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Title: POLYMORPHIC, SYMPATRIC FLAVONOID VARIANTS OF CLAYTONIA PERFOLIATA DONN FROM THE COLUMBIA RIVER GORGE

Abstract approved: Redacted for privacy
Dr. Kenton L. Chambers

Claytonia perfoliata is a west-American polyploid complex of common annuals (x=6). The species is polymorphic; exomorphic characters are often variable within local populations. In a transect of the Columbia River Gorge diploids, tetraploids, hexaploids, octoploids, and decaploids were collected, which varied in shape of basal and cauline leaves, degree of cauline leaf fusion, color of corolla and calyx, robustness of growth, size of flower parts and seeds, and foliage coloration. Progeny from field collections were grown in uniform regimes of light, temperature, and moisture. Each family of progeny was morphologically indistinguishable from its individual parent implying a high degree of self-fertilization in nature. The form and sequence of maturation of basal leaves was found to be an important character differentiating the families of progeny. Phenolic compounds from whole plants cultured under uniform greenhouse conditions were

extracted (from fresh plant material) in stages using acetone and methanol, and were separated using two-dimensional paper chromatography. The flavonoids of petal, sepal, leaf, stem, and whole plant methanolic extracts (from dried plant material) of wild populations were separated and isolated. Striking chromatographic differences between morphological forms, both within and between chromosome levels were found. Chromatographic spots were identified on the basis of a set of six spectra, color reactions, and R_f values. Though preliminary in scope, this study demonstrates the value and reliability of certain chemical, morphological, and cytological characters as valuable taxonomic criteria applicable to further study of C. perfoliata.

Polymorphic, Sympatric Flavonoid Variants of *Claytonia perfoliata* Donn from the Columbia River Gorge

by

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Typed by Susie Kozlik for John Michael Miller

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I. INTRODUCTION

Claytonia perfoliata Donn is a west-American polyploid complex of annuals having diploid, tetraploid, hexaploid, octoploid, and decaploid constituents (Miller, 1975). The complex as studied is circumscribed by these key characters: plants annual with a fibrous root system surmounted by a shortened caudex bearing a rosette of whorled basal leaves; flowering branches having a single pair of opposite leaves which may or may not be fused into a perfoliate disc; inflorescences ebracteate above the lowermost flower; seeds three, shining and bearing a prominent elaiosome; chromosome base number of x=6. Members of this species are common in natural and disturbed habitats in western North America from British Columbia south into Mexico, expecially in the spring of the year. The species is an introduced naturalized weed in Europe, Australia, and New Zealand. In California certain hexaploids develop weedy infestations of orchards (Swanson, 1964).

Conflicting interpretations of taxonomic characters coupled with the high degree of polymorphism exhibited by almost every entity and a lack of knowledge of the extent of polyploidy have led to widely differing classifications. Recent taxonomic treatments of *Claytonia*

perfoliata seem to have been developed with little attention being paid to breeding systems, morphological development, population structure, or the extent of environmental modification of the phenotype. No one has biochemical evidence to support one or another of the various proposed taxonomic systems for the species.

The present study is an attempt to gather data and to generate hypotheses regarding the origin and relationships of selected populations within the polyploid complex. It is hoped that these hypotheses will form a basis for future detailed studies of the entire group.

Because of the gregarious nature of the species and its wide distribution, the study area was confined to the Columbia River Gorge and lower Columbia Basin of Oregon and Washington. The methodologies used, including that of comparative flavonoid biochemistry, were chosen for their potential value in improving our knowledge of the sources and nature of the variation exhibited by Claytonia perfoliata.

II. LITERATURE REVIEW

I have chosen to recognize the species as a member of the genus Claytonia, contrary to the opinion of writers of some west-American floras (Hitchcock and Cronquist, 1973, Hitchcock et al., 1964, Peck, 1961, Munz, 1959) that it is better placed in the genus Montia (a concept first proposed by Howell in 1893). The placement of C. perfoliata in Claytonia is based on evidence from studies of pollen morphology, comparative morphology, and chromosome number by recent workers on the Portulacaceae (Lewis and Suda, 1968, Nilsson, 1967, Swanson, 1966). Munz (1974) in his recent "Flora of Southern California" also adopts Claytonia for C. perfoliata and its applied species.

Claytonia perfoliata occurs in many morphological forms which vary in basal leaf shape, degree of fusion of cauline leaves, color of corolla and calyx, robustness of growth, size of flower parts and seeds, and foliage coloration. Certain diploids, together with a few forms from other chromosome levels, are scarcely one centimeter tall when in flower. In contrast, some hexaploids, octoploids, and decaploids reach heights approaching five decimeters and produce abundant amounts of foliage. Frequently exomorphic characters vary within a single population, as has been described by Swanson (1964) for certain of the hexaploids. The high degree of polymorphism

exhibited by Claytonia perfoliata sens. lat., has stimulated a diversity of taxonomic treatments, and the synonomy for the species is large. Rydberg (1932) in his monograph of the Portulacaceae in North America treated the species to its most extensive splitting. He divided Claytonia perfoliata (which he placed in the genus Limnia) into 18 species. Some of these had been named earlier by Suksdorf (1923), whose newly described entities had come mainly from the Columbia River Gorge. Subsequent treatments by Munz (1959) and Hitchcock et al. (1964) went to the opposite extreme by lumping all taxa reported by Ryberg and others into a single species. Hitchcock et al. (1964 p. 245) attribute the remarkable variation within the species in the Northwest to "local ecological conditions and to genetic variation within freely interbreeding populations."

The most comprehensive study of Claytonia perfoliata was that of Swanson (1964), whose research largely involved a genecological investigation of two hexaploid forms. Swanson collected plants from approximately 130 field populations in California between the Oregon border and Kern County, primarily west of the Sierra Nevada crest. However, because of the immensity of the problem and the widespread nature of the species, Swanson's samples were admittedly inadequate to allow a full understanding of this complex taxon. His cytological survey indicated the existence of diploids, tetraploids, and hexaploids. Among these could be recognized forms in which a distinctive

morphology could sometimes be correlated with a particular chromosome level. Several such entities were reported. These included two very different diploids, one of which is the common Claytonia depressa Suksd. (more correctly called Claytonia rubra (Howell) Tidestr.), a taxon which is characteristic of drier Pinus, Pseudotsuga, and Juniperus woodlands of the West. A second diploid, endemic to the Channel Islands of California, was reported. Four distinct tetraploids and two hexaploid forms (deltoid-leaved and linear-leaved) were also found. No formal taxonomic treatment for these biological entities was proposed, however. The bulk of Swanson's work involved a study of habitats, breeding systems, hybridity, and ecotypic differentiation of the two hexaploid forms. Several experiments were conducted to study morphological development, flowering behavior, and seed size differences with respect to specific morphological features. The hexaploid entities studied by Swanson were autogamous but retained their ability to outcross.

Literature describing species variation and secondary plant substances in the Portulacaceae is scanty. Both Gibbs (1974) and Harborne (1967) fail to list the occurrence of any identified flavonoid compound for the Portulacaceae. The only study published involving flavonoids of Claytonia was that of Douglas and Taylor (1972), which described flavonoid variation in Claytonia lanceolata Pursh. R_f values

and color reactions were reported but no attempt was made to identify the flavonoid compounds in the species. The only record of the identification of secondary plant substances for Claytonia perfoliata was that of Mabry et al. (1963). Mabry and his co-workers established the existence of betacyanins in the species.

III. MATERIALS AND METHODS

Field Studies

The plants used in this investigation were collected from the Columbia River Gorge and lower Columbia Basin of Washington and Oregon. Populations of Claytonia perfoliata were sampled from Ellsworth, Washington on the west end of the Columbia Gorge to Boardman in north-central Morrow County on the east, a distance of 140 airline miles. The majority of the collections were made within two miles of the Columbia River.

During the spring of 1974 and 1975, 37 populations were sampled from a variety of habitats. Three collections of seeds and seed parents made in April, 1972 by Dr. Kenton L. Chambers and seven interesting populations from other points in Oregon and California are included in the present study. Individuals from all 47 populations were analyzed cytologically. Young inflorescences were fixed in the field using a mixture of chloroform:ethanol:glacial acetic acid (4:3:1 v/v). Rosettes were broken apart by hand, and young (whole) flowering branches bearing immature inflorescences were fixed. Bud samples were stained and stored in 70% aqueous ethanol at 0 ^{o}C until analyzed. Permanent microslides of dividing microspore-mother cells were prepared. Whole flower buds, prestained in alcoholic carmine, were squashed in 45% aqueous acetic acid and mounted in

Hoyer's medium. The procedure followed was that of Snow (1963). Voucher specimens and permanent microslides are deposited in the Oregon State University Herbarium (OSC). Meiotic preparations were studied with a Zeiss phase-contrast microscope. Preparations representing the five chromosome levels were photographed using an Exakta 35 mm camera with 45 mm extension tube attached to the microscope. The image was recorded on Kodak SO-410 high contrast photomicrography film. Cytologically analyzed representative plants (henceforth called the P_1 - parental generation) from the study populations were transplanted to the research greenhouses at Oregon State University. These plants successfully set seeds, which were collected and stored dry for five months.

During the spring of 1975, original collection sites as well as new populations were visited, where cytological material and vouchers were secured as before. Whole individuals from collections made during spring 1974 and 1975 were placed into paper bags and dried under warm air in the laboratory. Mass collections of corollas, calyces, cauline leaves (perfoliate discs), stems, and basal leaves were obtained. These separated plant parts were dried in the same way as the whole plants, above.

Greenhouse Experiments

Seeds and the resulting experimental progeny from collections are henceforth designated as the S_1 (putatively self-pollinated) generation. Care was taken to insure that the S_1 was grown under uniform environmental conditions. Seeds were planted in 3-inch peat moss cups using a uniform planting mixture of Bandini Oak Leaf Mould (processed Lithocarpus duff) and Perlite (2:1). Planting cups were placed in flats on the greenhouse bench. Plants were transplanted the fourth week into 5-inch plastic pots using a uniform planting mixture of Bandini Oak Leaf Mould:autoclaved topsoil:autoclaved sand (4:1:1). All cultures were grown in a research greenhouse at Oregon State University, Corvallis, Oregon (123°15'W, 43°30'N) on a single greenhouse bench. Watering and lighting were automatically controlled using a misting device and fluorescent light banks on tandem timers. Four light banks were placed over the bench, perpendicular to the bench axis. Each light bank consisted of three dual ballast units with four 48-inch fluorescent bulbs per unit. Each lighting unit had three types of bulbs; the two end bulbs were Sylvania F40GRO (Gro-Lux) while the inner bulbs were Sylvania F40D (Daylight) and Sylvania F40CW (Cool-white). The light produced in this particular experimental design is of respectable quality across the visible spectrum with emphasis at 660 nm. Uniform light quality (560 foot candles) across the entire

bench surface was also attained. Light intensity was measured on 18 December 1974 (a cloudy day) at 12:30 pm. PST using a General Electric Hectolux (color and cosine corrected) light meter. Daylength for the first 14 weeks was set at 12 hours. After 14 weeks, daylength was increased to 14 hours. Throughout the experiment the plants were misted only during the initial 12 hour light period. For the first four weeks misting was set for 1 minute intervals of continuous mist, once every hour. Misting of this nature resulted in an approximate daily precipitation of 5 mm (discounting evaporation from the rain gauge). After four weeks the misting interval was increased to 10 minutes of continuous mist, once every 50 minutes. Daily precipitation of this nature was approximately 50 mm (again discounting evaporation). After 8 weeks, misting was reduced to 5 minutes of continuous mist, once every 55 minutes, which resulted in a daily precipitation of 20 mm. Continuous temperature readings were recorded with an Electric Autolight Model 1000 Recording Thermometer. Temperature ranged from 59°F to 70°F during the daily light period and averaged 63°F. The nightly temperature ranged from 49°F to 59°F and averaged 54°F.

During the course of the experiment, morphological data were recorded. Progeny from individuals collected by Dr. Kenton L. Chambers during April of 1972 were cytologically analyzed (as before).

Cultures used in chemical analysis were uprooted just before flowering. This point in the developmental sequence was determined when fertile branches were fully extended but just prior to the opening of the first flower. Individuals used in artificial hybridizations were transferred to a portion of the bench devoid of misting and were watered by hand. Vouchers were secured after the developmental sequence seemed complete. All vouchers representing plants used in chemical analysis, artificial hybridizations, and morphological studies are depositied in the Herbarium of Oregon State University (OSC).

Artificial hybridizations were attempted between several hexaploid entities from the Columbia River Gorge. Every morning during the flowering period I selected various flowering branches of the seed parents and labeled them (using a small piece of white gum label) with the garden number of the pollen parent. As each flower opened, it was emasculated and pollinated. Pollination was achieved by removing a flower from the pollen parent with a forceps and lightly brushing the seed parent's stigma with this flower. Because of the small size of both seed and pollen parent flowers, it was difficult to manipulate single anthers. Emasculation however, was easily accomplished with a sharp forceps. Once pollinated, flowers usually closed (over a period of a few hours) and nodded. The small size of individual flowers did not permit bagging after pollination. When bagging was attempted, undue mechanical damage occurred, resulting

in flower death. After most of the flowers of a branch were pollinated but before the lowermost flowers of the raceme had released their seed, the entire branch was enclosed using small plastic polyethylene bags. Bags were secured with thread just below the cauline leaves (perfoliate discs). Any flowers not pollinated were removed from the inflorescence with a sharp forceps. This procedure did not seem to affect capsule development of other flowers on the same inflorescence. Bagging of the flowers of C. perfoliata is essential since seeds are generally forcefully ejected several feet from the parent plant by the pressure of the capsule on the slippery seeds (Willis, 1892). The number of capsules bagged as well as numbers of seeds set or ovules aborted were recorded (Table 2). Seeds were stored and will be planted after their dormancy requirement is met.

Chemical Studies

From individuals grown in culture and collected in the field, flavonoids and flavonoid glycosides, as well as other phenolic compounds, were extracted in stages, separated, and purified. Preliminary spectral analyses of purified flavonoids were initiated, and some tentative compound identifications were made.

Fresh plant material from greenhouse cultures was extracted according to a modification of the method of Egger (1969). Small pieces of stems and leaves were extracted twice in 20 ml of acetone

(24 hours each) at room temperature. The extracts were pooled, filtered through Whatman GF-A glass filter discs, and treated with double their volume of petrol ether (b. p. 40-60 °C) in a separatory funnel. The lower, aqueous acetone phase which appeared as a reddish or yellow liquid, was withdrawn and taken to dryness using a rotary evaporator. The upper petrol ether phase containing fats, chlorophylls and other lipophilic coloring material was discarded. The resulting sticky, brown residue was dissolved in 10 ml of reagent grade methanol and taken to a small volume (about 3 ml) using a stream of warm air. The resulting dark-yellow liquid was spotted on Whatman 3 mm chromatographic paper. Plant fragments were saved after filtering through glass and were dried on filter paper for 24 hours. The resulting fragments were ground into fine pieces by hand and placed in 10 ml of methanol. Extraction was carried out over two 24 hour intervals, replacing the methanol after 24 hours with fresh methanol. Extracts were pooled and taken to a small volume using a stream of warm air. The resulting yellow liquid was also spotted on Whatman 3 mm chromatographic paper.

Fresh material from plant populations collected in the field was dried in paper bags over a stream of warm air, ground into fine pieces by hand, and pre-extracted with petrol ether (b. p. 40-60 °C). The general procedure followed was that of Crawford (1973). Up to six successive petrol ether extractions were made at 24 hour

intervals. The resulting green liquid fractions were discarded.[1] After thus removing fats, chlorophylls, and other lipophilic coloring material from the sample, enough reagent grade methanol was added to cover the tissue. After 24 hours, the methanol extract was replaced with fresh methanol and the sample was extracted for an additional 24 hours. Extracts were pooled and concentrated to a small volume using a stream of warm air. The resulting yellowish liquid was spotted on Whatman 3 mm chromatographic paper.

Procedure for Paper Chromatography

The procedure for paper chromatography of flavonoids was that described by Mabry et al. (1970) with modifications by Crawford (1973). Extracts were spotted in the lower right-hand corner of Whatman 3 mm chromatographic paper (46 x 57 cm sheets) until the spot was 3 cm in diameter and displayed intense purple fluorescence in longwave ultraviolet light. Using descending chromatography, chromatograms were first developed in the long direction using tertiary-butanol:glacial acetic acid:distilled water (3:1:1 v/v) henceforth called TBA, then turned 90° and developed in distilled water:glacial

[1] A minimum of 1.0 g of dried whole plant material was needed to produce one satisfactory chromatogram (about eight small plants). Approximately 300 corollas (0.15 g) produced a satisfactory chromatogram. Generally 4 g of dried plant material was used. From the resulting pooled extracts, six chromatograms were produced.

acetic acid (85:15 v/v) henceforth called HOAc. Concentrations of flavonoid were visualized by viewing chromatograms with long-wave uv light with and without treatment with ammonia fumes. All spots were circled and R_f values were recorded for each. Spots from 17 chromatograms tentatively determined to be the same compound (by R_f values and color reactions) were cut from chromatograms, pooled, placed in Erlenmyer flasks, and eluted in reagent grade methanol twice over a 48 hour period. Extraction of flavonoid material from the chromatographic paper was hastened by placing the flasks on a mechanical shaker for 24 hours. The two eluates were pooled and taken to dryness using a rotary evaporator. The resulting residue was redissolved in methanol and taken down to a small volume (3 ml). The sample was then streaked on Whatman 3 mm chromatographic paper and rechromatographed in s single direction using the TBA solvent system. Bands were cut from the chromatograms and individually severed into small pieces. Pieces of each band were eluted for 15 minutes in spectroscopic-grade methanol and the resulting eluates were spectrally analyzed.

To test the entire chromatographic procedure, a sample of chromatographically pure Rutin (Quercetin-3-O-rhamnoglucoside) was subjected to the procedure as described.

Procedure for Spectral Analysis

Spectral analysis conformed to established procedures (Mabry et al., 1970). Flavonoids to be analyzed were eluted in spectral methanol for 15 minutes and the resulting eluates were divided into three small glass sample vials. Using a Beckman DB-G Spectrophotometer, spectra were obtained from a scan between 240 nm and 440 nm. A total of six spectra were produced for each flavonoid sample: (1) a spectrum of the flavonoid in methanol was first obtained, (2) aluminum chloride (5% in spectral methanol) was then added to the cuvette. After the addition of 5 drops the spectrum was determined, (3) 2 drops of 4N HCl were then added to the cuvette from part 2. After the spectrum was run, the solution was discarded. (4) 4 drops of sodium methoxide (2.5 g sodium metal added to 100 ml of spectral methanol) was then added to the cuvette containing the second portion of the flavonoid solution and the spectrum was immediately determined. The solution in the cuvette was then discarded. (5) To the third portion of the flavonoid, anhydrous powdered sodium acetate was added until a saturated solution was obtained, then the spectrum was determined. (6) To the cuvette containing the sodium acetate saturated flavonoid solution, powdered boric acid (H_3BO_3) was added (to neutralize the sodium acetate) and the sixth spectrum for the flavonoid was determined. From the set of six spectra, R_f values, and color

reactions from respective chromatograms, the basic structures of two compounds were determined. The identification of the sugar moieties of these compounds is planned as part of a further study.

