AN ABSTRACT OF THE THESIS OF

Karen E. Bledsoe for the degree of Master of Arts in Botany and Plant Pathology presented March 15, 1993. Title: Morphological and Cytological Variation in *Trillium albidum* Freeman (Liliaceae).

Abstract approved:

This work was performed on *Trillium albidum* Freeman (Liliaceae), to clarify the pattern of morphological variation between the small-flowered populations at the northern limit of its range (*T. parviflorum* Soukup) and the larger-flowered populations from southern Oregon and northern California. It was hypothesized that only a single species of sessile-flowered *Trillium* occurs in the range of *Trillium albidum*, from the Puget Sound area in Washington to Northern California. Eighteen sites were visited in Washington and Oregon. Morphological data taken from field-collected specimens included size of parts, ratios of length and width of parts, and color of parts. Rhizome shoot scars on herbarium specimens were counted to estimate relative plant age. Plant ovaries from the field were cold-treated and preserved for karyotyping. It was found that plant height, petal lenght, and leaf length positively correlated with rhizome shoot scars. Cluster analysis excluding size characteristics showed a tendency of populations to form groups corresponding to three geographical areas: Washington and northern Willamette Valley, central Willamette Valley to the Umpqua River, and populations south of the Umpqua divide. Principal components analysis revealed two groups: the

southern-most Oregon populations, and populations from the Umpqua River northward. Karyotypes give some additional support to these patterns through the limited geographical occurrence of particular chromosome types. Plants in Washington showed heterozygosity of chromosomes, and a limited karyotype. Chromosome B0 and the heterozygous combination of E4-8 was found only in plants in the Willamette Valley. One population Jackson County, Oregon has plants with chromosome E6, which was not found in other populations. Though population groupings were revealed by multivariate statistics, without further study it was not desirable to give them formal infraspecific names. The percentage of taxonomically ambiguous individuals was too high, and the amount of overlap in variation too great, to permit a formal species distinction between the southern and northern populations, though subspecies might be recognized after further study. Based upon these data, the recognition of *T. parviflorum* is not justified.

Morphological and Cytological Variation in *Trillium albidum* Freeman (Liliaceae)

by

Karen E. Bledsoe

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INTRODUCTION

Subject, purpose, and scope of the study.

Trillium albidum Freeman is a spring-flowering woodland herb of western Washington, Oregon, and California, whose range extends from Puget Sound, Washington, southward more or less continuously to Placer and Sonoma counties, California. Prior to its description by Freeman (1975) the species had been included within *T. chloropetalum* (Torrey) Howell, a taxon of the San Francisco Bay region of California. Although Freeman's reclassification of the aggregate species *T. chloropetalum* into four species, including *T. albidum*, was a marked improvement over earlier taxonomic treatments of the group, certain unanswered questions remain concerning intraspecific variation and taxonomic divisions within *T. albidum* itself. For example, Freeman's field studies of living populations did not extend north of Douglas County, Oregon, leaving incomplete his description of the species' variability in the northern part of its range. Small-flowered populations from southwestern Washington and adjacent Oregon were subsequently described by Soukup (1980) as a new species, *T. parviflorum*. However, the characteristics separating *T. parviflorum* from *T. albidum* appear to be minor and intergradient.

The present study was undertaken to clarify the pattern of morphological variation in *Trillium albidum* in the region between the small-flowered extremes at

the northern limit of its range (*T. parviflorum*), and the larger-flowered populations from southern Oregon and northern California (*T. albidum* Freeman). A further goal was to incorporate chromosome morphology data as a separate line of evidence that might reveal taxonomically significant variation within *T. albidum*.

Trillium species are known to show differentially staining regions in the mitotic chromosomes of somatic tissue after exposure to temperatures near 0° C (Bailey, 1954; Darlington and Shaw, 1959; Kurabayashi, 1952; Shaw, 1959). Chromosome banding patterns have been used in other studies for species differentiation (Bailey, 1954; Chinnappa and Morton, 1977), and in recognizing breeding patterns within *Trillium* species (Fukuda, 1967, 1969). For each of the five chromosomes in the haploid complement, different morphological types can be recognized by the size and position of lightly staining, allocyclic bands (Dyer, 1964). Intraspecific variation in chromosome morphology had been used to study the population genetics and evolution of species of *Trillium*, both in Japan (Kurabayashi, 1958) and the eastern United States (Serota, 1969a; Fukuda and Grant, 1980), and the technique had been applied in a preliminary way to the genus in the western United States (Kurabayashi, 1963; Fukuda, 1967; Fukuda and Channel, 1975).

The purpose of my study is threefold: (1) to describe the morphological and chromosomal variation of *Trillium albidum* populations, (2) to better to evaluate the taxonomic status of *T. parviflorum* and (3) to discover whether there is justification for dividing *T. albidum* into geographically separate subspecies.

Review of previous work.

The genus *Trillium* Linnaeus (Liliaceae) consists of about 40-50 species (Freeman, 1975; Samejima and Samejima, 1987), occurring primarily in temperate woodlands of the Northern Hemisphere. Sessile-flowered species of subgenus *Phyllantherum* are limited to North America, while pedicellate-flowered species of subgenus *Trillium* are found in both eastern Asia and North America. Species of *Trillium* are similar in general morphology, although their foliaceous bracts and floral organs may vary considerably in size, shape, and color within and between species. The overall relatively simple vegetative morphology, together with the sometimes overlapping variation in floral traits, make delineation of species a difficult task at best (Gates, 1917; Freeman, 1975). Species differentiation in *Trillium* is usually based upon morphology of reproductive parts, particularly the shape and color of the petals, anthers, ovaries, and stigmas (Bodkin and Reveal, 1982; Freeman, 1969; Patrick, 1984; Soukup, 1980)

The monograph of *Trillium* subgen. *Phyllantherum* by Freeman (1975) was based principally on morphology and geographical distribution. It included a thorough review and synthesis of the nomenclatural literature and synonymy of the subgenus. In delimiting the species, Freeman relied on morphological differences linked with definable distribution patterns. Included in his monograph were descriptions of three new species, *T. albidum, T. kurabayashii* Freeman, and *T. angustipetalum* (Torrey) Freeman. These taxa were segregated from the earlier-named species *T. chloropetalum*. Within this group, *T. albidum* is distinguished primarily by its (1) white petals, rather than purple; (2) its chiefly green androecium and gynoecium, rather than purple; and (3) its sweet floral scent, rather

than musky odor. It ranges from southwestern Washington to northern California. *Trillium chloropetalum* and *T. angustipetalum* are found only in California. *Trillium chloropetalum* was described by Freeman as having a purple androecium and gynoecium, with petal colors ranging from purple to yellow, green, or occasionally white. *Trillium angustipetalum* was described as having linear, dark purple petals, basally attenuate, shiny bracts, and a fetid odor. The fourth species, *T. kurabayashii* is found in southwestern Oregon and adjacent California, as well as disjunctly in the northern Sierra Nevada. It was described as having bronze-purple petals, wider than in *T. angustipetalum*, sessile, dull-green bracts, and a fetid floral odor. The key published by Freeman (1975) for the above taxa is as follows:

1a. Stamens about twice the length of carpels; flowers with sweet rose-like fragrance

2a. Anther connective greenish; filament usually green, sometimes purple; anther dehiscence lateral; gynoecium greenish (rarely with purple stigmas); petals white (rarely purple basally) to pink

T. albidum

2b. Androecium and gynoecium purple throughout; anther dehiscence introrse; petals varying from purple to yellow or white

T. chloropetalum

1b. Stamens only slightly longer than carpels; flowers with musky or fetid odor

3a. Petals linear, more than 7 (mostly ca. 9) times as long as wide, dark purple; bracts ovate, rounded or blunt, basally attenuate, often shiny underneath

T. angustipetalum

3b. Petals oblanceolate, less than 6 (mostly ca. 4.5) times as long as wide, brownish or greenish (lurid) purple; bracts ovate, slightly acuminate, sessile, dull green underneath

T. kurabayashii

Following Freeman's study, Soukup (1980) published a new species, *T. parviflorum*, which he separated from *T. albidum* on the basis of smaller overall size, smaller leaves, larger petal length-to-width ratio, purple fruit, a basal purple streak on the petals, and a spicy floral fragrance. The species' range as given by Soukup is southwestern Washington and northwestern Oregon as far south as Clackamas and Yamhill counties. He stated that *T. parviflorum* is a "remarkably constant entity over its entire, rather small distributional range. Its flower part dimensions show a narrower range of values than those of *T. albidum*" (Soukup 1980, p 332). However, acceptance of *T. parviflorum* as a distinct species has not been universal among botanists of the Pacific Northwest. The distinctions between it and *T. albidum* are not as sharp as Soukup described. In particular, questions have been raised as to whether size differences are valid in characterizing closely related *Trillium* species. Nevertheless, the name *T. parviflorum* quickly entered into popular literature (Dusek, 1980), adding to the general confusion surrounding sessile-flowered trilliums in the Pacific Northwest.

Absolute size may be one of the least reliable taxonomic characters in *Trillium* (Freeman, 1975). Life history studies in the genus (Kawano et al., 1986; Ohara and Utech, 1986a, 1986b) and other lilies such as *Erythronium* (Kawano et al., 1982) and *Calochortus* (Fiedler, 1987) have made the assumption that size is representative of plant age, and have assigned individuals to age classes

accordingly. A study by Nesom and LaDuke (1985) on *T. nivale* indicated that plants could be aged by the number of shoot scars on the rhizome. Hanzawa and Kalisz (1993) found a positive correlation between leaf area and plant age in *Trillium grandiflorum,* where age was determined by constrictions in the rhizome. These constricitons appear to correlate with the shoot scars described by Nesom and LaDuke (1985). While there is some disagreement as to what number of scars indicates a year's growth, relative age can be determined between individuals, at least. If a relationship between age and size does exist, it could be demonstrated by counting rhizome scars and testing the correlation between their number and the size of plant parts. If a correlation were found, it would be helpful in evaluating the importance of size that Soukup used as a diagnostic feature of *T. parviflorum.*

Karyotype composition has been useful in defining *Trillium* species and in judging evolutionary relationships between them (Serota and Smith, 1967). Bailey (1954) used patterns of chromosome banding as species indicators in *Trillium,* finding that differential patterns are not constant within a species but can be useful in distinguishing between species. Serota (1969a) used karyotypes, morphology, and ecology in studying the taxonomy of *Trillium* in North Carolina and Tennessee. In her work, cytology aided in determining that several taxa with limited distributions belonged to one large, discontinuous population. Serota (1969b) also used cytology to revise the taxonomy of *T. rugelii,* a sessile-flowered *Trillium* in North Carolina.

Freeman (1975) separated his new species *T. kurabayashii* not only by its morphology but also by a distinctive and uniform karyotype that had earlier been described by Kurabayashi (1963). In the same paper, Kurabayashi described the karyotypes of "*Trillium sessile*" (*T. chloropetalum* in the broad sense, not typical *T.*

sessile L. found in the eastern United States) from six stations in Washington, Oregon and California. Three of these stations fell within the range of *T. albidum* as described by Freeman. The three populations showed similar karyotypes in general, with minor variations between and within populations. Karyotypes of these populations were distinct, however, from those of *T. kurabayashii* in coastal Curry County, Oregon, and *T. chloropetalum* in the San Francisco Bay region. A goal of the present study is to expand the cytological study of Oregon populations beyond that begun by Kurabayashi (1963), and to define more completely the composite karyotype of *T. albidum*, including populations within the range of *T. parviflorum*.

If karyotypes of plants throughout the range are found to be similar, and variation in morphology can be accounted for by age or ecological factors, then division of *Trillium albidum* into two species would seem to be unwarranted. This study began with the hypothesis that only one species, *T. albidum*, exists, and that variation within the species is ecoclinal and lacks clear discontinuities that might indicate separate taxonomic entities. Eighteen populations of white, sessile-flowered *Trillium* were sampled throughout most of the natural range of the species in western Oregon and Washington. The morphological and karyotype variations among individuals and populations were compared. Three questions were addressed: (1) What are the morphological differences and similarities between populations and individuals? (2) To what factors can morphological variation between populations and individuals be attributed? (3) Does the pattern of karyotypes suggest one taxonomic unit or more?

MATERIALS AND METHODS

The species

As described by Freeman (1975), *Trillium albidum* has a range extending in a narrow band from Pierce County, Washington, southward through the Puget Trough and Willamette Valley, into low interior valleys along the Umpqua and Rogue River drainages. Its range widens in northern California, where plants grow both in the Coast Ranges and on the western slopes of the Sierra Nevada (figure 1). In Oregon and Washington, the species is confined to elevations of 600 m or less. In California, populations occur from ca. 800 m to 2000 m elevation in the mountains on either side of the Sacramento Valley but are absent from the valley itself (Freeman, 1969, 1975).

Plants of *Trillium albidum* are erect, single-stemmed herbs, with a stout, tuber-like rhizome and three large, sessile, ovate leaves subtending a single sessile flower. The stem is 30-55 cm tall; the leaves are 10-18 cm long. The three petals are white (rarely with purple or pink on the basal part), oblanceolate to obovate, 50-85 mm long. The gynoecium is green, rarely with purple stigmas. The anther connectives are green and the filament is green or occasionally purple; stamens are about twice as long as carpels. The odor of the flower is sweet, usually rose-like, often quite strong. According to Freeman (1975), *T. albidum* does not appear to intergrade with *T. angustipetalum* or *T. kurabayashii*; however, he tentatively ascribed some of the variation in *T. chloropetalum* in the vicinity of San Francisco Bay to past introgression with *T. albidum*.

Figure 1. Range map of *Trillium albidum* Freeman in western Washington and Oregon through Northern California, showing location of 18 sites sampled in this study. The dotted line denotes the range limits according to Freeman (1975). Study site names corresponding to acronyms are given on pp. 11 - 16.

