

AN ABSTRACT OF THE THESIS OF

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Title: Morphogenetic Studies of Intraspecific Hybrids of Microseris laciniata (Hook.) Sch. Bip.

Abstract approved

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Dr. Kenton L. Chambers

Microseris laciniata is a perennial, self-incompatible species classified in subgenus Scorzonella. It is morphologically the least specialized species in the genus, in contrast to the members of subgenus Microseris, which are morphologically advanced, self-compatible annuals.

First-generation hybrids were available from crosses involving eight populations of two subspecies of Microseris laciniata from southwestern Oregon and northwestern California. This study focused on the morphology and reproductive behavior of twelve different intraspecific crosses of this group.

One of the morphological characters investigated was the number of pappus-parts per achene. This trait was chosen not only for the ease with which it can be analysed, but also because it was used in recent studies on evolutionary genetics of Microseris. All the ten families of hybrids tested, except four, showed significant variation

in the distribution of pappus-part numbers within themselves. Most of the distribution patterns resembled the "high determining" genotypes reported in the literature.

The other morphological trait investigated was the number of phyllaries of the involucre. The phyllaries are an important character in distinguishing the two subspecies of M. laciniata. The proportion of outer versus inner phyllaries was tested statistically to determine whether there were significant differences among the siblings of each hybrid family. This characteristic was found to be uniform in each family, although different families seemed to differ among themselves. The results could not be compared with earlier published accounts, due to differences in cultural conditions for the plants.

The reproductive behavior of the intraspecific hybrids was studied with respect to (1) seed fertility, (2) pollen stainability, and (3) chromosomal behavior at meiosis.

The test plants were crossed in the greenhouse with siblings and sometimes with half-sib plants. The percentage of pollinations that yielded fruits with normal embryos was calculated. The results of these hand pollinations showed widely varying success in seed formation. Sometimes a particular sibling cross would yield one capitulum with no fertile fruits and a second capitulum with mostly normal fruits. There was no tendency for

F 1's within the same subspecies to be more fertile than F 1's between subspecies. The low seed productivity of many sibling crosses, despite their high pollen fertility, is suggestive of shared self-incompatibility factors among sister plants.

Pollen stainability was investigated for eight intraspecific and three interspecific hybrids of M. laciniata. Among the intraspecific hybrids studied only two had markedly reduced pollen stainability. Pollen germinability in vitro failed in several attempts. In vivo only a few pollen tubes were observed on the styles of two of the four hybrids crossed to their siblings, indicating a strong rejection reaction in all four hybrids tested. Chromosomal behavior at meiosis in intraspecific hybrids was mostly normal, but minor irregularities were seen especially from prophase to first anaphase. No gross structural changes in the chromosomes of M. laciniata were observed, despite the probable antiquity of the species and spatial isolation of its populations.

Morphogenetic Studies of Intraspecific
Hybrids of Microseris laciniata
(Hook.) Sch.-Bip.

by

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MORPHOGENETIC STUDIES OF INTRASPECIFIC
HYBRIDS OF MICROSERIS LACINIATA
(Hook.) Sch.-Bip.

I. INTRODUCTION

The genus Microseris includes two morphologically distinct groups of plants in western North America, consisting of 10 morphologically advanced annuals and six structurally less specialized perennials (Chambers 1955, Stebbins 1972, Bachmann 1979). A thorough biosystematic study has been done for the annual species by Chambers (1955) and a few scattered papers exist on the perennial species. Distinct evolutionary trends have been described in various morphological and cytological traits, with the perennials generally being more primitive and the annuals more advanced. The genus Microseris was arranged by Chambers into four subgenera. One perennial species forms a distinct subgenus unique to Australia and New Zealand. In North America, the annuals make up subgenus Microseris, and the perennials comprise subgenera Scorzonella and Apargidium. Microseris laciniata is probably the most primitive species among the perennials. It is divided into two subspecies that differ from each other principally in the shape and structure of the involucrel phyllaries.

A pattern of morphological intergradation among the annuals was described by Chambers in his 1955 treatise. A similar pattern exists for the perennials. Because the

species are not always clearly distinct morphologically, it is important to discover what genetic barriers, if any, exist among the perennials, as was done for the annuals by Chambers. A major purpose of the present study was to describe the intraspecific reproductive barriers within M. laciniata, as a standard for future studies at the interspecific level.

Numerous accessions of Microseris laciniata were collected by Chambers and grown in the greenhouse, where intraspecific hybridizations were then performed. These crosses by Chambers yielded the F 1 hybrids used in the present study. The following morphogenetic features of these hybrids were investigated:

1. Number of pappus parts per fruit
2. Number of phyllaries per capitulum
3. Fertility of the F 1 hybrids, including:
 - a. Seed production
 - b. Pollen stainability and germinability
4. Chromosomal behavior at meiosis

Studies on the genetic behavior within and between species of subgenus Scorzonella are necessary to fill the gaps in our understanding of the phylogenetic relationships of the entire genus. The purpose of this study is to focus on the morphology and reproductive behavior of intraspecific hybrids of Scorzonella's most primitive species, Microseris laciniata.

