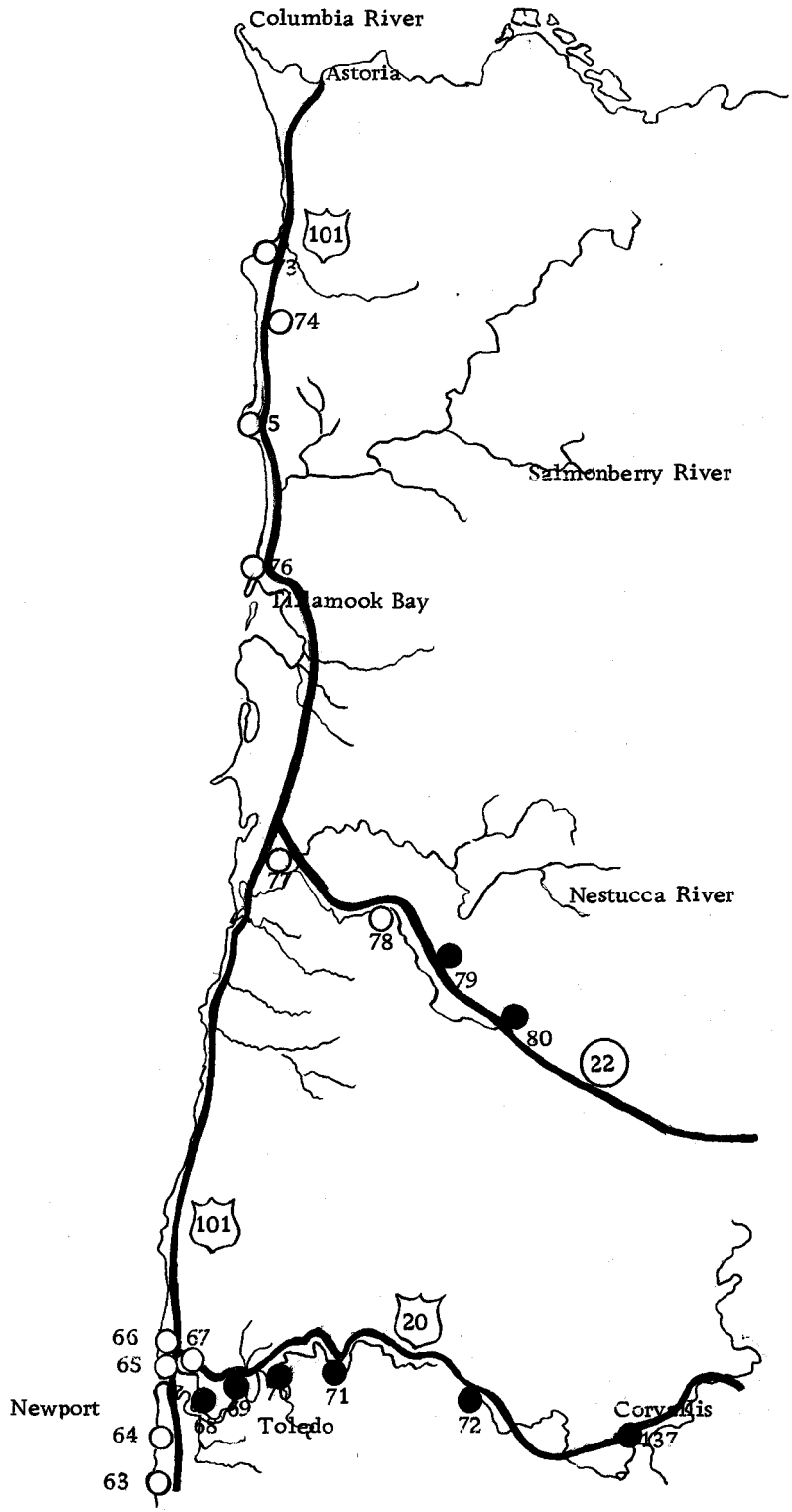

Figure 5. Distribution of tetraploid and hexaploid Achillea in northwestern Oregon. Solid circules=tetraploid populations (n=18), open circles=hexaploid populations (n=27).

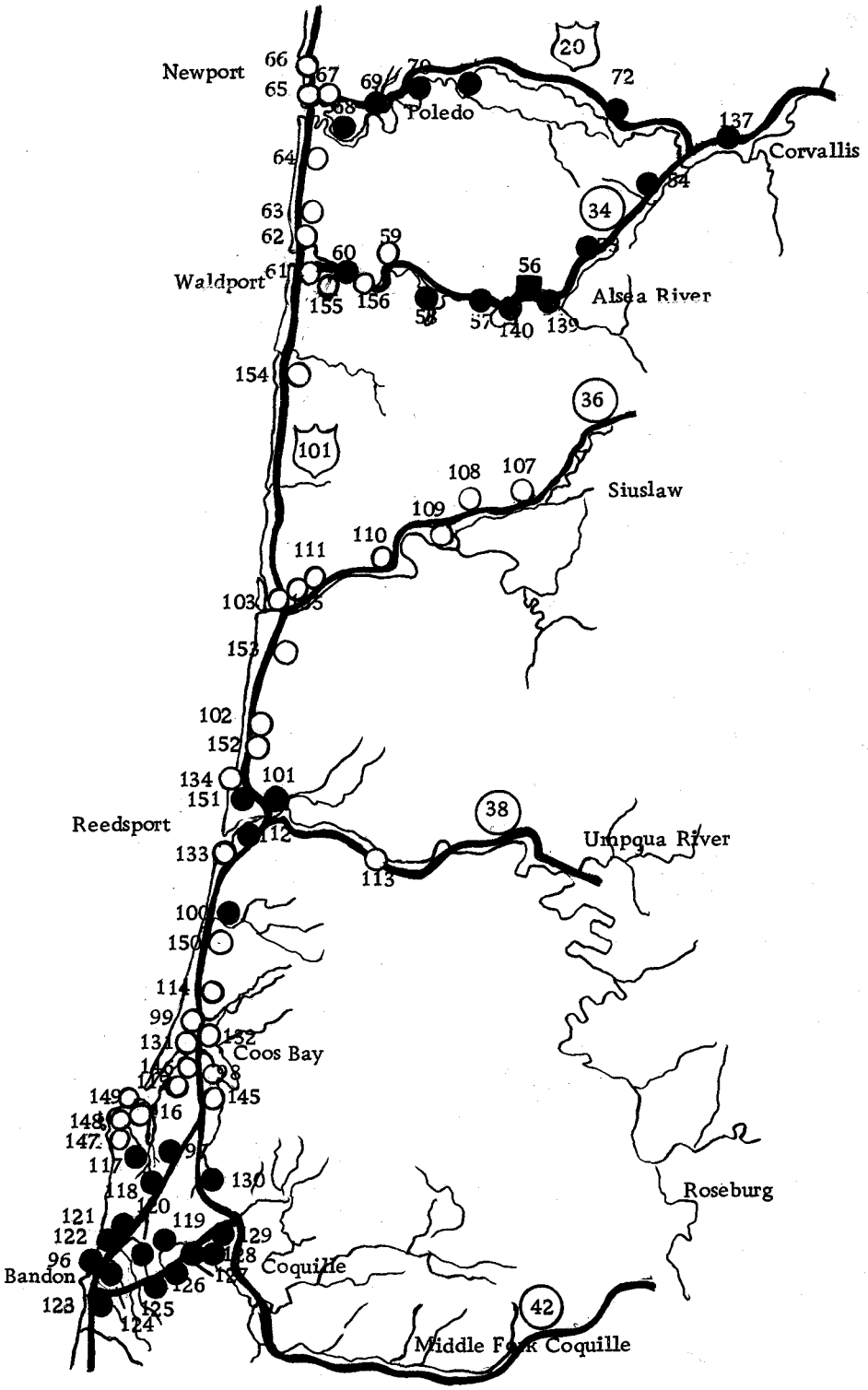

Figure 6. Distribution of tetraploid and hexaploid Achillea in western Oregon. Solid circles=tetraploid populations (n=18), open circles=hexaploid populations (n=27); squares=mixed populations; and triangle indicates pentaploid plant (2n=45).