IV. RESULTS

Cytological Studies

Meiotic preparations of individuals collected from the Columbia River Gorge revealed that polyploidy in Claytonia perfoliata was more complex than had been originally suspected. Prior to this study, reports in the literature, with one exception, gave the hexaploid level as the highest chromosome number in the species.[2] As shown in Table 1, the populations examined form a complete euploid series from diploid to decaploid. Meiosis in individuals from the 47 study populations was normal, and no clear evidence of aneuploidy was found. Bivalents vary in length, overall shape, and number of chiasmata. Figures 1a through 5c illustrate metaphase I for representative diploid, tetraploid, hexaploid, octoploid, and decaploid

[2] An octoploid Claytonia perfoliata has been reported (Nilsson, 1967) but its morphology is not known. According to Orjan Nilsson (personal communication) the plants were grown from seed supplied him by Dr. Patricia Holmgren, and he does not have vouchers or accession numbers to accompany the octoploid count. Dr. Holmgren (personal communication) recalls sending the seeds to Nilsson but does not remember where the seeds were collected. Nilsson (1967) said that the plants were from Seattle, Washington but this was "merely because the seeds were mailed from there," according to Dr. Holmgren. She thought that the seeds originated from one of Dr. C. L. Hitchcock's collections made during 1965 or 1966. However, Dr. Melinda Denton (personal communication) assures me that no specimens of Claytonia perfoliata were collected by Holmgren or Hitchcock during those years. According to Dr. Denton, Dr. Hitchcock does not recall the origin of Orjan Nilsson's octoploid seed.

Table 1. Populations of Claytonia perfoliata sens. lat. analyzed cytologically.

Chromosome number	Collection locale	Accession
2n=12 (as 6 pairs)	(WA) Skamania Co: Underwood; 1/2 mi. w. of the Klickitat Co. line on Hwy. 14.	325-S_1
2n=12 "	(WA) Skamania Co: (as #325-S_1).	Chambers 3385-S_1
2n=12 "	(OR) Deschutes Co: Indian Ford Campground; Hwy. 20, Deschutes National Forest.	335
2n=12 "	(OR) Deschutes Co: (as #335)	336
2n=12 "	(WA) Skamania Co: Underwood; Christ-Zada Cemetery, w. side of the White Salmon River.	413
2n=12 "	(OR) Hood River Co: Hood River Valley-Booth Hill; near a private airstrip.	420
2n=24 (as 12 pairs)	(CA) Humboldt Co: Clam Beach Co. Park.	364-S_1
2n=24 "	(OR) Sherman Co: Mouth of the Deschutes State Park.	383
2n=36 (as 18 pairs)	(OR) Benton Co: Corvallis.	293
2n=36 "	(WA) Clark Co: Ellsworth.	295

Table 1. Continued.

Chromosome number	Collection locale	Accession
2n=36 (as 18 pairs)	(WA) Clark Co: Washougal; 2 mi. e. on Hwy. 14.	298
2n=36 "	(WA) Skamania Co: Beacon Rock State Park.	301
2n=36 "	(WA) Klickitat Co: White Salmon; 1.8 mi. n. of Hwy. 14 on the lower White Salmon R. rd.	302
2n=36 "	(WA) Klickitat Co: (as #302)	304
2n=36 "	(WA) Klickitat Co: (as #302)	305
2n=36 "	(OR) Hood River Co: Viento State Park.	308
2n=36 "	(OR) Multnomah Co: Multnomah Falls-Dalton Point Boat Landing.	309
2n=36 "	(OR) Wasco Co: Rowena Crest Viewpoint; Mayer State Park.	315
2n=36 "	(WA) Klickitat Co: The Dalles Dam; 1/4 mi. s. of junct. of Hwy. 14 and U.S. Hwy. 197.	317
2n=36 "	(WA) Klickitat Co: Horsethief Lake State Park; junct. between park service rd. and Hwy. 14.	318

Table 1. Continued.

Chromosome number	Collection locale	Accession
2n=36 (as 18 pairs)	(WA) Skamania Co: Underwood; 1/2 mi. w. of the Klickitat Co. line on Hwy. 14.	322
2n=36 "	(WA) Skamania Co: (as #322)	324
2n=36 "	(OR) Benton Co: McDonald State Forest.	334
2n=36 "	(OR) Wasco Co: The Dalles; 7 mi. e. on Hwy. I-80N.	Chambers 3391-S_1
2n=36 "	(WA) Skamania Co: Underwood; east end of town.	Chambers 3387-S_1
2n=36 "	(OR) Sherman Co: Mouth of the Deschutes State Park.	382
2n=36 "	(OR) Sherman Co: (as #382)	384
2n=36 "	(OR) Sherman Co: Fulton Canyon-Hwy. 206; 2 mi. e. of the Deschutes R. bridge.	385
2n=36 "	(OR) Gilliam Co: J. S. Burres State Park, Hwy. 206 on the e. bank of the John Day River.	387
2n=36 "	(OR) Gilliam Co: Arlington; 2.4 mi. s. on Hwy. 19.	389

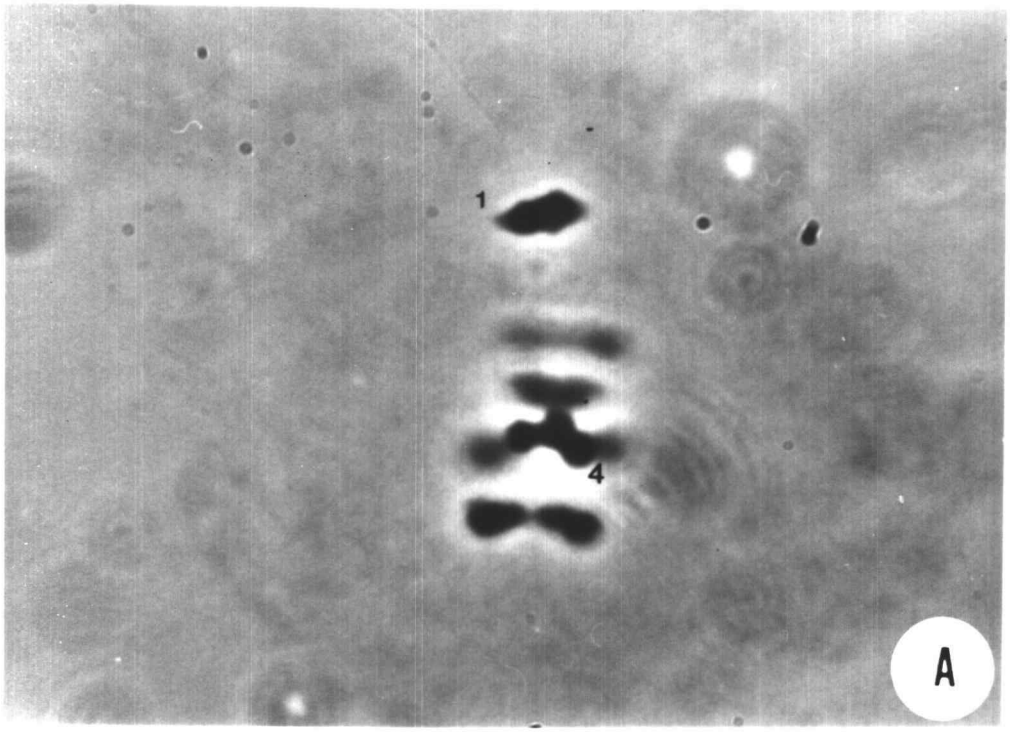
Table 1. Continued.

Chromosome number	Collection locale	Accession
2n=36 (as 18 pairs)	(OR) Morrow Co: Boardman; 4 mi. w. on Kunze Rd.	390
2n=36 "	(OR) Benton Co: Corvallis.	415A
2n=36 "	(WA) Klickitat Co: Lyle; 0.8 mi. e. on Hwy. 14.	407
2n=48 (as 24 pairs)	(OR) Hood River Co: Viento State Park.	306
2n=48 "	(OR) Hood River Co: (as #306)	307
2n=48 "	(OR) Wasco Co: Mosier; e. end of town.	313
2n=48 "	(OR) Wasco Co: Mema Triangulation Station; Rowena Crest.	401
2n=48 "	(OR) Wasco Co: (as #401)	402
2n=48 "	(CA) Humboldt Co: French Camp Ridge-Bald Hills Rd. 18.6 mi. se. of the Lady Bird Johnson Grove parking lot on BHR.	361
2n=48 "	(CA) Humboldt Co: Horse Mountain-Titlow Hill Rd., junct. of roads 5N10 and 6N01.	367

Table 1. Continued.

Chromosome number	Collection locale	Accession
2n=48 (as 24 pairs)	(CA) Humboldt Co: Horse Mountain-Titlow Hill Rd., 2.6 mi. se. of U. S. Hwy. 299.	369
2n=60 (as 30 pairs)	(WA) Klickitat Co: White Salmon; 1.8 mi. n. of Hwy. 14 on the lower White Salmon R. rd.	303
2n=60 "	(OR) Wasco Co: Mosier.	310
2n=60 "	(OR) Wasco Co: (as #310)	312
2n=60 "	(WA) Klickitat Co: Wishram; 3.6 mi. e. of junct. of Hwy. 14 and U. S. Hwy. 197.	329
2n=60 "	(OR) Wheeler Co: Clarno-Fossil Highway; Pine Creek (Peacock Canyon).	345
2n=60 "	(OR) Wasco Co: Mosier; 1 mi. e. on U. S. Hwy. 30. Common in orchards.	396

Figure 1a. Metaphase I of a dividing microspore-mother cell of diploid Claytonia rubra (Howell) Tidestr. (2n=12, as 6 numbered pairs) Miller #336-Indian Ford Campground/ Santiam Highway. Two focal planes are pictured X2000.


1
4
A

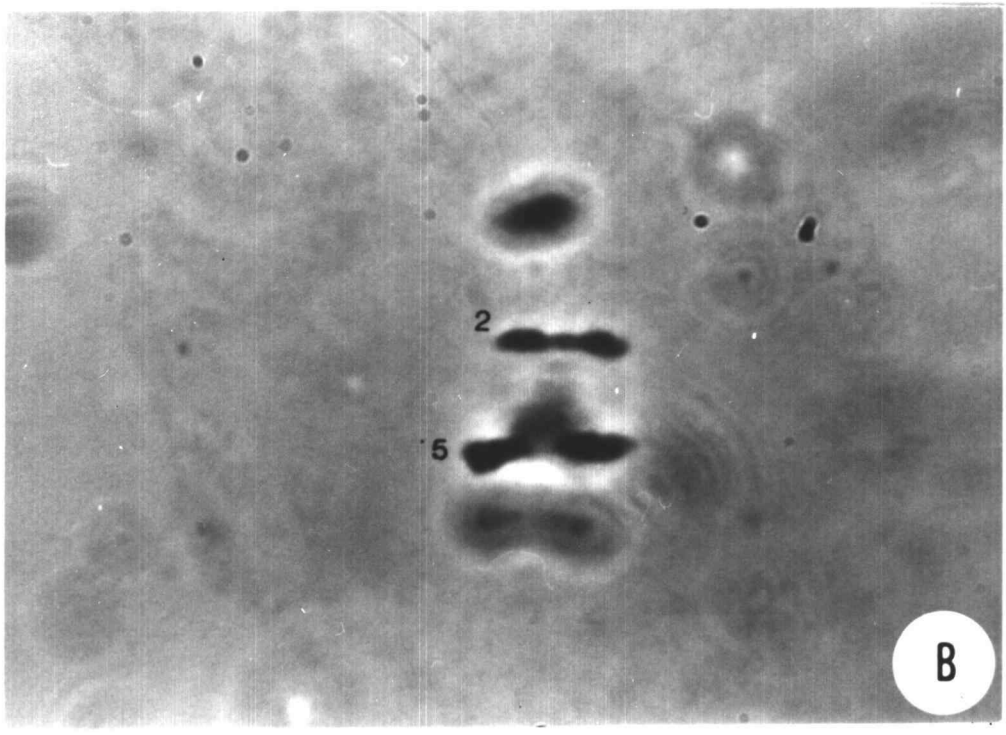

2
5
B

Figure 1b. Figure 1 (continued); a third focal plane (C) is pictured X2000.

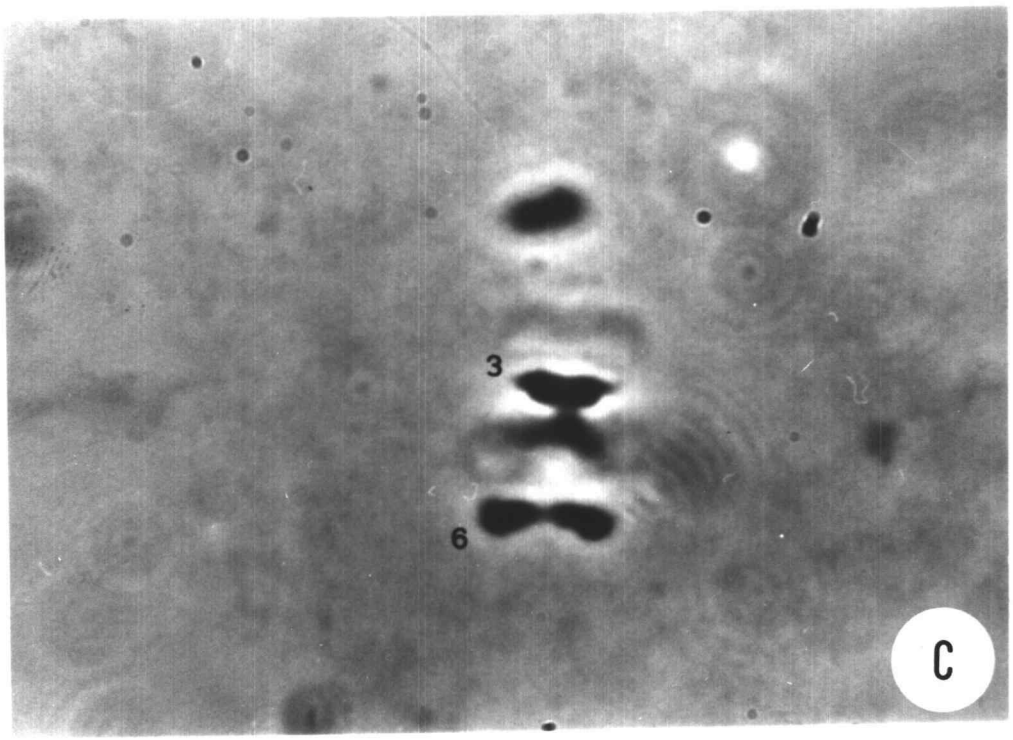

3
6
C

Figure 2b. Figure 2 (continued); a third focal plane (C) is pictured X2000.

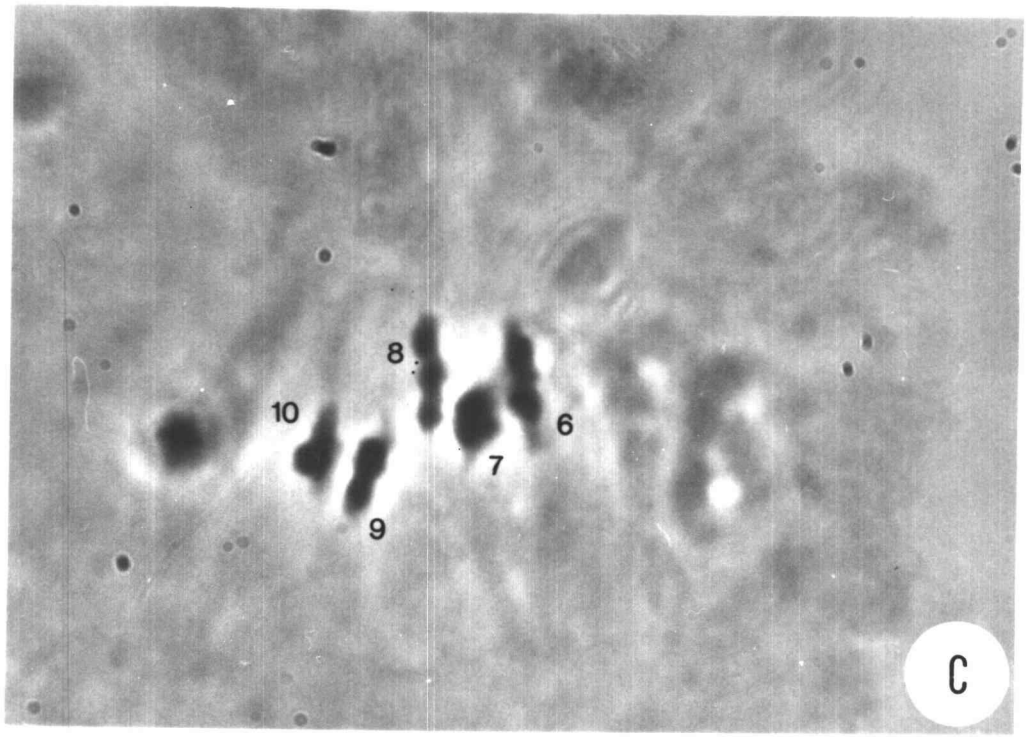
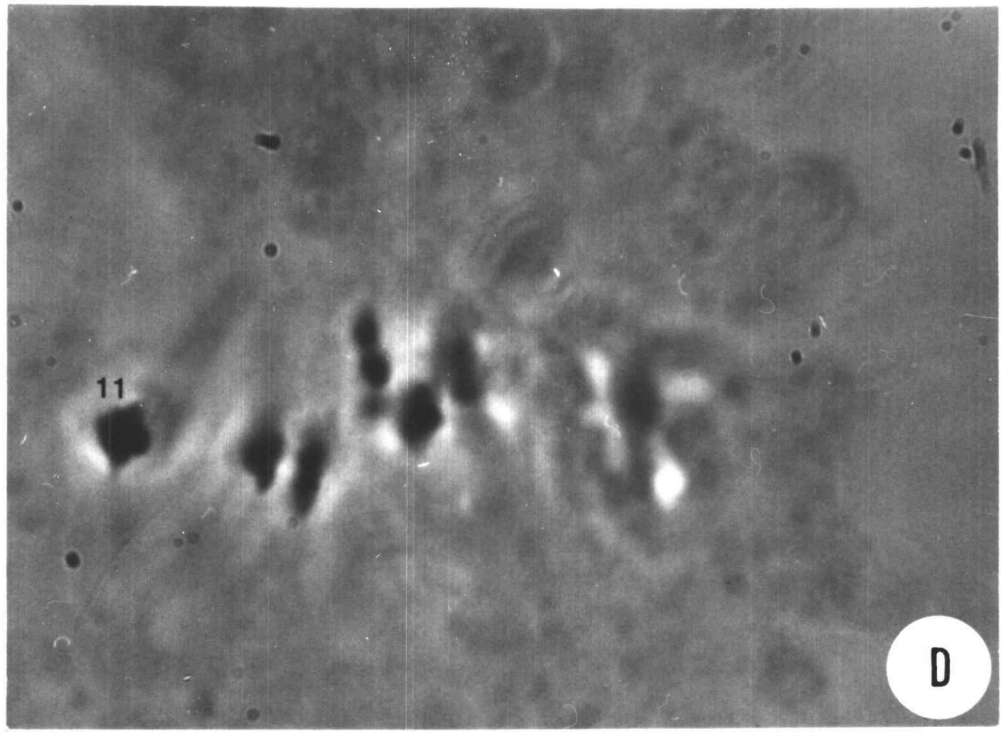

8
10
6
7
9
C

Figure 2c. Figure 2 (continued); the fourth and fifth focal planes (D and E) are pictured X2000.