II. LITERATURE REVIEW

The genus Microseris was described by David Don in 1832; it is classified in the tribe Cichorieae, family Asteraceae (Compositae). The tribe Cichorieae was recognized in 1789 by Jussieu in his *Genera Plantarum*. The tribal boundaries have never been much of a problem to taxonomists. Indeed, the Cichorieae is considered "the most distinctive and easily recognizable subdivision of the family Compositae" (Stebbins 1953). This consistency in tribal characters has even induced certain taxonomists to treat the group as a separate family.

The older treatments of the Cichorieae were artificial and based on only a few diagnostic characters. These treatments are well explained in a paper by Stebbins (1953) on the classification of the tribe Cichorieae. Stebbins was the first to arrange the Cichorieae genera in a natural and phylogenetic way. For that purpose he utilized not only a variety of morphological characters but included information from geographic distribution and chromosomal affinities as well. Stebbins places Microseris in subtribe Microseridinae, close to Cichorinae and Stephanomeriinae. In his classification, subtribe Microseridinae includes the following genera: Microseris, Phalacroseris, Apargidium, Agoseris, Krigia, Pyrropappus and Picrosia. In more recent treatments Apargidium has been merged with Microseris and

Nothocalais has been given generic status (Chambers 1957, 1960).

The genus Microseris is considered a difficult one taxonomically, because of the complex morphological variability of its species. The causes of this variability began to be understood through the work of Chambers in 1955, which included studies on breeding systems, interspecific hybridizations, and chromosomal behavior. In his treatise, Chambers reviewed the previous taxonomic treatments of the genus, and discussed its relationship to the allied genera Agoseris and Nothocalais. He described the genus as a whole, separating the perennial species (as "handsome, tall-stemmed plants with showy heads of dandelion-like flowers") from the annuals, which are less conspicuous, low growing rosette plants (Chambers 1955). He made a division into four subgenera, one of which was further divided into three sections. This last was subgenus Microseris, around which his biosystematic work centered.

Infrageneric Classification of
Microseris (Chambers 1955)

<u>Genus</u>	<u>Subgenus</u>	<u>Section</u>	<u>Species</u>
	Scorzonella		M. laciniata M. paludosa M. sylvatica M. howellii M. nutans
MICROSERIS	Apargidium.....		M. borealis
	Moniermos		M. scapigera
		Calocalais....	M. linearifolia
		Brachycarpa...	M. decipiens M. heterocarpa
	Microseris		M. douglasii M. acuminata M. campestris M. bigelovii
		Microseris....	M. elegans M. pygmaea

Microseris laciniata, along with the other perennial species was placed by Chambers (1955) in subgenus Scorzonella. The following key (Chambers, 1957) distinguishes the six recognized taxa of this subgenus:

Pappus parts 15-30, bright white, the awns soft, plumose.

1. M. nutans

Pappus parts 6-12, the awns rather stiff, minutely spiculate, or if subplumose then the pappus tawny.

Pappus paleae 5-10 mm. long, fimbriate at the apex about the base of the awn; awns subplumose, tawny.

2. M. sylvatica

Pappus paleae usually less than 5 mm. long, tapering into the awn or emarginate; awns minutely

spiculate to barbellulate, white or tawny.

Outer phyllaries ovate-lanceolate to broadly ovate or circular, acute to cuspidate, usually glabrous dorsally, all over 2.5 mm. broad.

3a. M. laciniata ssp. laciniata

Outer phyllaries linear to ovate lanceolate, acute to attenuate, often scurfy puberulent dorsally, the smallest ones not over 2.5 mm. broad.

Involucre narrowly or broadly campanulate, 25- to 75-flowered, 10-25 mm. high; coastal, in Oregon and California.

Pappus palea 0.5-1.5 mm. long, deltoid or ovate, the awns white or tawny.

3b. M. laciniata ssp. leptosepala

Pappus palea 2.0-4.0 mm. long, lanceolate, the awns tawny.

4. M. paludosa

Involucre cylindrical or narrowly campanulate, 15- to 25 flowered, 8-18 mm. high; Siskiyou Mountains.

5. M. howellii

The species of subgenus Scorzonella are not sharply separable. Their taxonomy depends on correlation of certain morphological characters, combined with different patterns of distribution and ecological preference. Intergradation is particularly evident between the two subspecies of Microseris laciniata (Chambers 1957).

Scorzonella is considered the most primitive subgenus of Microseris, which in turn is the least specialized member of the tribe Cichorieae. Within it, Microseris laciniata is probably the most primitive species (Chambers 1957, Stebbins 1972).

