# CYTOGEOGRAPHY OF *ACHILLEA MILLEFOLIUM* IN OKLAHOMA AND ADJACENT STATES

WILMA PIREH AND RONALD J. TYRL

The circumboreal *Achillea millefolium* L. (Asteraceae: Anthemidae) is one of the most extensively studied polyploid complexes. Comprising diploids, tetraploids, hexaploids, and octoploids ($x=9$), the complex is cytologically most diverse in southeastern Europe and southwestern to central Asia. Investigations by Schneider (1958) and Ehrendorfer (1952b, 1953, 1959a–d) indicate that the complex in Eurasia is composed of isolated diploid cytotypes and extensive polyploids of hybrid origin. Species names have been applied to these ploidy levels; Ehrendorfer (1952b) recognized four diploid, one tetraploid, two hexaploid, and one octoploid species.

Studies of polyploidy and geographical distribution in North America west of the Sierra-Cascade crest have been extensive beginning with the now classical studies of Turesson (1939) and Clausen, Keck, & Hiesey (1938, 1940, 1948). These and other cytogeographical studies by Lawrence (1947), Ehrendorfer (1952b, 1973) and Tyrl (1969, 1975) revealed only tetraploids ($n = 18$), hexaploids ($n = 27$), and their pentaploid, septaploid, and octoploid hybrids. Hexaploid *Achillea* principally occupies coastal habitats from Alaska to Baja California, while the tetraploid occurs in the interior except for the coastal areas of northwestern Washington and southwestern Oregon -northwestern California where it replaces the hexaploid cytotype.

Although the distributions of *Achillea* cytotypes have been studied extensively in the Pacific Coast states, relatively few counts have been made of populations in central and eastern North America. Widely spaced counts by Turesson (1939), Ehrle (1958), Mulligan & Bassett (1959), Turner et al. (1961), DeJong & Longpre (1963), Löve & Solbrig (1964), Löve & Löve (1966), Löve & Ritchie (1966), Hedberg (1967), Smolinski et al. (1967), Jones (1968), Suda & Argus (1969), Ehrendorfer (1973), and Gervais (1977) indicate that tetraploids predominate but with hexaploids occasionally occurring such as those found in Illinois (Smolinski et al., 1967), Ontario (Mulligan & Bassett, 1959), and Quebec (Ehrendorfer, 1973).

The studies by Tyrl (1969, 1975) indicated that although broad generalization concerning cytotype distribution in *Achillea* may be made, the distribution of tetraploids and hexaploids is often much more complex at the population level. Frequently hexaploid plants or populations are found in areas containing primarily tetraploids and vice versa. Populations comprising both $4x$ and $6x$ plants occur. In addition, the discovery of tetraploid plants producing unreduced gametes suggests active formation of hexaploids in North America. The objective of this study was to determine the chromosome number of *Achillea* plants growing in Oklahoma and adjacent states in order to determine if similar, complex cytogeographic patterns occur in the interior.

## MATERIALS & METHODS

Chromosome counts were made of 218 populations in Oklahoma and adjacent states (Pireh, 1978). A population sample normally consisted of material from three or four plants growing in road right-of-ways and adjacent fields. Each locality was assigned an accession number and located by range, township, section as well as mileage from a permanent landmark.

Heads in various stages of flowering were fixed in chloroform, 95% ethanol, glacial acetic acid (6:3:1) for a minimum of 24 hours, then washed and stored in 70% ethanol. Heads were stained in bulk, using hydrochloric-alcoholic carmine stain for 24 hours at 60° C and then washed in 70% ethanol. Excised anthers were squashed and mounted in Hoyer's Medium and examined with phase-contrast optics. Metaphase chromosomes were counted, being easily observed in microspores undergoing the first post-meiotic mitosis. Counts were obtained from three or four microspores per plant.

Somatic cell counts were also made. Achenes were germinated on moist filter paper in petri dishes. The fresh root tips were pretreated with a saturated aqueous solution of paradichlorobenzene for 3 hours at about 60° C, fixed in 95% ethanol and glacial acetic acid (3:1), washed in 70% ethanol, and then stained, squashed, and mounted in 1% aqueous acetocarmine. The chromosomes of three or four cells per root tip were counted.

## RESULTS

The 293 chromosome counts obtained from 218 populations in this study are combined with those of earlier reports (cited above) in Figure 1. All plants examined by us, with one exception, were tetraploid ($2n = 36$). Meiotic divisions were regular, all microspores having 18 chromosomes and exhibiting uniform cytoplasmic staining and morphology. One plant from Caddo County in western Oklahoma was an aneuploid with a somatic chromosome number of 34. Meiosis was normal with spores having 17 chromosomes.

## DISCUSSION

Tetraploid *Achillea* appears to predominate in central and eastern North America; the additional chromosome counts reported here confirm the broad distributional pattern described by Ehrendorfer (1973). The hexaploids found in the St. Lawrence River Valley and along the Atlantic Coast are believed to be recent introductions from Europe, being found near seaports or closely resembling commonly imported cultivars. Additional information, however, is needed regarding the occasional hexaploid plants reported from the interior of the continent (Mulligan & Bassett, 1959; Smolinski et al., 1967; Ehrendorfer, 1973). Their status as native plants or escaped ornamentals is yet unresolved; verification of their chromosome numbers is needed, as well as extensive sampling of adjacent populations.