11
D

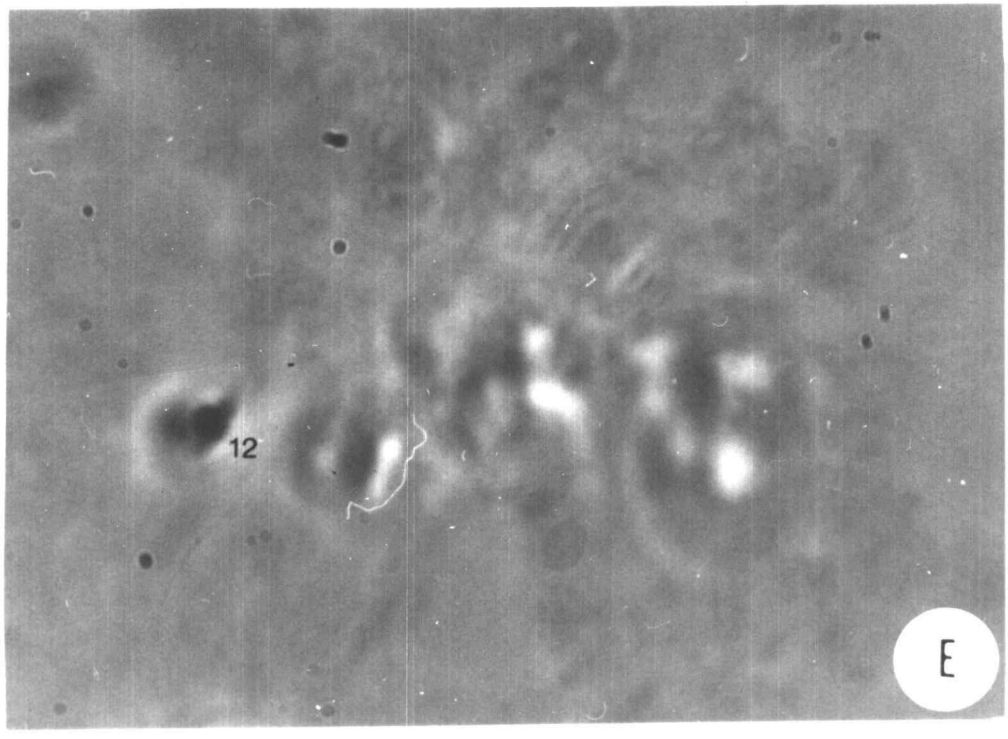
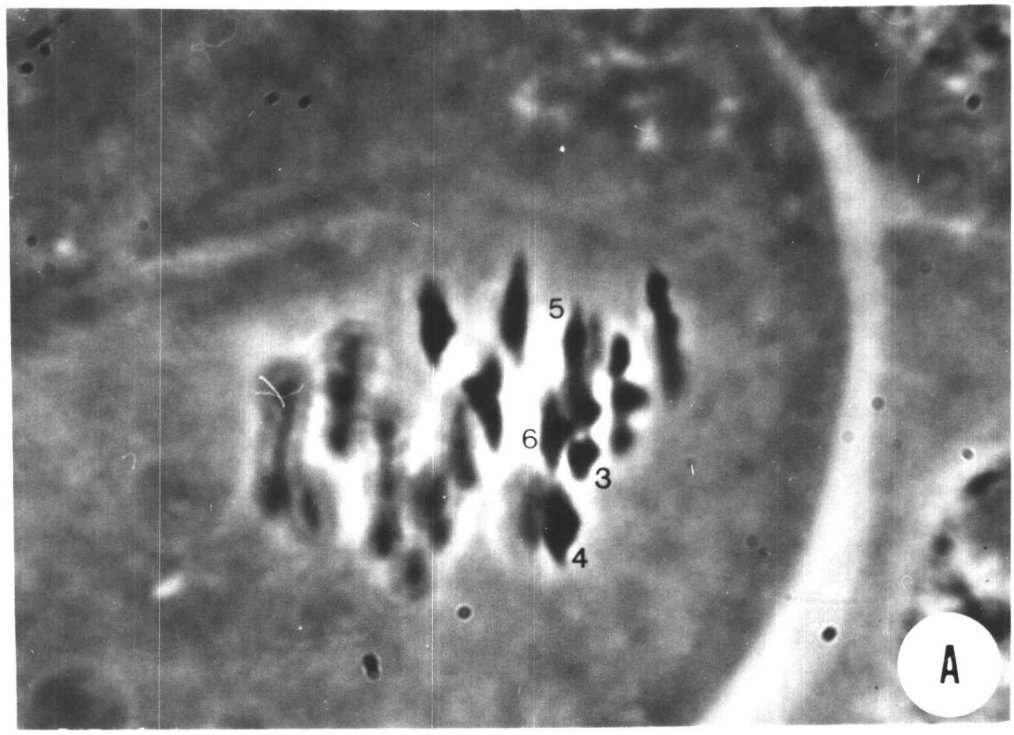

12
E

Figure 3a. Metaphase I of a dividing microspore-mother cell of hexaploid Claytonia perfoliata Donn (2n=36, as 18 numbered pairs) Miller #389-Arlington. Two focal planes (A and B) are pictured X2000.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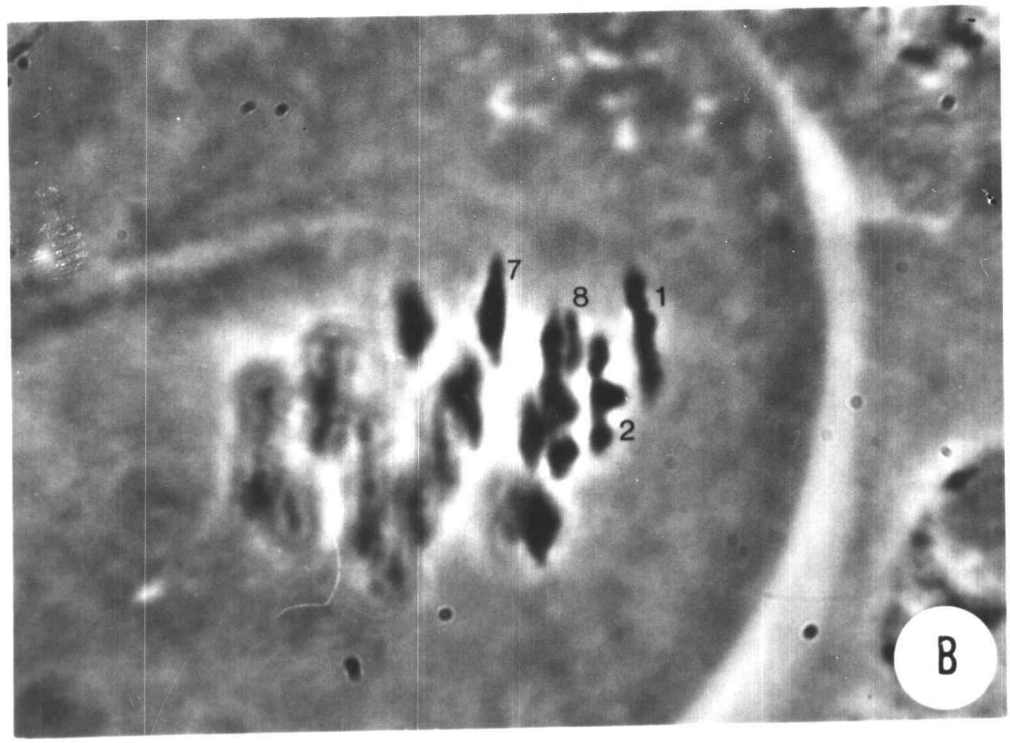
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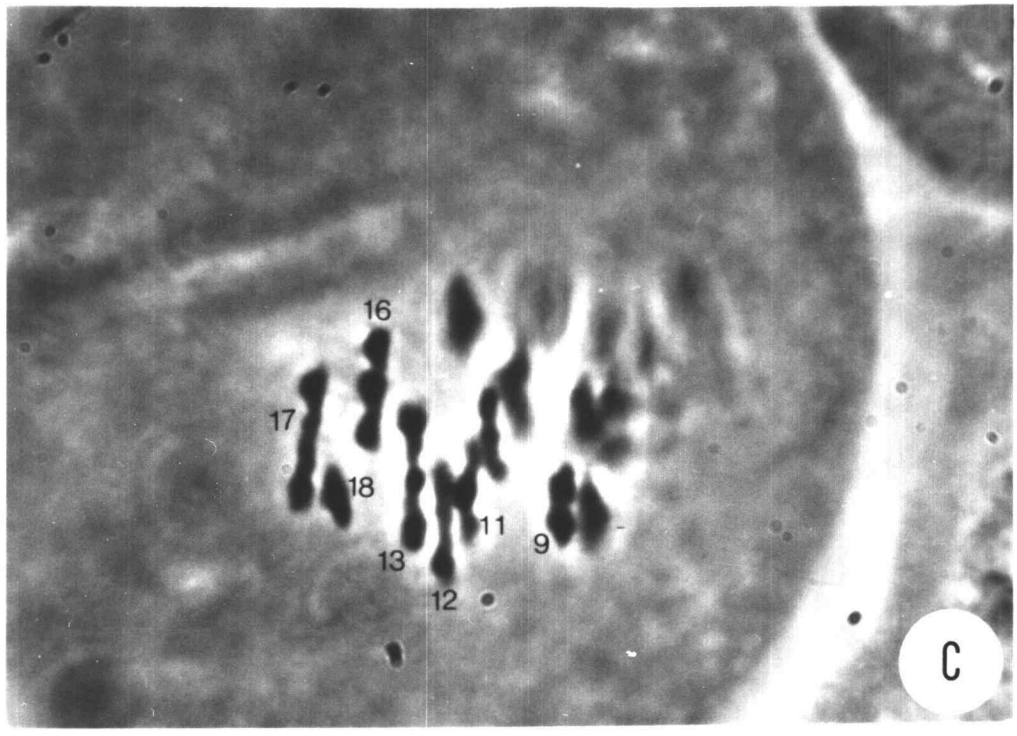
Figure 3b. Figure 3 (continued); the third and fourth focal planes (C and D) are pictured X2000.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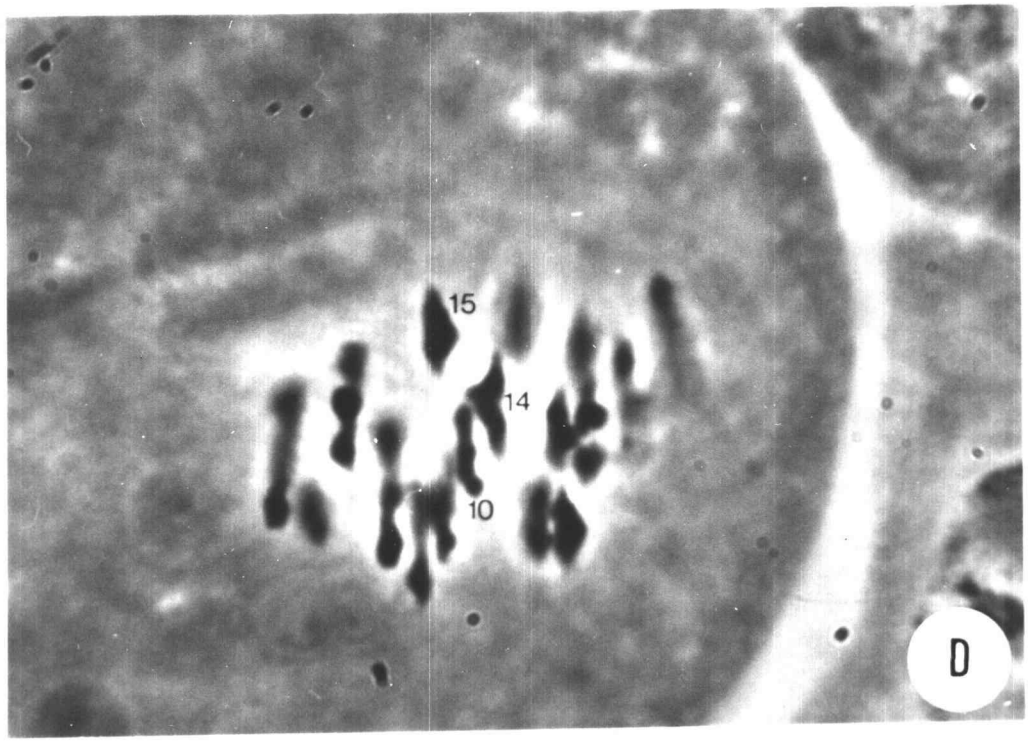
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Figure 3c. Figure 3 (continued); a fifth focal plane (E) is pictured X2000.

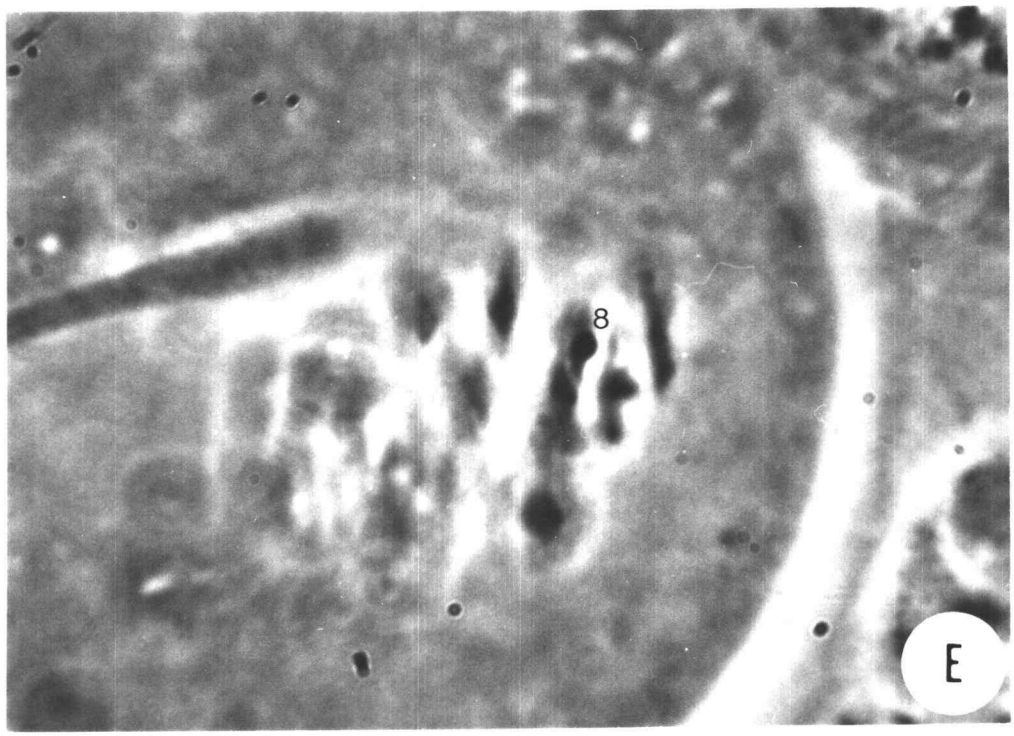
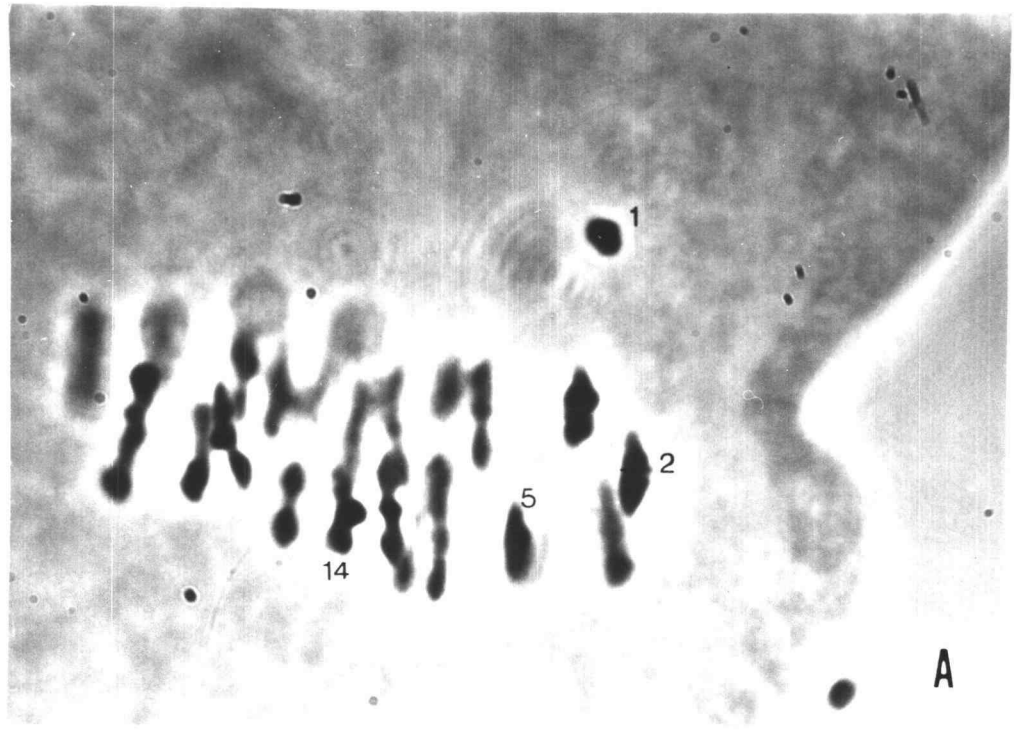