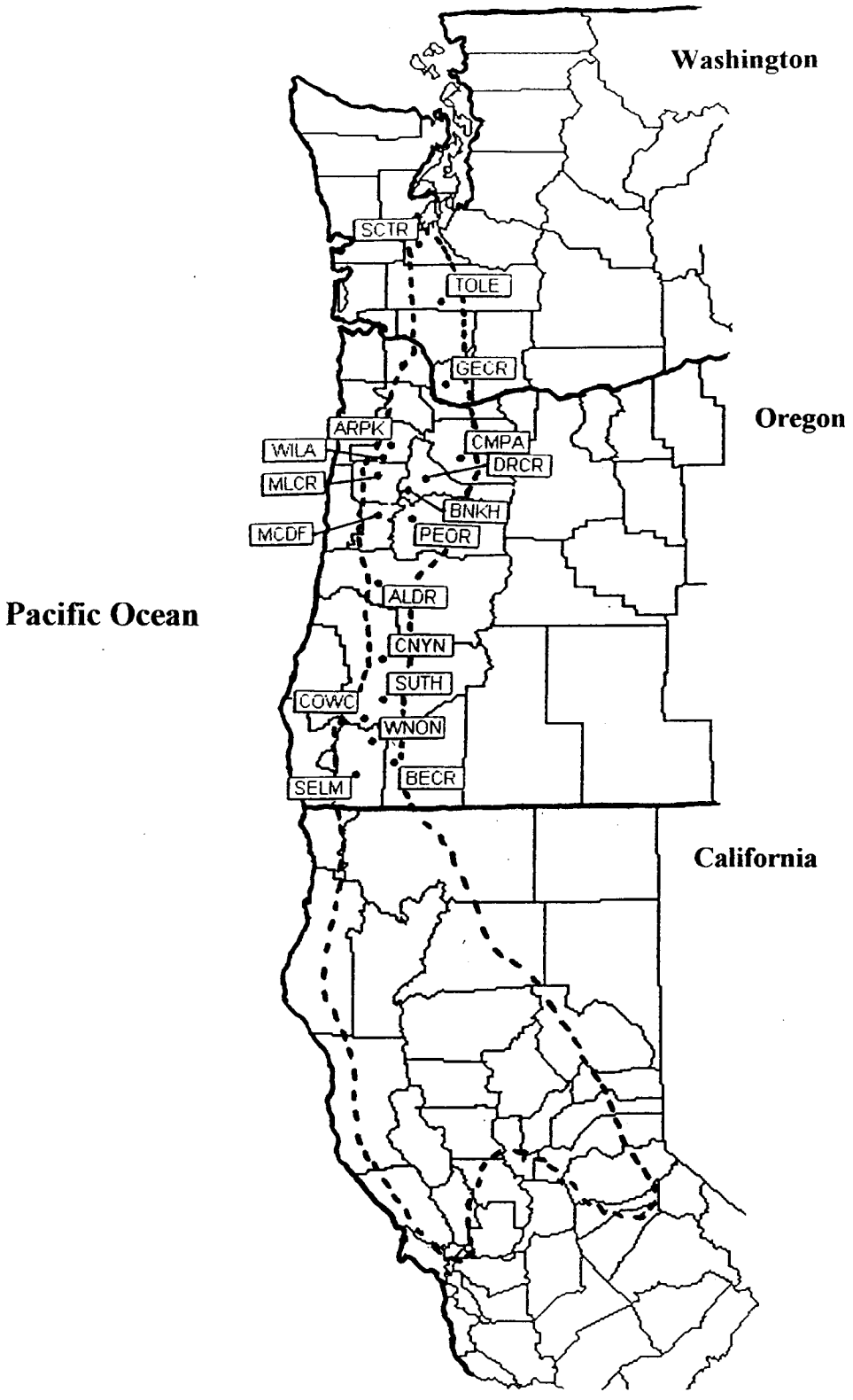

Washington
Oregon
California
Pacific Ocean
SCTR
TOLE
GECR
ARPK
WILA
MLCR
MCDF
CMPA
DRCR
BNKH
PEOR
ALDR
CNYN
SUTH
COWC
WNON
SELM
BECR

Figure 1

According to Soukup, the northern populations of *Trillium albidum* constitute *T. parviflorum*. *T. parviflorum* occurs with *T. albidum* in Marion and Polk counties, where they hybridize; all populations south of Polk County should consist entirely of *T. albidum*, therefore. Soukup separated *T. parviflorum* on the basis of its having leaves 8.5-11.5 cm long, linear or lanceolate petals 28-40 mm long, a basal purple streak on the petals, a shorter scape 18-24 cm tall, ovaries red or purple basally, fruits maroon rather than green, and a spicy odor as opposed to a rose-like odor in *T. albidum*.

Selection of the study sites

Using herbarium label data, personal sightings, and suggestions from other botanists, 18 populations in Washington and Oregon were located and used for this study (figure 1). Care was taken to locate sites that included the area of putative hybridization (Soukup, 1980) as well as to sample throughout the range in the two states. I attempted to find populations that were large enough to permit sampling of 10-20 plants and removal of up to 10 plants for cytological study. For each population, I removed no more than 10 percent of the plants and took voucher specimens (flower parts and bracts) from no more than 50 percent. The Canyon Creek population (CNYN) was too small to allow this large a sample, but it was included because of the significance of its geographical position.

Description of study sites

Several common factors characterize the 18 sites studied. Elevation varied from 200-600 m. Twelve of the 18 sites were in association with small streams. The plants themselves grow primarily in shaded areas, under a canopy of climax or second-growth trees, typically Oregon white oak (*Quercus garryana*), ash (*Fraxinus latifolia*) and Douglas fir (*Pseudotsuga menziesii*). The plants may spread to sunnier areas along clearings, stream banks, and roadsides, though plants in sunnier or drier locations may be smaller than average or to grow in thick clumps from a single rhizome rather than the typical one to three stems. Descriptions of specific sites are as follows:

Scatter Creek Wildlife Refuge (SCTR). Thurston County, WA. Township 16 North, Range 3 West, Section 27. Associated species are *Alnus rubra, Fraxinus latifolia, Quercus garryana,* and *Urtica dioica. Trillium* populations at this site were found in stands of mixed hardwoods and were frequently associated with *Urtica.* The area was low and flat and subject to flooding in some years. The site had formerly been pasture. Tracks, droppings, and browsed *Trillium* stems indicated that deer still disturb the populations. There were few signs of human disturbance.

Toledo Road (TOLE). Lewis County, WA. Township 11 North, Range 4 West. Associated species are *Alnus rubra, Fraxinus latifolia, Quercus garryana,* and *Cytisus scoparius.* Several small populations were found along a road

in wooded areas between home sites. The sites appeared to be frequently disturbed by trampling and in some areas by grazing. Plants at this site tended to be small.

Gee Creek Rest Area (GECR). Clark County, WA. Township 4 South, Range 4 West, Section 38. Associated species are *Thuja plicata, Alnus rubra, Oemleria cerasiformis, Acer circinatum, Asarum caudatum, Trillium ovatum*, and *Viola glabella*. The site was characterized by large, old trees, possibly second growth, with *Trillium ovatum* and *Trillium albidum* occupying low, wet areas near a creek. Footpaths through the woods, and the presence of small children bearing armfulls of *Trillium* indicated frequent disturbance at this site. *Trillium ovatum* was far more common than *Trillium albidum*.

Airport Park (ARPK). Yamhill County, OR. Township 4 South, Range 4 West, Section 28. Associated species are *Fraxinus latifolia, Alnus rubra, Viola glabella, Erythronium oregonum*, and *Trillium ovatum*. The plants sampled in this area were primarily from less accessible sites in the park, away from footpaths and less subject to disturbance. The area is characterized by a closed canopy of hardwood trees mixed with young conifers. A creek runs through the area.

Willamina (WILA). Yamhill County, OR. Township 5 South, Range 7 West, Section 44. This site is on private land adjacent to the Yamhill River. Associated species are *Fraxinus latifolia, Claytonia sibirica, Hydrophyllum* sp., *Pseudotsuga menziesii*, and *Alnus rubra*. A *Trillium* population was located on the steep banks of the river and on flat areas below the banks. Most plants were under shrubs. A 4-merous *Trillium albidum* was found here.

Camp Adams (CMPA). Clackamas County, OR. Township 4 South, Range 6 East, Sections 6 and 31. This site is near the city of Molalla. Associated species are *Fraxinus latifolia, Thuja plicata, Berberis aquifolium, Rubus ursinus, Urtica dioica,* and *Viola glabella.* Plants were sampled in more remote areas of this youth camp, near a stream bank and in thick, second growth woods consisting of mixed hardwoods and conifers. The area appeared to be visited only occasionally by humans.

Drift Creek (DRCR). Marion County, OR. Township 7 South, Range 1 East, Section 9. This site is on private property along Drift Creek Road, adjacent to Silver Falls State Park. Associated species are *Pseudotsuga menziesii, Rubus ursinus*, and *Viola glabella.* This site contained plants showing signs of frequent browsing by deer. Human disturbance was occasional, though the owners of the property were attempting to maintain the integrity of particular sites containing native plants. Douglas-fir was the primary tree species and was believed by the owners to be second growth. The prior owners had used the land for cattle grazing.

Mill Creek Park (MLCR). Polk County, OR. Township 6 South, Range 6 West, Section 75. Associated species are *Pseudotsuga menziesii, Rubus ursinus, Urtica dioica, Salix* sp., and *Viola glabella. Trillium* populations were found near the banks of the creek under the canopy of Douglas-fir; a few plants were found under willows as well. The park is in the Coast Range and is surrounded by farmland and timber land.

Bunker Hill (BNKH). Marion County, OR. Township 8 South, Range 5 West, Section 33, southwest corner. This site is on private land. Associated species are *Symphoricarpos alba, Claytonia sibirica, Quercus garryana,* and *Pseudotsuga menziesii.* The area had been grazed until twenty years prior to the study. The area surrounding the site has had some smaller trees removed and a footpath put in, but the *Trillium* are seldom disturbed except by deer, which browse quite heavily on blooming plants. Consequently, though the area has dense populations of *Trillium,* few of the plants were in flower. Some clones on the edge of the woods, close to a landscaped area, had twenty or more stems emerging from the same rhizome. Chemical fertilizers may account for this extraordinary feat. The canopy consisted of mixed Oregon white oak and young Douglas fir.

MacDonald Forest (MCDF). Benton County, OR. Township 11 South, Range 11 West, Section 19. Associated species are *Pseudotsuga menziesii, Fraxinus latifolia, Alnus rubra, Urtica dioica, and Hydrophyllum* sp. The populations were found along Oak Creek near the southern entrance to the forest. The canopy was composed primarily of Douglas-fir, with ash and alder present near the stream banks. It was not determined whether the trees were second-growth or old-growth. The forest itself contains several stands of mature old-growth forest.

Peoria (PEOR). Linn County, OR. Township 12 South, Range 4 West, Section 15. This site is on private property near the town of Peoria. Associated species are *Pseudotsuga menziesii, Fraxinus latifolia, Alnus rubra,* and *Hydrophyllum* sp. Plants were found along a stream, in a narrow band near the banks. The property owners protect the area from disturbance.

Alderwood State Park (ALDR). Lane County, OR. Township 16 South, Range 6 West, Section 28. This site is located along the banks of Long Tom Creek. Associated species are *Pseudotsuga menziesii, Dicentra formosa,* and *Calypso bulbosa*. *Trillium* was found along footpaths and well off the trails within 15 m of the stream banks. Many of the plants were in more open areas compared with other populations.

Sutherlin Rest Area (SUTH). Douglas County, OR. Township 24 South, Range 6 West. Associated species are *Alnus rubra, Rhus diversiloba, Dodecatheon* sp., and *Delphinium* sp. Plants were scattered on a hillside in open woods, often under Rhus. The association may be a result of plants being over-picked in more open areas. A small stream flowed through the area.

Canyon Creek (CNYN) Douglas County, OR. Township 30 North, Range 6 West. Associated species are *Pseudotsuga menziesii, Alnus rubra,* and *Viola* sp. This small population was located on a side road above an entrenched creek. The plants occupied a narrow strip of woods between the road and the small canyon.

Cow Creek Rest Area (COWC). Douglas County, OR. Township 32 South, Range 6 West, Section 35. Associated species are *Pseudotsuga menziesii, Alnus rubra, Rubus ursinus, Dodecatheon* sp., and *Delphinium* sp. Plants were found under heavy shrub cover, often associated with *Rhus*. A 4-merous plant was found at this site.

Winona Road (WNON). Josephine County, OR. Township 38 South, Range 6 West, Section 36. Associated species are Pse*udotsuga menziesii, Alnus rubra, Rubus ursinus, Dodecatheon* sp., and *Delphinium* sp. A population of *Trillium* occupied mixed, open woods on a hillside above a creek. Plants were also found in sunny locations near the creek.

Bird's Eye Creek (BECR). Jackson County, OR. Township 38 South, Range 7 West, Section 19, Northeast quarter of Northwest quarter. Associated species are *Pseudotsuga menziesii, Alnus rubra, Trillium ovatum, Hydrophyllum* sp., and *Viola* sp. Extremely robust specimens nearly one m in height were found at this site. The population occupied the floor of a steep-walled gully adjacent to a stream, under a canopy of large Douglas-fir. Residents of the area believed the site had not been logged in at least 40 years and had not been grazed by cattle.

Lake Selmac (SELM). Josephine County, OR. Township 38 South, Range 7 West, Section 18. Associated species are *Pseudotsuga menziesii, Alnus rubra, Rhus diversiloba,* and *Trillium ovatum*. The type specimens of *Trillium albidum* were collected in 1967 by Freeman from near this area. The artificial lake was added since that time, from water flowing out of Deer Creek. *Trillium* was found under a Douglas-fir canopy. Those plants nearest the paths were the most heavily picked by visitors, and they appeared smaller and sparser than those in more thickly wooded areas. Robust specimens were found growing in thick stands of *Rhus*.

Field studies.

Sites were sampled in late March and early April of 1986 and 1987. Plants chosen for measurement were at least one m apart, a distance chosen to prevent sampling of two clones from the same rootstock (Kurabayashi, 1963). Plants were collected in the order in which they were encountered in a walk through the population, until at least 10, and up to 20, were sampled. For later morphological analysis, one leaf, one petal, and one sepal were selected at random from each plant, removed, and pressed. Plant height, color of reproductive parts, and flower scent were recorded in the field. Color was noted as "red present," "red faint," or "white." Scent was recorded as "spicy," "non-descript," or "rosy." A total of 272 plants were sampled for the morphological analysis. Plants in bud, to be used for cytological examination, were selected in a second sampling at each site in the same manner as those chosen for morphological analysis. Because these plants had to be in bud, it was not possible at the time of the study to perform cytological and morphological analysis on the same plant. Plants were cut at the soil line, the stems put in water, and the plants transported in an ice chest until they could be refrigerated.

Laboratory studies and analysis

Morphology: Length and width in mm were measured on each of the pressed samples of leaves, petals, and sepals. The number of nerves (major veins) on each leaf was counted, as this was a feature Soukup used in describing *T.*

parviflorum. Herbarium specimens from several universities were examined to located specimens with roots intact. Shoot scars on the rhizomes of herbarium specimens were countable in 35 specimens, and these were scored to give relative plant ages. Counts were approximate. Many of the rhizomes were broken, contorted, or caked with soil, and scars were difficult to count.

Simple correlations were run using the morphological data, in order to determine the relationship between plant age, and plant size. Relative plant age, as determined from shoot scars, was tested against plant height, leaf length, and petal length as measured on the herbarium specimens by calculation of correlation coefficients (r).

In order to determine the multivariate structures of the morphological data, the entire set of data was analyzed using CLUSB4, a program using a divisive clustering algorithm to determine the minimum variance partition of n sample units with p attributes into k clusters. The algorithm is designed to produce a non-hierarchical cluster structure in which the within-cluster sum-of-squares is minimized. The number of clusters produced in the final solution is specified by the user. The algorithm was originally designed by George Diehr (School of Business Administration, University of Washington). C. David McIntire (Department of Botany, Oregon State University) and Scott Overton (Department of Statistics, Oregon State University) used the algorithm to design the original CLUSB as a mainframe program and later designed the PC version used in this study. CLUSB-4 was used to initially examine the field data in this study.