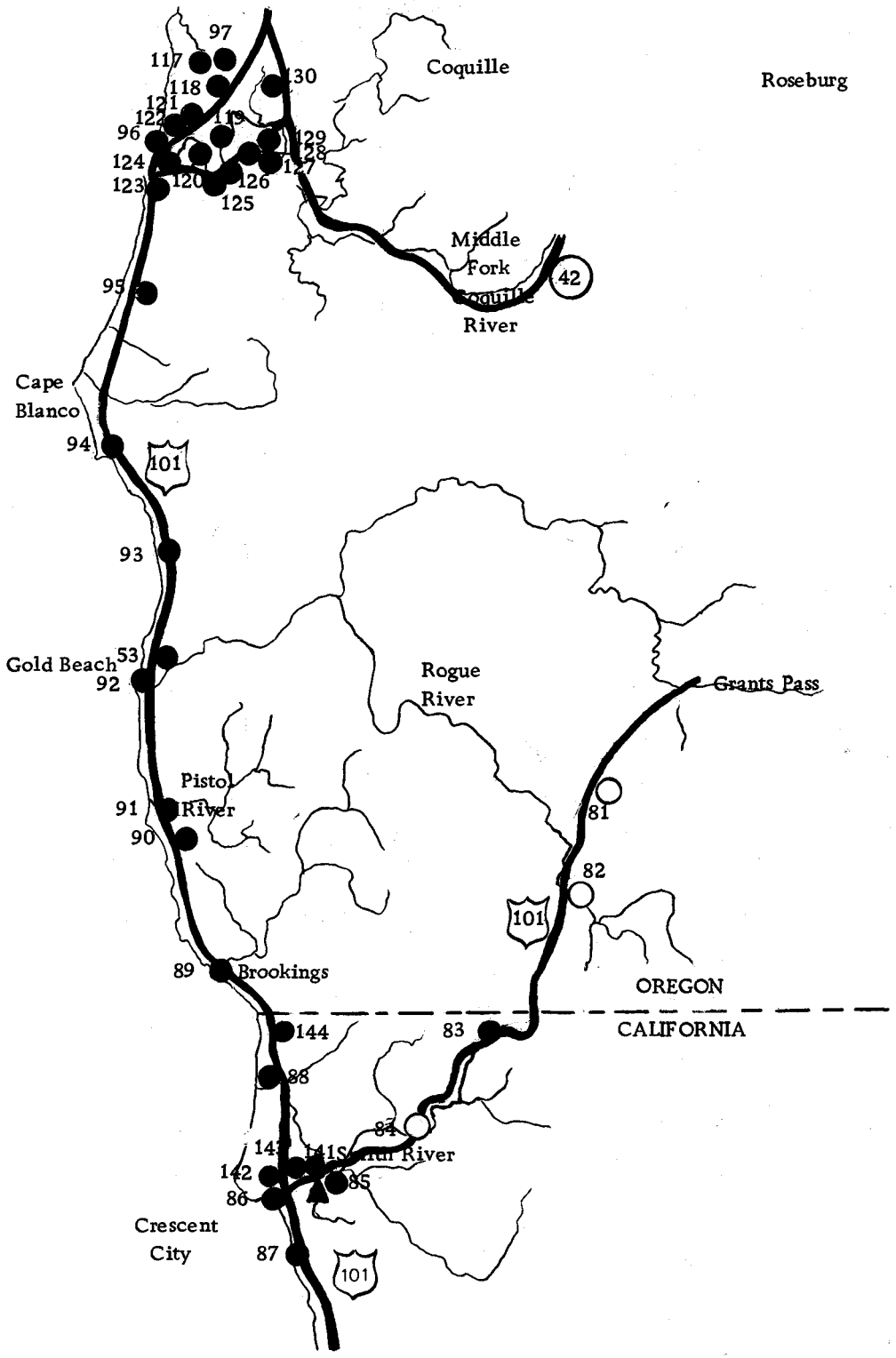

Figure 7. Distribution of tetraploid and hexaploid Achillea in southwestern Oregon and northern California. Solid circles=tetraploid populations (n=18); open circles=hexaploid populations (n=27); squares=mixed populations; and triangle indicates pentaploid (2n=45).

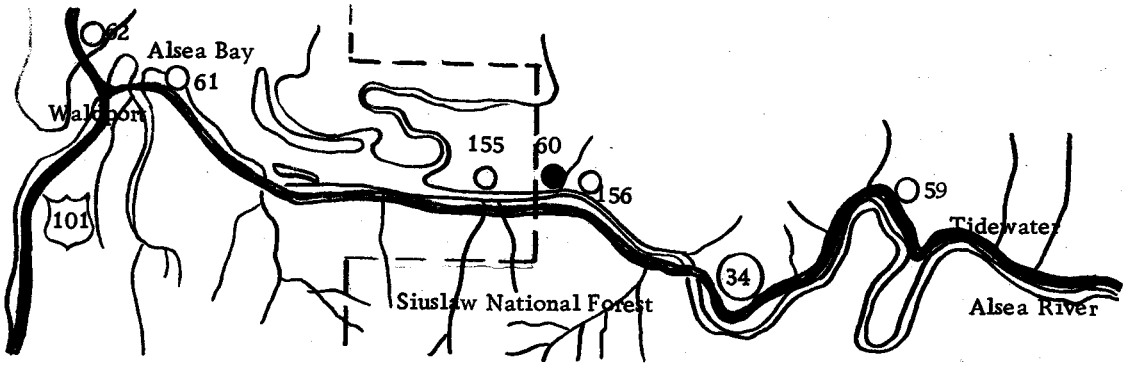

Figure 8. Distribution of tetraploid and hexaploid Achillea in Alsea River valley, Oregon. Solid circles=tetraploid populations (n=18); open circles=hexaploid populations (n=27); squares=mixed populations.

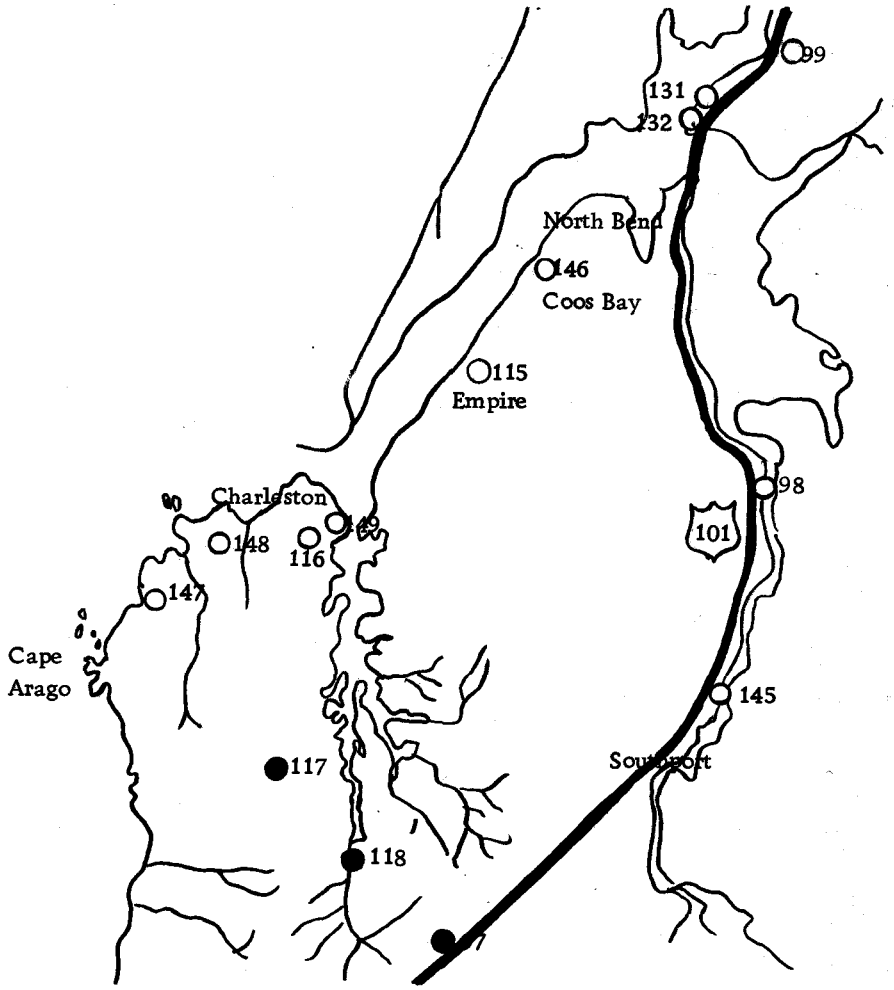

Figure 9. Distribution of tetraploid and hexaploid Achillea in central coastal Coos County, Oregon. Solid circles= tetraploid populations (n=18); open circles=hexaploid populations (n=27); squares=mixed populations.

tetraploid populations (101, 112).