8
E

Figure 4a. Metaphase I of a dividing microspore mother cell of octoploid Claytonia perfoliata Donn (2n=48, as 24 numbered pairs) Miller #402-Mema/Rowena Crest. Two focal planes (A and B) are pictured X2000.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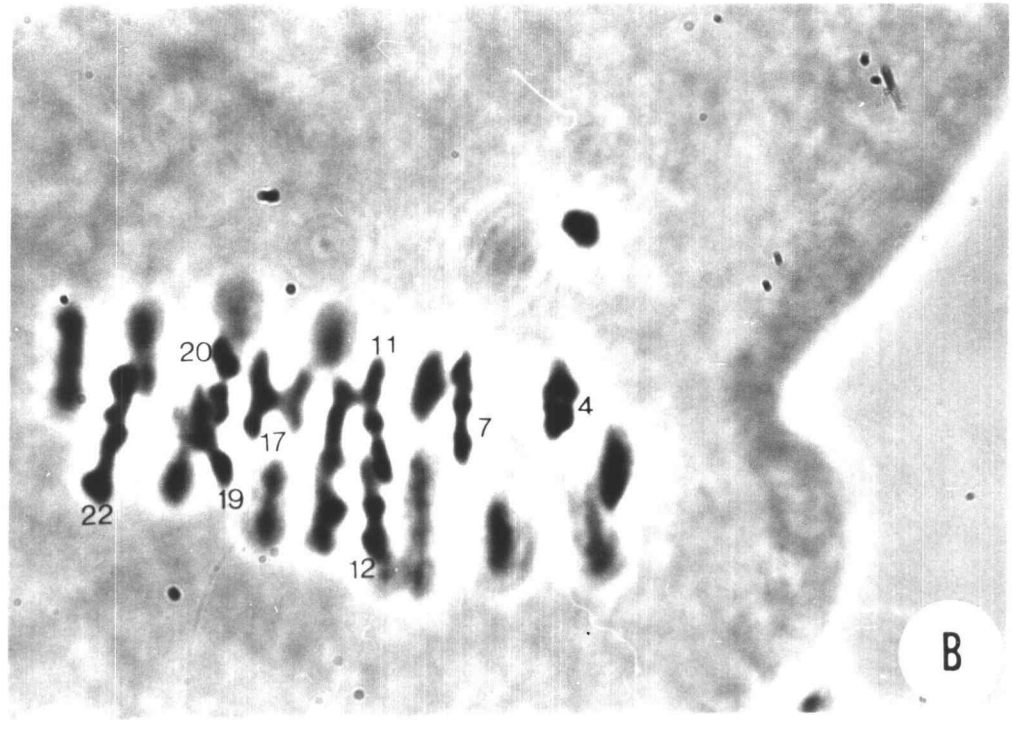
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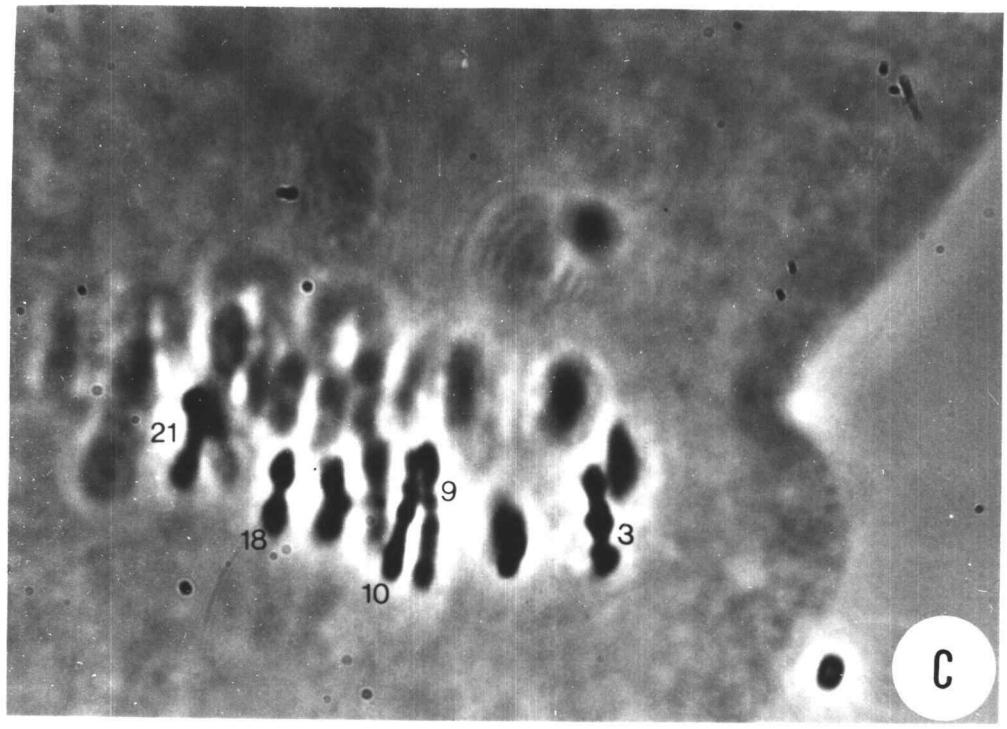
Figure 4b. Figure 4 (continued); the third and fourth focal planes (C and D) are pictured X2000.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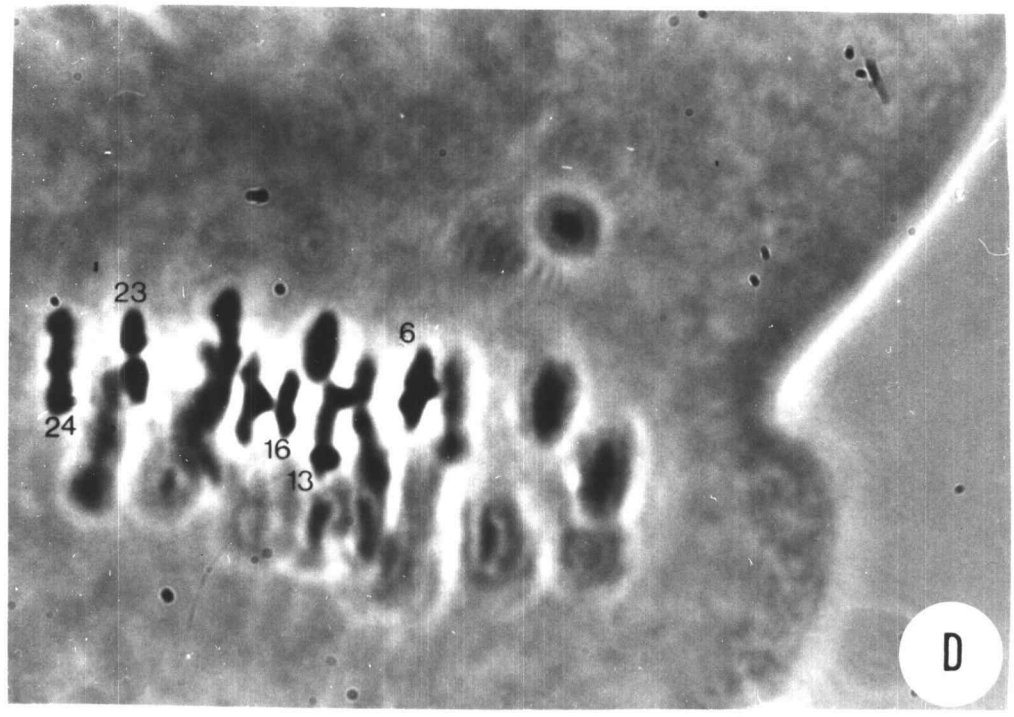
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Figure 4c. Figure 4 (continued); a fifth focal plane (E) is pictured X2000.

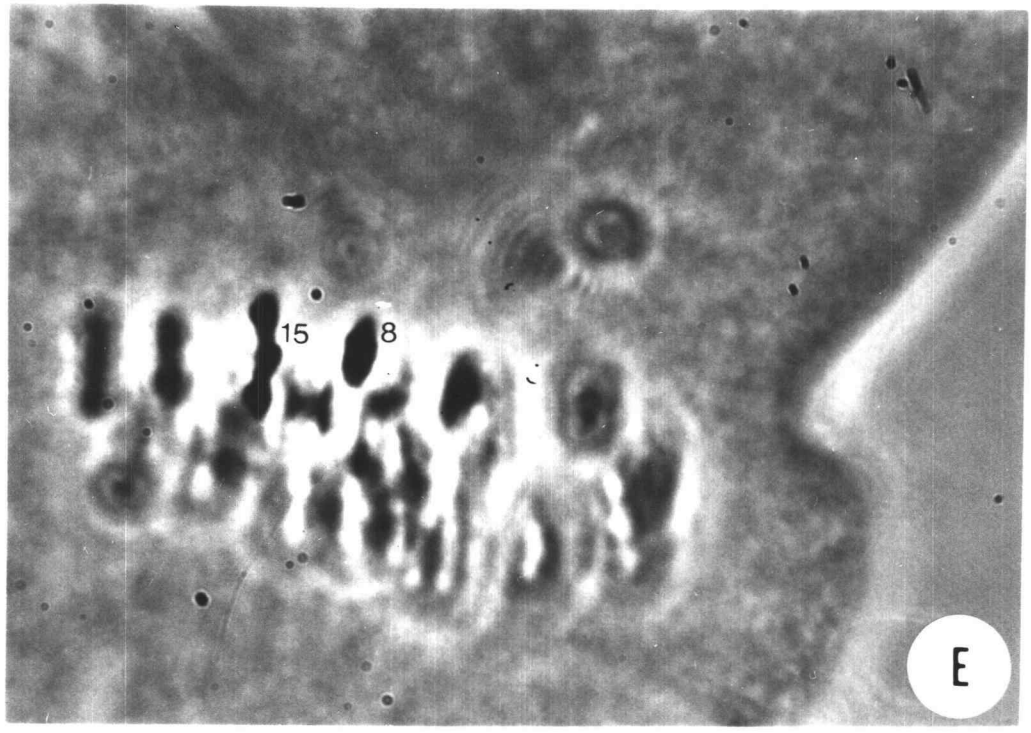
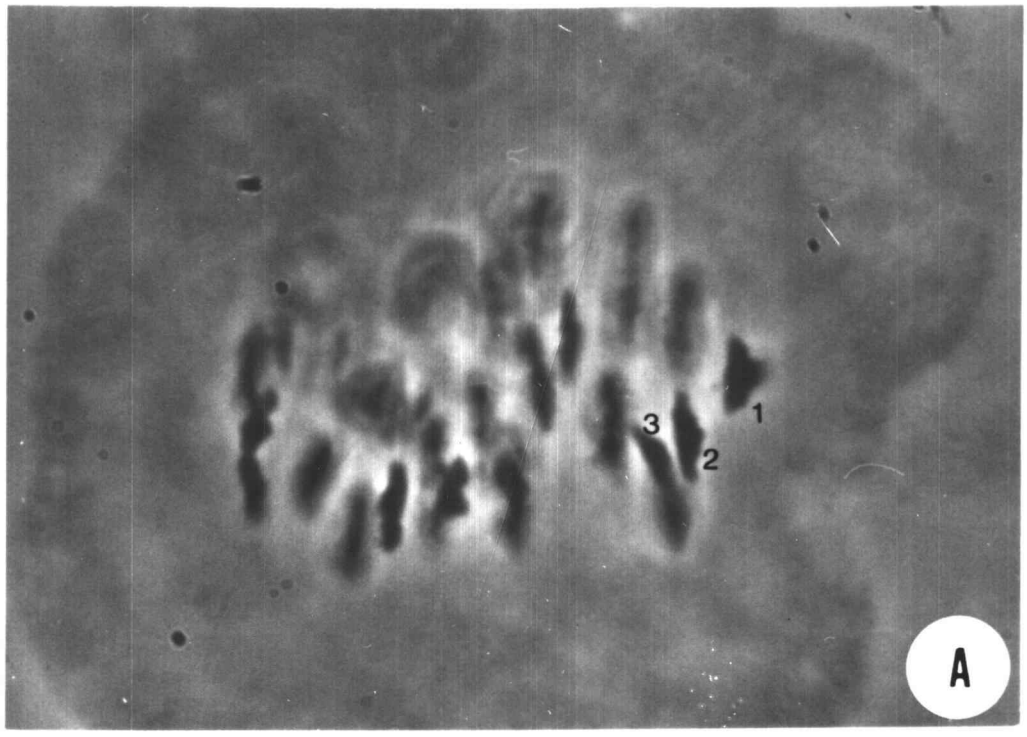

15
8
E

Figure 5a. Metaphase I of a dividing microspore mother cell of decaploid Claytonia perfoliata Donn (2n=60, as 30 numbered pairs) Miller #396-Mosier orchard. Two focal planes (A and B) are pictured X2000.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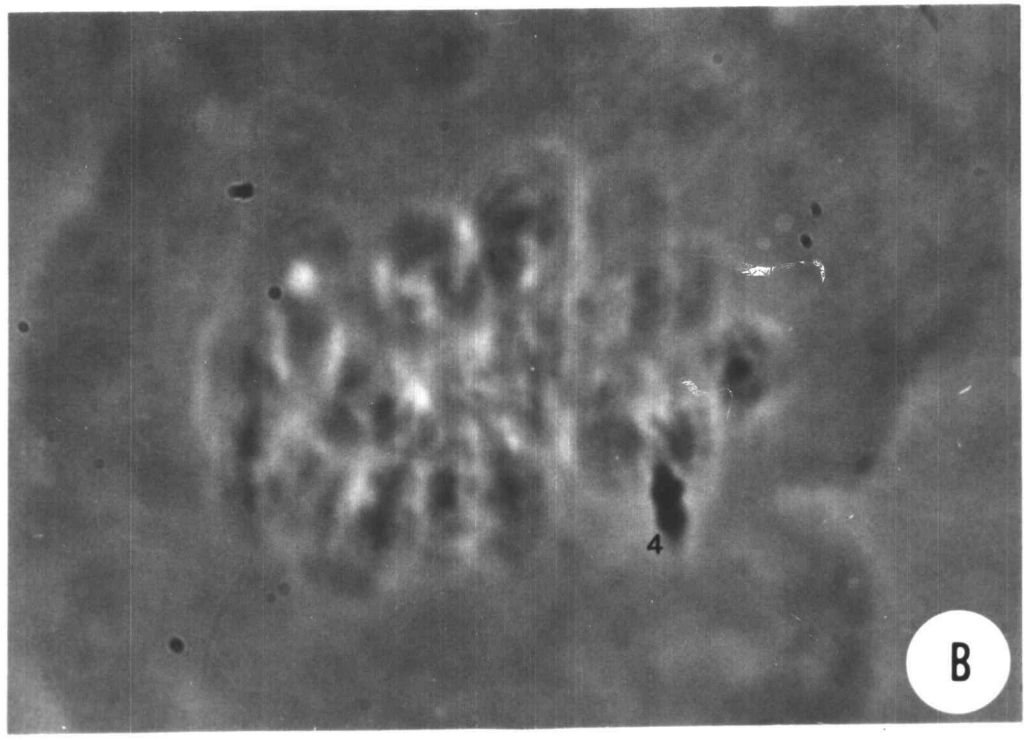
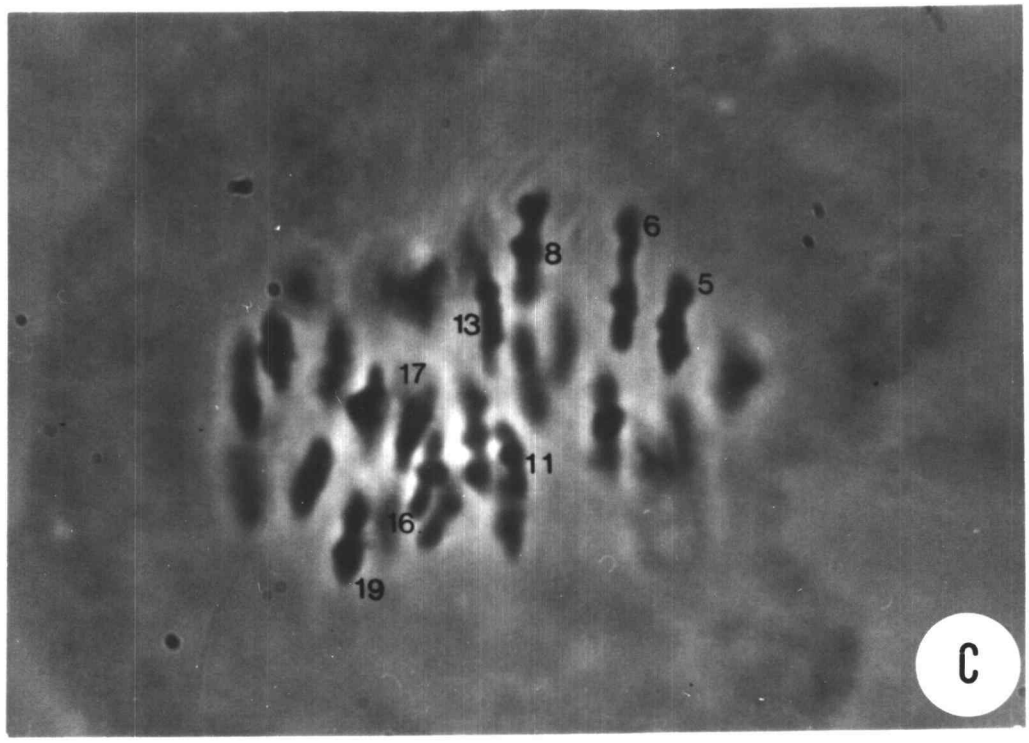

4
B

Figure 5b. Figure 5 (continued); the third and fourth focal planes (C and D) are pictured X2000.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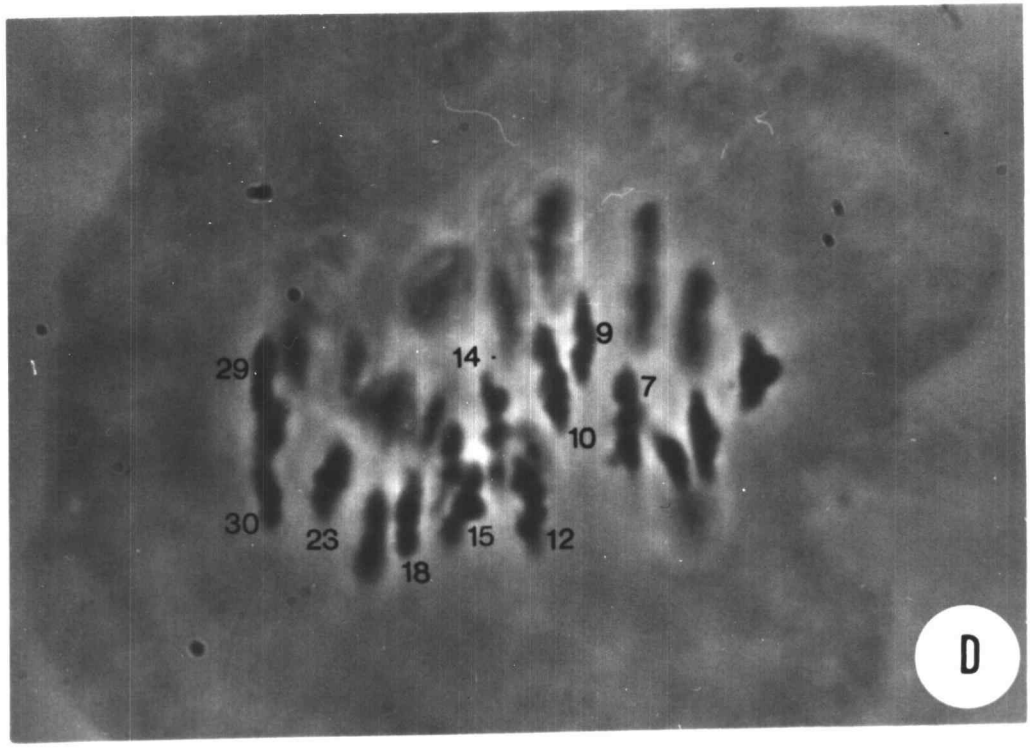
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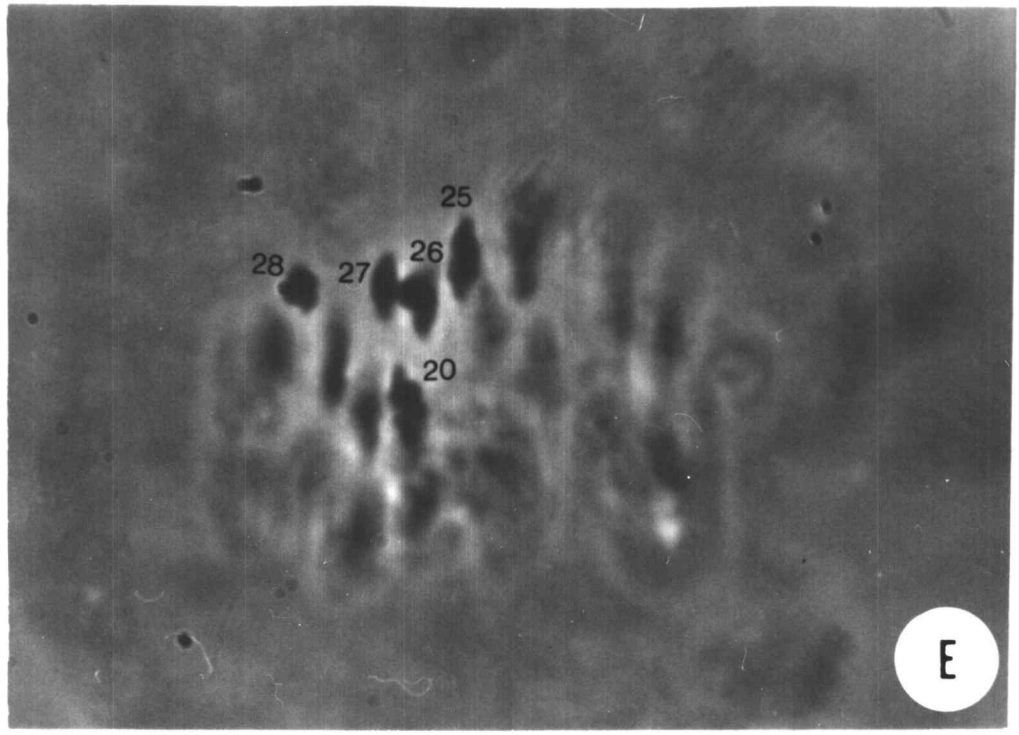
D