Further analysis was performed using NTSYS-pc, a numerical taxonomy and multivariate analysis system for the personal computer, developed by F. James Rohlf of State University of New York. The purpose of the system of programs is

to find and display structure in multivariate data, using a variety of modules to carry out a number of analytical algorithms. NTSYS-pc was used in order to compare the results of cluster analysis with that of CLUSB-4, and to apply principle components analysis to the data.

For both of these programs, data was assembled into a table, and data applying to color and fragrance was quantified arbitrarily. As the shapes of the leaf and petal were important in Soukup's description of *T. parviflorum,* the shapes of these parts were quantified by using (1) the ratio between length and width, which describes the broadness of the leaf, and (2) a shape index, which was the distance from the tip of the leaf or petal divided by the overall length of the leaf or petal, which indicated at what point the plant part was most broad. For the purpose of this study, the following variables were used: leaf length, leaf length/leaf width, leaf shape index, number of major nerves (veins) on the leaf, petal length, petal width, petal length/petal width, petal shape index, sepal length, sepal width, plant height, color of ovary, color of stigma, color of petal, fragrance.

In order to quantify the color and fragrance data for analysis, the following numerical values were arbitrarily established: for color, 0 = red or purple color strongly present, 1 = red or purple color faint, but present, 2 = no red or purple color present; for fragrance, 0 = spicy scent easily identified, 1 = fragrance sweet, but cannot be identified as either spicy or rosy, 2 = rosy scent easily identified.

Cytology: Plants from the field were cold-treated prepared by a method similar to that used by Serota and Smith (1967). Plants were transported from the field in water in an ice chest and were put in a refrigerator as soon as possible, where they were kept at 0° C for 96 hours to despiralize the heterochromatin. Ovaries were

removed, placed in modified Carnoy's fixative (3 parts 100% ethyl alcohol, 1 part propionic acid, 1 part chloroform) for 24 hours at room temperature. Ovaries were transferred through dilutions of ethyl alcohol, then stored in 70% ethyl alcohol at -10° C. Ovules were squashed in aceto-carmine with a drop of Hoyer's solution for permanency. Chromosomes were photographed as soon as possible after the slides were prepared. Slides were examined for cells in metaphase, where the chromosomes were sufficiently spread apart so as to make identification of individual chromosomes possible. Photographs were made using a Zeiss 2.5 X 6 cm format and Kodak T Max (400 ASA) sheet film. Tracings were made from the negatives on a light table and used to construct karyotypes. Each chromosome was individually traced, and location of heterochromatin was noted, so as to make identification possible. Identification of the chromosome types followed the classification and terminology of Kurabayashi (1963) shown in figure 2, so that Kurabayashi's results could be compared with those this study.

Figure 2. Schema of chromosomes of *Trillium albidum*, after Kurabayashi (1963) (n = 5). This is the karyotype used for identifying chromosomes. Segments of heterochromatin are denoted by dotted lines. The five chromosome types are denoted by letters, while the heterochromatin patterns are identified by numbers. Only those patterns used by Kurabayashi in his 1963 study that pertain to *T. albidum* are shown.

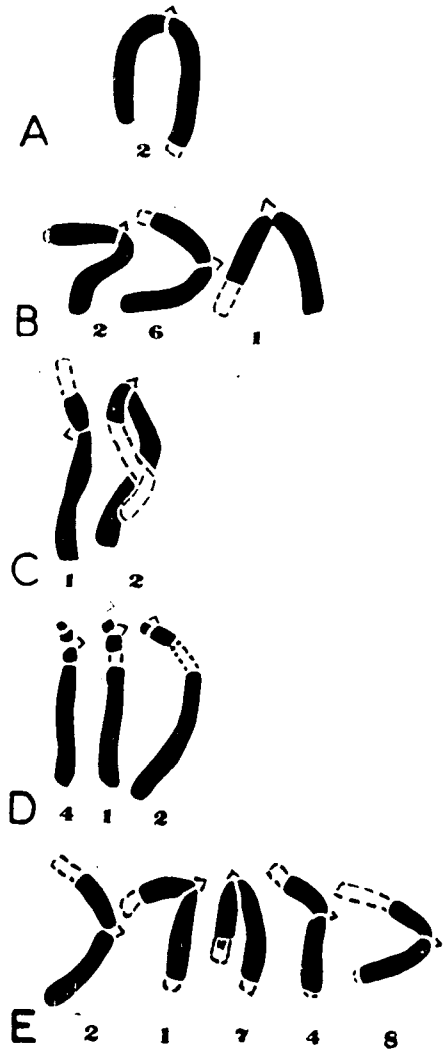


Figure 2

RESULTS

Field observations.

For each plant observed in the field, comparisons were made with the positive diagnostic traits in Freeman's description of *Trillium albidum* and Soukup's description of *Trillium parviflorum.*

Elliptic petal shape, characteristic of *T. albidum,* was seen on at least a few individuals in all populations, whereas linear petals, characteristic of *T. parviflorum,* were seen primarily in the Washington and Willamette Valley populations, rarely in the southern Oregon sites. Oblanceolate petals were seen occasionally in all populations (figures 3a-3r).

Both three- and five-nerved leaves were recorded in all samples, though Soukup describes three-nerved leaves as being a characteristic of *T. parviflorum,* and five nerved leaves a characteristic of *T. albidum.* Seven-nerved leaves were found at SUTH, BECR, and SELM.

Nearly all of the plants with reddish color on the base of the petals were found in the Willamette Valley populations. Though Soukup states that plants from Washington also have petals with a color streak, I found this feature faintly in only three plants from Washington. Rarely, plants bearing petals with at least slight coloration were seen as far south as SUTH.

The southern limit for plants with a spicy fragrance was BNKH. Plants with a rosy fragrance were found only as far north as MLCR. Several chi-square tests were performed to see if petal color and fragrance were independent, the null

Figures 3a through 3r. Petal shapes from petals collected at the study sites, showing the wide variation in petal shape and size within and between populations.

3a. Scatter Creek (SCTR)

3b. Toledo Road (TOLE)

3c. Gee Creek (GECR)

3d. Airport Park (ARPK)

3e. Camp Adams (CMPA)

3f. Willamina (WILA)

3g. Mill Creek (MLCR)

3h. Bunker Hill (BNKH)

3i. Drift Creek (DRCR)

3j. MacDonald Forest (MCDF)

3k. Peoria Road (PEOR)

3l. Alderwood State Park (ALDR)

3m. Sutherlin Rest Area (SUTH)

3n. Canyon Creek State Park (CNYN)

3o. Cow Creek Rest Area (COWC)

3p. Winona Road (WNON)

3q. Lake Selmac (SELM)

3r. Bird's Eye Creek (BECR)

Figure 3 extends from page 23 to page 40.