Hexaploid plants were found in the midst of tetraploid populations in several areas. A mixed population was found in the Alsea River valley (56-1, 2, 3). Initially a single hexaploid plant (56-1) was detected. The tetraploid plants of this population were noted to be producing a number of tetraploid pollen grains. Hexaploid populations (81, 82, 84) were found in southwestern Oregon surrounded by tetraploids (Figure 7). Further counts to determine the extent of the hexaploid distribution and the presence of hybrids in this area were not made.

Chromosomal investigations of the coastal river valleys revealed that in some, tetraploid plants extended almost to the coastal bluffs. For example, in the Yaquina River valley, tetraploids were found 8.0 miles inland from the mouth of the river on bluffs overlooking Yaquina Bay and at Toledo (Figure 6). In the Siuslaw and Nestucca River valleys, hexaploid plants were found 26 and 16 miles inland, respectively (Figures 5 and 6).

The absence of Achillea was noted on a transect along U. S. Highway 20 in Lincoln County in the area of Pioneer Summit (R10W, T10S). Chrysanthemum leucanthemum, which occupys a habitat similar to Achillea, was also absent. It was observed that the highway had been relocated and the right of way extensively cleared. On either side of this area, Achillea and Chrysanthemum were present

in large populations in open fields. A similar situation was observed in southwestern Curry County along U. S. Highway 101 between Cape Ferrelo and the Pistol River. Achillea and Chrysanthemum were notably absent but were abundant in adjacent areas, and the highway route had been altered. Achillea was also found to be absent in the area encompassed by a secondary road from Oregon Highway 38 to Loon Lake in Douglas County (Figure 2). Prostrate vegetative growth or the remnants of previous years' stems were not detected. Construction work was not in progress and the roadside vegetation appeared to have been undisturbed for several years.

Little correlation could be made between environmental conditions and chromosome level. The tetraploids and hexaploids occupied essentially the same types of habitats in diverse climatic and edaphic conditions, ranging from exposed coastal bluffs to the protected margins of forests. The plants were abundant in cleared highway rights of ways, along untended fence rows, and in fallow fields. It was noticeable that the plants were not found under a dense vegetative canopy, but rather along the open margins. Geographical or ecological barriers between the two levels were not detected.

V. DISCUSSION

Distributional Patterns

The results of this investigation indicate that the distributions of the tetraploid and hexaploid chromosome forms of Achillea in western Oregon are in a state of change. In contrast to previous studies reporting more or less clear-cut patterns, it is felt that the distributions of the two levels are not distinct, but rather are variable and overlapping. There are several lines of evidence for this conclusion. First is the alteration of the tetraploid and hexaploid populations in the Alsea and Umpqua River valleys, and on the coast between Bandon and Florence. The intrusion of the coastal hexaploids in some river valleys, i.e., the Siuslaw, and the presence of tetraploids in close proximity to the coast in others, as in the Yaquina, constitute a second indicator for this state of fluctuation. A third is the presence of mixed populations; for example, accession 56 in the Alsea River valley, two hexaploid populations among the tetraploids in southwestern Oregon, and a pentaploid plant in the midst of a tetraploid population in northwestern California.

In accounting for this variation in distributions, as reported here, several factors may be considered. First is the conceptual scope of the investigation. Previously reported patterns were based on counts from widely separated sites. Turesson (1939) made

collections from ten locations along the Pacific Coast from British Columbia to California. Clausen, Keck, and Hiesey (1938, 1940), Lawrence (1947), and Ehrendorfer (1952b) made approximately 170 more counts on the same area. While these counts are sufficient to establish a distributional map for western North America, such as is presented in Figure 1, they are inadequate for a much smaller geographic unit such as southwestern Oregon. Restriction of the transect areas and frequent sampling of the populations reveals the distributions of the two chromosome forms at a population or even at the plant level, and from this, there emerges the varying and overlapping pattern.

A second factor affecting reported distributional patterns is that of time. In the intervening years between the last study (Ehrendorfer, 1952b) and this one, the tetraploid and hexaploid plants which were allopatric could have become sympatric, due to the adaptable nature and ease of migration of Achillea. Factors contributing to this "weedy" or colonizing nature are several. Genetic variability characterizes Achillea in such ecological and physiological characters as flowering time, periodicity, and phenotypic plasticity. Its breeding system, involving obligate outcrossing, favors genetic recombination and thus genetic flexibility. Vegetative propagation by rhizomes and perenniality aid in its establishment. Reports of the rapid spread of a plant species are not uncommon in the literature; for

example, there are the reports on the movement of Senecio squalidus Willd. in the early nineteenth and middle twentieth century in England (Salisbury, 1961).

Together with changes through time, there is the even more important factor of the formation of suitable habitats for the establishment of Achillea. Lawrence (1947) noted in his study the lack of geographical or physiological barriers between the two chromosome forms; and Turesson (1939) indicated its weedy nature and the significance of man's activities in aiding its dispersal. Field observations made during this study disclosed that Achillea was found in open areas, along the cleared rights of ways, at the margins of forests, in fallow fields, and in abandoned open areas; but very seldom was it found in under the forest canopy. Robinson (1966) has quantitatively determined one controlling factor of Achillea habitats, reporting that it is not found on test plots in Pinus ponderosa forests where insolation, the degree of solar radiation, is less than ten percent (as measured by a Wagar-type insolation grid). This signifies the limits of ecological potential for the plant with respect to one factor.

The northern coastal mountain area, where this overlapping of Achillea chromosome distributions is most pronounced, is characterized (Dennis, 1966) by dense coniferous forests of Pseudotsuga menziesii, Abies grandis, and Picea sitchensis except where interrupted by man or fire. Man's activities of lumbering, farming,

highway and railroad construction, and certain conservation practices in the coastal mountains and valleys have created a vast number of habitats for Achillea. Plants are no longer barred from migration; therefore, there is frequent contact between the two chromosome forms as detected in this investigation.

Migration of a species involves dispersal and establishment of progeny. Studies by Salisbury (1961) reveal that viable seeds of Achillea have been obtained from the excreta of birds and horses, suggesting animals as a possible dispersal mechanism. The large number of achenes that a single plant is capable of producing, the prolonged flowering period, and the wide range of environmental tolerances contribute to its dispersibility. Dispersal may also be facilitated by open rights of ways and hillside cuts. Salisbury (1961) states that seeds contained in mud may be picked up in the tread of tires and carried for considerable distances. An indication that this may occur in Achillea is the presence of a single isolated tetraploid clonal clump, accession 60, growing in the graveled highway margin between hexaploid populations.

Following dispersal, establishment of Achillea in new habitats is facilitated by its vegetative, reproductive, and genetic characteristics. The forementioned characters of phenotypic plasticity, periodicity, and time and length of flowering contribute to establishment. Vigorous vegetative growth and perenniality reduce its

dependency on seed production for immediate survival. Outcrossing, good chromosome pairing, and high chromosome number result in a stable--yet flexible--system for immediate and continued establishment.

Taking into consideration these factors, the sequence of distributional events occurring in western Oregon may be hypothesized. The hexaploid plants were confined to the open coastal dunes and cliffs, being unable to penetrate into the coastal mountain forests because of the insolation factor determined by Robinson. Similarly the tetraploids were confined to the open areas east of the coastal mountains, also unable to move into the forests. The presence and abundance of Achillea is reported by early explorers and botanists. Coville in 1897 reported Achillea to be abundant throughout the Pacific Northwest and to be used by various indian tribes. Utilization of the forests of the Coast Mountains and early lumbering techniques opened up new habitats for Achillea, allowing migration of both the tetraploids and hexaploids.

In some areas the tetraploids are progressing toward the coast more rapidly than the hexaploids are progressing inland and in other areas the reverse is occurring. This study was not comprehensive enough to determine whether there was contact between the two levels at all points. That there is contact is demonstrated by the detection of mixed populations, the alternation of chromosome levels,

and the presence of pentaploid hybrids.