Figure 5c. Figure 5 (continued); the fifth and sixth focal planes (E and F) are pictured X2000.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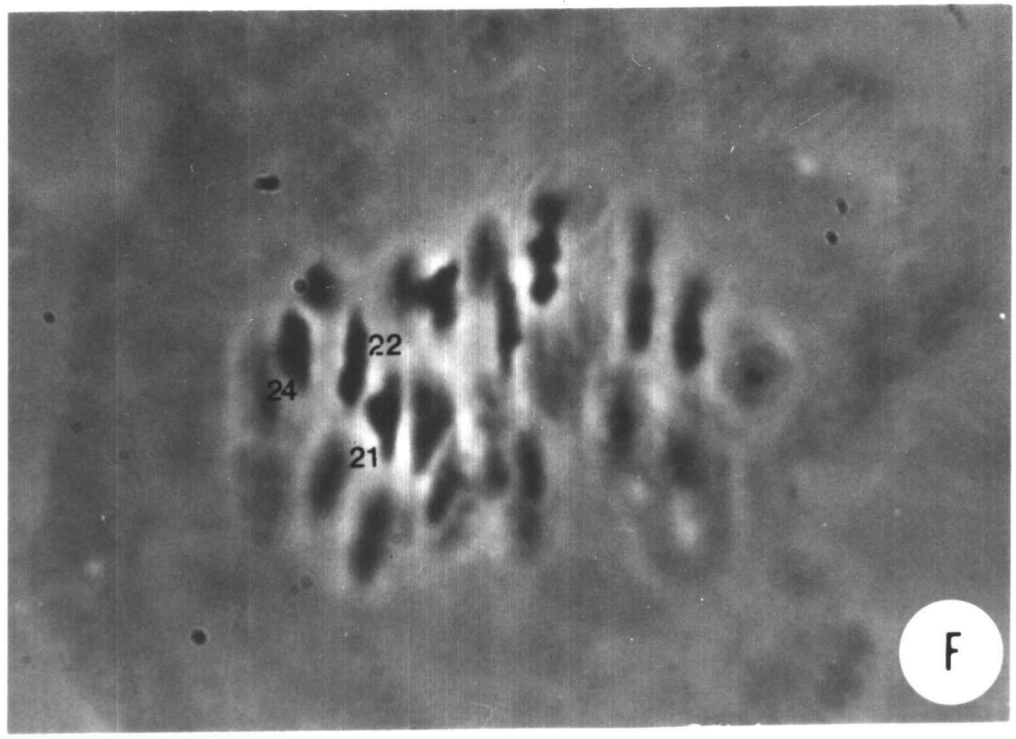
E


22
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F

Table 2. Seed set resulting from self-pollinations and artificial hybridizations between hexaploids of Claytonia perfoliata Donn.

Seed parent[1]	Pollen parent	Capsules bagged	Seeds set	Seeds[2] inviable	Ovules aborted
318-1.4	⊗	12	30	0	6
318-1.4	301-1.1	2	(failure due to mechanical damage)		
318-1.4	305-1.2	1	2	0	1
301-1.1	KLC3391-1.2	12	23	0	13
301-1.1	318-1.4	5	15	0	0
301-1.1	KLC3387-1.3	3	8	0	1
301-1.1	⊗	18	53	0	1
305-1.2	318-1.4	5	15	0	0
305-1.5	KLC3387-1.4	5	15	0	0
KLC3387-1.3	⊗	18	54	0	0
KLC3387-1.3	301-1.1	1	1	2	0
KLC3391-1.2	305-1.2	3	6	0	3

[1] #318 refers to the original source of the plant (accession data are in Table 1). "-1." refers to the S_1 generation. ".4" refers to the individual used in the hybridization.

[2] Seeds classified as inviable were not black and shining, but instead were usually purplish, reddish, or wrinkled.

microspore-mother cells. All the pollen grains that were examined were tricolpate, which conforms to earlier reports (Nilsson, 1967, Swanson, 1966).

The results, in terms of seed-set, of self-pollinations and artificial hydridizations attempted between selected hexaploids are recorded in Table 2. The expected number of seeds from each pollination is three. Nearly all self- and cross-pollinations gave a high percentage of normal seeds. Future research will be directed at fertility and meiotic behavior of hybrids from these and other crosses.

Reproductive Biology

Claytonia perfoliata is prevailingly autogamous, as judged from previous reports (Swanson, 1964) and my own observations in both the field and the greenhouse. With the exception of being dwarfed, each S_1 generation grown under uniform environmental conditions in the greenhouse was indistinguishable morphologically from its respective parent. This similarity of the S_1 to its parent is well expressed in the shape of the basal leaves (Figure 9c and Figure 10a, 10c, and 10e). Natural populations representing siblings of the individuals collected during the spring of 1974 were revisited in 1975. In all cases, the plants were morphologically the same as those sampled the preceding year.

The mechanism for selfing is evident in the flower structure of the species. Certain tetraploids and hexaploids have a corolla that is exceeded by the calyx. Autogamy is likely to be obligate in these plants for two reasons: (1) the petals can never fully open, and the stamens therefore cannot fully reflex from the stigma before anthesis, and, (2) plants having these minute corollas generally flower and set seed in middle or late March, before potential insect pollinators are active. In contrast to the petal morphology of these putatively obligate selfers, the flowers of various other entities from the hexaploid, octoploid, and decaploid levels often have conspicuous petals which are two or three times the length of the calyx (e.g. accession numbers 293, 295, 301, 302, 303, 304, 305, 310, 396, 361, 367, 369, 415a). As described below, these flowers appear to be facultatively autogamous but are capable of being outcrossed by insect visitors as well.

Observations of insect pollinators were carried out at a hexaploid population of C. perfoliata near Lyle (407) and a second population containing sympatric hexaploids and decaploids (White Salmon 302, 303, 304, 305). At White Salmon, both the pink-flowered hexaploids and white-flowered decaploids were seen to be visited by various Diptera and Hymenoptera, especially bees and bumblebees. Swanson (1964) reported similar observations both on populations in the field and in his outdoor growth chambers at Berkeley, California.

Although the decaploids at White Salmon were inodorous the pink-flowered hexaploid plants (305) produced noticeable floral fragrance detectable at least one meter from the source. The fragrance of certain hexaploid flowers was not confined to field populations, in fact. Both the pink-flowered hexaploid individuals (305-S_1) and white-flowered hexaploid individuals (318-S_1) in greenhouse culture produced a noticeable odor.

The most interesting insect activity was observed at Lyle. This population of hexaploids (407) was growing mixed with Hydrophyllum capitatum Dougl. var. thompsonii (Peck) Const., which on my first visit had only immature inflorescences. Leptocornis rubrolineatus Barber (Hemiptera), together with numerous Diptera and Hymenoptera including bees and bumblebees, was observed to visit the flowers of the hexaploid C. perfoliata. The bees and bumblebees visited the flowers only occasionally, while the Diptera and Leptocornis remained on or in close proximity to the C. perfoliata plants. It appeared that the Leptocornis (Western Boxelder Bug) was sucking fluids from the inside of the Claytonia flowers. It would achieve this by crawling on top of a flower and inserting its proboscis into the corolla. Upon withdrawing its proboscis, with several pollen grains attached, the insect would proceed to another flower and resume the same activity. Ten days later, when I revisited the Lyle hexaploid population, the Hydrophyllum was in full bloom. Various Hymenoptera were seen on

flowers of this species, but they were not routinely visiting the Claytonia as before. The Leptocornis and various Diptera were observed on plants of both the Hydrophyllum and Claytonia.

Some mention should be made of seed dispersal and seed strategy of the Claytonia perfoliata populations studied. The forceful release of Claytonia seed from the capsule has been known for many years (Willis, 1892). In light of the distribution of ruderal C. perfoliata along roads in the study area, it seems likely that its seeds are further transported by moving automobiles, in clumps of mud and tire treads--a mode of long-range dispersal already suggested by Swanson (1964).

Variation in seed size was conspicuous between diploids, hexaploids, and decaploids. The smallest seeds (Figure 6b and 6c) originated from two populations that I suspect are obligately autogamous: Claytonia rubra (Underwood #325) and a hexaploid Claytonia perfoliata whose corollas are exceeded by the calyx (Underwood KLC #3387). Decaploids produced the largest seeds, with an average weight of 2.0 mg (Figure 6d and 7d)--slightly heavier than the largest hexaploid seeds reported by Swanson (1964). Correlations between seed size and other morphological features are presented in the following section.

Under uniform conditions in the greenhouse seeds germinated sporadically over a period of several days, whether they were

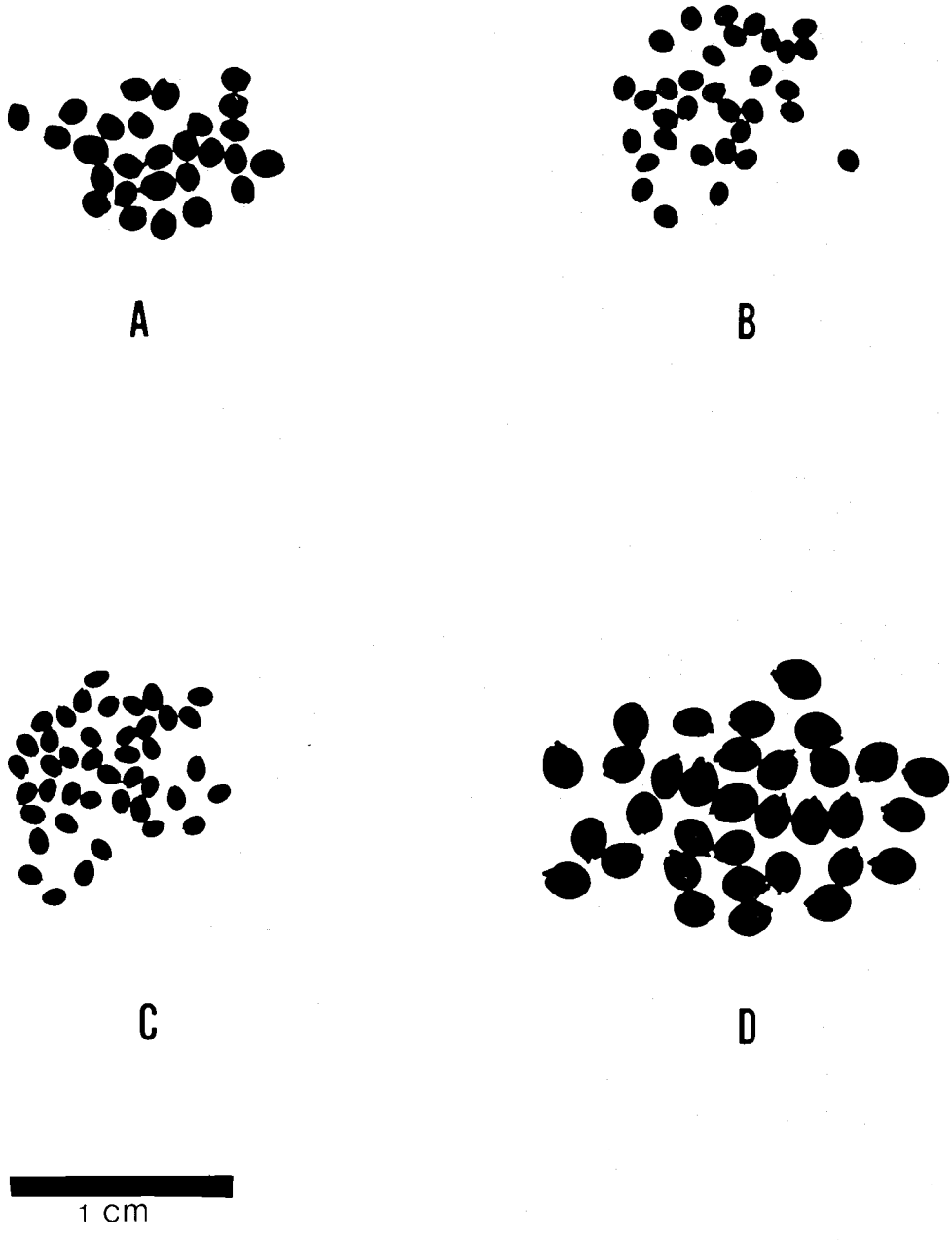


Figure 6. Variation in seed size between a diploid (C-Underwood #325), two hexaploids (A-Corvallis #293, B-Underwood KLC #3387) and a decaploid (D-White Salmon #303).

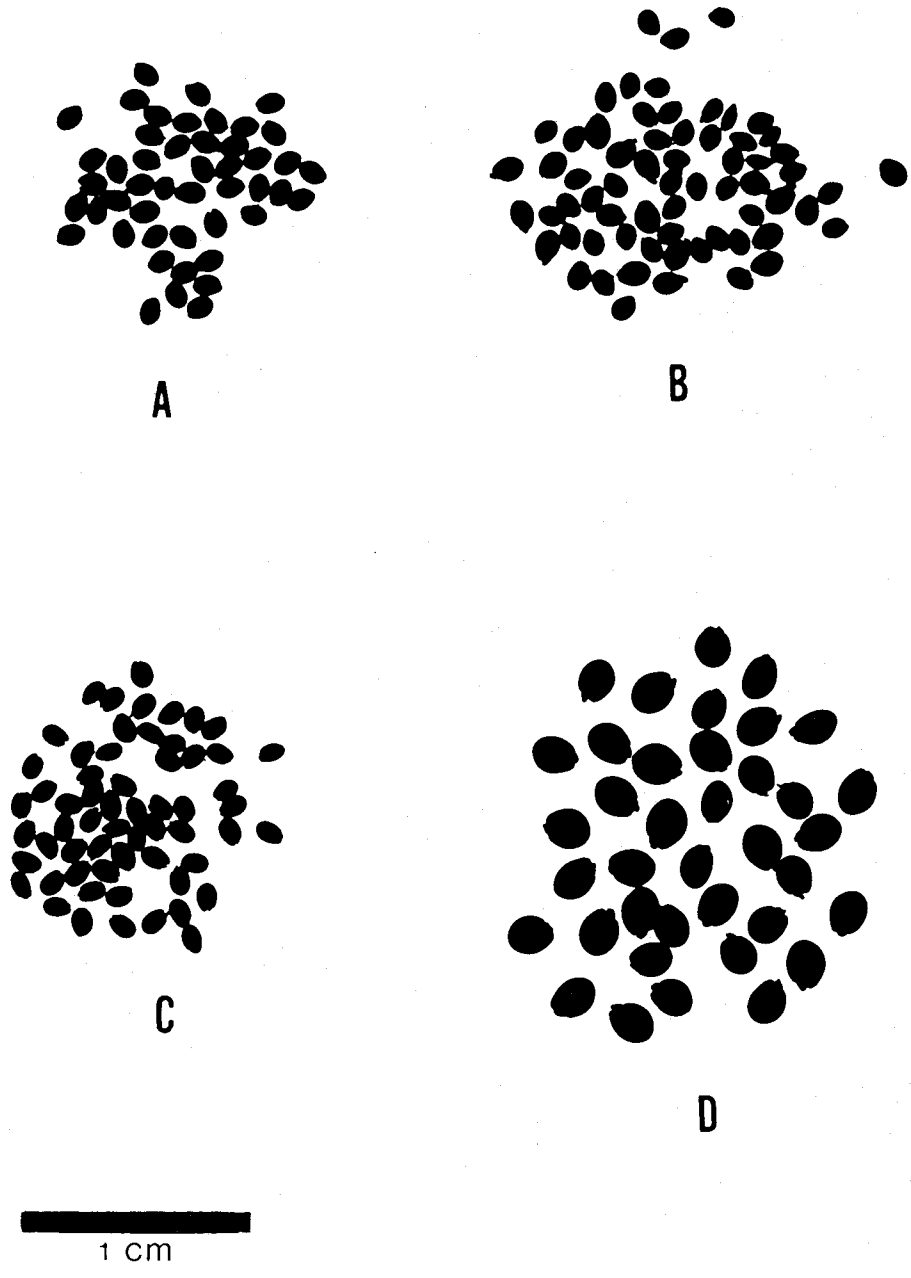


Figure 7. Variation in seed size between three hexaploids (A-Ellsworth #295, B-White Salmon #305, C-Beacon Rock #301) and a decaploid (D-Mosier #310).

germinated on filter paper or in peat moss cups. The possible strategy of this sporadic germination is discussed in a later section; it is a phenomenon that deserves further investigation.

Morphological Variation and Plant Development

Claytonia perfoliata in the Columbia Gorge and lower Columbia Basin consists of a limited number of recognizable entities, which mostly can be distinguished from one another on the basis of simple morphological characters. Chromosome numbers in a population can usually be predicted from features such as basal leaf shape, flower size, and petal color. Only two entities, a hexaploid and tetraploid, have characters so similar that taxonomic distinction is difficult. However, on the basis of habitat type, even these two entities can be told apart. A detailed discussion of habitats follows in the next section.