Microseris laciniata has pale, fleshy, biennial tap-roots, which also characterize the other perennial species. Each root arises near the base of a rosette leaf and is associated with a developing shoot in the leaf axil (Chambers 1955). The two subspecies can be distinguished from each other by the shape, nervation and pubescence of their outer involucral phyllaries. The distinction between the two subspecies was well described by Chambers (1957):

Plants of Microseris laciniata ssp. laciniata are characterized by generally large heads with broad, ovate-lanceolate to circular-cuspidate, glabrous outer phyllaries, while ssp. leptosepala usually possesses small and narrow outer phyllaries that are linear to lanceolate or deltoid and often covered with white-furfuraceous or black-villous pubescence.

Microseris is gradually becoming better known bio-systematically. Many of its species have been intercrossed and progeny have been obtained for genetic studies. Populations of Microseris, at least those found in western North America, have been recorded by Chambers from field and herbarium studies and representatives are available for interested researchers. All this, plus the ease with which it can be grown in cultivation, make the genus a well suited organism for cytological, biochemical and many other studies in evolutionary genetics.

The use of angiosperms in evolutionary genetics was stressed by Stebbins:

For comparing subspecies, species, and occasionally genera, they (the angiosperms) are superior to any group of animals, since hybridization is easier and barriers of reproductive isolation between morphologically and ecologically diverse populations are, in general, less strongly developed. The poverty of their fossil record is to some extent compensated by the presence of intercrossable populations that differ with respect to at least some characters similar to those that separate major groups. (Stebbins 1974)

In the last few years, evolutionary geneticists have searched for a well suited angiosperm for certain microevolutionary studies that would enable them to make extrapolations on major evolutionary trends of plants and even animals. According to K. Bachmann (1979), personal communication), Microseris has most of the necessary qualifications for the task, and it may very well be the genetic model of the near future.

One of the modern lines of evolutionary genetics using Microseris as a model deals with comparative studies in nuclear DNA amount. Studies have shown that DNA content per nucleus varies over 100-fold among vascular plants, but that this variation is not necessarily correlated with major levels of evolutionary advancement. However, studies within more restricted taxa such as subtribes or genera have shown that the distribution of DNA amount sometimes forms microevolutionary patterns of either increase or decrease in DNA correlated with the relative advancement of

the group. In Microseris, most of the perennial species have 1.6-2.8 times the nuclear DNA content of the annuals (Price 1976, Bachmann et al. 1979). One of the questions that arose from this finding concerned the evolutionary and ecological significance of DNA amount. There was evidently a pattern of diminution of nuclear DNA paralleling the evolution of the advanced annuals from the more primitive perennials. Was the decrease of DNA amount of the annuals essential to their attaining the morphological specializations required for a shift from mesic to arid environments? One line of evidence favoring this suggestion has to do with the positive correlations found between DNA content and minimum mitotic cycle time, duration of meiosis, cell size, and minimum life cycle (Price and Bachmann 1976):

The annual species of Microseris in response to selection for a more rapid developmental rate may have lost expendable sequences of DNA because of their quantitative effects in cell size, mitotic cycle time and duration of meiosis. The low DNA amount of the annuals has resulted in a highly specialized organism of restricted evolutionary potential, probably caused by a loss in the number of regulatory genes. (Price 1976)

Studies on the amount of ribosomal RNA were also done in subtribe Microseridinae with results similar to the studies of nuclear DNA amount. These experiments supported the idea that Microseris laciniata was the modern species

most like the ancestor of the four annual species of Microseris (Hemleben, Bachmann and Price 1978).

Another line of research deals with scanning and transmission electron microscopy of pollen morphology of subtribe Microseridinae done by Feuer and Tomb (1977). This survey not only confirmed the tribal relations established by Stebbins, but also separated the annual species of Microseris from the perennials. The annuals were found to have "small lacunae with low ridges of short spines with less well defined bases and relatively large polar thickenings." The perennials have "significantly larger pollen with well defined lacunae, high ridges and small polar thickenings. The spines are large, conical and show well developed spine bases" (Feuer and Tomb 1977). Except for the difference between the annuals and the perennials, pollen morphology did not allow for intrageneric distinctions. However, the comparison of the eight genera studied supported Stebbins' classification of the Cichorieae as a natural and cohesive group.