In contrast is the distributional pattern of the hexaploid chromosome form in central California. This area is characterized by extensive grasslands and little or no overstory. The hexaploid Achillea occupies numerous habitats in the coastal mountains, San Joaquin Valley, and Sierran foothills, extending well inland (Figure 1). This pattern suggests that the forested coastal mountains of Oregon were instrumental in restricting the hexaploid form to coastal habitats.

Pollen Measurements

The utilization of pollen grain size as an indicator of ploidy level in Achillea proved to be partially successful. In general, there is a discontinuity in the pollen grain diameters of the tetraploid and hexaploid chromosome form, but it must be noted that considerable overlap does occur. These results coincide with reports of researchers working in other genera, in which pollen size may be employed to determine polyploidy, but only with reservations. As mentioned previously, Bell (1954), Dean (1966), and Heckard (1960) indicated that intermediate size classes prevented distinct separations of various chromosome levels. In contrast is the work published by Ehrendorfer (1952b) and Mulligan and Bassett (1959) stating that there are definite ranges of mean pollen diameters in Achillea and making no mention of overlapping variations. Gould (1957) also

reports a distinct correlation between ploidy level and pollen grain size in Andropogon L.

Pollen grain size provides an excellent means of detection of unreduced grains at the tetraploid level. These grains have diameters of approximately twice normal size and are easily distinguished at low magnifications.

Measurement of the internal diameter of a binucleate grain is easily accomplished and is believed to be somewhat more accurate than external measurements involving the spines of the exine.

In considering possible causes of an overlap between the two chromosome levels, several factors are apparent. The first of these is the problem of measuring all pollen grains at similar stages of development. This was resolved by measuring the grains at the binucleate stage immediately following the first post-meiotic division, using as a cytological marker the degree of staining. However, the grains remain binucleate for a time and may possibly undergo volume changes, thus affecting measurements.

A second source of variation is experimental error in the preparation of material and apparatus, and in measurements. While it is known that once dry, pollen grain exines remain constant in size and shape (Faegri and Iversen, 1964), there have been few reports with regard to size changes of fresh material in various chemical mixtures such as those used in killing-fixing solutions.

Experimentation was not attempted as to the effect on pollen grain diameter of varying the proportions of chemicals in the killing-fixing solution.

A third source of error is the measurement of aborted or malformed pollen grains rather than viable ones. Characters such as the stainability of the grain's cytoplasm, its having a circular shape in equatorial view, and its size being comparable to other cells were used as indicators of normalacy. An associated factor is the anther sac area in which the measured grains were located. The physiological condition of the plant and environmental changes during microsporogenesis could possibly affect microspore size. Grains were measured from various portions of the sacs, and emphasis was placed on selecting good binucleate grains.

Pollen grain size appeared to be relatively constant in a single plant, with ranges of two or three microns. Means of plants within a population generally varied only one or two microns, even between plants in extremely contrasting habitats such as deep shade and bright sun. At the tetraploid level it was noted that the means of plants from Coos and Curry Counties, Oregon, were generally two or three microns higher than tetraploids from the northern Coast Mountains and the interior, although the ranges were approximately the same (Appendix II). This variation was not observed among populations of hexaploids.

Pentaploid Hybrid

An indication of probable contact between tetraploid and hexaploid chromosome levels was the discovery of a pentaploid plant, accession 85-4, in coastal Del Norte County, California. This individual formed primarily 18 bivalents and 9 univalents at meiotic metaphase I. This pairing implies a high degree of homology between the chromosomes of the two levels. Almost all of the pollen grains appeared to be viable, the cytoplasm staining readily. Chromosome numbers in the pollen grains ranged from 18 to 27 with the majority of cells having between 20 and 26. Pollen diameters ranged from those indicative of a tetraploid plant to those of a hexaploid, with the majority being intermediate. This variation in chromosome number and in grain size would be expected with unequal segregation of chromosomes in a plant having irregular meiosis. The plant was robust and morphologically indistinguishable from surrounding tetraploid plants.

The detection of this plant confirms reports (Schneider, 1958; Ehrendorfer, 1959a) that partially fertile pentaploid hybrids do occur naturally in Europe and in North America. Cytological examination of experimentally produced pentaploid hybrids revealed a similar pairing situation, and Schneider (1958) pointed out that experimental backcross progeny showed a decrease in fertility

because of genome disproportion and cryptic factors. Persistence of the pentaploid in nature would be primarily vegetative due to these factors reducing fertility. Undoubtedly many other hybrids exist throughout the contact areas, but go undetected because they lack morphological distinctness.

The discovery of a pentaploid plant in this area of northern California was unexpected. Previous distributional studies suggested that this region contained only tetraploids with hexaploids occurring farther north or south along the coast. Two solutions are possible to the question of how this plant originated. The first is that scattered hexaploid plants or populations do occur through this area and have not been detected. Indications of this are the presence of hexaploid populations at Selma, Oregon (81), Cave Junction, Oregon (82), and in the Smith River valley, California (84). A second solution is that a hexaploid plant may have developed from tetraploid progenitors (by processes to be discussed below) and then hybridized with surrounding tetraploid plants to produce the pentaploid hybrid.

Cytogenetic Patterns

From researches on the nature and occurrence of polyploidy, ideas as to its mechanisms have been developed (Stebbins, 1950; Swanson, 1957). Mechanisms that may be involved in the formation of diploid gametes include: ameiosis, formation of restitution nuclei,

and somatic doubling of chromosomes. Polyploid plants may originate as the result of the fusion of two diploid gametes, the somatic doubling of chromosomes, or the fusion of diploid and haploid gametes. Ehrendorfer's (1959e) observations on the meiotic behavior of Achillea reveal that the processes involved in the development of polyploids do occur in the A. millefolium-complex. Meiotic irregularities are of four types: derangements in synapsis, in chromosome reproduction, in spindle action, and in cell wall formation. Data compiled in this study confirm these reports. Anthers of various tetraploid plants were observed to contain unreduced pollen grains (n=36) among normal reduced grains. These tetraploid grains are easily distinguished in that their diameters are approximately two times the size of diploid grains. Investigations of the meiotic errors responsible for their formation were not made at this time.

These tetraploid grains appear to be viable, as judged from morphological appearance and stainability. Should they be functional, they could contribute to the formation of higher polyploid levels in the genus. The fusion of two tetraploid gametes, for example, would give rise to an octoploid plant (2n=72). Lawrence (1947) reported the natural occurrence of a single plant from Fresno County in central California. A cytological survey of the area might disclose that tetraploid plants there are able to produce unreduced pollen grains and eggs. That unreduced eggs do occur has been reported

in Europe (Ehrendorfer, 1960). The low frequency of occurrence of octoploids is probably due to selective factors such as the gametophytic screen in megasporogensis and differential growth rates of pollen grains. In Europe an octoploid species, A. pannonica Scheele, is present, however.

In a similar fashion, a hexaploid zygote (2n=45) may be produced by the fertilization of a diploid egg (n=18) by a tetraploid pollen grain (n=36). A high frequency of multivalents would be expected in such an autoploid cross. This condition could be reduced by selection for normal bivalent pairing in subsequent generations, or more directly by genetic control of pairing.

There is some evidence to suggest that change from one chromosome level to another may have occurred in Achillea in western North America. First, there is a lack of major barriers to genetic exchange between the various ecotypes at both tetraploid and hexaploid levels. The work of Clausen et al. (1938, 1940, 1948, 1951, 1955) shows that a close genetic relationship and high interfertility exist between the tetraploid ecotypes. A close relationship exists also between those races at the hexaploid level, although some weak barriers are present. These relationships imply a common origin for the tetraploid ecotypes and for the hexaploids. Second, attempts to hybridize the Eurasian hexaploid, A. millefolium sensu stricto, with the North American hexaploid, A. borealis from the

Pacific Coast, have been unsuccessful (Clausen, Keck, and Hiesey, 1940; Ehrendorfer, 1952a; Hiesey and Nobs, 1952). This complete lack of viable progeny strongly suggests the absence of close genetic relationships between the two hexaploid forms, and therefore may indicate that the North American form is not directly derived from the European. Associated with these results is evidence from the meiotic chromosome pairing in the pentaploid hybrid mentioned above. This consisted of 18 II's and 9 I's, which denotes a relatively high degree of homology between the tetraploid and hexaploid genomes. Reduction in the number of multivalents expected may be due to genetic stabilization of meiosis in the genus (Schneider, 1958).