Further study of putative hexaploids along the Arctic coast is also warranted. Determining ploidy level by measuring pollen grain diameters, Mulligan & Bassett (1959) reported hexaploids as present only along the northern coasts of Alaska, the Yukon, the Northwest Territories, Ontario, Quebec, and Newfoundland. Gervais (1977), however, has discovered that known tetraploids from northern Quebec possess pollen grains with diameters equal to or greater than those of hexaploid plants.

The presence of only $4x$ plants from diverse climatic and edaphic regimes, and the absence of meiotic irregularities in the continental interior support the hypothesis advanced by Ehrendorfer (1952b, 1973) and Tyrl (1969, 1975) that evolutionary activity in the genus is centered in the Pacific Northwest, especially the Klamath and Olympic Mountains. In these areas, the occurrence of numerous tetraploid plants producing both reduced ($n = 18$) and unreduced ($n = 36$) microspores, the occurrence of solitary hexaploid plants among tetraploids having unreduced spores, and the occurrence of septaploids ($2n = 63$) and octoploids ($2n = 72$) among tetraploids and hexaploids producing unreduced spores suggest that functional unreduced gametes are responsible for increases in ploidy level in *Achillea*. For example, the union of 2x and 4x gametes results in the

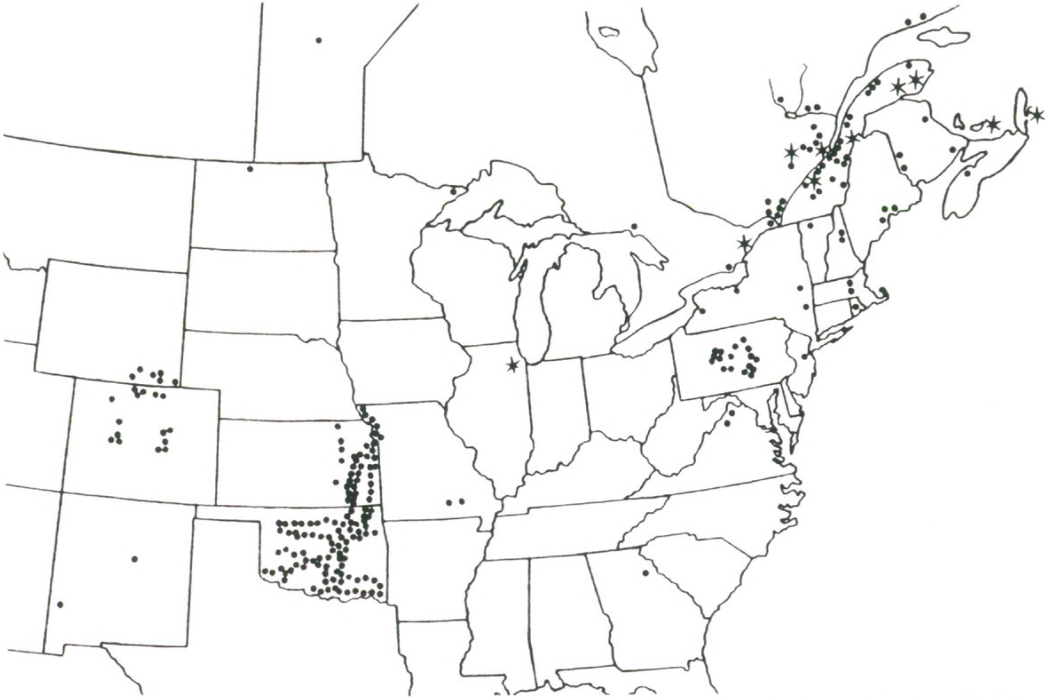

Figure 1. Distribution of *Achillea* cytotypes in the central and eastern United States and adjacent Canada. Solid circles, tetraploid populations ($n = 18$); solid stars, hexaploid populations ($n = 27$).

formation of a 6x zygote ($2n = 54$). The coastally restricted hexaploids appear to be indigenous to western North America and to arise from previously established tetraploid progenitors. The extensive observations of $4x$–$6x$ intergradation in morphology, ecology, and environmental responses support this conclusion (Clausen et al., 1940, 1948; Hiesey, 1953; Hiesey & Nobs, 1952). In additiion, the limited success in crossing Eurasian and North American hexaploids indicates a distinct genetic relationship (Clausen et al., 1940; Ehrendorfer, 1952a; Hiesey & Nobs, 1970). In contrast, crosses between old and new world tetraploids are successful. Nondisjunction apparently is not a factor in south-central North America. Presumably as a consequence, the meiotic system of *A. millefolium* is evolutionally conservative in this region.

ACKNOWLEDGEMENTS

We thank Dr. James R. Estes of the University of Oklahoma for his review of the manuscript.

LITERATURE CITED

Clausen, J., D. Keck & W. M. Hiesey. 1938. Chromosome number and environment. Carnegie Inst. Wash. Year Book. **37:**220–221.