The third line of study that utilizes Microseris as a model in evolutionary genetics deals with traits of the inflorescence and of the mature fruit that can be easily quantified and that are relatively independent of environmental influences. These traits are: number of achenes per head, number of pappus parts per achene, and number of phyllaries per head. The trait examined most thoroughly

so far is the number of pappus parts of the annual species of Microseris. Bachmann et al. found that all North American annual species of Microseris had a five-part pappus and that one South American species, M. pygmaea, had a ten-part pappus. Hybridizations between M. bigelovii, a North American species, and M. pygmaea resulted in a progeny that segregated for the genetic determination of either the five or ten basic parts. Each natural population contained a predictable proportion of achenes with aberrant pappus part numbers. These deviations from the basic number followed a Poisson distribution for numbers added to five or deleted from ten (Bachmann and Chambers 1977). The pappus parts originate on the ovary from points located above the ten provascular bundles that are formed during the development of the floral primordium. Either all these sites develop into pappus parts, or only alternate sites develop. There appears to be a genetic mechanism that triggers one or the other form, but so far it has not been explained by a simple Mendelian model (Bachmann and Chambers 1977). In M. laciniata, the distribution was much like that of the M. pygmaea (10 pappus parts) X M. bigelovii (15 pappus parts) hybrids. It appears that in the evolution of the annuals from the perennials, there was a canalization from a highly variable to a precise number of pappus parts. Similar character fixation also has occurred in the number and arrangement of involucrel

phyllaries (Bachmann et al., 1979).

Quantitative characters of the flowering heads of Microseris laciniata were scored for three seasons of growth. On some plants the average number of pappus parts per achene varied on different heads during a single growing season, and significant plant to plant variation was also observed. The variation was explained as an interplay between developmental factors favoring a high (10) or low (5) basic pappus number (Bachmann and Price 1978). Pappus-part number was plotted with respect to the spatial distribution of the achenes on each individual capitulum. The frequency and spatial distribution of achenes with different numbers of pappus parts in M. laciniata were found not to be entirely random (Bachmann and Price 1978). A diagram of a cross section of a head of Microseris was designed in order to quantify the intuitive observation that the marginal achenes tend to have fewer pappus parts than the central ones. Each achene was plotted as to its place in the "genetic spiral" (developmental sequence) on the head. This analysis of the capitulum did not indicate any direct dependence of the pappus-part number on the position of an achene in the capitulum genetic spiral, but it did show there is a highly significant correlation of increasing pappus number in the sequence from earlier formed to later formed achenes.

Morphological data on intraspecific hybrids are given in the present work, with the hope that they will shed some light on the mechanism of pappus inheritance, and thus on the biosystematics of the perennial species of Microseris. As Stebbins pointed out, intraspecific studies done in a microevolutionary level are obligatory to the better understanding of transpecific evolution, which in ultimate analysis permits extrapolations of larger evolutionary trends for both plants and animals (Stebbins 1974).

III. MATERIALS AND METHODS

Accessions

Living material of the two subspecies of Microseris laciniata was collected in southwestern Oregon and northwestern California sites by Chambers and cultivated in the Oregon State University greenhouse. Intra- and interspecific crosses were made between selected plants of different populations, and the F 1 hybrids were planted and grown to maturity. The number of siblings in the F 1's ranged from one to six. The parental populations are listed below, arranged by subspecies and by an identifying accession number. Voucher specimens are deposited in the Oregon State University Herbarium.

Subspecies laciniata:

- 229 - Jackson Co., Oregon: Sams Valley Road, which parallels Hwy 234 to the N. of Lower Table Rock perhaps 1 mi.; the locality being 0.6 mi. W. of the spot where these two roads join and 1.1 mi. W. of the junction of Hwy. 234 with N-S road between the two Table Rocks.
- 263 - Josephine Co., Oregon: Wilderville Road west of Murphy, on north side of Applegate River, 0.8 mi. west of junction with New Hope Road. R 4 W., T. 37 S., Sect. 14.
- 284 - Humboldt Co., California: About 1.5 mi. E. of the Freeway overpass, on road from Garberville to Harris.
- 290 - Humboldt Co., California: 0.9 mi. west of Bridgeville, on Calif. Hwy. 36.

Subspecies leptosepala:

- 283 - Del Norte Co., California: Gasquet Flat, just north of the power station by Hwy. 199.
- 293 - Trinity Co., California: 0.8 mi. from Hwy. 96, on gravel road to Weitchpec School House.
- 294 - Siskiyou Co., California: Road along Salmon River, 1.3 mi. east of Lewis Creek bridge (12.6 mi. east of junction with Hwy. 96, between Sommes Bar and Forks of the Salmon).
- 295 - Humboldt Co., California: R 4 E., T. 10 S., Sec. 18.

Additional accessions:

The following species were also included in the pollen stainability and merosis studies:

- 168 - Microseris paludosa: Presidio of San Francisco; Lincoln Blvd. at its junction with the southern cross-road to Washington Blvd., 0.9 mi. n. of the 25th Ave. entrance to the Presidio.
- 261 - Microseris paludosa: Monterey Co.: Point Lobos State Park.
- 234 - Microseris nutans: Wasco Co.: Warm Springs Indian Reservation; Hwy. 26, 18.9 mi. NW. of bridge over Deschutes River, and a short distance S. of bridge over Warm Springs River.
- 304 - Microseris sylvatica: Santa Clara Co., Mt. Hamilton. Range on Arroyo Mocho - Patterson Pass road.