Cytological markers, such as the number of chromosomes having satellites, may be used in studies of polyploidy. Gates (1942) believed such evidence to be useful in the detection of polyploid series. Ehrle (1958) reported tetraploid Achillea to have four distinct satellites in somatic cells. The hexaploid, if the result of autoploidy, would be expected to have three satellites in its microspores. This number was observed in grains of a few plants, but the majority of microspores were observed to have two satellite chromosomes. This may be due to two factors. One is the failure to distinguish all of the satellites, due to the crowding of the chromosomes in mitotic metaphase; second is that one satellite may be suppressed in the presence of the other two. This phenomenon would be similar to

that reported by McClintock (1934) and Navashin (1934) in other genera.

The presence of a single hexaploid plant, in a population of tetraploids producing a large number of unreduced gains (accession 56), indicates the probable autoploid origin of the hexaploid chromosome form. That more heteroploid populations were not immediately detected may be due to the self-incompatibility of plants of this genus; the establishment of a hexaploid population in an area would be dependent upon the presence of more than one hexaploid individual to effect cross-pollination.

The similarities observed in morphological characters, habitats, and environmental responses are further evidence for a close phylogenetic relationship between the two ploidy levels. The characters used to differentiate the two forms taxonomically are superficial, inadequate for field recognition, and are highly modifiable by different environmental conditions, as indicated by observations on transplant material in the greenhouse.

The taxonomic treatment of the evolutionary complex present in Achillea, is dependent upon the researcher's opinion as to the emphasis to be placed on chromosomal discontinuities without associated morphological breaks. In a genetic system such as this, the biological species concept does not require that species be delimited on the basis of chromosome number, but rather a single

species should be recognized, indicating that it is highly polymorphic with two chromosome levels and a series of phenotypically plastic ecotypes.

Cytogeographic Patterns

By relating distributions of the tetraploid and hexaploid chromosome forms in Achillea to past environmental and geological conditions, a sequence of events in the development of the present cytogeographic pattern may be hypothesized. The Oregon coastal mountain ranges may be divided, on a geological basis, into two areas each with its distinctive flora. Smith (1933) described the two areas as the northern and southern coast ranges. The northern region, extending northward from the Coquille River, is composed of Tertiary and Quaternary rock formations; while the southern, from the Coquille River south, contains older Cretaceous metamorphic rocks, serpentines and argillites, and Paleozoic and Mesozoic igneous and sedimentary rocks. This southern coast area has also been included in a larger geological area described as the Klamath Mountains (Diller, 1902; Baldwin, 1945, 1964). Boundaries of this region, one of the oldest areas in the Pacific Northwest (Condon, 1910), are generally described as either the Coquille River, Coos Bay, or the Rogue River on the North; and the South Fork of the Trinity River in California on the South (Diller, 1902).

Vegetation of the two areas is different (Dennis, 1966; Weidemann, 1966). The northern region is characterized by coniferous forests containing the following species, among others: Pinus contorta, Picea sitchensis, Abies grandis, Pseudotsuga menziesii, Thuja plicata, Gaultheria shallon, and Vaccinium ovatum. The southern region or Klamath Mountains possess an open coniferous and broad-leaf evergreen forest, including: Ceanothus thrysiflorus, Rhododendron occidentale, Garrya spp., Quercus spp., Arctostaphylos spp., Arbutus menziesii, Chamaecyparis lawsoniana, and Lithocarpus densiflora. Coniferous forests are dense and continuous in the northern region and, where absent because of lumbering or fire, are replaced by dense stands of Alnus and Acer. In the southern area, the forests are not as dense, being naturally interrupted in some areas where shrubs or bunch-grasses predominate.

Distributional studies indicate that the tetraploid chromosome form occupies coastal habitats in Coos and Curry Counties, Oregon, and Del Norte and Humboldt Counties, California, replacing the hexaploid form which is distributed north and south along the Pacific Coast. This region of tetraploid occurrence coincides closely with the geologically defined Klamath Mountains.

The origin or origins of the two chromosome forms in western North America is one problem yet to be fully resolved. Taking into

account the presence of continental connections and climatic changes in the Pacific Northwest (Cain, 1944; Stebbins, 1950; Whittaker, 1961; Baldwin, 1965), it is speculated that tetraploid achilleas, migrating from Eurasia via the known Bering Sea land bridge, became established in the area of what is now the Klamath Mountains. With the advent of glaciation in the Pleistocene epoch, intermediate populations to the North were lost. At the end of this cold period, Achillea was able to radiate outward. That the Klamath Mountains were an area of refuge is indicated by the many endemics and relics reported to occur in them. The vegetational history of these mountains was discussed by Whittaker (1961). With regard to Achillea, Ehrendorfer (1952b, p. 131) states that the tetraploids from this region, "...probably represent segments of a relatively primitive racial entity, which may have existed in that area fairly unchanged for very long periods."

Tetraploid plants found at the northern limits of the coastal distribution, which also corresponds to the northern limits of the Klamath Mountains, were noted to be producing a large number of unreduced pollen grains. Hexaploid plants could be produced in these marginal areas by the previously discussed fertilization of diploid egg and tetraploid pollen grain. The hexaploids, once established, could be dispersed along the coastal bluffs and sand dunes northward, being restricted to the coastal areas by the presence of available habitats with sufficient insolation.

This hypothesis, that the hexaploids, now distributed along the Pacific Coast, had their origins in the tetraploids of the Klamath Mountains, contradicts Ehrendorfer's theory (1965) that both the tetraploid and hexaploid forms came from northeastern Asia, with subsequent spread across the North American continent. Strong reproductive barriers between the old and new world hexaploids, the lack of ecotypic hybridization barriers at both levels, and the absence of hexaploids in the continental interior and on the coast in the Klamath Mountains region strongly suggests that the hexaploids are indigenous to North America. Experimental studies on the cytogenetics and crossability of the hexaploids reported along the northern coasts of Alaska and Canada (Mulligan and Bassett, 1959) may be of aid in resolving this problem.

Following the establishment of hexaploid populations, their rapid movement back into the Klamath Mountains region may have been limited by factors such as self-incompatibility. Single hexaploids plants dispersed among tetraploids, could reproduce only vegetatively or by forming pentaploid hybrids by outcrossing with the tetraploids. For similar reasons, the tetraploids may not have been able to move readily into areas occupied by hexaploids along the coast. That dispersion in the coastal areas is possible for both the tetraploids and the hexaploids is indicated by the present overlapping pattern of the two forms in the Bandon--Coos Bay--Reedsport

area. In general, more or less distinct distributions of the tetraploid and hexaploid chromosome forms of Achillea developed, with the hexaploids restricted to the coast and the tetraploids to the Klamath Mountains and the interior. With the advent of man and his creation of open communities, the dispersal of each chromosome form was greatly facilitated, resulting in the overlapping pattern revealed in the present study.

VI. SUMMARY

The results of this cytogeographic investigation of the tetraploid and hexaploid chromosome forms of Achillea millefolium L. reveal that the distributions of the two forms are not as simple as previously reported. Instead, they are rather complex, consisting of overlapping distributions, mixed populations, disjunct populations, and apparent migration of one form into areas occupied by the other. Pentaploid hybrids were detected in nature, morphologically resembling their tetraploid and hexaploid progenitors, and having meiotic pairing of eighteen bivalents and nine univalents. Anaphase segregation of the univalents was random. The maximum internal diameter of viable binucleate pollen grains can be utilized as an indicator of ploidy level in the genus, although with some reservations. The distribution of the coastal tetraploid in southwestern Oregon can be correlated with the distinctive geological region termed the Klamath Mountains. Plants of this area, especially those at its distributional limits, were observed to be producing a large number of unreduced pollen grains that were apparently viable.

From these results, several inferences and conclusions are drawn. The first is that the distributions of the two chromosome forms are not static, but rather dynamic and in a state of transition. Throughout the investigated area, different stages in this transition

may be observed. In some areas there are distinct distributions with the hexaploid form on the coast and the tetraploid form inland. In other areas there is contact of the two levels with mixed or disjunct populations; and in others, hybridization and the establishment of pentaploid hybrids occurs.