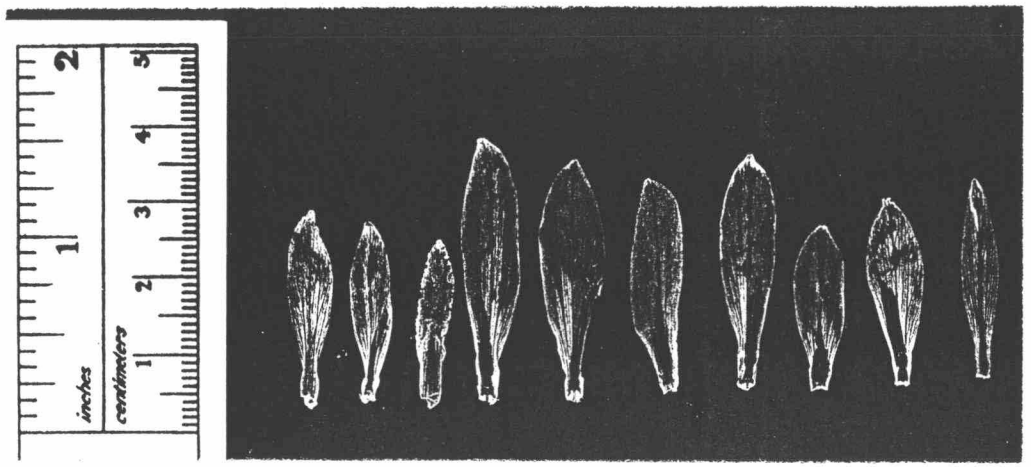


Figure 3a

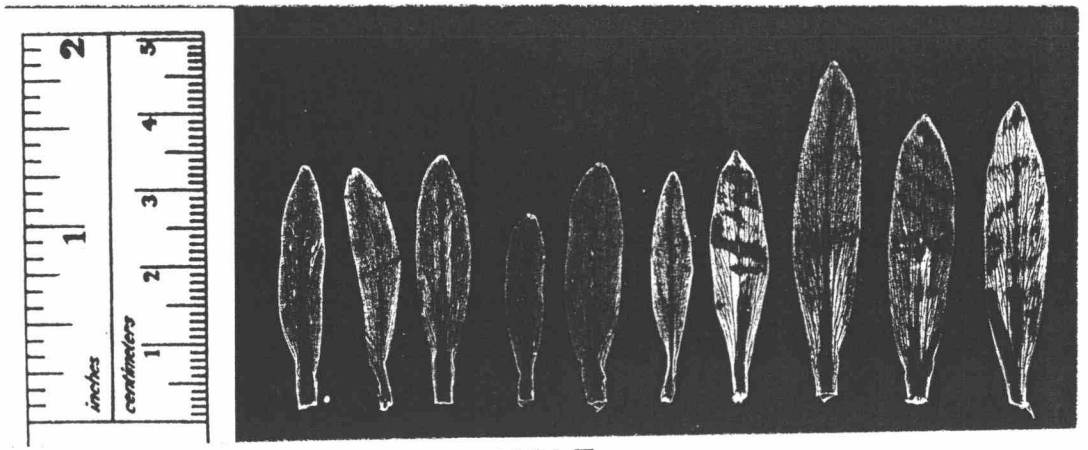


Figure 3b

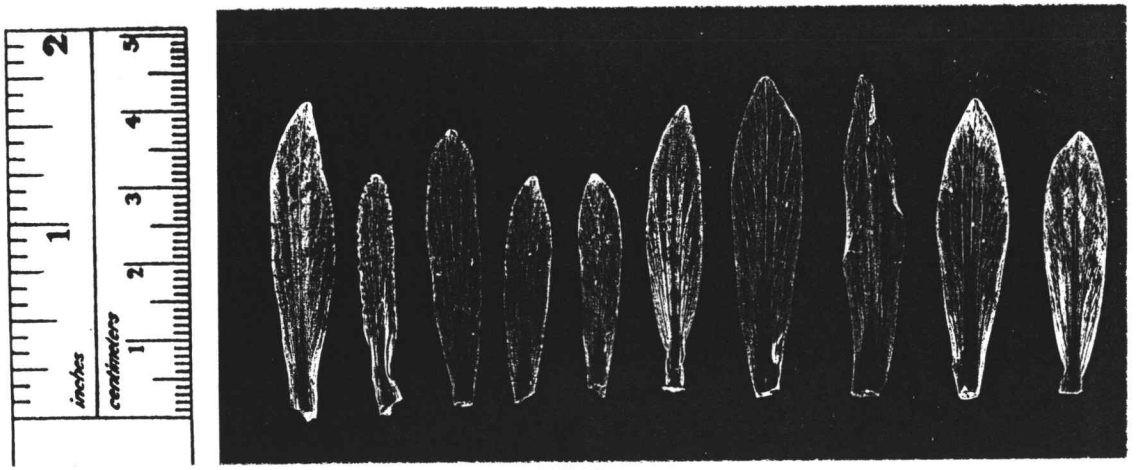


Figure 3c

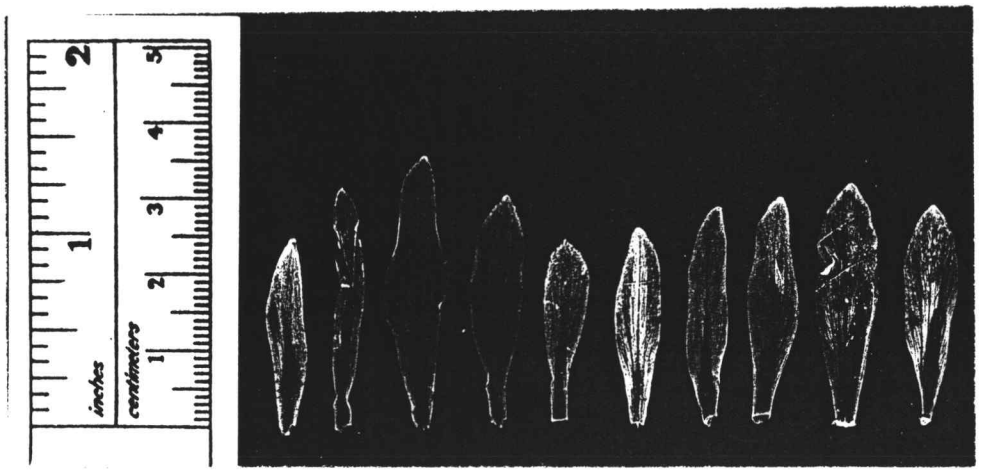


Figure 3d

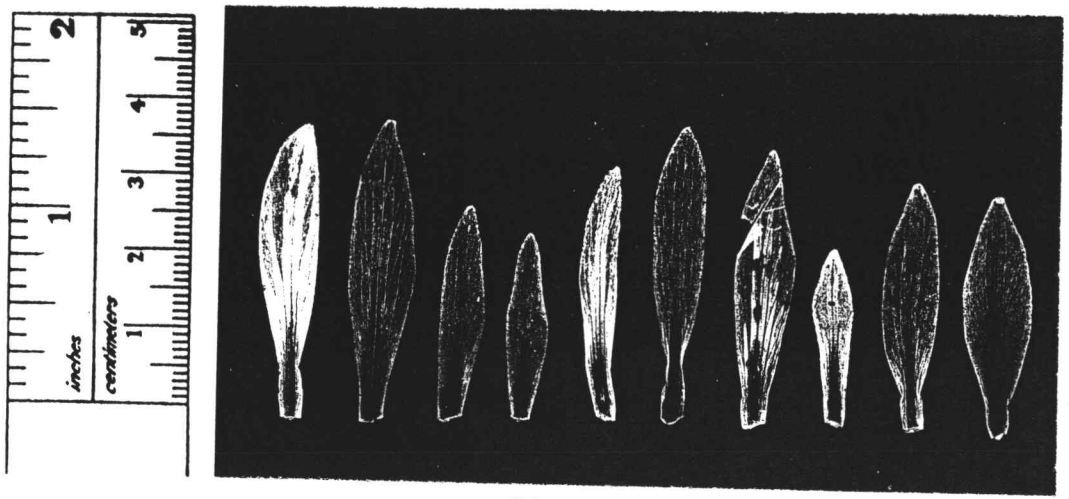


Figure 3e

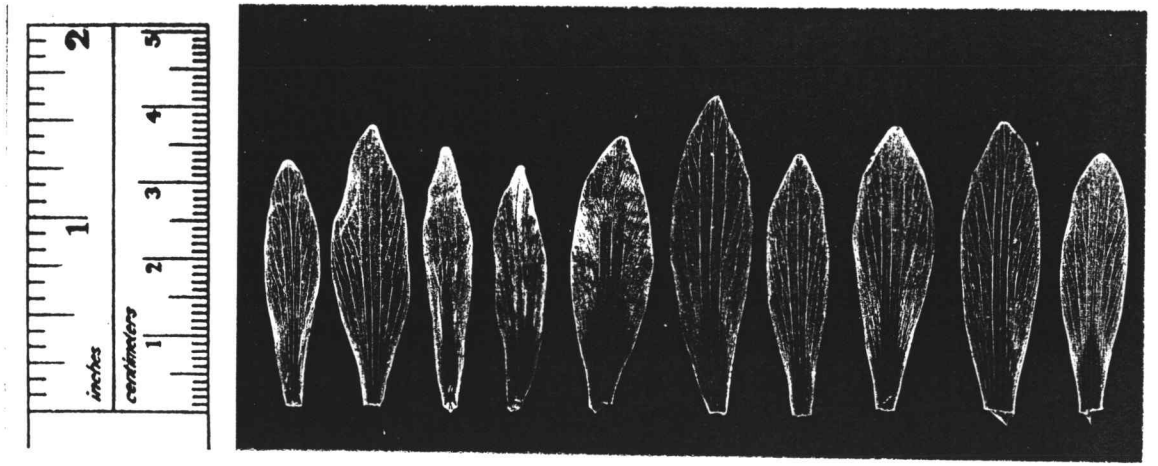


Figure 3f

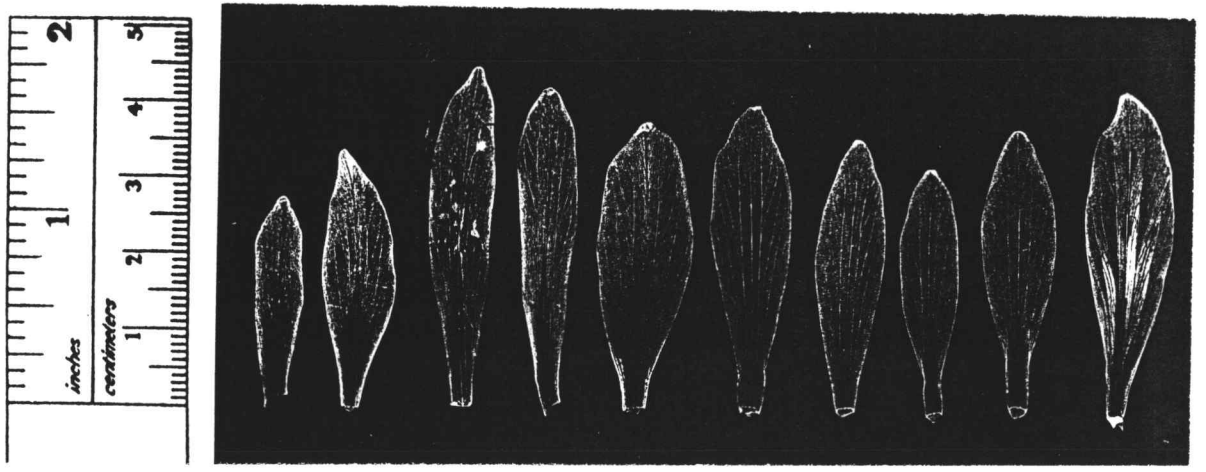


Figure 3g

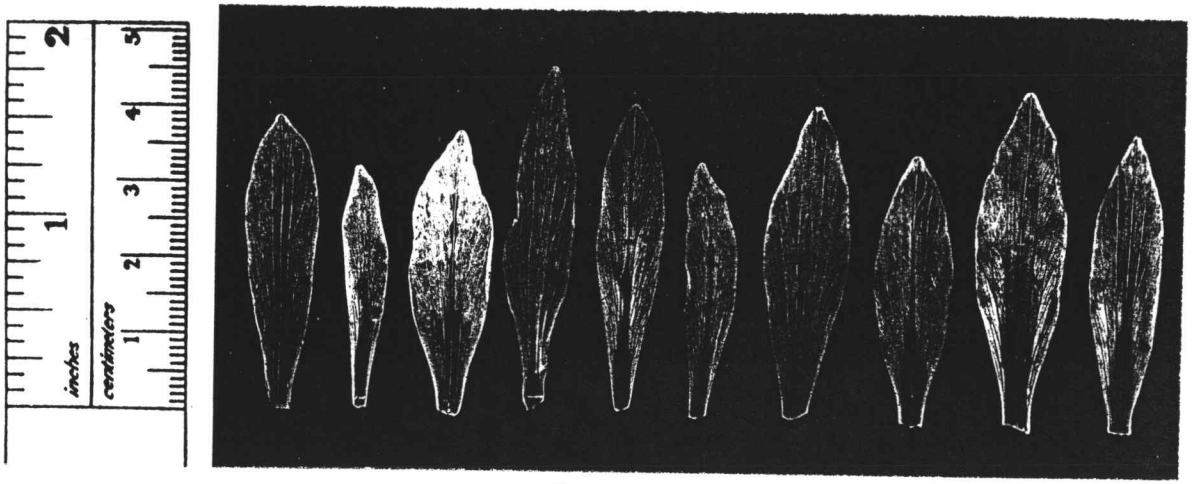


Figure 3h

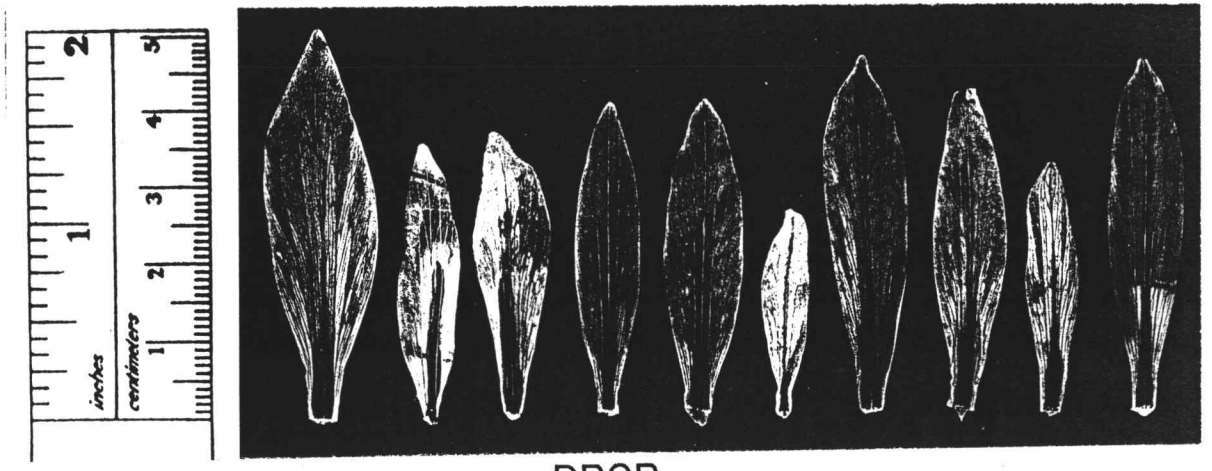


Figure 3i

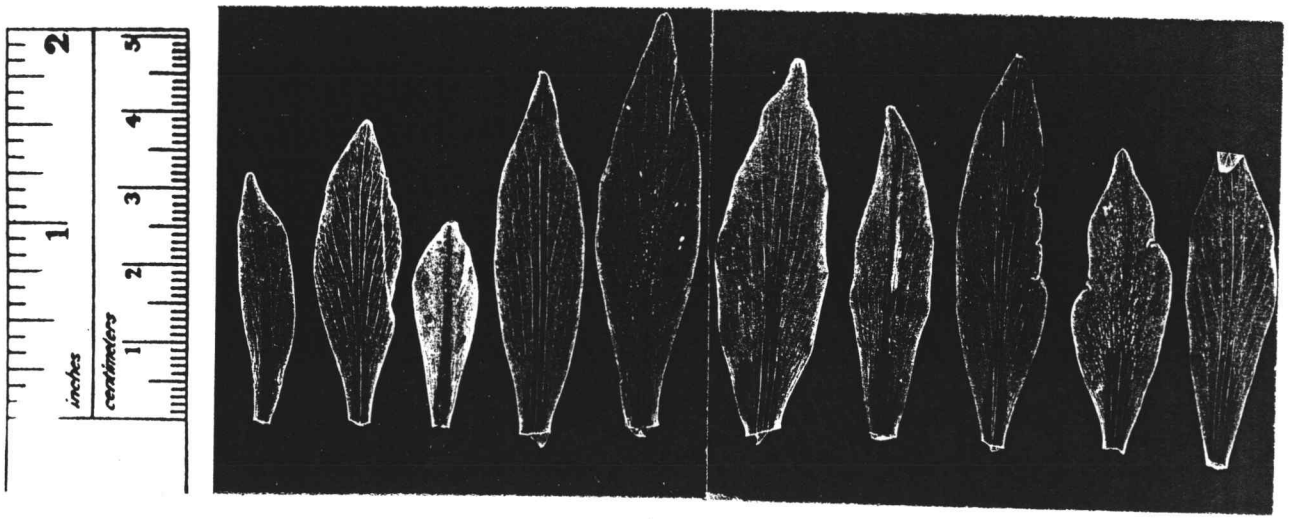


Figure 3j

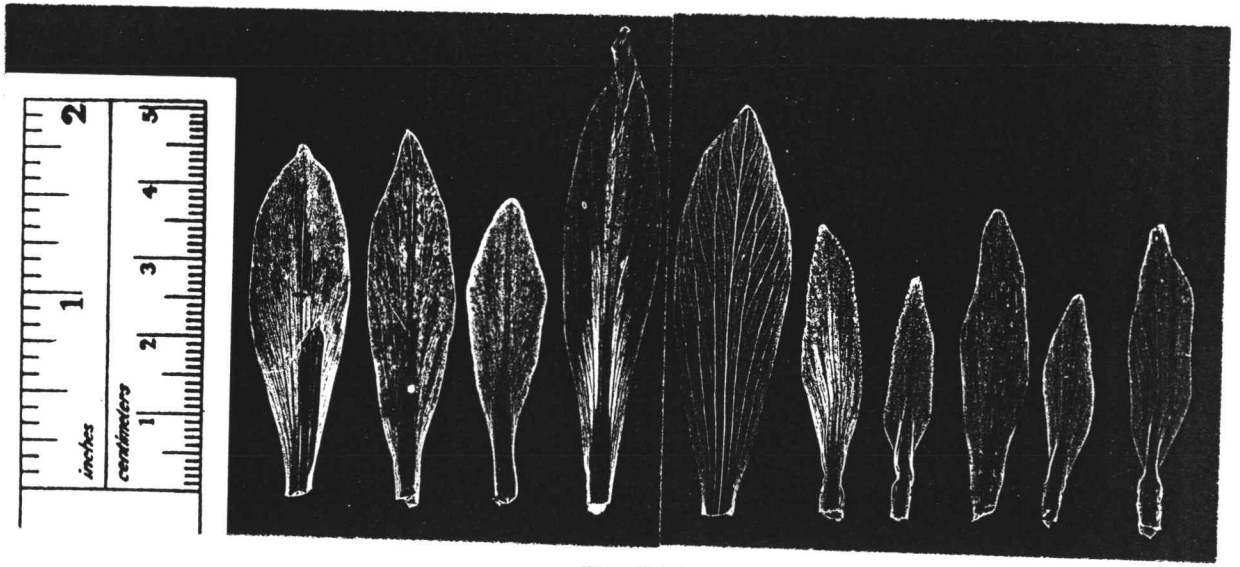


Figure 3k

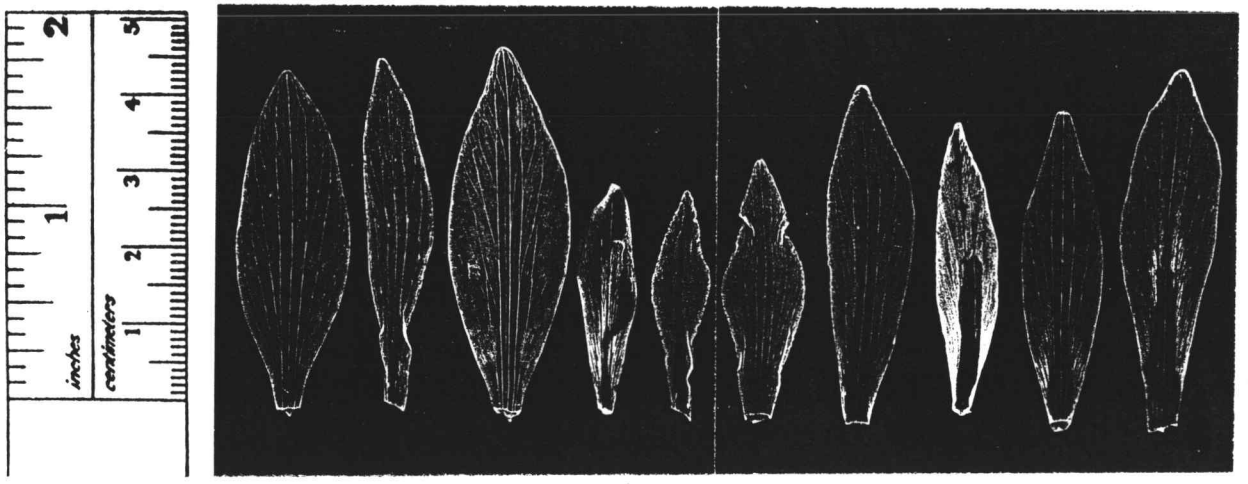


Figure 3l

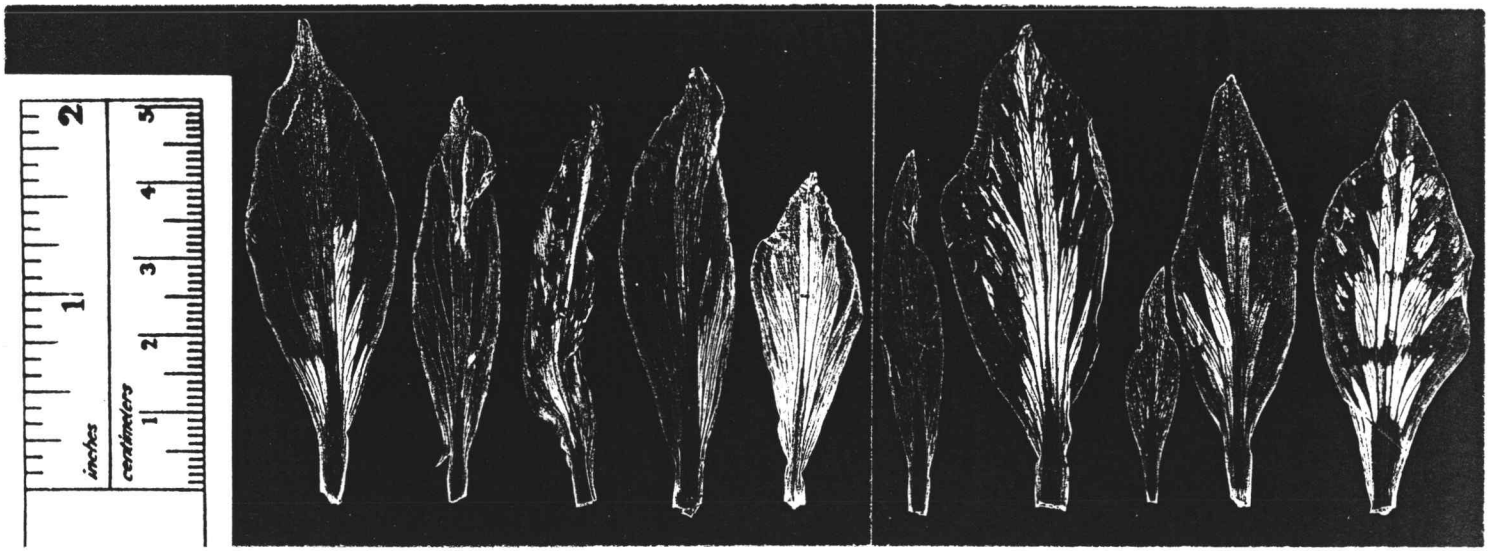


Figure 3m

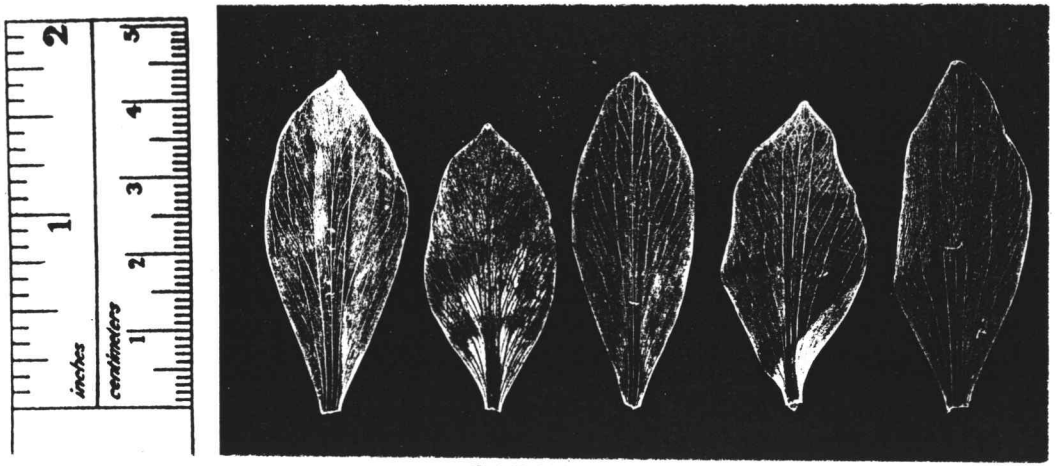


Figure 3n

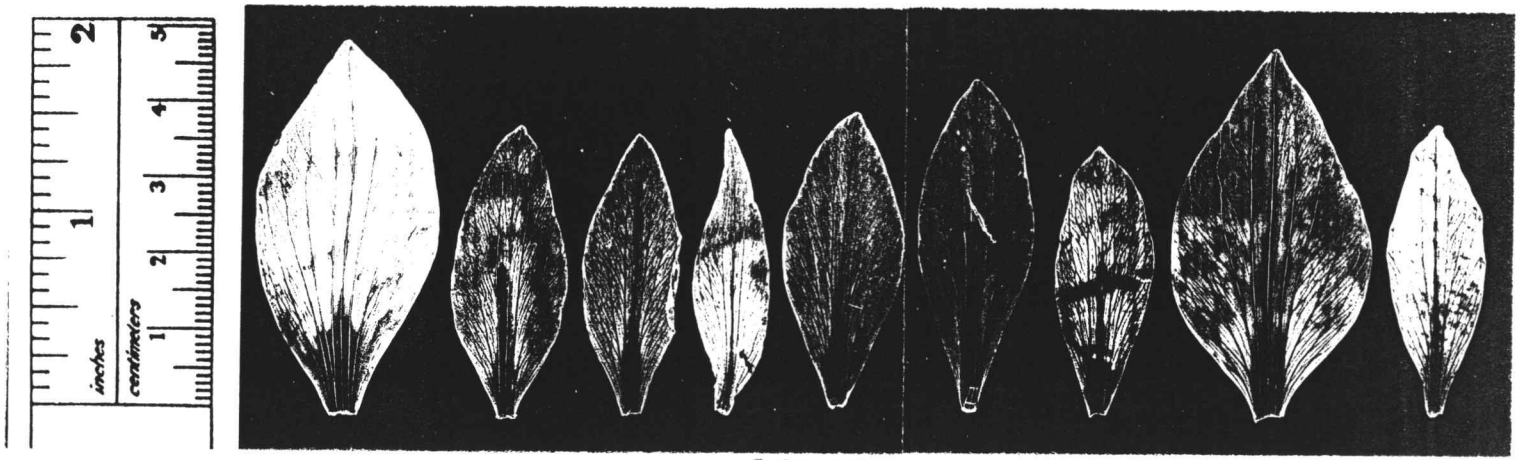


Figure 3o