The following hybrids were used in this study; the pistillate parent is listed first (Fig. 1):

Microseris laciniata ssp. laciniata X Microseris laciniata ssp. laciniata

(229-3 X 284-1), (229-3 X 290-1), (284-1 X 290-1), (290-1 X 229-3), (290 X 263-9).

M. laciniata ssp. leptosepala X M. laciniata ssp. leptosepala

(283-1 X 295-1), (293-4 X 283-1), (294-5 X 283-1).

M. laciniata leptosepala X M. laciniata laciniata

(283-1 X 290-1), (293-1 X 229-3), (293-1 X 284-6), 295-1 X 229-3).

M. laciniata laciniata X M. laciniata leptosepala

(263-9 X 293-1)

Number of Pappus Parts

The fruit of Microseris is an achene whose ovary is crowned by a tuft of appendages, referred to as the "pappus parts." These pappus parts develop above ten provascular bundles, and in Microseris laciniata their number varies from five to ten or rarely more (Bachmann and Price 1978). As noted above, this variable pattern found in the primitive M. laciniata is in contrast to the more stable one found in the advanced species of annuals. In order to correlate the patterns of pappus number to the group's evolution, it is desirable to have information on the inheritance of this trait. For this, mature heads of the various F 1 plants were collected throughout the growing season and individually placed in carefully labeled petri dishes. The number of pappus parts per achene was determined for each head. The counting was made easier by placing each achene on a piece of colored paper under a microscope and pressing its pappus part flat with the index finger.