A second inference is that the North American hexaploid form has its origins in the tetraploid form, through the formation of unreduced or tetraploid pollen grains and the subsequent fertilization of diploid eggs. Indications of this are the presence of reproductive barriers between American and Eurasian hexaploids; the high degree of chromosome pairing in the pentaploid hybrids; the existence of mixed populations, with a single hexaploid plant among tetraploid plants that are producing tetraploid grains; and morphological similarities indicative of an autoploid system of evolution.

From the distributional patterns developed, it is speculated that the tetraploid form, established during Tertiary times in the Klamath Mountains, gave rise to the hexaploid form along the geologic margins of the area. Further dispersal of the chromosome forms occurred, with the hexaploid being restricted to the coastal areas and the tetraploid to the interior.

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APPENDICES

Appendix I. Collection data and chromosome numbers for Achillea accessions.

Accession number	Chromosome number	County	Range	Township	Section	Notes
51	18	Deschutes	12E	14S	9	Down river from Lower Bridge 0.9 mi. in Deschutes River canyon, L. R. Estes 77.
52	--	Curry	14W	38S	7	Meyer's Creek bridge, 7.0 mi. S of Gold Beach, elev. 100 feet, L. R. Dennis.
53	18	Curry	14W	36S	30	Rocky bank of Rogue River, 0.5 mi. E of Gold Beach, L. R. Dennis 2814.
54	18	Benton	6W	12S	29	200 feet from junction of Ore. Hwy. 34 and Rock Creek Road on latter, along fence row.
55	18	Benton	7W	13S	15	Roadside ditch along Ore. Hwy. 34, shaded south bank, 200 feet W of Charles Nelson mailbox.
56	18, 27	Benton	8W	14S	8	Isolated population W of Maltby Cr. bridge on Ore. Hwy. 34, north roadside ditch.
57	18	Lincoln	9W	14S	1	0.5 mi. W of Fall Cr. on Ore. Hwy. 34 at entrance to farm turnoff, steep south-facing bank.
58	18	Lincoln	10W	13S	36	Small isolated population, north end of Dead End Spur bridge on Ore. Hwy. 34.
59	27	Lincoln	10W	13S	28	Steep south-facing bank, 0.3 mi.W of Tidewater city limits on Ore. Hwy. 34.
60	18	Lincoln	11W	13S	25	Large clonal clump, 0.5 mi. E of Siuslaw Nat. Forest boundary marker on Ore. Hwy. 34.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
61	27	Lincoln	11W	13S	20	Steep north-facing bluff 25 ft. above Alsea Bay, 0.6 mi. W of Waldport city limit sign on Ore. Hwy. 34.
62	27	Lincoln	12W	13S	18	Roadside ditch along U. S. 101 1.4 mi. N of Alsea Bay bridge, sandy soil of dune cut.
63	27	Lincoln	12W	12S	25	Small plants, exposed coastal bluff at beach trail start. Seal Rock State Park.
64	27	Lincoln	12W	12S	7	Ocean Wayside State Park entrance.
65	27	Lincoln	11W	11S	17	Sandy exposed area 200 ft. from junction of OSU Marine Science Lab and U. S. Hwy. 101 on former.
66	27	Lincoln	11W	11S	8	Fence row in front of Coast Guard Station, Newport.
67	27	Lincoln	11W	10S	9	South-facing bluff. Yaquina Bay Hwy. 3.3 mi. E of Coast Guard Station.
68	18	Lincoln	11W	11S	10	Exposed west-facing bluff, Yaquina Bay Hwy. 8.1 mi. E of Coast Guard Station.
69	18	Lincoln	11W	11S	18	Yaquina Bay Hwy. 0.6 mi. SE of junction with U. S. Hwy. 20.
70	18	Lincoln	10W	10S	26	Small population north-facing bank on U. S. Hwy. 20 0.3 mi. W of Sam Creek Rd. junction.
71	18	Lincoln	9W	11S	10	Large population, grassy hillside 0.4 mi. E of Eddyville city limit sign.
72	18	Benton	7W	11S	23	Fence row 14.4 mi. E of Eddyville on U. S. Hwy. 20.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
73	27	Clatsop	10W	5N	9	Rocky soil, roadside cut U. S. Hwy. 26, 1.6 mi. S of U. S. Hwy. 101 junction.
74	27	Clatsop	10W	5N	19	Exposed coastal bluff opposite "The Rocks" on Cannon Beach Loop Road.
75	27	Tillamook	10W	3N	18	0.6 mi. N of Oswald West State Park boundary marker on U. S. Hwy. 101, exposed coastal bluff, side of Neahkahnie Mt.
76	27	Tillamook	10W	1N	17	Sand dunes beneath Coast Guard Lookout, entrance Tillamook Bay.
77	27	Tillamook	10W	4S	13	Roadside bank Ore. Hwy. 22 0.4 mi. E of Hebo Ranger Station.
78	27	Tillamook	9W	5S	28	0.5 mi. NW of Little Nestucca River bridge on Ore. Hwy. 22.
79	18	Yamhill	8W	5S	33	Large population in field 0.5 mi. S of South Yamhill River bridge on Ore. Hwy. 22.
80	18	Polk	7W	6S	13	Ore. Hwy. 22, 1.5 mi. S of junction with Ore. Hwy. 18.
81	27	Josephine	8W	38S	14	Clearing in area of old logging, 2.1 mi. S of Selma on U. S. Hwy. 199.
82	27	Josephine	9W	40S	36	9.2 mi. S of Cave Junction on U. S. Hwy. 199 in open area. Clonal clumps.
83	18	Del Norte	4E	18N	20	Roadside cut in narrow east-west valley 6.2 mi. S of California Quarantine Station on U. S. Hwy. 199.
84	27	Del Norte	1E	17N	24	Sandy bank, flooded by Smith River in 1964 at east end of bridge on U. S. Hwy. 199.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
85	2n=45, 18	Del Norte	1W	16N	1	South entrance to Webber Grove State Park on U. S. Hwy. 199 along fence row extending north from Monument Ranch entrance gate.
86	18	Del Norte	1W	16N	29	Southwest-facing coastal bluff. 110 ft. W of corner of Taylor and 6th St., Crescent City, California.
87	18	Del Norte	1E	14N	20	Exposed west-facing coastal bluff directly opposite False Klamath Rock.
88	18	Del Norte	1W	18N	26	1.2 mi. N of Rowdy Creek bridge on U. S. Hwy. 101.
89	18	Curry	14W	40S	36	Harris Beach State Park, dead-ended old highway section, large population.
90	18	Curry	14W	38S	29	East-facing slope of grassy sand dune 0.8 mi. S of Carpenterville turnoff on U. S. Hwy. 101.
91	18	Curry	14W	39S	4	Roadside bank 5.7 mi. S of Pistol R. bridge on abandoned U. S. Hwy. 101.
92	18	Curry	15W	36S	25	Sand dune bluff on Gold Beach frontage road 1.1 mi. N of junction with U. S. Hwy. 101.
93	18	Curry	14W	34S	6	Coastal bluff 0.5 mi. S of Brush Creek bridge on U. S. Hwy. 101.
94	18	Curry	15W	32S	29	Pseudotsuga cutover area 1.2 mi. N or Port Orford city limit sign on U. S. Hwy. 101. Small plants among dense undergrowth.
95	18	Coos	15W	30S	13	Small roadside population 2.2 mi. N of Coos County line on U. S. Hwy. 101.
96	18	Coos	14W	28S	17	North end of Coquille River bridge on U. S. Hwy. 101.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
97	18	Coos	14W	27S	21	Regeneration area of Picea and Pseudotsuga, 0.8 mi. N of Charleston turnoff on U. S. Hwy. 101.
98	27	Coos	13W	25S	33	Roadside ditch opposite S. P. RR marker 770 on U. S. Hwy. 101 300 ft. S of First Street intersection, Coos Bay.
99	27	Coos	13W	24S	15	Marshy area in dune area 3.5 mi. N of Coos Bay bridge on U. S. Hwy. 101.
100	18	Douglas	12W	22S	31	Opposite mileage marker 220, 0.6 mi. N Douglas County line on U. S. Hwy. 101.
101	18	Douglas	12W	21S	27	North end of Umpqua River bridge on U. S. Hwy. 101.
102	27	Douglas	12W	20S	5	Steep bluff behind Carter Lake boat ramp entrance sign 0.6 mi. S of Lane County line on U. S. Hwy. 101.
103	27	Lane	12W	18S	27	Vacant lot behind Snack Bar Cafe, 12th and U. S. Hwy. 101, Florence.
104	18	Wallowa	44E	3S	--	4.0 mi. in on Aneroid Trail, Wallowa Nat. Forest, J. R. Estes 87.
105	--	Wallowa	44E	3S	3	2.0 mi. in on Hurricane Trail, Wallowa Nat. Forest, J. R. Estes 89.
106	18	Wallowa	45E	3S	9	Base of east terminal moraine, edge of Wallowa Lake, J. R. Estes 91.
107	27	Lane	8W	17S	18	Small population 4.5 mi. W of Greenleaf Creek bridge on Ore. Hwy. 36.
108	27	Lane	9W	17S	16	Exposed road bluff cut 0.2 mi. W of Green Creek bridge on Ore. Hwy. 34.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
109	27	Lane	10W	17S	23	0.3 mi. W of Thompson Creek bridge on Ore. Hwy. 36.
110	27	Lane	10W	18S	8	Opposite lumber mill office 1.1 mi. E of Tiernan P. O. on Ore. Hwy. 36.
111	27	Lane	11W	18S	16	Marshy area along Siuslaw River 0.1 mi. E of Cushman city limit sign.
112	18	Douglas	12W	22S	1	Roadside turnout area 1.7 mi. E of Reedsport city limit sign on Ore. Hwy. 38.
113	27	Douglas	11W	22S	1	Open area 200 ft. from Umpqua River along W boundary of Umpqua Wayside Park.
114	27	Coos	13W	24S	15	Marshy bank of North Slough Canal S of Hauser on U. S. Hwy. 101.
115	27	Coos	13W	25S	30	0.2 mi. N of Pigeon Point Rd junction with Charleston-Empire road on latter.
116	27	Coos	14W	26S	11	Base of hill, Bandon-Charleston road 0.3 mi. SW of Cape Arago State Park turnoff.
117	18	Coos	14W	27S	8	Large population, clear-cut area 1.1 mi. N of Coquille-U. S. Hwy. 101 Rd. and Bandon-Charleston road on latter.
118	18	Coos	14W	27S	14	0.3 mi. N of junction of U. S. Hwy. 101 and Coquille-Bandon road.
119	18	Coos	13W	27S	27	Beaver Hill Lookout Rd.0.4 mi. SE of junction with U. S. Hwy. 101.
120	18	Coos	13W	28S	6	North Bank Rd. 1.0 mi. N of junction with Riverton Ferry Rd., 300 ft. N of George Welsh mailbox.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
121	18	Coos	14W	28S	23	North Bank Rd. 5.5 mi. W of Riverton Ferry Rd., 200 ft. E of F. E. Berry mailbox.
122	18	Coos	14W	28S	9	Roadside rubble pile, North Bank Rd. 1.0 mi. W of Randolph.
123	18	Coos	15W	28S	25	Bandon Beach Loop Rd. Exposed coastal bluff.
124	18	Coos	14W	28S	9	South Bank Rd. 0.6 mi. E of Prosper.
125	18	Coos	14W	28S	15	South Bank Rd. 0.7 mi. NW of junction with Ore. Hwy. 42S.
126	18	Coos	14W	28S	23	Coquille River bluffs, 0.7 mi. E of South Bank Rd. junction on Ore. Hwy. 42S.
127	18	Coos	13W	28S	19	Coquille River flood plain 3.0 mi. W of Riverton on Ore. Hwy. 42S.
128	18	Coos	13W	28S	5	Roadside bluff, Ore. Hwy. 42S, 0.8 mi. E of Riverton.
129	18	Coos	13W	28S	3	Clonal clump, edge of pavement, 0.2 mi. E of Fat Elk Creek bridge on Ore. Hwy. 42S.
130	18	Coos	13W	27S	10	0.3 mi. N of Coaledo on Ore. Hwy. 42.
131	27	Coos	13W	25S	3	Rock jetty in Haines Inlet of Coos Bay, 50 ft. E of "Dead End" sign.
132	27	Coos	13W	25S	3	Exposed bay bluff 0.2 mi. N of end of Coos Bay bridge.
133	27	Douglas	13W	22S	14	Exposed coastal bluff, Winchester Bay observation point.
134	27	Douglas	12W	21S	4	Ridge top 2.0 mi. N of Gardiner city limit sign on U. S. Hwy. 101.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
135	27	Lane	12W	18S	24	Large population at east end of Ore. Hwy. 36 bridge over north fork of Siuslaw River.
136	--	Siskiyou	5E	17N	16	Start of Twin Valley Trail, E side of Young's Valley, elev. ca. 4000 ft. R. L. Carr.
137	18	Benton	5W	11S	34	Grass embankment behind Girls Athletic Building. Oregon State University.
138	--	Harney	35E	33S	--	Undisturbed habitat, east side of Steens Mts., cliff ledge overlooking Alvord Desert, J. R. Estes 94-96.
139	18	Benton	8W	14S	8	Isolated population W of Maltby Cr. bridge on Ore. Hwy. 34, roadside ditch.
140	18	Benton	8W	14S	8	Roadside ditch along Ore. Hwy. 34 300 ft. W of Calhoun mailbox, 0.2 mi. W of accession 139.
141	18	Del Norte	1W	16N	1	South entrance to Webber Grove State Park on U. S. Hwy. 101 along fence row extending north from Monument Ranch entrance gate.
142	18	Del Norte	1W	16N	20	Roadside bank along U. S. Hwy. 101 0.2 mi. S of junction with U. S. Hwy. 199.
143	18	Del Norte	1W	16N	17	Roadside bank along U. S. Hwy. 101 0.2 mi. N of junction with U. S. Hwy. 199.
144	18	Del Norte	1W	18N	17	Roadside bank along U. S. Hwy. 101 5.4 mi. S of Winchuck River bridge.