The morphological characters which correlate with chromosome number in the 15 entities studied are (1) corolla color--pink versus white, (2) corolla length--1.5-6.5 mm, and (3) mature basal leaf shape--linear, narrowly rhombic, broadly rhombic, deltoid, depressed deltoid, ovate, and spatulate (with a circular blade). The range of variation of mature basal leaves is illustrated in Figure 8. Other morphological characters including calyx color (when plants are in fruit) and calyx length, are sometimes useful. Corolla color, corolla

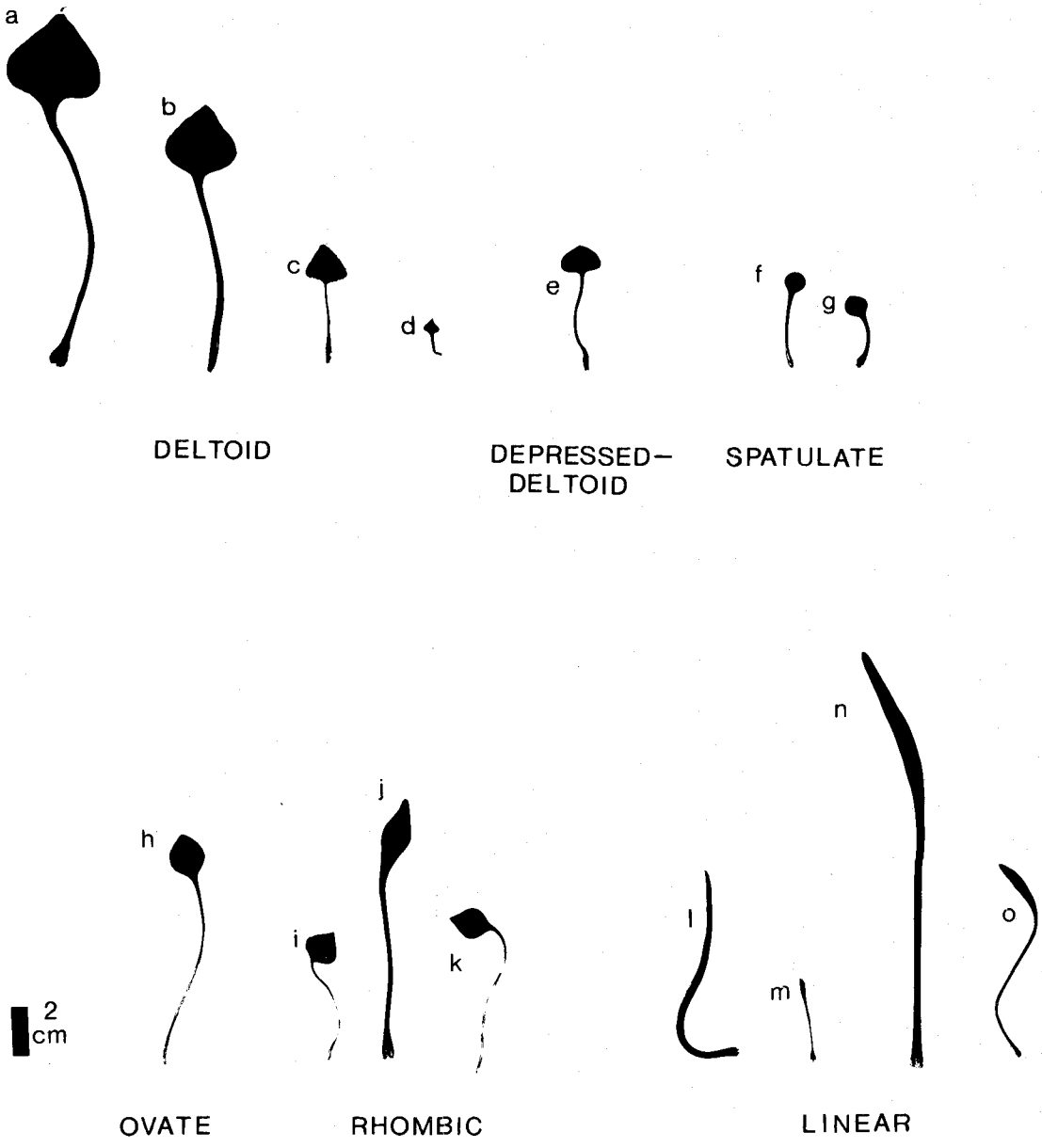


Figure 8. Representative mature basal leaves of the Claytonia perfoliata entities studied (note exceptions l and o which are juvenile basal leaves); a=entity #9, b=entity #11, c=entity #1, d=entity #1, e=entity #6, f=entity #8, g=entity #2, h=entity #7, i=entity #10, j=entity #3b, k=entity #10, l=juvenile basal leaf of entity #11, m=entity #4, n=entity #3a, o=juvenile leaf of entity #10.

length, and mature basal leaf shape were identical or closely similar among parents from the field, progeny from these parents the succeeding year, and progeny from respective parents grown under uniform conditions in the greenhouse. In contrast, some of the characters chosen by Rydberg (1932) in his taxonomy of Claytonia (Limnia) perfoliata not only varied among putative selfed siblings but even between organs of a single individual. In particular, the degree of fusion of the cauline leaves, elongation of the raceme axis, and number of pedicels per node of the raceme varied on single diploid and hexaploid entities.

A synopsis of character: chromosome-number correlations is found in Table 3. Foliage coloration, in particular an organ-specific accumulation of a water soluble "beet-red" color (probably due to an accumulation of betacyanins), was inherited in the progeny grown under uniform conditions in the greenhouse. Three entities are worthy of mention. The common diploid Claytonia rubra has conspicuous accumulations of betacyanin on the leaf undersides. The Columbia Gorge octoploid also has noticeable coloration on the leaf undersides, but the color is more purple than "beet-red." Of particular interest is a peculiar calyx ornamentation among the pink-flowered hexaploids. The common ruderal linear-leaved entity has variegated calyces. When the plants are mature, the summits of the calyces appear chlorotic, the center portion "beet-red," and the base green. A less

Table 3. The fifteen entities studied, showing correlation of morphological characters with ploidy level.

Entity	Corolla color	Corolla length (mm)	Calyx color	Calyx length (mm)	Mature basal leaf shape	Accessions
DIPLOIDS						
(1) C. rubra: Underwood, Hood River Valley, Christ-Zada Cemetery	white	3.0	reddish	2.5	deltoid	325-S_1, 335, 336, KLC 3385-S_1, 413, 420
TETRAPLOIDS						
(2)* Mouth of the Deschutes River, Clam Beach	white	2.0-2.5	green to red-tinged	2.5-3.0	spatulate (with circular blade)	383, 364-S_1
HEXAPLOIDS						
(3a) Corvallis, Ellsworth, White Salmon, Underwood	pink	4.0-4.5	variegated	2.5-3.0	linear	293, 295, 305, 324
(3b) White Salmon, Multnomah Falls-Dalton Point, McDonald State Forest, Corvallis	pink	4.0-4.5	green to red-tinged	2.5-3.0	narrowly rhombic	302, 304, 309, 334, 415A
(3c) Beacon Rock, Underwood	pink	3.5-4.0	red in fruit	2.0-2.5	linear	301, 322
(3d) Lyle	pink	4.0-4.5	green to red-tinged	2.5-3.0	deltoid	407

Table 3. Continued.

Entity	Corolla color	Corolla length (mm)	Calyx color	Calyx length (mm)	Mature basal leaf shape	Accessions
(3e) John Day River-J. S. Burres State Park, Rowena Crest	pink	3.0-3.5	green to red-tinged	2.5	linear	315, 387
(4) Horsethief Lake State Park	white	2.5	green to red-tinged	1.5-2.0	linear	318
(5)* Underwood	white	1.5-2.0	green	2.5-3.0	narrowly rhombic	KLC 3387-S_1
(6)* Washougal, Viento State Park	white	2.5-3.0	green	2.5-3.0	depressed deltoid	298, 308
(7) The Dalles, Mouth of the Deschutes River	white	2.5-3.0	green	2.5	ovate	KLC 3391-S_1, 317, 384
(8)* "Sagebrush entity": Mouth of the Deschutes River, Fulton Canyon, Arlington, Boardman	white	2.5	green	2.5	spatulate (with circular blade)	382, 385, 389, 390
OCTOPLOIDS						
(9) Horse Mountain, French Camp Ridge	pink	5.0	variegated	3.0	deltoid	361, 367, 369

Table 3. Continued.

Entity	Corolla color	Corolla length (mm)	Calyx color	Calyx length (mm)	Mature basal leaf shape	Accessions
(10) Viento State Park, Mosier, Mema-Rowena Crest	pink	3.0-3.5	red-tinged	2.0-2.5	broadly rhombic-acuminate	306, 307, 313, 401, 402
DECAPLOIDS						
(11) White Salmon, Mosier, Mosier orchard, Clarno, Wishram	white	6.0-6.5	green	5.0	deltoid	303, 310, 312, 329, 345, 396

* Putatively obligate selfers; calyx equal to or exceeding the corolla.

common linear-leaved hexaploid has calyces deep red in fruit. Both types of calyx ornamentation were inherited in selfed progeny grown in the greenhouse under uniform conditions. As individuals of Claytonia perfoliata mature in nature, "beet-red" pigments become evident. Variation in seed size and weight is also notable among the study populations. Figures 6 and 7 illustrate seed variation among selected diploids, hexaploids, and decaploids. The seeds of tetraploid and octoploid C. perfoliata fall within the range of the seeds illustrated. Robustness of growth, which appeared to be environmentally and developmentally controlled in part, is discussed in a later paragraph.

Claytonia perfoliata, when grown under uniform greenhouse conditions, has distinctive developmental patterns which may or may not be genetically and environmentally controlled. A synopsis of the ontogenetic changes in basal leaf shape is presented in Table 4.

Seeds, when tested in soil in uniform greenhouse culture, germinated sporadically over a ten-day interval. Those which were placed on filter paper discs under the misting device on the greenhouse experimental bench, exhibited similar behavior. It seems probable that germination of seeds in nature is also sporadic, but further experiments and observations are necessary to prove this. The end result of sporadic seed germination perhaps can be seen in populations of a single entity in the field. Most of the study populations,

Table 4. A synopsis of 2X, 4X, 6X, and 8X ontogeny from seed germination to flowering of the S_1 generation in uniform greenhouse culture.

Entity	Number of plants cultured	Shape of first juvenile leaves	Shape of mature basal leaves	Average time from germination to flowering
DIPLOIDS				
(1) Underwood 325-S_1	8	rhombic-deltoid	deltoid	No data available
(1) Underwood KLC 3385-S_1	8	rhombic-deltoid	deltoid	No data available
TETRAPLOIDS				
(2) Clam Beach 364-S_1	10	linear	spatulate	No data available
HEXAPLOIDS				
(3a) Corvallis 293-S_1	6	linear	linear	90 days
Ellsworth 295-S_1	6	linear	linear	90 days
White Salmon 305-S_1	10	linear	linear	65 days
Underwood 324-S_1	4	linear	linear	No data available
(3c) Beacon Rock 301-S_1	6	linear	linear	74 days
Underwood 322-S_1	3	linear	linear	58 days
(3e) Rowena Crest 315-S_1	6	linear	linear	45 days
(4) Horsethief Lake State Park 318-S_1	6	linear	linear	45 days
(5) Underwood KLC 3387-S_1	5	narrowly rhombic	narrowly rhombic	60 days
(6) Washougal 298-S_1	6	spatulate	depressed deltoid	90 days

Table 4. Continued.

Entity	Number of plants cultured	Shape of first juvenile leaves	Shape of mature basal leaves	Average time from germination to flowering
(6) Viento State Park 308-S_1	6	spatulate	depressed deltoid	52 days
(7) The Dalles KLC 3391-S_1	5	linear to spatulate	spatulate to ovate	33 days
OCTOPLOIDS				
(10) Viento State Park 306-S_1	8	rhombic	rhombic-acuminate	No data available
Viento State Park 307-S_1	8	narrowly rhombic	narrowly rhombic-acuminate	52 days
Mosier 313-S_1	6	rhombic	rhombic-acuminate	No data available

regardless of chromosome level, were composed of hundreds of plants, some juvenile, others mature and robust. Regardless of age, plants seemed to flower and produce seed (plants were classed as mature when their final stage in basal leaf shape was completely developed).

Each entity of Claytonia perfoliata displays a distinctive sequence of maturation of its basal leaves. This entire sequence of basal leaf shapes is usually visible in populations of mixed juvenile and mature plants. The main basal leaf sequences from entities collected in nature are illustrated in Figures 9 and 10. The number by each leaf refers to the location of that leaf on the shortened caudex (#1 would be the first leaf produced).

Certain environmental modifications of plant development were observed in field populations. Individuals found in "safe" microhabitats (niches not choked with competitors and having adequate moisture and rich soil) were usually robust and had fully mature basal leaves. Plants collected in "stress" microhabitats (having a water deficit, poor soil, or competition) were dwarfed and had developed a strong "beet-red" coloration. Basal leaf maturation seemed to be in an arrested stage. However, regardless of plant stature or final basal leaf shape, plants from both the field and greenhouse flowered and produced seed. More importantly, corolla color, corolla size, calyx color, and calyx size of depauperate individuals were virtually indistinguishable from similar features of robust, "normal" plants.

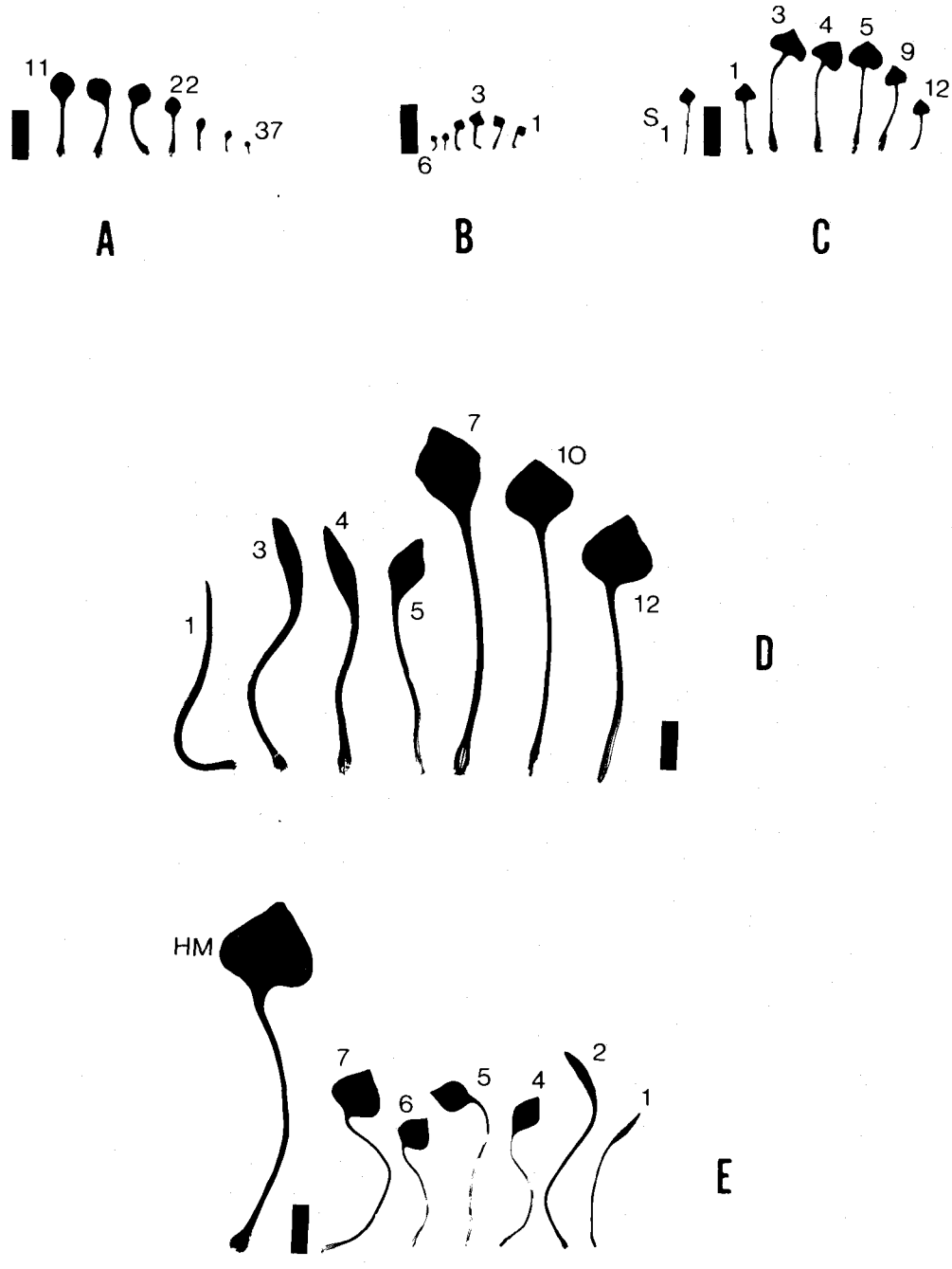


Figure 9. Basal leaf sequences of diploids, tetraploids, octoploids, and decaploids. Numbers refer to the location of the leaf on the shortened caudex (#1 would be the first leaf produced). A=#383 (entity #2), B=#413 (entity #1), C= KLC #3385 (entity #1), D=#396 (entity #11), E=#401 (entity #10), HM=Horse Mountain octoploid #369 (entity #9). The black measuring bar = 2 cm.

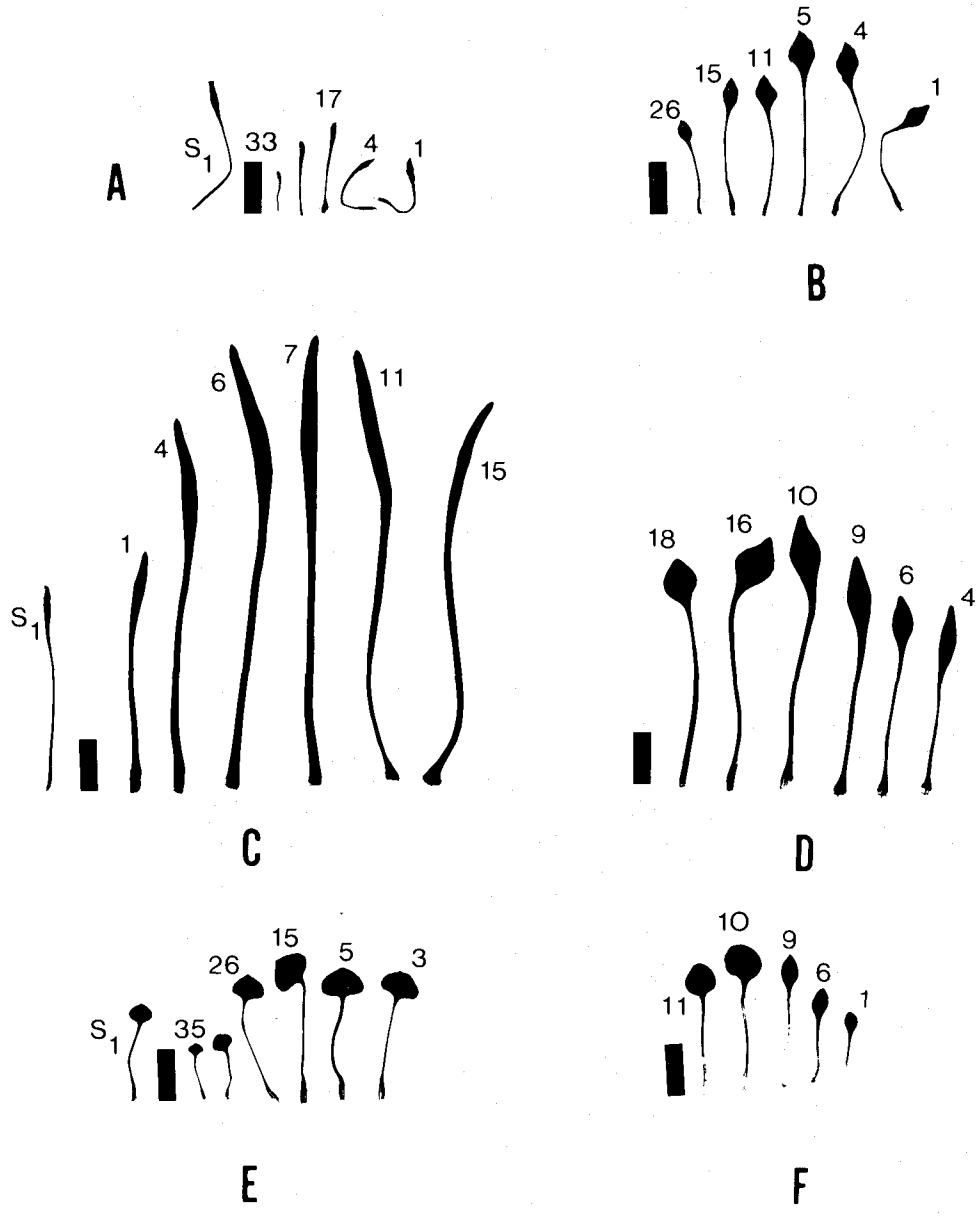


Figure 10. Basal leaf sequences of hexaploids. Numbers refer to the location of the leaf on the shortened caudex (#1 would be the first leaf produced). A=#318 (entity #4), B=KLC #3387 (entity #5), C=#293 (entity #3a), D=#415A (entity #3b), E=#308 (entity #6), F=#382 (entity #8). The black measuring bar = 2 cm.