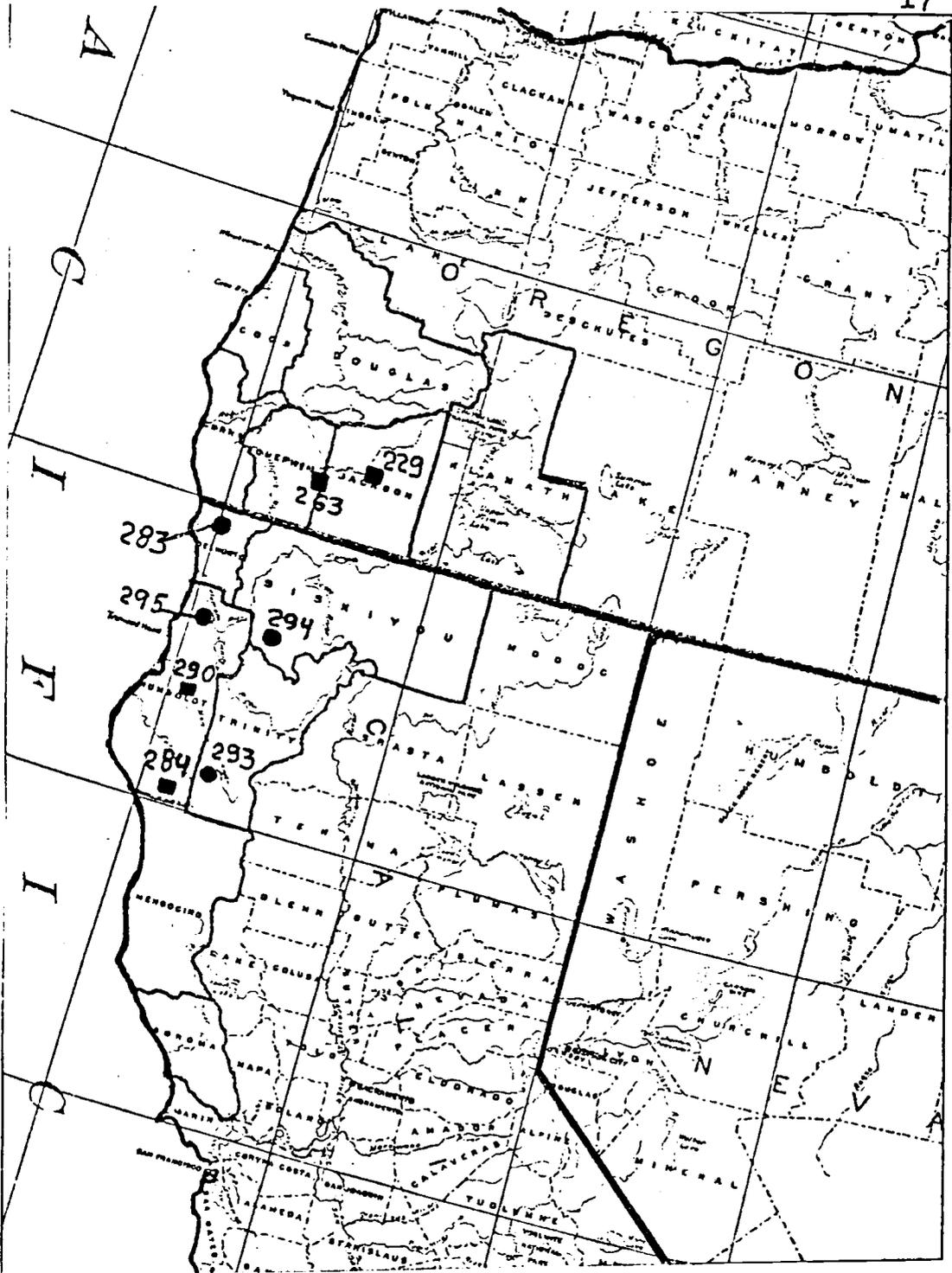


Fig. 1. Partial map of Oregon and California showing the eight populations of *Microseris laciniata* studied. The squares represent *ssp. laciniata* and the circles *ssp. leptosepala*.

Forty-nine heads and over three thousand achenes were scored for numbers of pappus parts.

Number of Phyllaries

The phyllaries are the character used to distinguish the two subspecies of M. laciniata. The word "phyllary" comes from the Greek "phyllarion," the diminutive of "phyllon" or leaf. Phyllaries are modified leaflets forming the involucre bracts that subtend the flower heads (capitula) of family Compositae.

The involucre bracts of Microseris laciniata constitute two more or less distinct series of broader outer phyllaries surrounding the elongated inner ones. The inner and outer may intergrade in length and shape, and in some cases no clear distinction between them is possible.

In a study of M. laciniata done by Bachmann and Price (1978) the average number of phyllaries in 23 out of 27 plants studied was found to be close to 21. The other four plants had numbers of 26.0, 26.1, 29.5 and 30.7, respectively. A high between-plant variance was observed and interpreted as an indicator of a genetic component in the variability.

For the present study, phyllaries of intraspecific hybrids of Microseris laciniata were counted. The materials were taken from the same heads used for the pappus-part counts and fertility studies. The plant

material that had become hard and stiff was softened by soaking it in warm water for a few minutes prior to the counting. The number of outer and inner phyllaries were recorded for 44 heads of various M. laciniata hybrids.

Fertility of the F 1 Hybrid Families

Seed Production

The intraspecific hybrids in the garden were crossed with sibling plants whenever possible or with their closest available relative. The pollination method used was the "violin-playing" technique developed by Chambers for Microseris. It consisted of removing a floret with a fine forceps and brushing the pollen-covered style across the clean, moist V-shaped stigmas of the sibling plant. Once pollinated, the head was tagged and the approximate number of successful pollinations recorded. Since anthesis occurs gradually in the capitulum of composites and only newly opened florets are receptive, it was usually necessary to repeat the operation on each head for several days. After maturation of the achenes, each tagged head was picked and placed in an individually labeled petri-dish. Since M. laciniata is self-incompatible, the fertility was scored by simply dividing the number of pollinations performed by the number of fertile fruits formed. The fertile achenes from these crosses that represent the F 2's have been kept for further studies.

Pollen stainability

Flowering heads of the hybrids were fixed for two days in a solution of chloroform, 95% ethanol and glacial acetic acid in a 4:3:1 ratio. They were transferred to 70% ethanol for long-term storage in a refrigerator. Pollen was sampled separately for five individual florets per head. The unopened florets were placed on a clean glass slide, in two drops of aniline blue. Cutting off both ends of the florets made it easier for unwanted tissues to be removed, and further cutting and squashing of the floret released the pollen grains. A cover slip was placed on the mass of floating pollen grains to avoid evaporation and thus desiccation. Pollen was scored as viable if the cytoplasm was plump and completely stained. Inviabile pollen grains were judged to be those with shrunken or poorly stained cytoplasm. Scoring of pollen fertility was accomplished for most of the hybrids in this study.

Pollen germinability

Pollen germination in vivo was observed for two hybrids of M. laciniata, under the U.V. microscope. An unopened floret was hand emasculated by ripping the corolla with a needle and removing the five filaments with a fine forceps. The emasculation was found necessary, because a large amount of pollen is freed from the filaments at anthesis and sticks on the outer walls of the style. These

fluoresce in the U.V. microscope and interfere with the observations. After emasculation, the florets were left in a moist petri-dish for 24-36 hours until the gynoecium was completely mature and the stigmas opened. After that, they were pollinated and left in the moist petri-dishes for 4-24 hours. The styles were then removed and placed on a clean glass slide in a drop of aniline blue, covered with a plastic coverslip and squashed flat. The slides were then inspected in the U.V. microscope for the occurrence of pollen tubes.

Pollen germination in vitro was attempted by methods described by Brewbaker (1957, 1963), which consisted of putting fresh pollen in sucrose solution with and without boron, in a hanging droplet or petri-dish. Various sucrose concentrations were tried, but none was successful. Only a couple of pollen tubes were seen in a group of two hundred. Pollen material from both hybrids and from the parents were used, both showing a high percentage of viability by common staining methods. It has been previously noted in the literature that the trinucleate pollen of Compositae does not germinate readily in vitro.