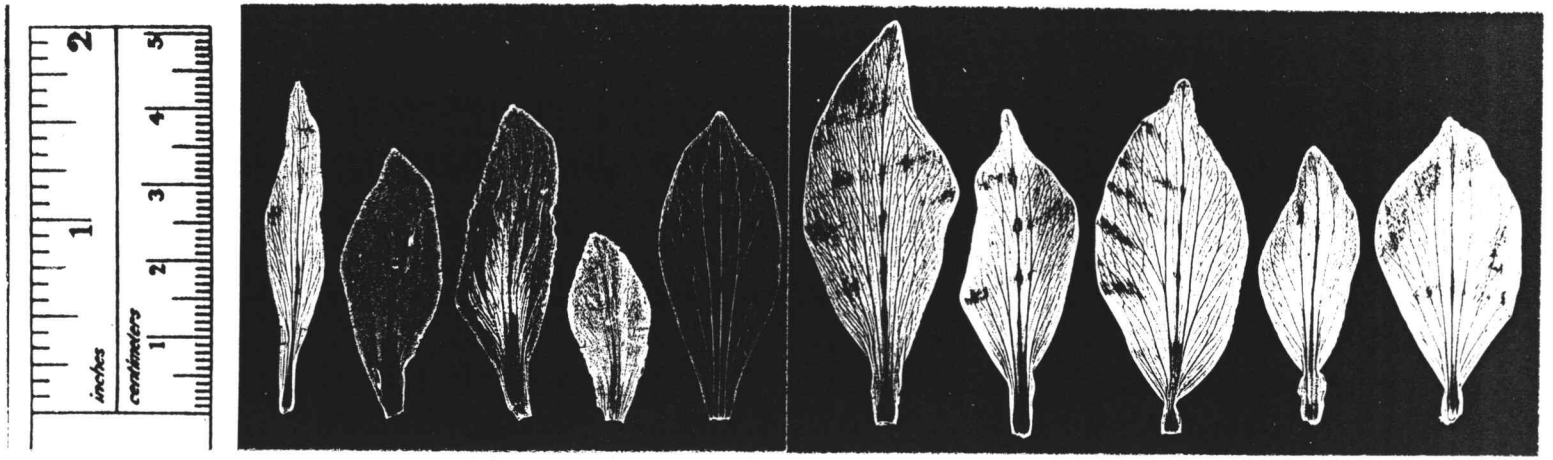
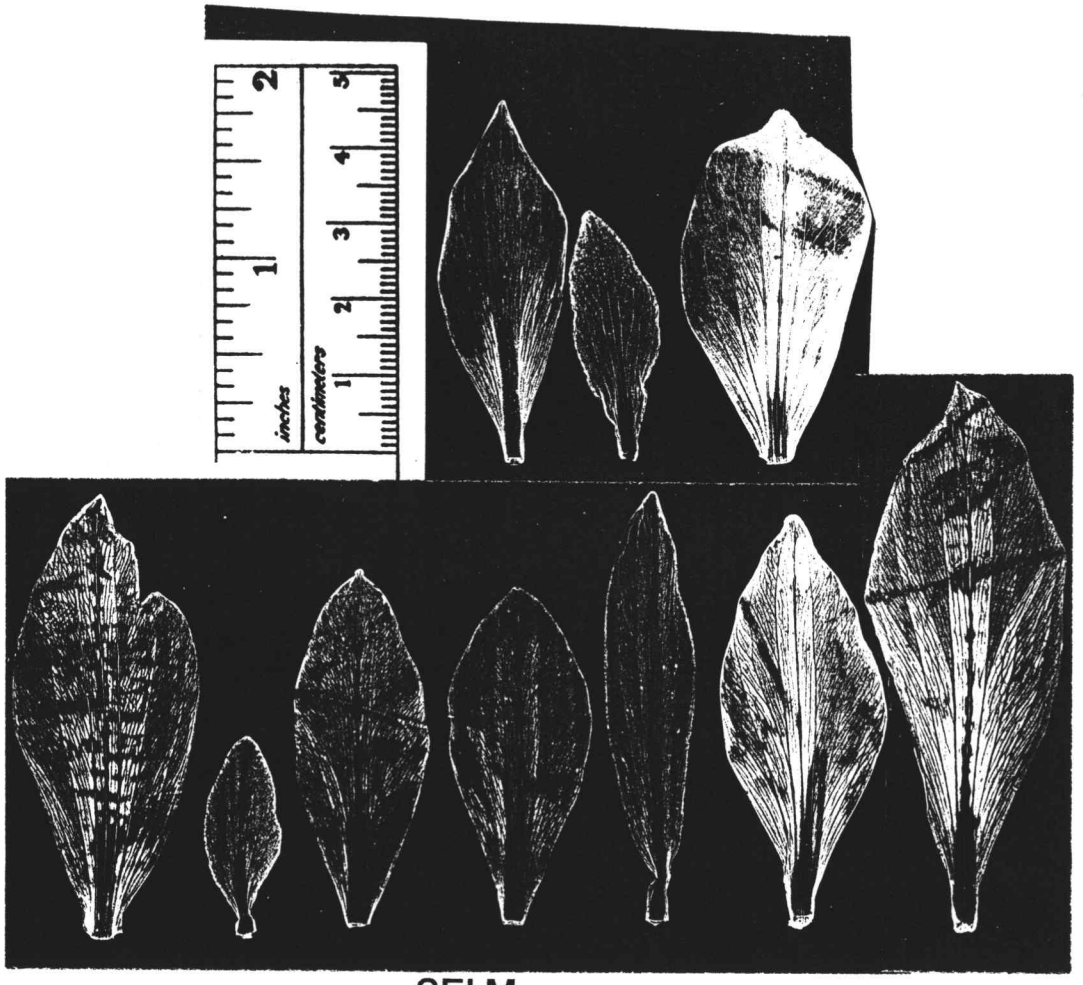


Figure 3p



SELM

Figure 3q

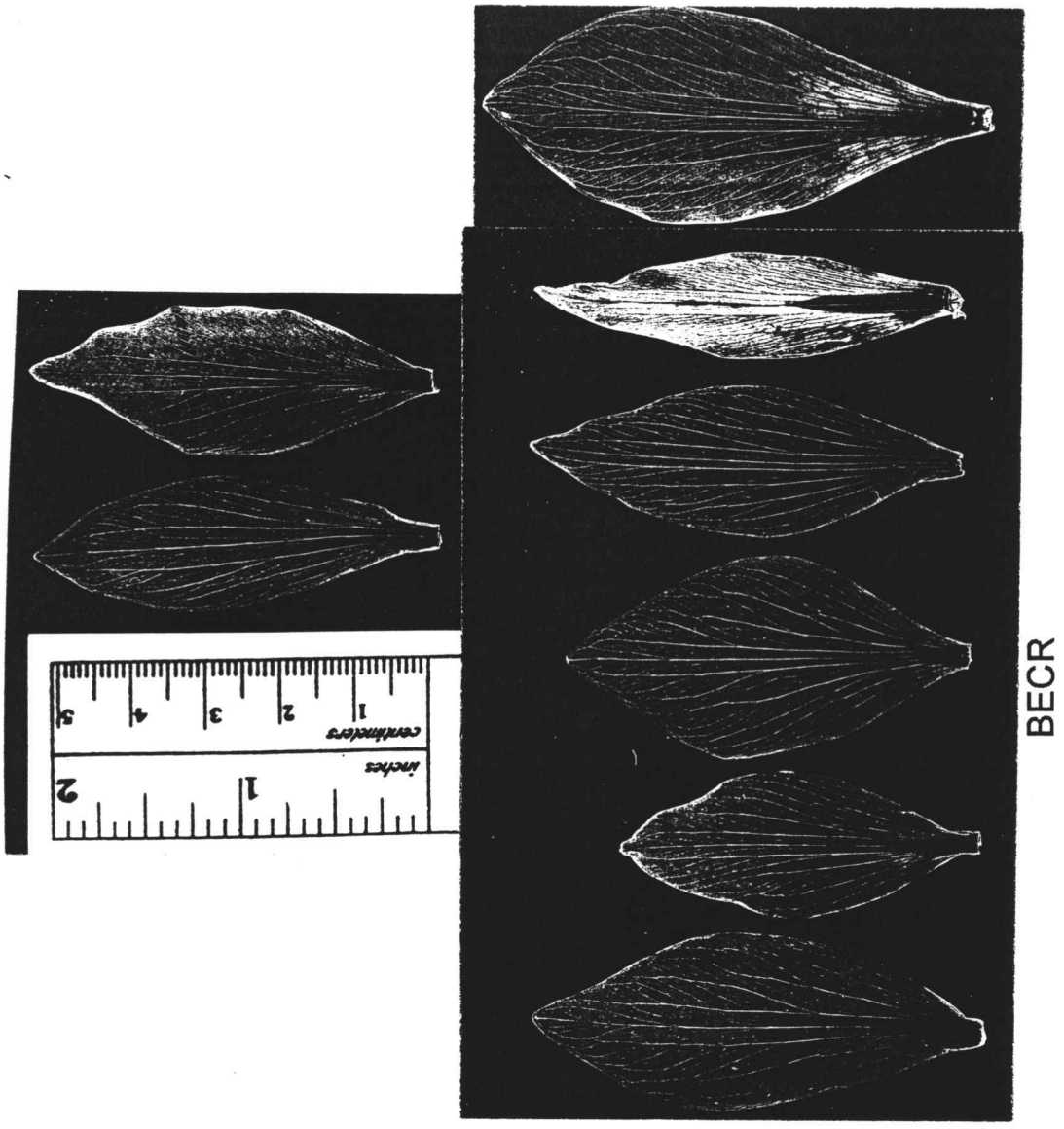


Figure 3r

hypothesis being that the traits are independent. First, a two-way frequency table was drawn up, row variables given as "color present" and "no color present", and column variables given as "rosy" and "not rosy". When analyzed for goodness of fit, $X^2 = 15.80$. At a significance level of .05, and degrees of freedom = 1, null hypothesis is not supported. The test was repeated with a three-way table, with row variables given as "color present", "color faintly present" and "no color present", and column variables given as "spicy" "sweet" and "rosy". For this table, $X^2 = 24.63$. At a significance level of .05, and degrees of freedom = 4, null hypothesis is not supported.