Appendix I. (continued)

Accession number	Chromosome number	County	Range	Township	Section	Notes
145	27	Coos	13W	26S	23	Small population along railroad right of way in swampy ditch 0.2 mi. S of S.P.R.R. marker 773.
146	27	Coos	13W	25S	21	Isolated clump 500 ft. E of intersection of Ocean and Bulter in residential Coos Bay.
147	27	Coos	14W	26S	9	Exposed coastal sand dune 500 ft. N of shower facilities at Sunset Bay State Park.
148	27	Coos	14W	26S	4	200 ft. N of entrance to Bassendorff Beach Park.
149	27	Coos	14W	26S	2	Shaded roadside bank cut 0.3 mi. W of Charleston drawbridge.
150	18	Coos	13W	23S	26	Small population along U. S. Hwy. 101 1.0 mi. S of Tenmile Creek bridge.
151	18	Douglas	12W	21S	15	Roadside bluff on U. S. Hwy. 101 halfway up grade N of Gardiner opposite entrance to paper processing plant.
152	27	Doulgas	12W	20S	17	Small roadside population on U. S. Hwy. 101 0.9 mi. S of entrance to Carter Lake State Park boat launching facilities.
153	27	Lane	12W	19S	27	Roadside ditch along U. S. Hwy. 101 1.0 mi. S of Siltcoos River bridge.
154	27	Lincoln	12W	15S	3	Small coastal bluff population at entrance to Cape Perpetua Visitor Center on U. S. Hwy. 101.
155	27	Lincoln	11W	13S	26	Isolated plant 0.7 mi. W of Siuslaw National Forest boundary marker on Ore. Hwy. 34.
156	27	Lincoln	11W	13S	25	Isolated plant 0.3 mi. E of Siuslaw National Forest boundary marker on Ore. Hwy. 34.

Appendix II. Measurements on internal diameter of Achillea pollen.
Ten good grains measured for each accession.