Chromosomal Behavior at Meiosis

Buds of the various intraspecific hybrids and two interspecific hybrids of M. laciniata times M. paludosa and M. sulvatica were fixed in a solution of chloroform,

95% ethanol, and glacial acetic acid in a 4:3:1 ratio. After 48 hours, the bud samples were transferred to 70% ethanol. The young buds of Microseris have florets of different sizes, each size characterizing a particular stage in meiosis. A pair of florets with the dividing microspore mother cells in the desired stage, were squashed and mounted in a drop of 45% aqueous acetic acid and Hoyer's medium. The permanent microslides were deposited in the herbarium of Oregon State University. These slides were studied on a Zeiss phase-contrast microscope with 1000 X magnification. Photographs of dividing cells were taken with a Exacta camera on a microscope adapter, using Kodak Tri-X film of ASA 32.

IV. RESULTS

Number of Pappus Parts per Fruit

The various individual plants that resulted from each cross were statistically tested in order to find out whether or not their pappus part distributions were the same. Previous studies of pappus-part counts in Microseris laciniata revealed that each individual plant had a repetitive distribution pattern (Bachmann and Chambers 1977). Moreover, the patterns of pappus parts were the result of two opposing genetically determined tendencies, one for high and the other for low pappus-part numbers (Bachmann and Price 1978).

Chi-square tests done to compare distributions in pappus-part number in the siblings from each cross did show significant variation in six out of ten families (Table 1).

Figure 2 represents the pappus-part distribution in the siblings of each cross studied. These graphs seem to indicate that whenever a difference in pappus-part distribution occurred among siblings it was due to the presence of one or two deviant individuals. In four out of ten crosses there were no significant differences in pappus-part distribution among siblings. Three additional crosses were sampled for one sibling only and thus could not be submitted to a Chi-square test.

Table 1. Chi-square tests to compare distribution in pappus parts in siblings of 10 hybrid crosses. Null hypothesis: no between-sibling difference

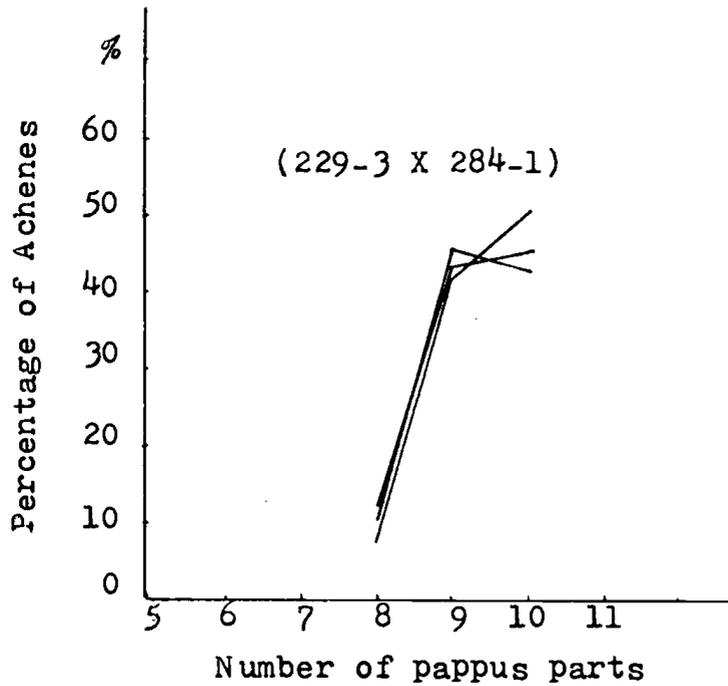
alpha = .95

Hybrid Crosses	χ^2	D.F.	Crit.Val.	Sig.	N(achenes)
(229-3 X 284-1)	1.381	4	9.49	+	250
(229-3 X 290-1)	187.16	16	26.30	-	431
(284-1 X 290-1)	201.11	10	18.31	-	282
(290-2 X 263-9)	135.00	15	25.00	-	381
(293-4 X 283-1)	301.742	8	14.51	-	345
(294-5 X 283-1)	5.14	4	9.49	+	247
(263-9 X 293-1)	108.00	4	9.49	-	111
(283-1 X 290-1)	6.835	4	9.49	+	157
(293-1 X 284-6)	100.00	21	32.67	-	831
(295-1 X 229-3)	2.543	3	7.81	+	205