When looking at the Willamette Valley populations alone, it appeared that in this region color and fragrance may independent in this region. Rosy-scented plants with colored petals were found at MLCR and BNKH. Spicy-scented plants with all-white petals were found at BNKH. At CMPA, nine out of ten plants had a nondescript scent, and nearly equal numbers of all-white and colored-petaled plants were seen. Chi-square tests were performed on data from the Willamette Valley sites in the same way as the total data was analyzed. In a two-way table, with row and column variables as given, $X^2 = 1.04$. At a significance level of .05, and degrees of freedom = 1, null hypothesis is supported. However, when a three-way table was constructed, with variables as given above, $X^2 = 12.74$. At a significance level of .05, and degrees of freedom = 4, null hypothesis is not supported, though at a significance level of .01 the null hypothesis is supported. Therefore, it may be said that petal color and fragrance are somewhat independent within the Willamette Valley region.

Morphology.

Simple linear correlations between number of shoot scars on the rhizome and selected morphological characteristics was moderate. The strongest correlation was between leaf length and shoot scars ($n = 35$, $r = 0.63$). Correlation between plant height and shoot scars was weaker ($n = 35$, $r = 0.55$). Correlation between petal length and root scars was moderate ($n = 35$, $r=0.59$).

In the initial multivariate analyses that I attempted on the morphological data set, including all the field and laboratory observations, each individual plant (clone) was considered as a separate unit (data not shown). Using CLUSB-4 and specifying from three to five clusters in separate runs, a nearly random assemblage of individuals appeared in the trees produced, with no clear geographic grouping of the morphological data. Cluster analysis of the same set of morphological data for individuals was repeated with NTSYS-pc to see if a slightly different algorithm would yield different results. As was the case with CLUSB-4, there was not clear geographic separation of morphological traits (analyses not shown).

After failing to detect any clustering of the individual plants by their morphological attributes, I decided to calculate the population averages for all the morphological traits used. The resulting data table (table 1), in which all characteristics are given equal weight, was then subjected to cluster analysis using average taxonomic distances by the NTSYS-pc program, producing a standard dendrogram (figure 4). The clusters produced show a high level of similarity, with poor differentiation, except that two of the Washington populations cluster together. The pattern of clusters generally bears little relation to the geographic distribution of the study sites. For example, while two populations from southwestern

Table 1. Table of averages and standard deviations of 14 morphological characteristics measured on plants from the 18 study sites shown in figure 1. Averages of traits were used in multivariate analysis using NTSYS-pc.

Leaf length: in mm

Leaf len/w: ratio of leaf length to width

Leaf shape: ratio of leaf length to distance from the tip of the leaf to the widest part.

Number nerves: number of principal nerves in leaf

Petal length: in mm

Petal len/w: ratio of petal length to width

Petal shape: ratio of petal length to distance from the tip of the petal to the widest part.

Sepal length: in mm

Sepal len/w: ratio of sepal length to width

Plant height: in mm

Color stigma: 0 = red	1=faint redness	2=green
Color ovary: 0 = red	1=faint redness	2=green
Color petal: 0 = red	1=faint redness	2=white
Fragrance: 0 = spicy	1= non-descript	2=rosy

Site	site acronym		leaf length	leaf len/w	leaf shape	number nerves	petal length	petal len/w	petal shape	sepal length	sepal len/w	plant height	color stigma	color ovary	color petal	fragrance
Scatter Creek Wildlife Refuge	SCTR	AV	92.27	1.03	0.60	3.27	25.95	4.26	0.34	19.59	3.65	213.64	0.59	2.00	2.00	0.32
		SD	0.12	0.12	0.05	0.69	4.48	0.57	0.06	2.95	0.51	46.57	0.58	0.00	0.00	0.47
Toledo Road	TOLE	AV	98.50	1.12	0.60	3.25	35.69	4.88	0.36	24.69	4.13	259.38	0.00	2.00	2.00	0.13
		SD	12.12	0.10	0.04	0.66	5.55	0.43	0.06	2.91	0.49	55.73	0.00	0.00	0.00	0.33
Gee Creek Rest Area	GECR	AV	106.33	1.09	0.61	4.11	34.94	4.70	0.36	25.11	3.89	295.00	0.17	2.00	1.61	0.00
		SD	15.19	0.10	0.03	0.99	5.17	0.65	0.10	3.91	0.60	59.72	0.50	0.00	0.49	0.00
Airport Park	ARPK	AV	94.67	1.21	0.60	4.17	28.67	4.70	0.36	20.92	4.46	214.33	0.17	2.00	0.25	0.33
		SD	9.54	0.13	0.06	0.99	2.78	1.15	0.11	3.86	1.28	39.45	0.37	0.00	0.60	0.47
Wilamina	WILA	AV	112.18	1.08	0.60	4.53	36.94	4.03	0.40	29.88	4.30	252.35	0.35	2.00	0.76	0.59
		SD	12.27	0.10	0.05	0.85	4.04	0.55	0.03	2.81	0.46	33.87	0.68	0.00	0.81	0.49
Camp Adams	CMPA	AV	92.45	1.23	0.62	4.10	34.40	4.83	0.41	25.85	4.24	211.00	0.50	1.70	1.20	0.70
		SD	15.99	0.08	0.07	0.99	5.77	0.78	0.08	4.20	0.51	32.56	0.74	0.71	0.87	0.46
Mill Creek Park	MLCR	AV	93.21	1.02	0.66	3.57	34.50	4.22	0.39	26.43	4.23	240.29	0.43	2.00	0.86	0.71
		SD	14.33	0.06	0.05	0.90	6.90	0.73	0.07	3.94	0.48	78.90	0.49	0.00	0.91	0.80
Drift Creek Road	DRCR	AV	104.55	1.19	0.60	4.60	39.40	4.50	0.41	29.85	4.04	190.50	0.10	1.75	1.25	0.70
		SD	13.20	0.16	0.04	0.80	5.72	0.73	0.06	4.16	0.26	46.95	0.30	0.43	0.77	0.46
Bunker Hill	BNKH	AV	110.60	1.13	0.64	4.60	40.00	4.12	0.44	31.93	4.61	262.67	0.07	1.80	0.80	0.60
		SD	18.48	0.16	0.07	0.80	4.26	0.91	0.08	4.23	0.56	69.03	0.25	0.54	0.83	0.71
MacDonald Forest	MCDF	AV	115.47	1.21	0.63	4.60	43.40	4.69	0.42	32.93	4.45	287.93	0.67	2.00	2.00	1.40
		SD	19.10	0.12	0.05	0.80	10.68	1.06	0.08	5.51	0.59	51.79	0.79	0.00	0.00	0.61
Peoria	PEOR	AV	107.40	1.13	0.61	3.93	40.53	3.67	0.47	31.33	4.10	263.00	0.27	1.87	1.53	1.60
		SD	14.90	0.14	0.04	1.00	8.07	0.79	0.06	5.51	0.55	52.08	0.68	0.50	0.62	0.49
Alderwood State Park	ALDR	AV	98.87	1.22	0.60	4.47	37.00	3.82	0.45	28.13	4.08	255.93	0.53	2.00	1.93	1.73
		SD	15.38	0.18	0.05	0.88	7.43	0.68	0.04	5.03	0.58	63.21	0.72	0.00	0.25	0.44
Sutherlin Rest Area	SUTH	AV	120.80	1.22	0.65	5.40	51.60	3.97	0.49	36.80	4.77	319.80	0.80	2.00	1.90	1.70
		SD	13.69	0.13	0.05	0.80	8.85	1.27	0.03	6.88	0.70	48.20	0.98	0.00	0.30	0.46
Canyon Creek State Park	CNYN	AV	112.60	1.13	0.60	4.60	42.40	2.38	0.45	38.80	3.87	248.00	2.00	2.00	2.00	2.00
		SD	1.50	0.07	0.05	0.80	2.94	0.31	0.05	7.70	0.17	27.13	0.00	0.00	0.00	0.00
Cow Creek Rest Area	COWC	AV	94.70	1.25	0.63	4.00	42.40	2.69	0.49	34.30	3.99	224.00	1.80	2.00	1.80	1.60
		SD	20.02	0.18	0.01	1.34	6.07	0.52	0.04	4.86	0.60	81.51	0.60	0.00	6.00	0.49
Winona Road	WNON	AV	91.94	1.01	0.65	4.88	37.41	2.70	0.45	32.71	4.22	286.18	2.00	2.00	2.00	1.59
		SD	7.96	0.13	0.07	0.47	7.43	0.86	0.09	5.57	1.23	38.70	0.00	0.00	0.00	0.49
Bird's Eye Creek	BECR	AV	136.44	0.96	0.62	5.25	56.00	2.66	0.43	44.94	3.94	326.88	2.00	2.00	2.00	1.38
		SD	17.65	0.11	0.05	0.66	6.60	0.76	0.04	5.25	0.69	56.20	0.00	0.00	0.00	0.48
Lake Selmac	SELM	AV	114.87	1.17	0.62	5.13	49.20	2.60	0.45	40.00	3.98	273.20	2.00	2.00	2.00	2.00
		SD	17.63	0.17	0.03	0.88	10.27	0.65	0.04	8.20	0.96	66.21	0.00	0.00	0.00	0.00

Table 1

Figure 4. Dendrogram of cluster analysis performed on matrix of population averages, all morphological characteristics included.

Abbreviations of population names are given Table 1. Scale at the top indicates average taxonomic distance between objects in the unweighted pair group clustering method used.

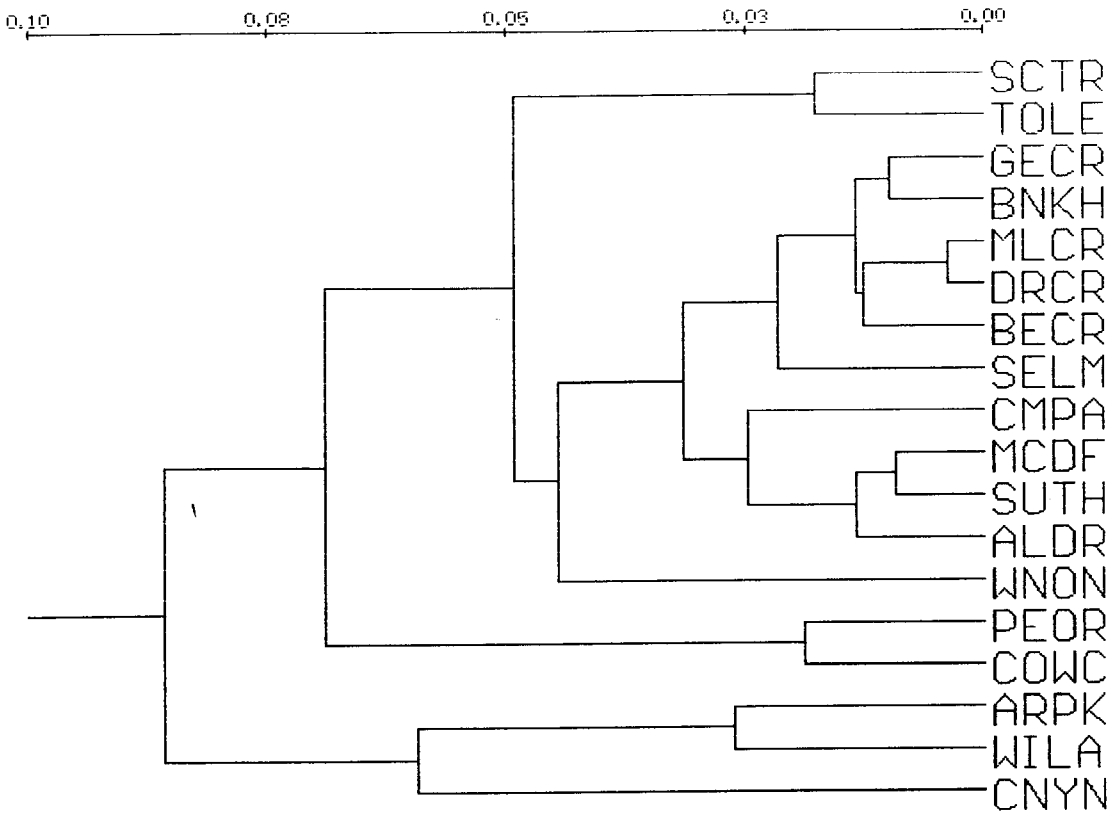

0.10
0.08
0.05
0.03
0.00
SCTR
TOLE
GECR
BNKH
MLCR
DRCR
BECR
SELM
CMPA
MCDF
SUTH
ALDR
WNON
PEOR
COWC
ARPK
WILA
CNYN

Figure 4

Washington (SCTR, TOLE), supposedly representing *Trillium parviflorum*, cluster together, the third Washington population (GECR) is in a cluster with populations from Marion, Polk, Jackson, and Josephine counties. The three populations from Douglas County (SUTH, COWC, CNYN) appear at widely separated parts of the dendrogram. Two populations from Yamhill County (ARPK, WILA) cluster together but are not closely connected with those from Clackamas, Marion, and Polk counties. The Lake Selmac population (SELM), which represents the nomenclatural type of *T. albidum*, is in a cluster that includes populations from Clark County, Washington, and Marion and Polk counties, Oregon.

Since Freeman (1975) states that size is an unreliable taxonomic characteristic in *Trillium*, and the correlation between rhizome shoot scars and size of plant parts indicates that plant age may affect size, I decided to analyze the data again after eliminating data pertaining strictly to size. The choice of morphological traits was reduced to include only data relating to shape of parts, floral scent, and color of floral parts, with leaf length, petal length, sepal length, and plant height removed. Number of nerves in the leaves was also retained as a characteristic (table 1). The reduced data were subjected to cluster analysis by the NTSYS-pc program, and a dendrogram was produced. The three clusters (figure 5) segregate into three geographic groups at a relative similarity of about 1.0. The first group includes populations from southwestern Washington and northwestern Oregon (lower Willamette Valley) as far south as Clackamas and Yamhill counties. The second group extends from Marion and Polk counties in the Willamette Valley, south to Canyon Creek in the Umpqua River drainage of southern Douglas County. The third group includes the site on Cow Creek, in southern-most Douglas County, and the three populations occupying the Rogue and Illinois river drainages of Josephine

Figure 5. Dendrogram of cluster analysis performed on a reduced set of averaged morphological traits.