———. 1940. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Publ. Carnegie Inst. Wash. **520:**1–452.

———. 1948. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Publ. Carnegie Inst. Wash. **581:** 1–129.

DeJong, D. C. D. & E. K. Longpre. 1963. Chromosome studies in Mexican compositae. Rhodora **65:**225–240.

Ehrendorfer, F. 1952a. Cytology of *Achillea* hybrids. Carnegie Inst. Wash. Year Book. **51:**124–125.

———. 1952b. Cytotaxonomic studies of *Achillea*. Carnegie Inst. Wash. Year Book. **51:**125–131.

———. 1953. Systematische und Zytogenetische Untersuchungen an Europasichen Rassen des *Achillea millefolium* Komplexes. Österr. Bot. Z. **100:** 583–592.

———. 1959a. *Achillea roseo-alba* Ehrendf., *spec. nov.*, eine hybridogene, di- und tetraploide Sippe des *Achillea millefolium* Komplexes. Österr. Bot. Z. **106:**363–368.

———. 1959b. Differentiation—hybridization cycles and polyploidy in *Achillea*. Cold Spring Harbor Symp. Quant. Biol. **24:**141–152.

———. 1959c. The results of comparative phylogenetic studies on *Galium*, *Knautia*, and *Achillea*. Congr. Internatl. Bot. 9th. **2:**102.

______. 1959d. Mechanisms of micro-evolution in diploid and polyploid races of *Achillea*. Ber. Deutsch. Bot. Ges. **72**:11–12.

______. 1973. New chromosome numbers and remarks on the *Achillea millefolium* polyploid complex in North America. Österr. Bot. Z. **122**:133–143.

EHRLE, E. B. 1958. A cytotaxonomic study of the genus *Achillea* in Pennsylvania. Rhodora **60**:7–9.

GERVAIS, C. 1977. Cytological investigation of the *Achillea millefolium* complex (Compositae) in Quebec. Canad. J. Bot. **55**:796–808.

HEDBERG, O. 1967. Chromosome numbers of vascular plants from arctic and subarctic North America. Ark. Bot., Ser 2. **6**:309–326.

HIESEY, W. M. 1953. Comparative growth between and within climatic races of *Achillea* under controlled conditions. Evolution **7**:297–316.

______. & M. A. NOBS. 1952. Genetic structure of climatic races and species of *Achillea*. Carnegie Inst. Wash. Year Book. **51**:122–125.

______& ______ 1970. Genetic and transplant studies on contrasting species and ecological races of the *Achillea millefolium* complex. Bot. Gaz. **131**:245–259.

JONES, S. B. 1968. Chromosome numbers in southeastern United States Compositae. II. Bull. Torrey Bot. Club **95**:488–494.

LAWRENCE, W. E. 1947. Chromosome numbers in *Achillea* in relation to geographic distribution. Amer. J. Bot. **34**:538–545.

LÖVE, A. & O. T. SOLBRIG. 1964. IOPB chromosome number reports. I. Taxon **13**:99–110.

______ & D. LÖVE. 1966. Cytotaxonomy of the alpine vascular plants of Mt. Washington. Univ. Colo. Stud., Ser. Biol. **24**:1–74.

______ & J. C. RITCHIE. 1966. Chromosome numbers from central northern Canada. Canad. J. Bot. **44**:429–439.

MULLIGAN, G. A. & I. J. BASSETT. 1959. *Achillea millefolium* complex in Canada and portions of the United States. Canad. J. Bot. **37**:73–79.

PIREH, W. 1978. Cytogeography and reproductive biology of *Achillea millefolium* in Oklahoma and adjacent states. M.S. Thesis. Oklahoma State University, Stillwater, OK.

SCHNEIDER, I. 1958. Zytogenetische Untersuchungen an Sippen des Polyploid-Komplexes *Achillea millefolium* L. *s. lat.* (Zur Phylogenie der Gattung *Achillea* I.) Österr. Bot. Z. **105**:111–158.

SMOLINSKI, S. J., C. L. BELL, L. BELL & L. BAUER. 1967. The isolation of Achillin from *Achillea millefolium*. Lloydia **30**:144–149.

SUDA, Y. & G. W. ARGUS. 1969. IOPB chromosome number reports. Taxon **18**:221.

TURESSON, G. 1939. North American types of *Achillea millefolium*. Bot. Not. **1939**:813–816.

TURNER, B. L., J. H. BEAMAN & H. F. L. ROCK. 1961. Chromosome numbers in the Compositae. V. Mexican and Guatemalan species. Rhodora.**63**: 121–129.

TYRL, R. J. 1969. Cytogeography of *Achillea millefolium* in Western Oregon. Brittonia **21**:215–223.

______. 1975. Origin and distribution of polyploid *Achillea* (Compositae) in Western North America. Brittonia **27**:187–196.

W.P.
DEPARTMENT OF BIOLOGY,
JUNDISHAPURE UNIVERSITY,
AHWAZ, IRAN

R.J.T.
HERBARIUM,
OKLAHOMA STATE UNIVERSITY,
STILLWATER, OKLAHOMA