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
51-3	18	18.7	20.7	20.0	0.53	0.17
53-2	18	16.7	19.3	17.6	1.05	0.33
53-4	18	17.3	20.7	19.2	0.99	0.31
54-1	18	19.3	22.7	20.8	0.77	0.24
54-2	18	18.0	20.0	18.9	1.25	0.40
55-1	18	16.7	23.3	19.9	0.86	0.27
55-2	18	19.3	23.3	20.8	0.94	0.30
55-3	18	19.3	22.0	20.5	0.61	0.19
56-1	27	16.7	20.0	18.4	0.74	0.23
56-2	18	17.3	22.0	19.8	1.09	0.34
56-3	18	18.0	20.7	19.5	1.04	0.33
57-1	18	18.0	20.0	19.2	0.52	0.16
57-2	18	17.3	19.3	18.3	0.54	0.17
58-1	18	16.0	18.7	17.3	1.04	0.33
59-1	27	19.3	22.0	20.5	0.66	0.21
59-2	27	22.0	22.7	22.3	1.32	0.42
60-1	18	16.0	18.7	17.2	0.84	0.27
61-1	27	20.0	22.0	20.8	1.22	0.39
61-2	27	22.7	26.7	25.1	1.10	0.35
62-1	27	20.7	24.0	22.3	0.82	0.26
63-1	27	20.0	22.7	21.7	0.79	0.25
64-1	27	20.0	22.7	21.3	1.11	0.35
65-1	27	20.0	24.0	21.8	0.71	0.22

Appendix II. (continued)

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
65-2	27	20.7	22.7	21.9	1.26	0.40
66-1	27	19.3	23.3	21.7	0.95	0.30
67-2	27	20.0	24.7	22.1	0.80	0.25
68-1	18	18.0	20.7	19.2	1.12	0.35
68-2	18	18.0	20.7	19.2	1.09	0.34
69-1	18	16.0	19.3	17.9	0.99	0.31
70-1	18	18.0	20.0	19.1	1.16	0.38
70-2	18	18.7	20.7	19.3	0.47	0.15
71-1	18	18.0	20.0	19.1	0.48	0.15
71-2	18	17.3	20.0	18.8	1.12	0.35
72-1	18	18.0	20.0	18.9	0.48	0.15
73-1	27	22.0	28.0	25.2	1.63	0.52
74-1	27	22.0	25.3	24.0	1.21	0.38
75-1	27	19.3	21.3	20.4	1.10	0.35
76-1	27	19.3	22.7	20.7	0.78	0.25
77-1	27	19.3	22.7	20.8	1.12	0.35
78-1	27	20.0	23.3	21.3	0.95	0.30
79-1	18	18.7	20.7	19.8	0.48	0.15
80-1	18	16.7	19.3	18.1	1.09	0.34
81-1	27	22.0	26.0	24.5	1.04	0.33
82-1	27	20.0	21.3	20.8	0.40	0.13
82-2	27	20.0	23.3	21.9	0.92	0.29
83-1	18	18.7	22.0	20.1	0.94	0.30

Appendix II. (continued)

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
83-2	18	18.7	21.3	20.1	1.17	0.37
83-3	18	20.0	23.3	21.8	0.75	0.24
83-4	18	18.7	21.3	20.0	0.71	0.22
83-5	18	20.0	23.3	21.8	0.89	0.28
84-1	27	20.0	22.7	21.2	0.77	0.24
85-1	--	18.0	20.0	19.2	0.52	0.16
85-2	18	17.3	20.0	18.9	0.54	0.17
85-3	18	18.7	21.3	20.0	0.57	0.18
85-4	20-26	20.0	22.0	21.2	0.46	0.15
86-1	18	19.3	22.7	21.5	1.01	0.32
86-2	18	20.7	23.3	22.0	0.57	0.18
86-3	--	17.3	20.0	18.5	0.70	0.22
86-4	18	20.7	24.0	22.5	0.93	0.29
87-1	18	18.7	21.3	20.3	0.63	0.20
87-2	18	18.0	20.7	18.9	0.63	0.20
88-1	18	18.0	20.0	18.8	1.09	0.34
89-1	18	18.0	20.0	18.8	1.14	0.36
90-1	18	18.7	22.0	20.1	0.96	0.30
91-1	18	18.0	20.7	19.2	0.80	0.25
92-2	18	19.3	22.0	20.5	1.17	0.37
92-3	18	18.7	20.0	19.2	0.32	0.10
93-1	18	18.7	21.3	19.9	0.81	0.26
94-1	18	18.7	20.7	19.8	1.18	0.37

Appendix II. (continued)

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
95-1	18	18.7	20.7	19.5	1.12	0.35
95-2	18	18.7	20.7	20.1	0.51	0.16
96-1	18	20.0	23.3	21.8	1.11	0.35
97-1	18	19.3	23.3	20.7	0.91	0.29
98-1	27	21.3	25.3	23.9	0.90	0.28
99-1	27	20.0	21.3	20.5	0.40	0.13
100-1	18	18.7	23.3	21.7	0.48	0.15
101-1	18	18.0	21.3	19.9	1.07	0.34
102-1	27	21.3	24.7	23.2	1.17	0.37
103-1	27	20.0	23.3	21.7	0.79	0.25
104-1	18	18.0	21.3	20.0	0.88	0.28
106-1	18	17.3	18.7	17.7	1.14	0.36
107-1	27	20.7	23.3	22.4	1.16	0.37
107-2	27	20.0	22.0	21.3	0.47	0.15
107-3	27	22.7	24.0	23.3	0.41	0.13
108-1	27	22.0	24.7	23.5	0.70	0.22
108-2	27	20.0	23.3	22.0	0.88	0.28
109-1	27	20.7	23.3	21.5	0.66	0.21
109-2	27	21.3	24.0	22.5	1.22	0.39
110-1	27	21.3	24.0	22.3	0.68	0.22
110-2	27	21.3	23.3	22.3	0.42	0.13
111-1	27	21.3	24.0	22.5	1.17	0.37
111-2	27	22.7	26.7	24.1	1.07	0.34

Appendix II. (continued)

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
112-1	18	22.7	26.7	24.1	1.07	0.34
112-2	18	22.0	26.7	24.4	1.00	0.32
113-1	27	22.0	26.0	23.2	1.09	0.34
113-2	27	20.0	22.7	21.3	0.82	0.26
114-1	27	21.3	25.3	23.3	1.06	0.34
114-2	27	19.3	22.0	20.4	0.95	0.30
115-1	27	24.0	26.7	25.1	1.32	0.42
115-2	27	23.3	26.0	24.7	1.30	0.41
116-1	27	20.7	23.3	21.9	1.17	0.37
116-2	27	20.0	25.3	21.2	0.51	0.16
117-1	18	20.7	24.0	22.4	0.71	0.22
118-1	18	17.3	22.7	20.0	0.97	0.31
119-1	18	18.0	20.7	19.7	1.11	0.35
120-1	18	18.0	20.7	19.8	0.75	0.24
120-2	18	17.3	20.0	18.9	1.14	0.36
120-3	18	18.7	22.0	19.9	1.09	0.34
121-1	18	19.3	21.3	20.1	0.46	0.15
121-2	18	17.3	19.3	18.7	1.16	0.37
122-1	18	18.0	20.0	19.2	0.62	0.20
123-1	18	19.3	22.7	21.2	1.12	0.35
123-2	18	19.3	22.0	20.5	1.17	0.37
124-1	18	20.0	21.3	20.9	0.35	0.11
124-2	18	18.7	21.3	20.4	0.62	0.20

Appendix II. (continued)

Accession No. plant	Chromosome number (N)	Size Range (μ) Smallest	Largest	Mean	Standard deviation	Standard error
125-1	18	18.0	21.3	19.3	1.06	0.34
125-2	18	20.0	20.9	20.3	1.27	0.40
125-3	18	18.7	21.3	20.0	0.66	0.21
126-2	18	16.7	19.3	18.1	1.07	0.34
127-1	18	18.0	20.7	19.3	0.57	0.18
127-2	18	18.7	20.7	20.1	1.19	0.38
127-3	18	20.7	23.3	21.7	0.58	0.18
128-1	18	20.0	22.7	21.2	1.19	0.38
128-2	18	18.7	22.0	20.8	1.09	0.34
129-1	18	20.0	22.7	21.1	1.18	0.37
130-1	18	18.0	20.7	19.3	0.62	0.20
130-2	18	18.0	21.3	19.7	0.91	0.29
131-1	27	21.3	23.3	22.3	0.48	0.15
132-1	27	20.0	23.3	22.0	1.06	0.34
133-1	27	22.7	24.7	23.8	0.58	0.18
133-2	27	22.0	26.0	23.2	0.99	0.31
134-1	27	19.3	21.3	22.0	0.53	0.17
134-2	27	21.3	23.3	22.1	0.46	0.15
135-1	27	19.3	22.0	20.9	1.18	0.37
135-2	27	18.7	22.7	20.5	0.87	0.28
135-3	27	20.7	22.7	21.8	0.54	0.17