Abbreviations of population names are given in table 1. Scale at the top indicates average taxonomic distance between objects in the unweighted pair group clustering method used.

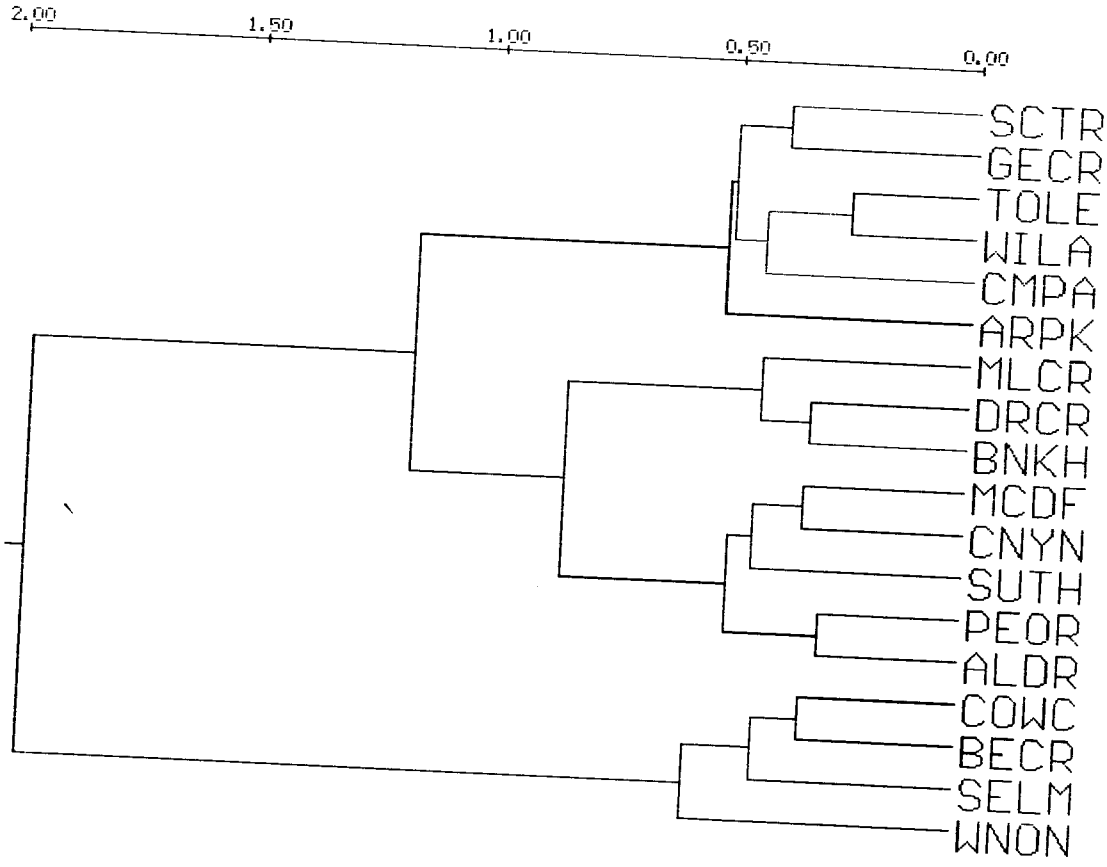

2.00
1.50
1.00
0.50
0.00
SCTR
GECR
TOLE
WILA
CMPA
ARPK
MLCR
DRCR
BNKH
MCDF
CNYN
SUTH
PEOR
ALDR
COWC
BECR
SELM
WNON

Figure 5

and Jackson counties. These southernmost populations are well segregated from the Northwestern Oregon and Southwestern Washington populations.

Three dimensional projections of principle components analysis were performed with NTSYS-pc, again using the average morphological values for the 18 populations. Data were standardized prior to computing the correlations, using the default settings for the program, in which the mean of each variable is subtracted from the linear transformation and the difference is divided by the standard deviation. Average population values were first analyzed using all 14 measured morphological characteristics (figure 6). In a second PCA run, the same four size characteristics (leaf length, petal length, sepal length, and plant height) were removed from the data as was done in the cluster analysis, and the data were again subjected to a principle components analysis (figure 7).

Cytology

In the karyotypes observed (table 2) all plants in all populations were homozygous for chromosome A2. Out of 193 karyotypes examined, including the data of Kurabayashi (1963), only one individual from a population near Portland showed chromosome types C2 and D2. All other individuals were homozygous for C1 and D1. Only chromosomes B and E showed variation.

Karyotypes from the three Washington populations showed remarkable consistency and homogeneity. Chromosome types B1 and B2 were predominant and generally appeared in homozygous combinations. Chromosome B6 appeared in only two karyotypes. Only chromosome type E2 appeared in these populations.

Figure 6. Projection of principle components analysis performed on matrix of population averages, all morphological characteristics included.

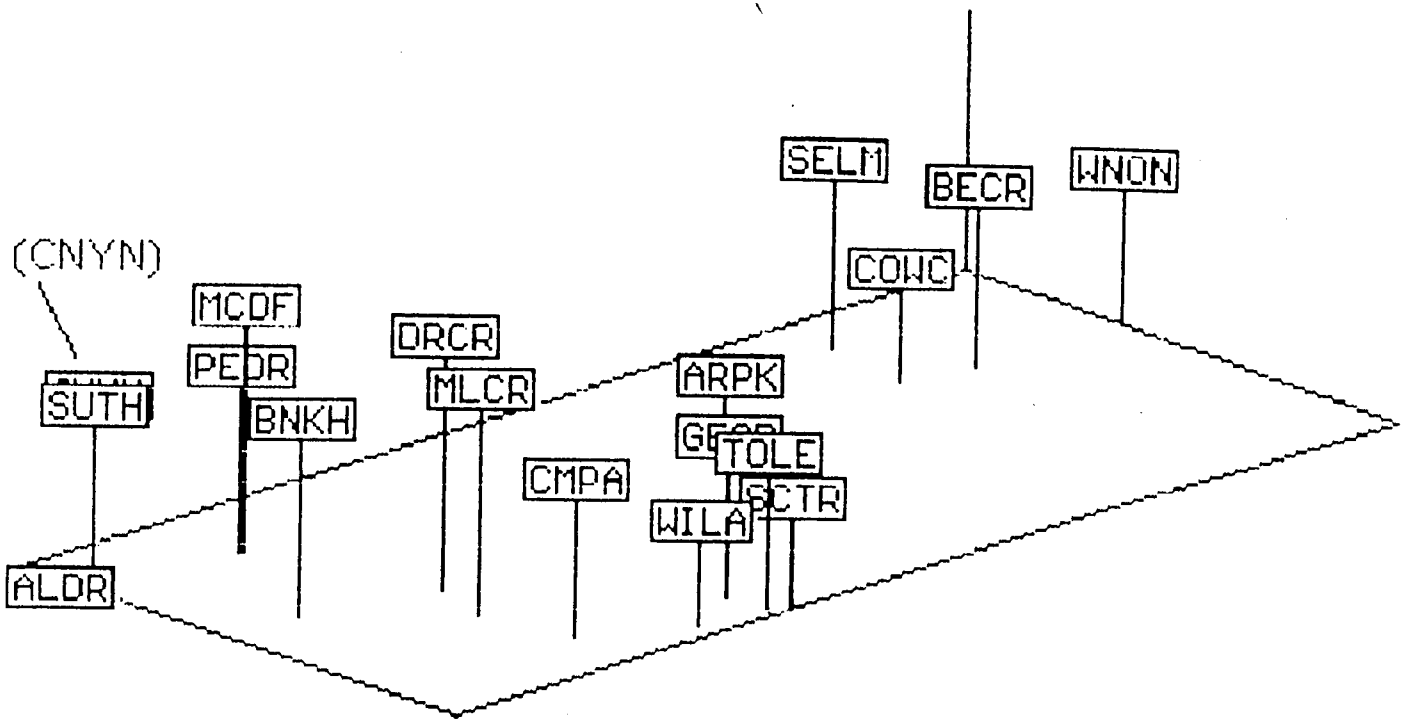

SELM
BECR
WNON
(CNYN)
COWC
MCDF
PEDR
DRCR
MLCR
ARPK
SUTH
BNKH
TOLE
CMPA
WILA
ALDR

Figure 6

Figure 7. Projection of principle components analysis performed on a reduced set of averaged morphological traits.

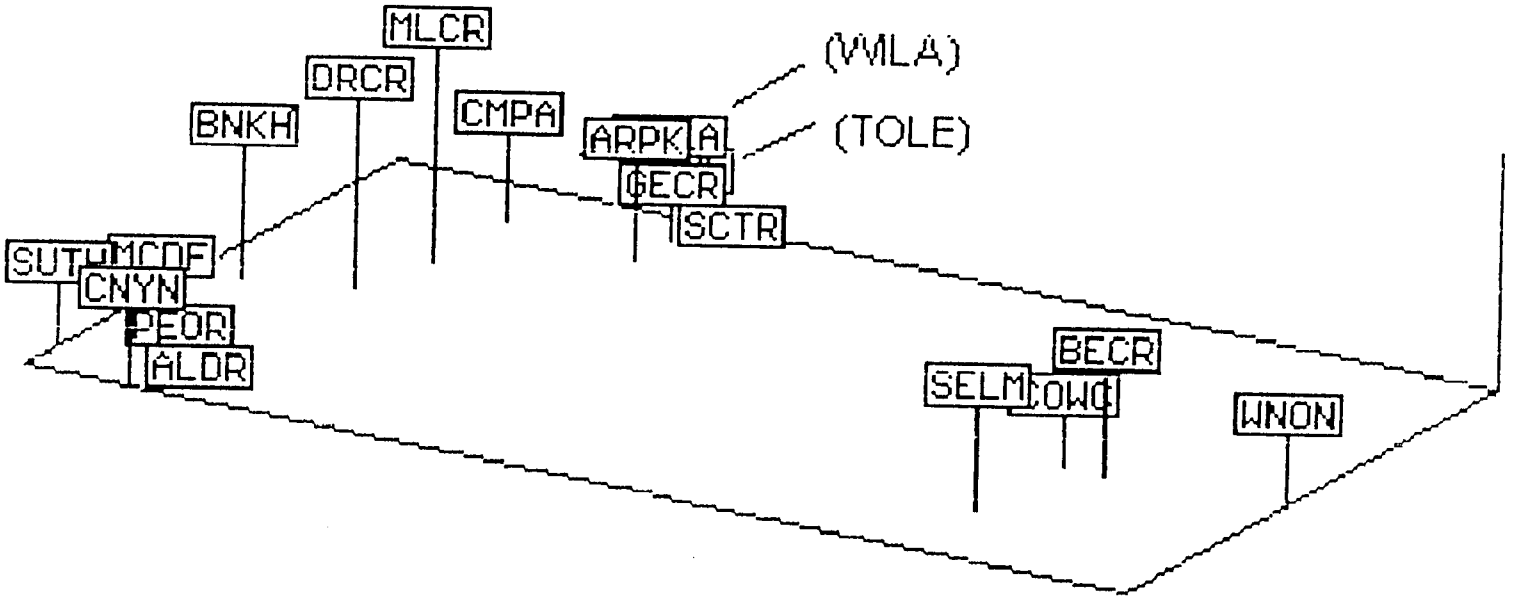

MLCR
DRCR
BNKH
CMPA
(VMLA)
ARPK
A
(TOLE)
SCTR
CNYN
ALDR
BECR
SELM
WNON

Figure 7

Table 2. Karyotypes of plants collected in the field for cytological study, with the data of Kurabayashi (1961), denoted by (K), included for comparison.

Genotype numbers are arbitrarily assigned for each population and differ between populations. Chromosome designations (e.g., B1, B2, E4, E8) follow the terminology and chromosome taxonomy of Kurabayashi (see figure 2). The two numbers connected by a dash (e.g., B 1-1, E 1-7) show the diploid karyotypes of a given genotype (e.g., B 1-1 = homozygous for chromosome type B1; E 1-7 = heterozygous for chromosome types E1 and E7). The number of clones sampled per population is reported as n.

Usable karyotypes could not be obtained for all study sites.

Table 2 extends from page 50 to page 52.

sample name	type	B	E	freq.	n	freq. of homozygosity in the B chromosome	freq. of homozygosity in the E chromosome
Thurston (K)	1	1-1	2-2	39	39	1.00	1.00
SCTR	1	2-2	2-2	1			
	2	1-1	2-2	7	10	0.86	1.00
	3	1-6	2-2	2			
TOLE	1	1-2	2-2	2	3	0.33	1.00
	2	2-2	2-2	1			
Portland (K)	1	1-1	2-2	9			
	2	1-1	1-7	3			
	3	1-1	7-7	4			
	4	1-2	1-1	1	31	0.74	0.65
	5	1-2	1-7	6			
	6	1-6	7-7	1			
	7	2-2	1-1	3			
	8	2-2	1-7	2			
	9	2-2	2-2	1			
	10	2-2	7-7	1			
ARPK	1	0-6	2-2	2			
	2	0-1	2-2	1			
	3	0-1	1-2	1	6	0.17	0.67
	4	2-2	4-8	1			
	5	1-2	1-1	1			
WILA	1	1-1	1-2	1			
	2	0-1	1-2	1			
	3	0-2	1-1	1	5	0.40	0.40
	4	6-6	4-8	1			
	5	0-2	5-5	1			
CMPA	1	0-2	1-1	1			
	2	0-6	1-2	1	4	0.00	0.50
	3	0-1	1-2	1			
	4	0-1	1-1	1			
Molalla (K)	1	0-0	1-1	5			
	2	0-0	4-8	1			
	3	0-1	1-1	4			
	4	0-2	1-1	1	20	0.70	0.70
	5	1-1	1-1	5			
	6	1-1	5-5	2			
	7	1-2	1-5	1			
	8	2-2	1-1	1			

sample name	type	B	E	freq.	n	freq. of homozygosity in the B chromosome	freq. of homozygosity in the E chromosome
MLCR	1	0-0	2-2	2			
	2	0-1	1-1	1			
	3	1-6	2-2	2			
	4	1-1	1-2	1			
	5	1-1	2-2	2	13	0.69	0.77
	6	0-1	4-8	1			
	7	6-6	2-2	1			
	8	6-6	1-1	1			
	9	6-6	1-2	1			
	10	2-2	2-2	1			
DRCR	1	2-6	2-5	1			
	2	0-2	1-2	1			
	3	0-6	4-8	1	8	0.00	0.75
	4	0-6	1-1	2			
	5	0-6	5-5	2			
	6	0-2	5-5	1			
BNKH	1	0-2	5-5	3			
	2	1-6	1-1	1			
	3	1-1	1-1	2	8	0.25	0.88
	4	0-2	1-1	1			
	5	0-2	1-2	1			
PEOR	1	6-6	2-2	1			
	2	2-2	5-5	2			
	3	1-2	1-5	1	7	0.43	0.57
	4	0-1	1-5	1			
	5	1-2	2-5	1			
	6	0-2	5-5	1			
ALDR	1	1-6	1-1	2			
	2	6-6	2-2	3	6	0.43	0.85
	3	2-6	1-2	1			
	4	1-2	2-2	1			
SUTH	1	1-6	2-2	1			
	2	6-6	1-1	1			
	3	1-2	2-2	1	5	0.60	0.80
	4	2-2	1-2	1			
	5	2-2	1-1	1			
CNYN	1	6-6	5-5	2	2	1.00	1.00

sample name	type	B	E	freq.	n	freq. of homozygosity in the B chromosome	freq. of homozygosity in the E chromosome
COWC	1	1-1	1-1	1			
	2	1-1	1-2	1			
	3	6-6	5-5	1			
	4	6-6	5-5	1	7	0.57	0.86
	5	2-2	2-2	1			
	6	1-2	1-1	1			
	7	1-2	2-2	1			
WNON	1	1-2	1-2	1			
	2	2-6	1-2	1			
	3	2-2	2-2	5	9	0.11	0.78
	4	2-6	1-1	1			
	5	6-6	2-2	1			
BECR	1	2-2	2-6	3			
	2	1-2	6-6	1			
	3	1-2	2-2	2			
	4	1-2	1-1	1	10	0.40	0.70
	5	6-6	1-1	1			
	6	2-6	1-2	1			
	7	2-2	6-6	1			
SELM	1	2-6	1-2	1			
	2	1-2	1-2	1	3	0.00	0.33
	3	1-6	2-2	1			
				Total:		193	

All populations sampled within the Willamette Valley, except Portland (From Kurabayashi, 1963) and ALDR, had at least one individual with chromosome type B0, a chromosome which showed no heterochromatin banding. This chromosome did not appear in karyotypes from individuals south of the Willamette Valley, nor was it seen in the Washington populations. Chromosome B6 appeared occasionally in all northern Willamette Valley populations except Molalla (Kurabayashi, 1963) and was characteristic of all the more southern samples as well. Chromosome E5 was seen in 12 karyotypes from the Willamette Valley and in only three individuals from populations south of this area. Chromosomes E7 (seen only by Kurabayashi), E4, and E8 were found in some Willamette Valley populations but in none south of Marion County. Chromosomes E4 and E8 occurred only in heterozygous combinations.