The most striking observations of edaphic effects on plant development were made in the decaploid population at Mosier (310 and 312). Three "forms" were distinguishable. Under Quercus and Pinus were numerous robust deltoid-leaved individuals with large white corollas. In a sand pile near the roadway were more white-flowered types, but these had rhombic leaves. Growing in the road tar were still more white flowered types, just 4 cm tall with linear leaves. There is a strong presumption that these are all modifications of one plastic biotype (or polyploid genotype).

Flowering in Claytonia perfoliata, a long-day plant (Swanson, 1964), seems to be photoperiodically controlled and reflective of ecotypic differentiation. Further experimentation using populations of the same entity from different habitats is necessary if one is to detect genetic differences of an ecotypic kind.

Habitats

Claytonia perfoliata was collected in a variety of habitats from the Columbia Gorge and lower Columbia Basin. The diversity of climates and habitats there may be responsible for the large number of entities found. Certain entities were collected in specialized distinctive habitats while others were found growing everywhere (ruderals).

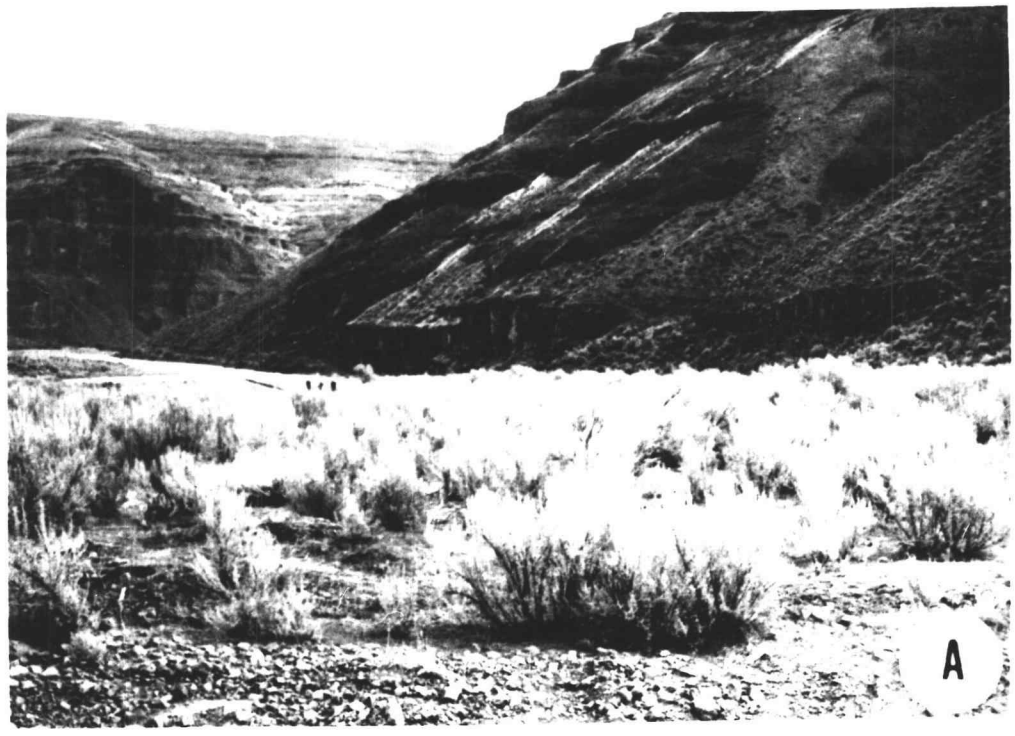
The diploid entity (#1) was only found in disturbed areas under Pseudotsuga or mixed Pseudotsuga and Quercus groves. Contrary to

expectations, C. rubra was not found under Pinus ponderosa at the eastern end of the Gorge. Instead, small populations were found in the central portion of the Columbia Gorge. At Underwood, C. rubra was found in 1972 growing sympatrically with hexaploids and decaploids on an old burn. Just a few hundred meters above the Underwood burn in the Christ-Zada Cemetery, C. rubra was collected in 1975 on freshly raked grave mounds.

The ruderal tetraploids were found growing in gravel surrounding parking stalls and picnic tables at the Mouth of the Deschutes State Park. On the west end of the Columbia Gorge, entity #6 was found in habitats similar to that of the tetraploids.

Most of the hexaploid entities collected were ruderal or semi-ruderal in areas of natural disturbance. Ruderal C. perfoliata was found along roads, in waste piles of dirt, in ditches, or around areas of human habitation. Semiruderals were found growing in crevices in raw basalt rock, in river silt, on rockslides, on burns, in sand, and in over-grazed woodlands. Figure 11 (part B) illustrates an over-grazed Pinus-Quercus woodland where hexaploids were found. One hexaploid entity (#10) was found in a relatively undisturbed habitat under Artemisia tridentata Nutt. and Purshia tridentata (Pursh) DC. This so-called "sagebrush entity" of the lower Columbia Basin was collected in mid-March. Attempts to collect flower bud material for chromosome counting in April the previous year had failed, since the

Figure 11. Picture A depicts an Artemisia tridentata brushland; a specialized habitat of hexaploid Claytonia perfoliata. Picture B illustrates an overgrazed Pinus ponderosa-Quercus garryana woodland; a habitat of ruderal hexaploid and decaploid Claytonia perfoliata.


A


B

Figure 12. The habitat of octoploid Claytonia perfoliata. Pictured is a north-facing basalt talus slope.

plants had already matured their seed. Adaptation to this highly specialized habitat may represent the filling of an unoccupied niche, a niche which allows plants to flower and set seed before grasses and other annuals take up all of the available moisture. Figure 11 (part A) illustrates the unique habitat of the "sagebrush entity."

Octoploids grew only in areas of natural disturbance on the Oregon side of the Columbia Gorge. Octoploid individuals were collected only from mossy pockets on steep, densely-shaded, north-facing talus slopes or on cliff faces. Figure 12 illustrates the octoploid habitat. In sharp contrast, the octoploid entity from Northern California was ruderal on overgrazed serpentine balds under Quercus garryana.

Decaploids, wherever collected, were ruderal. They occurred sympatrically with pink-flowered hexaploids on a burn near White Salmon. Near Mosier, decaploids were found as well developed infestations in orchards.

Flavonoid Chemistry

Of the many substances that a plant synthesizes, the flavonoids are the most important for chemotaxonomic investigations (Alston, 1967). Flavonoid compounds are useful genomic markers which often are inherited as simple Mendelian codominants (Alston, 1964). Studies in several groups of plants have shown that flavonoid ensembles can

give useful evidence on the ancestry of polyploids (Smith and Levin, 1963, Stebbins et al., 1963). The occurrence, in alloploids, of novel flavonoids in addition to compounds that are shared with the parental diploids, has been demonstrated (Torres and Levin, 1964, Levin, 1968, Levy and Levin, 1974). The results of chromatographic studies of selected diploid, tetraploid, hexaploid, octoploid, and decaploid Claytonia perfoliata sens. lat. suggest that their flavonoid constituents may provide data to support hypotheses concerning polyploid origins and relationships.

The fidelity of the chromatographic and spectrophotometric procedures for research on Claytonia was tested using Quercetin-3-O-rhamnoglucoside (Rutin). R_f values of 0.41 (TBA) and 0.55 (HOAc) were experimentally determined. Mabry et al. (1970) report R_f values of 0.44 (TBA) and 0.56 (HOAc) for Rutin. The spectrum of Quercetin-3-O-rhamnoglucoside in methanol and methanol + sodium methoxide reported by Mabry et al. (1970) was confirmed (to within 1 nm of the spectral maxima) following purification by one-dimensional paper chromatography.

Biotypes of C. perfoliata have complex two-dimensional chromatographic spot patterns. Figures 13 through 15 illustrate the putative flavonoid spot patterns from diploid, tetraploid, hexaploid, octoploid, and decaploid whole plant extracts. A synopsis of detectable spots is found in Table 5.

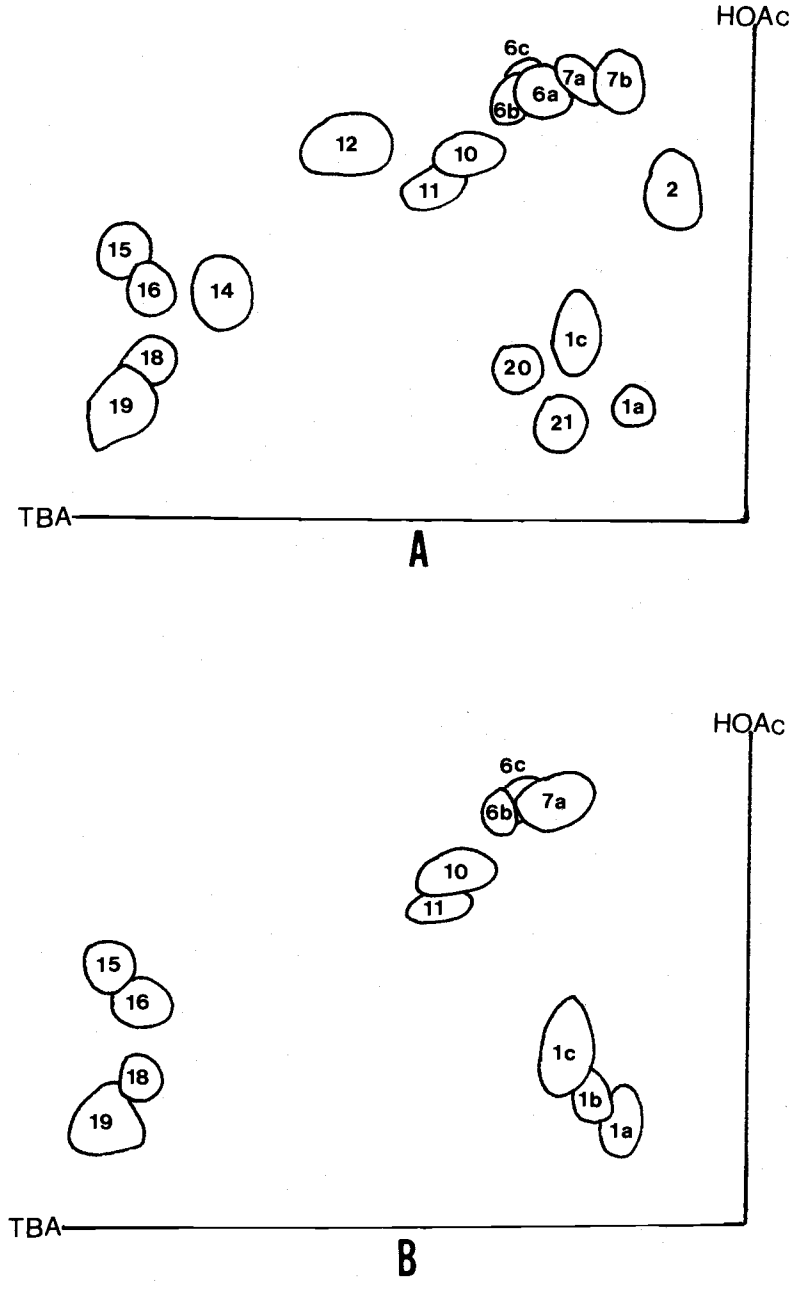


Figure 13. Two-dimensional chromatographic spot pattern of a diploid (A=Claytonia rubra (Howell) Tidestr. #420 - Hood River Valley) and a tetraploid (B=#383 - Mouth of the Deschutes).

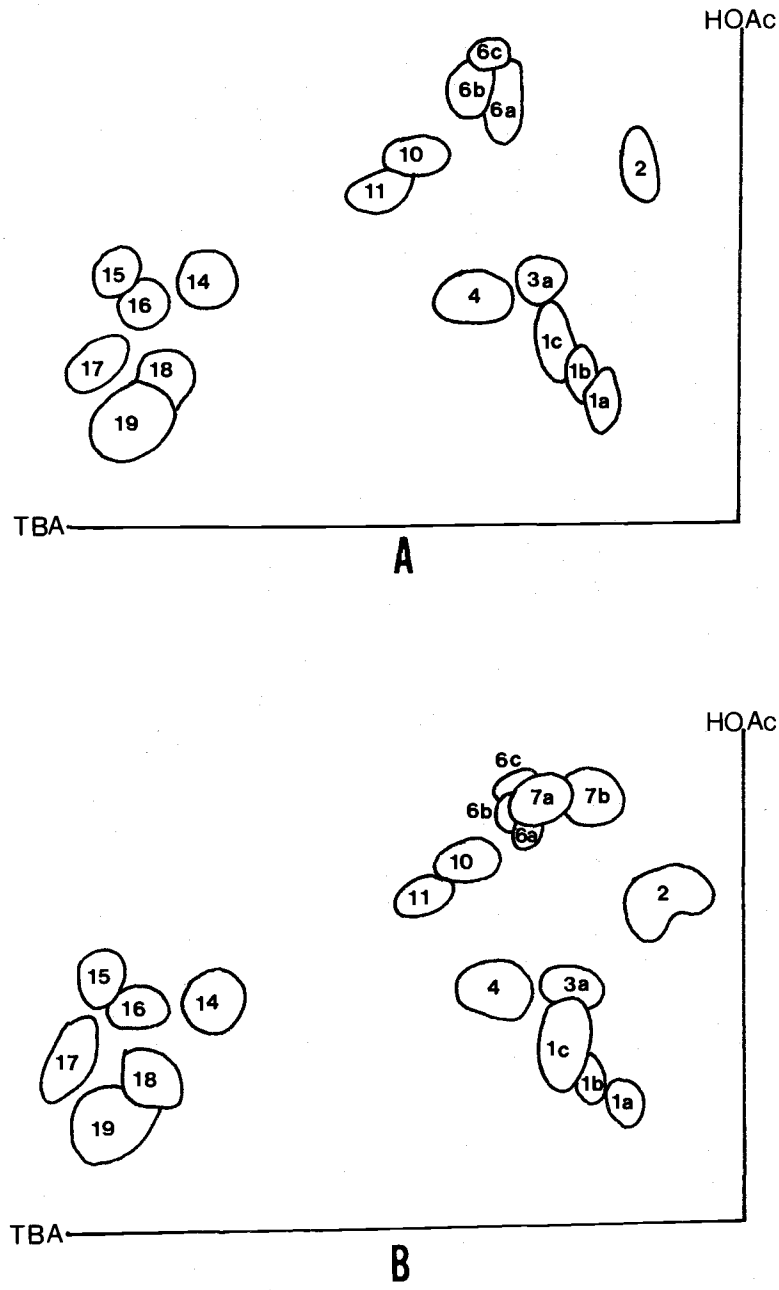


Figure 14. Two-dimensional chromatographic spot patterns of two linear-leaved, pink-flowered hexaploid entities (A= #293 - Corvallis, B=#387 - J. S. Burres State Park/ John Day River).

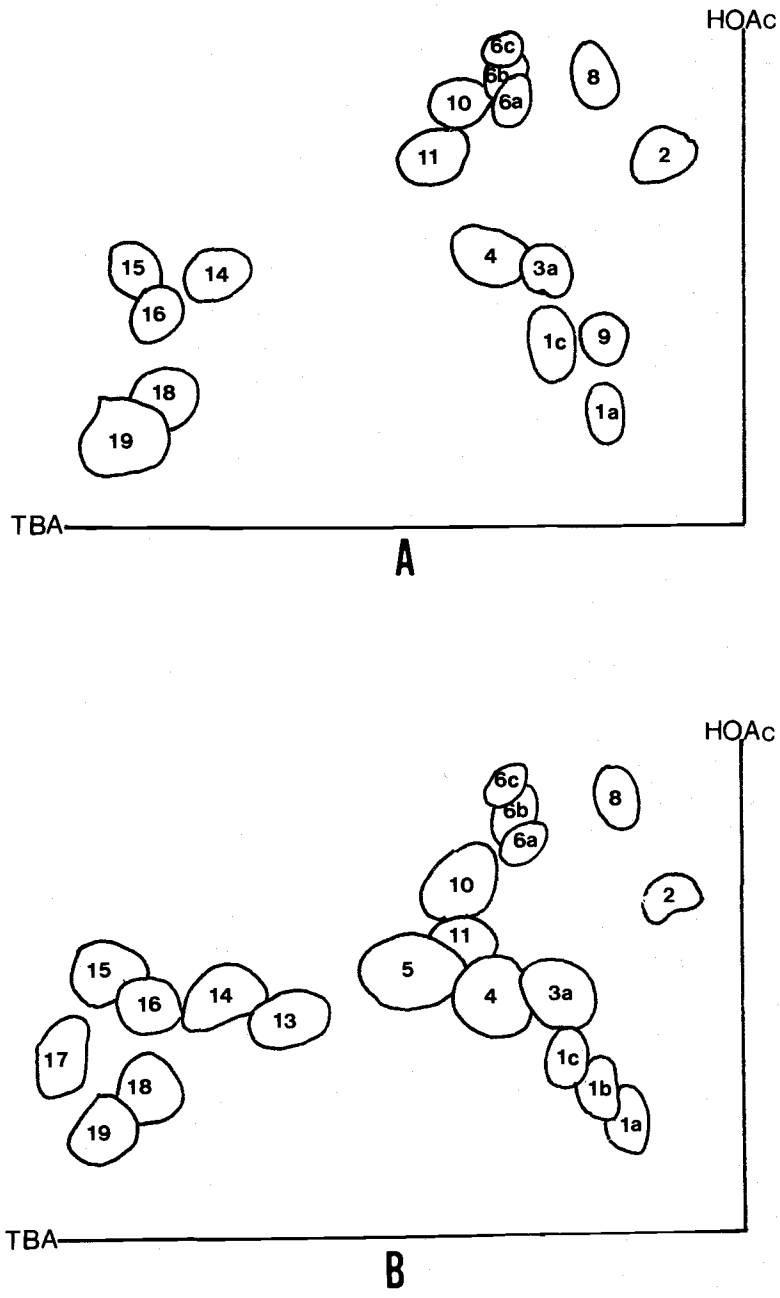


Figure 15. Two-dimensional chromatographic spot pattern of an octoploid (A=#401 - Mema triangulation station/Rowena Crest) and a decaploid (B=#396 - Mosier orchard).

Table 5. Chromatographic properties of putative flavonoids of Claytonia perfoliata (diploids, tetraploids, hexaploids, octoploids, and decaploids).

Spot Designation	R_f values[1] TBA	HOAc	Color Reactions[2] UV	UV + NH_3
1a	.21	.21	YO	lt-G
1b	.26	.24	YO	lt-G
1c	.29	.32	YO	lt-G
2	.12	.21	RO	RO
3a	.28	.52	dk-P	dk-G
3b	.26	.50	dk-P	dk-G
3c	.17	.50	dk-P	dk-G
4	.40	.56	dk-P	dk-G
5	.56	.63	dk-P	dk-G
6a	.29	.72	BG	BG
6b	.30	.77	B	B
6c	.30	.87	G	BG
7a	.36	.80	dk-P	dk-P
7b	.27	.81	dk-P	dk-P
8	.19	.84	lt-BG	lt-BG
9	.22	.35	RP	RP
10	.41	.71	lt-BG	lt-BG
11	.50	.64	YG	YG
12	.60	.77	dk-P	dk-G
13	.51	.43	dk-P	dk-B
14	.67	.47	dk-P	dk-B
15	.78	.51	B	B
16	.74	.50	lt-BG	lt-BG
17	.86	.38	Y	Y
18	.70	.24	B	B
19	.77	.15	B	B
20	.43	.25	B	B
21	.35	.16	Y	Y

[1] R_f values represent an average from five chromatograms.