In populations from south of the Willamette Valley, chromosomes B1, B2, and B6 appeared with nearly equal frequency. Chromosome E6 appeared in karyotypes from BECR plants but was found in no other population. The karyotypes of the southern Oregon populations appear similar to those of Washington populations, except that they have a greater frequently of heterozygosity compared with the usually homozygous Washington genotypes.

DISCUSSION

At the beginning of the study, it was hypothesized that only a single species of white, sessile-flowered *Trillium*, *T. albidum*, occurs in the region of interest, that is, Washington and Oregon west of the Cascade Range. The characteristics which Soukup (1980) chose to differentiate populations in the northern part of this range as a separate species, *T. parviflorum*, largely involve the smaller size of parts, both vegetative and reproductive, combined with subtle color and odor features. Seldom are these evident on herbarium specimens, and sometimes these features are even difficult to evaluate on living plants. Given the characteristic variability of *Trillium* species in general, it seems unlikely that flower color, odor, petal size and shape, leaf size, or plant height would be so invariant in *T. albidum* as to support the taxonomic splitting of *T. parviflorum*. Nonetheless, it is worthwhile to examine carefully the question of whether the morphological and cytological variation in *T. albidum* is correlated with the geographical distribution of its populations.

T. albidum has a south-to-north linear range, extending from California where its several closest relatives occur (*T. chloropetalum, T. angustifolium, T. kurabayashii*), to a northern limit in southwestern Washington, where its available habitats are probably restricted by climatic and biotic factors, such as light, season length, and mean annual temperature. Over such a continuous latitudinal range, it would be possible for a clinal pattern of morphological and genetic variation to develop, for example through adaptation to gradients in historic and environmental factors of various kinds, including human disturbance. Alternatively, the variation need not be strictly clinal, but might show a pattern of interruptions or discontinuities. Such gaps may be due to rapid historic and environmental changes

along the south-to-north gradient or to physical barriers (e.g. mountain ridges separating river drainage basins) that strongly reduce gene flow through pollen exchange or seed dispersal. The rate at which *Trillium* populations will spread even under ideal conditions is limited, as the seeds are distributed by ants (Mesler and Lu, 1983). The morphological and karyological data obtained in this study help to clarify population patterns in that part of the species' range north of California. They cast doubt on size characteristics--leaf length, plant height, size of sepals and petals--as indicative of genetic differences. Rather, such traits are relatively plastic and hence strongly modified by plant age and by habitat factors affecting plant growth. Indeed, it was observed at the BNKH site, which was more closely studied than the rest, that rhizomes which produce large, robust plants, will, if grazed, produce many smaller, non-flowering clones the next year. This was a casual observation, however, incidental to the rest of the study, and thus there is no numeric data to accompany it; it would be worth further study.

The other morphological characteristics that were examined, relating to the shapes of leaves, sepals, and petals, colors of petals, stigmas, and ovaries, and floral odor, when analyzed by clustering and principal components algorithms, show a structuring of the study populations into two or three geographical groups arranged along the north-south axis of the species' range. Karyological observations give some additional support to this pattern, through the limited geographical occurrence of particular chromosome types, at least in the limited sample of populations and individuals reported here.

The observations made in this study on geographically significant morphological and karyological patterns can be summarized as follows. In Washington, plants have white petals that tend to be narrow (broadly linear), plus a

spicy or nondescript floral scent, and usually three nerves on the leaves. Homozygosity of chromosome types was usual, indicating inbreeding or frequent cloning. In the Willamette Valley, particularly in the northern part, plants often have a reddish streak on the petals, though many have all-white petals. Leaves have three or five nerves. The floral fragrance can be spicy, nondescript, or rose-like. Petals tend to be narrowly elliptic. Chromosome types B0 and genotype E4-8, were found here and nowhere else. Heterozygosity in chromosomes is common, indicating more frequent reproduction by sexual outcrossing. In southern Oregon, plants have all-white petals which are usually broadly elliptic (though narrow petals are also seen), and floral scent may be non-descript, but is more often distinctly rose-like. Chromosome type E6 is found in the BECR population. Heterozygosity of chromosomes is usual, indicating frequent sexual reproduction (Kurabayashi, 1963; Handel, 1983).

I showed, through estimations of relative plant age by counting rhizome shoot-scars, that leaf length, petal length and plant height are positively correlated with age. The later study by Hanzawa and Kaliz (1993) appears to confirm this, though the correlation there is between leaf area and plant age. I did not investigate possible correlations of the size features within and between habitats in field populations, although such studies would help our understanding of the plastic growth responses of *Trillium* plants to environmental factors. Experiments might also be done with plants grown under controlled conditions. Extraneous factors such as browsing by deer (and by humans!) probably have a profound effect on the growth of individuals in nature. In terms of the search for consistent, useful taxonomic characters by which to define species and infraspecific taxa, the size of plants and their parts is probably of little value. As described in the previous

section, when size traits were factored into the clustering analyses, geographic patterns were for the most part obscured. In principal components analyses, geographic patterns were visible, but were more distinct when size traits were removed from the data.

Cluster analyses that exclude size characteristics (figure 5) show a strong tendency of the study populations to form three groups. These correspond to three geographical areas: Washington and northern Willamette Valley, central Willamette Valley to the Umpqua River region, and southern Oregon in the Rogue River region. Principal components analysis of the same data set (figure 7) reveals two groups, one consisting of the four southern-most Oregon populations, the other three consisting of all populations from the Umpqua River region northward. This second group is clearly subdivided into three smaller groups: (1) the northernmost populations, including all three populations from Washington and the two northernmost populations on Oregon, (2) mid-Willamette Valley populations, from Yamhill county to Marion county, and (3) the southernmost populations of this region, from Benton county southward to northern Douglas county. The distinctness of the four southern Oregon populations, which are geographically isolated from those farther north by the mountain divide between the Rogue and Umpqua rivers, is seen in figure 6, as well, where the first major split in the dendrogram separates their cluster from a cluster formed by the remaining populations. This correlates well with changes in geology in this region, which is older and more stable than the northern regions. The southern Oregon populations fall largely into the Klamath Mountains Province described by Franklin and Dryness (1973).

Karyotypes of *Trillium* show patterns that also reflect their geographical distribution, though not as clearly as the morphological data. Homozygosity in pairs

of B chromosomes is frequent in Washington. Kurabayashi's (1963) data show 39 identical karyotypes from one population in Washington. I obtained only three karyotypes from TOLE and none that were identifiable from GECR. The data from SCTR show a tendency toward homozygosity in the B chromosome. In pairs of E chromosomes, homozygosity appeared to be the rule. In these populations, chromosomes B1 and B2 were predominant, both in my data and that of Kurabayashi. Chromosome B6 was seen in only two plants. Of all the E chromosomes, only E2 was found in Washington.

The genetic situation in Oregon and southward may be that sexual reproduction is either more frequent or more successful among these plants, or that there is a better chance of individuals from one population pollinating plants of another population. In Oregon, frequency of homozygosity is less, from 0 to 0.74 for the B chromosome and from 0 to 0.87 for the E chromosome. The exception seen in Table 2 is the CNYN site, but note that only two karyotypes were obtained for this site. Chromosome types unique to this region are B0, E7 and the heterozygous combination of E4-8. In populations from south of the Willamette Valley, chromosomes B1, B2, and B6 appeared with similar frequencies. Chromosome E6 appeared to be unique to the BECR site. This was the only chromosome type unique to this area.

The greater morphological uniformity and chromosomal homozygosity that I observed in *Trillium* populations at the northern limit of the range in Washington state, probably correspond to a marked reduction in genetic variation there. These populations may be showing an "edge effect" in having reduced sources for new genes (gene flow is only possible from a single direction) and in being subjected to environmental conditions representing the limits of adaptation of the species. In

observing the karyotype data, the trend toward homozygosity in the Washington populations suggests either frequent cloning of plants within that region, or that all the current populations in Washington descend from a limited number of ancestors. The Columbia river, which divided the Washington from the Oregon populations, is an effective geographical barrier for a plant which relies upon ants for seed dispersal (Mesler and Lu, 1983). As well, depletion of genotypic variability can be the consequence of long establishment of a population at a given site (Handel, 1983). Trilliums are self-compatible (Nesom and LaDuke, 1985), and while self-pollination may not be the most frequent breeding system (Serota, 1969a), it would contribute to homozygosity. Populations of *T. albidum* become established in moist woods composed of mixed conifers and deciduous trees, usually *Pseudotsuga menziesii* and *Quercus garryana* or *Fraxinus latifolia* (Kurabayashi, 1963; personal observations). In such sites, sexual reproduction tends to be high, but as conifers become dominant in the canopy and the spring light level is reduced, sexual reproduction and seedling establishment become less, and cloning by rhizomatous budding, though also reduced, becomes the dominant form of reproduction (Kurabayashi, 1963; personal observations). Thus, after establishment, the number of genotypes in a population tends to decline due to reduced recombination and to death of individuals. The lowered range of genotype variability seen at some of the study sites may thus be due in part to the age of the populations. Alternatively, the age of the forest rather than the plants themselves may affect the genotypes. Those same forest conditions may inhibit sexual reproduction by reducing seed germination. Thick forest litter and lower light conditions may be unfavorable to seedlings. Disturbance may also be a factor affecting sexual versus asexual reproduction. Deer frequently browse plants in all populations. Where deer

browsing was the heaviest, cloning was frequent (personal observations). Presumably, human disturbance could have the same effect.

Interesting parallels exist between my study and that by Serota (1969) on *Trillium* species in the southeastern United States. Serota used floral fragrance, presence of anthocyanins, and geographical isolation to distinguish between *T. cuneatum*, *T. luteum*, and hybrids of the two in North Carolina and Tennessee. In her study, presence of floral anthocyanins was shown to correlate with a spicy scent. Hybrids, if they had any trace of reddish pigments, were either spice-scented or had no scent. *Trillium luteum*, which lacks anthocyanins, was reported to be lemon-scented.

Any taxonomic conclusions to be drawn from this work, possibly leading to a revised classification of the sessile-flowered *Trillium* of western Washington and Oregon, necessarily contain a subjective element. The objective pattern showing subdivision of the populations into one major and two lesser morphological--hence very likely genetic--groups (figures 6 and 9) does not by itself demand a certain taxonomic treatment. That is, even though the population groupings exist and can be revealed by multivariate statistics, it may not be desirable, in practical terms, to give them names in a formal infraspecific classification. In the practice of plant taxonomy, one must consider that populations may differ from each other statistically, yet the percentage of intergradient or otherwise morphologically ambiguous individuals may be so large that a taxonomic split, while possible, is not practical or useful. In the case of *Trillium*, if the morphological differences characterizing population groups are minor and developmentally variable (plastic), as in the case of dividing *T. parviflorum* from the larger group of *T. albidum* it proves difficult to apply the classification to plants existing in nature. This seems

would also be the case if differences involving size of plant parts (stem, leaves, perianth) were used as descriptors of subspecies. Characteristics which correlate better with the geographical pattern of populations include petal shape and color, together with floral fragrance. Difficulties with the practical use of these traits are, first, that they are mostly lost in dried specimens; second, that judging their states is often subjective (especially flower scent); and third, they often vary within and between populations such that individuals in a single population might often be assigned to different subspecies. This seems to have been the case with efforts by botanists to decide what plants in the Willamette Valley belong to "*T. parviflorum*," because individuals far south of the described range of this "species" have characteristics linking them to it.

The sharpest break in the morphological north-south cline in *Trillium albidum* occurs in southern Douglas County, on the divide between the drainage basin of the Umpqua River and that of the Rogue River. This break was not recognized, however, until morphometric analyses were performed during the present study. The nomenclatural type specimens of *T. albidum* came from the Rogue River region.

If a separate subspecies were to be recognized comprising all the populations north of the Rogue-Umpqua divide, it would have to be provided with a new subspecific epithet or with the epithet "parviflora" reduced from the species to the subspecies level. Viewing this problem in practical terms, it is my conclusion that it would be impractical at this time to divide *T. albidum* into two subspecies, given the information we have about its morphological variation. I believe that the percentage of taxonomically ambiguous individuals is too high, and the amount of overlap in variation too great, to permit a formal taxonomic split between the

southern and northern population groups. One must ask the question, "If I were given a plant of *Trillium* to classify, without knowing its geographical source, what is the probability that I could identify it correctly to subspecies on its morphology alone?"

I believe that the plants under study here do not possess a pattern of morphological variation that favors the recognition of the species *T. parviflorum* Soukup. Further analysis based upon the genetics of the plants would be necessary before I would be prepared to recognize new subspecies or varieties within the species *T. albidum*. If infraspecific taxa were eventually to be described, however, they might correspond with the three morphological subgroups geographically defined as: southwestern Washington and adjacent Oregon, the central Willamette Valley to the Rogue-Umpqua divide, and the Klamath Mountains to the Coast Range and Sierra Nevada of California.

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