[2] color codes: YO=yellow-orange, lt-G=light green, RO=red-orange, dk-P=dark-purple (absorbing), dk-G=dark-green, dk-B=dark-brown, BG=blue-green, lt-BG=light blue-green, B=blue, G=green, RP=red-dish-purple, YG=yellow-green, Y=yellow.

Tentative identifications of two flavonoid compounds, a chalcone and flavone (a Quercetin glycoside) were based on uv spectral comparisons with the published spectral maxima (Mabry, et al., 1970), R_f values, and color reactions. The spectral maxima for these two compounds are found in Table 6. The sugar moieties of these putative flavonoid glycosides have not been identified. Further work with sugar identification is underway.

Of considerable significance to questions of the development and function of different organs were visual quantitative and qualitative differences in chromatographic patterns and intensities of individual spots between petal methanolic extracts and extracts from other plant organs. Fifteen hundredths of a gram of decaploid petals produced a well-defined chromatographic spot pattern having resolution superior to that of a pattern produced from methanolic extracts of 4 g of decaploid whole plant material. Calyx, cauline leaf, basal leaf, and stem extracts had conspicuous spot differences from petal extracts. Figures 16, 17, and 18 illustrate the chromatographic differences between methanolic extracts of 0.15 g of decaploid (#396-Mosier orchard) plant organs and floral appendages, respectively.

Table 6. Identifications and spectral maxima of two Claytonia perfoliata flavonoids.

Spot Designation	Identity	Spectral Maxima (nm)[1]					
		MeOH	$AlCl_3$	$AlCl_3$+HCl	NaOMe	NaOAc	NaOAc+ H_3BO_3
1a	Chalcone?	264sh 308sh 331	268sh 308sh 335	268sh 313sh 335	255 314sh 380	305sh 331	243sh 305sh 332
3a	Quercetin glycoside	258 269sh 295sh 359	273 304sh 370sh 407	270 300sh 365sh 405	na	na	na

[1] sh=shoulder or inflection, na=insufficient compound for spectral analysis.

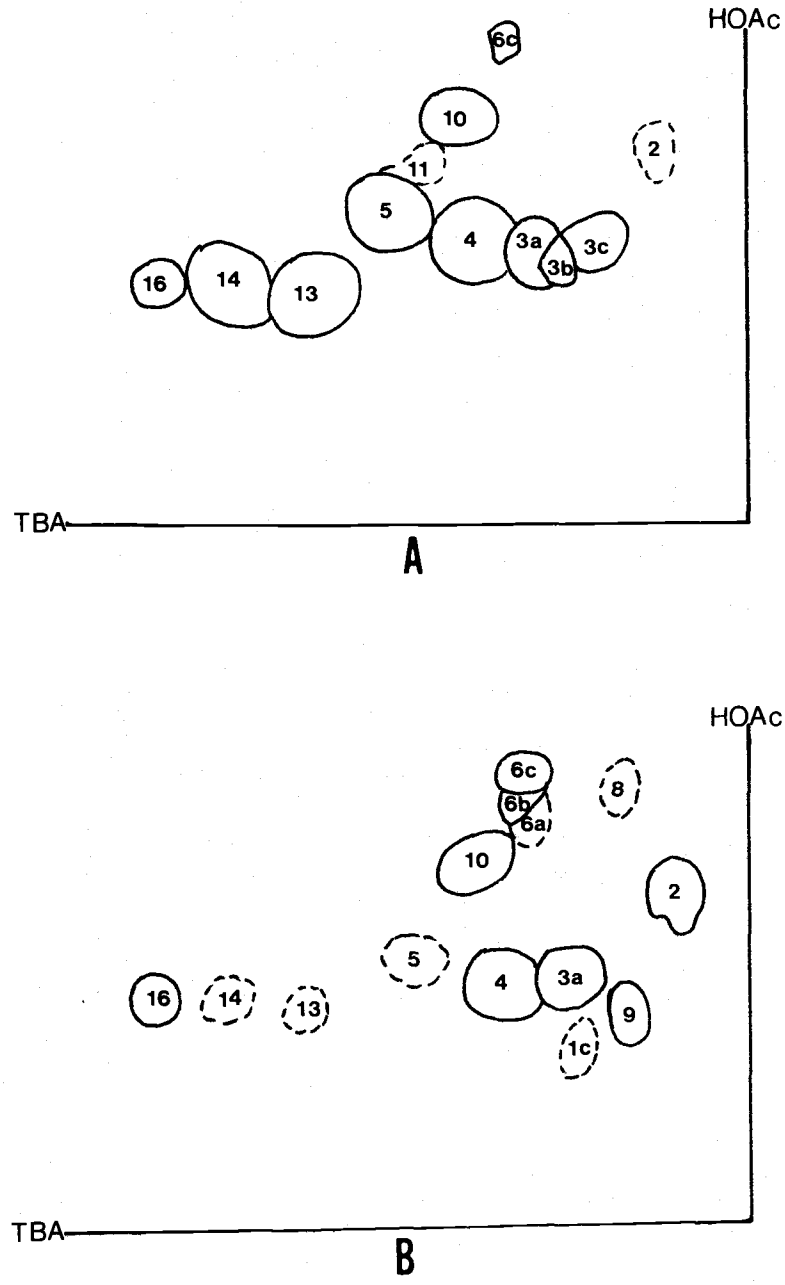


Figure 16. Two-dimensional chromatographic spot pattern of 0.15 g of decaploid (#396 - Mosier orchard) floral appendages (A=petals, B=sepals). Dotted circles represent compound localizations at the limit of visual detection.

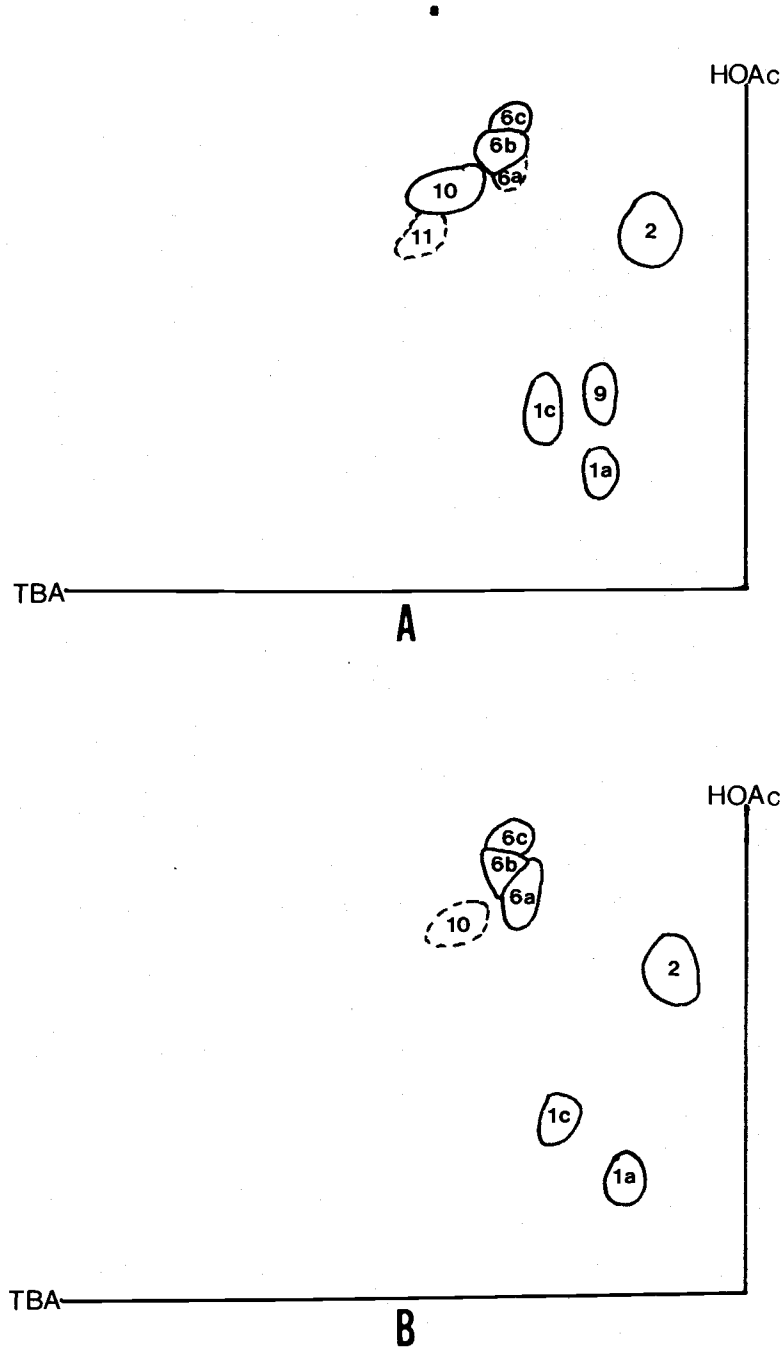


Figure 17. Two-dimensional chromatographic spot pattern of 0.15 g of decaploid (#396 - Mosier orchard) leaves (A=cauline leaves, B=mature basal leaves). Dotted circles represent compound localizations at the limit of visual detection.

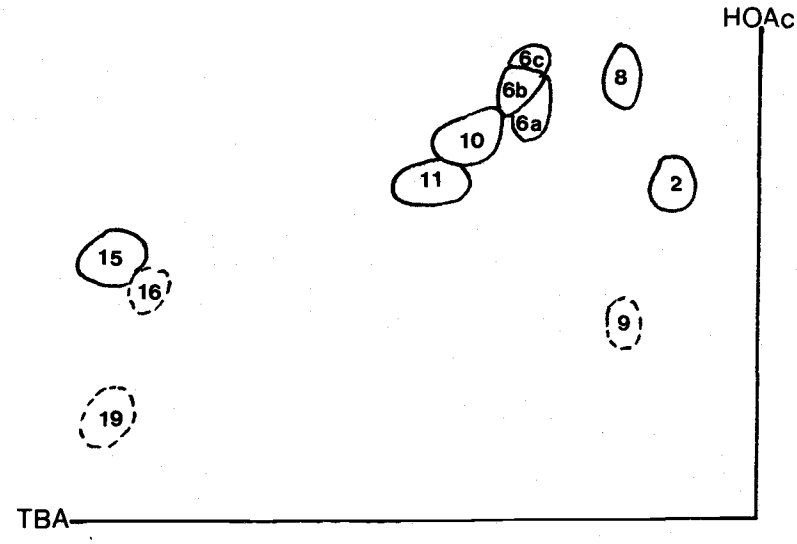


Figure 18. Two-dimensional chromatographic spot pattern of 0.15 g of decaploid flower stems (#396 Mosier orchard). Dotted circles represent compound localizations at the limit of visual detection.

V. DISCUSSION AND CONCLUSIONS

Claytonia perfoliata is not a simple polyploid complex. Based on the findings of the present investigation and the studies of Swanson (1964), several races, recognizable morphologically, cytologically, and ecologically, become evident: (1) two morphologically distinct and geographically isolated diploids, (2) five morphologically distinct and geographically isolated tetraploids, (3) as many as ten hexaploid entities (which include pink-flowered facultatively autogamous ruderals, white-flowered facultatively autogamous ruderals, white-flowered obligately autogamous ruderals, and the white-flowered obligately autogamous "sagebrush entity", (4) two morphologically distinct and geographically isolated octoploids (which occupy widely differing habitats), and (5) a distinctive decaploid. Field studies of these and other possible races are incomplete; hence, a satisfactory taxonomic disposition of the entities comprising C. perfoliata cannot be made at the present time.

Prevailing autogamy has provided C. perfoliata with frequent opportunities to increase its chromosome number. The following mechanisms for doubling of the chromosome number in plants of this species are hypothesized: (1) autoploidy from the products of the union of unreduced gametes of the same organism, (2) alloploids derived from products of the union of two unreduced gametes in F_1 hybrids

between widely differing entities at the same or different chromosome levels, (3) alloploids derived from the union of a reduced gamete of one organism with an unreduced gamete of another, and (4) alloploids derived from simple crosses between two chromosome levels (e.g., hexaploid X decaploid = direct octoploidy). The occurrence within the study area of several repeating biotypes such as the hexaploid "sagebrush entity" indicate that autogamy is a successful means of reproduction, and that seed dispersal is adequate to spread a given biotype widely in its appropriate habitat.

It is clear that a limited degree of outcrossing in the breeding system (possibly insect mediated), coupled with polyploidy, is responsible for much of the genetic polymorphism of the complex. Our lack of understanding of the relationships among the polyploids, the nature of plant development and population structure, breeding system, and the extent of phenotypic variation due to environmental influences, has been responsible for the widely differing taxonomic treatments of the taxon. Consequently, "key" characters have been misinterpreted (e.g. Rydberg, 1932) or rejected entirely (Hitchcock et al., 1964). It is notable that mature basal leaf shape coupled with corolla size and corolla color was used to predict the chromosome number of many of the biotypes studied. The occurrence of a morphologically distinctive population in a specialized habitat (e.g. C. rubra, the hexaploid "sagebrush" entity, or the Columbia River Gorge octoploid) supported

my chromosome number predictions. Additionally, floral characters were stable among individuals of the same entity, whether grown in the greenhouse or observed in the field in "stress" microhabitats. Conversely, mature basal leaf shape was found to be an environmentally and developmentally "plastic" character. Much of the so-called polymorphism in a single population (at least those populations reported here) is readily explained by the following: (1) populations normally consist of hundreds of plants, some mature and others juvenile, (2) the basal leaf shape of a single organism changes drastically with increasing age of the organism, (3) the sequence of basal leaf maturation is arrested in individuals growing under "stress conditions," however, and regardless of their basal leaf shape, the plants proceed to flower, and (4) chromosome races of C. perfoliata frequently occur sympatrically with one another.

The chromatographic profiles of C. perfoliata with 28 detectable spots (of which ten are dark, uv-absorbing spots) indicate a rich flavonoid chemistry. Techniques of comparative flavonoid biochemistry will be important in any future investigation concerning origin and relationship of chromosome races. Furthermore, a clear understanding of morphological development is essential if one is to gain meaningful biochemical data. The organ/floral appendage-specific accumulation (or biosynthesis) of flavonoid compounds is almost certainly developmentally related and under genetic control. Any further attempt to

compare extracts from plants at differing stages in ontogeny must be accomplished with care. Most of the dark, uv-absorbing flavonoid compounds of decaploid C. perfoliata are localized in petal tissue. It follows that extracts of plants having few inflorescences or undeveloped flowers might contain undetectable amounts of such compounds. The occurrence of large amounts of dark, uv-absorbing flavonoids in C. perfoliata petals is no doubt functionally significant. Harborne (1967) reports that petal "color" in many plants having ivory, cream, or white flowers is due to the presence of common flavonols and flavones. More importantly, Harborne suggests that the dark, uv-absorbing flavones provide flowers with a satisfactory means of attracting pollinating insects. It is peculiar that the facultatively autogamous decaploid C. perfoliata contains large amounts of these compounds. Their presence in the petal tissue might reflect the waning ancestral, large-flowered allogamous condition. Conversely, the decaploid entity might represent an evolutionary experiment which has, as its objective, to escape autogamy. How then, do the petal flavonoids of diploid, tetraploid, hexaploid, and octoploid C. perfoliata compare? It is attractive to hypothesize that petal flavonoid analysis, coupled with artificial hybridizations between entities might lend conclusive insight into the problems of origin and relationship within this putative polyploid "pillar" complex.

BIBLIOGRAPHY

Alston, R. E. 1964. The genetics of phenolic compounds. In: J. B. Harborne (editor), Biochemistry of Phenolic Compounds. pp. 171-204. Academic Press, New York.

_____ 1967. Biochemical systematics. In: T. Dobzhansky, M. K. Hecht, and W. C. Steere (editors), Evolutionary Biology 1:197-305. Appleton-Century-Crofts, New York.

Crawford, D. J. 1973. Isolation and identification of common flavones and flavonols from plant materials for chemotaxonomic studies. Phytochemical Bulletin 6(1):4-9.

Douglas, G. W. and R. J. Taylor. 1972. The biosystematics, chemotaxonomy, and ecology of Claytonia lanceolata in western Washington. Canadian Journal of Botany 50:2177-2187.

Egger, Kurt. 1969. I. Plant phenol derivatives. In: Egan Stahl (editor), Thin Layer Chromatography. pp. 687-706. Springer Verlag, Berlin.

Gibbs, R. D. 1974. Chemotaxonomy of Flowering Plants. McGill-Queens University Press, Montreal.

Harborne, J. B. 1967. Comparative Biochemistry of the Flavonoids. Academic Press, London. 383 p.

Hitchcock, C. L. and A. Cronquist. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle. pp. 107-108.

_____, A. Cronquist, M. Ownbey, and J. W. Thompson. 1964. Vascular Plants of the Pacific Northwest, Part 2. University of Washington Press, Seattle. pp. 236-246.

Howell, Thomas. 1893. A rearrangement of the American Portulacaceae. Erythea 1:2. pp. 36-39.

Lewis, Walter H. and Yutaka Suda. 1968. Karyotypes in relation to classification and phylogeny in Claytonia. Annals of the Missouri Botanical Garden 55(1):64-67.

Levin, D. A. 1968. The genome constitutions of eastern North American Phlox amphiploids. Evolution 22:612-632.

Levy, M. and D. A. Levin. 1974. Novel flavonoids and reticulate evolution in the Phlox pilosa-Phlox drummondii complex. American Journal of Botany 61(2):156-167.

Mabry, T. J., A. Taylor, and B. L. Turner. 1963. The betacyanins and their distribution. Phytochemistry 2:61-64.

_____, K. R. Markham, and M. B. Thomas. 1970. The Systematic Identification of Flavonoids. Springer Verlag, Berlin. 354 pp.

Miller, J. M. 1975. In: IOPB chromosome reports. Taxon (in press).

Munz, P. A. 1959. A California Flora. University of California Press, Berkeley. pp. 300-304.

_____ 1974. A Flora of Southern California. University of California Press, Berkeley. pp. 708-715.

Nilsson, Orjan. 1967. Studies in Montia L. and Claytonia L. and allied genera: III. Pollen morphology. Grana Palynologica 7(2):346.

Peck, Morton E. 1961. A Manual of the Higher Plants of Oregon. Binfords and Mort, Portland. pp. 299-302.

Rydberg, P. A. 1932. Monograph of the Portulacaceae. In: North American Flora 21(4):302-313.

Smith, D. M. and D. A. Levin. 1963. A chromatographic study of reticulate evolution in the Appalachian Asplenium complex. American Journal of Botany 50:952-958.

Snow, Richard. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Technology 38(1):9-13.

Stebbins, G. L., B. L. Harvey, E. L. Cox, J. N. Rutger, G. Jelencovic, and E. Yagil. 1963. Identification of the ancestry of an amphiploid Viola with the aid of paper chromatography. American Journal of Botany 50:830-839.

Suksdorf, Wilhelm. 1923. Washingtonische pflanzen III. Werdenda 1:11.

Swanson, J. R. 1966. A synopsis of the relationships in Montiodeae (Portulacaceae). Brittonia 18:229-241.

_____ 1964. Claytonia (Montia) perfoliata: A Genecological and Evolutionary Study. Ph. D. Dissertation; University of California, Berkeley.

Torres, Andrew M. and D. A. Levin. 1964. A chromatographic study of cespitose Zinnias. American Journal of Botany 51: 639-643.

Willis, J. C. 1892. On the distribution of seed in Claytonia. Annals of Botany 6:382-383.