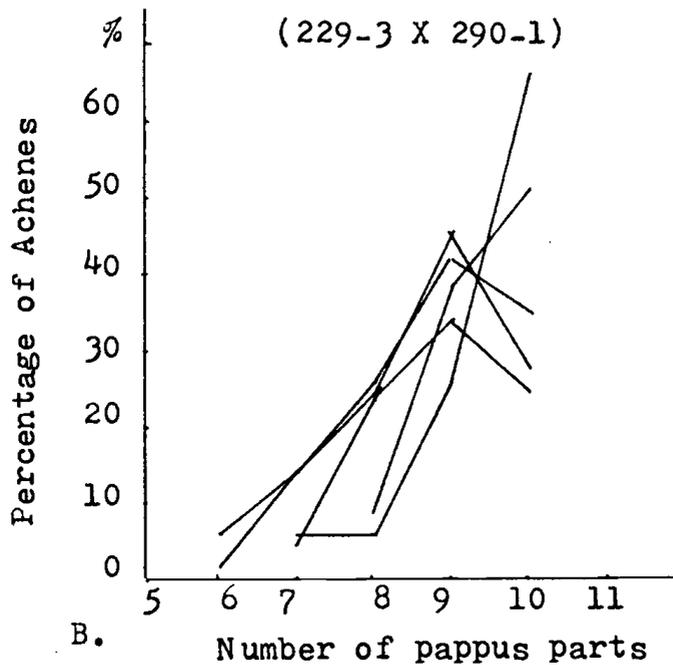
The four hybrid families that showed uniformity in pappus-part distribution were:

1. (229-3 X 284-1)
2. (294-5 X 283-1)
3. (283-1 X 290-1)
4. (295-1 X 229-3)

Figure 3 shows the characteristic distribution for each of these crosses when the pappus data for the siblings are combined. The assumption is made here that the siblings are genotypically alike with respect to this morphological trait. One must keep in mind, however, that there were only a few siblings counted for these hybrids, and the chance that this uniformity in pappus-part distribution is

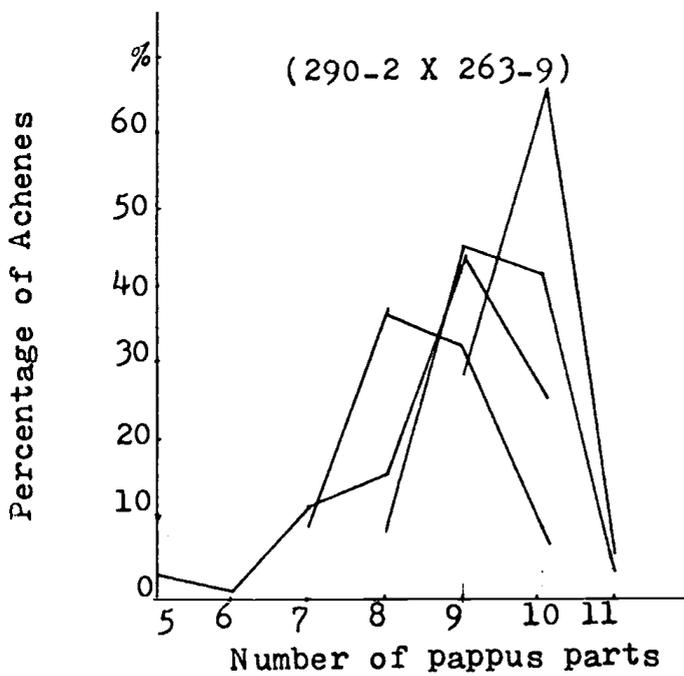
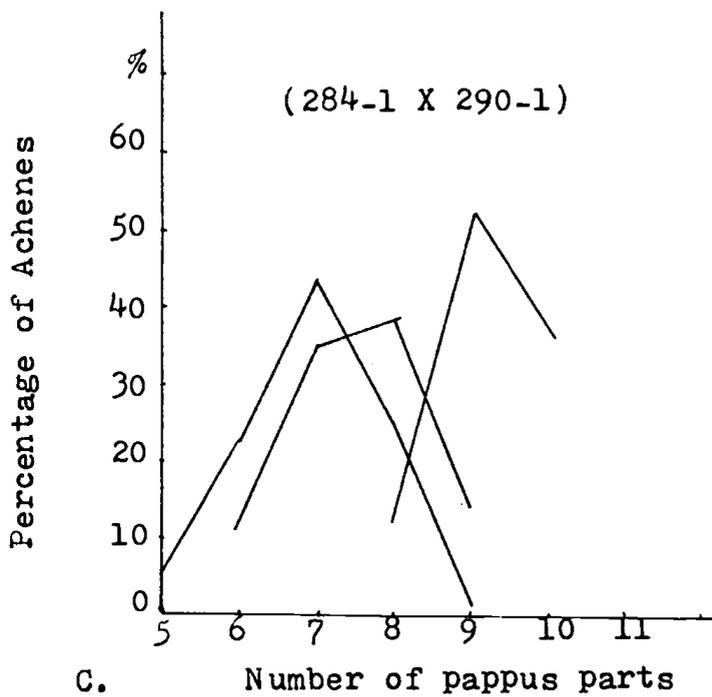


A.



B.

Fig. 2. Number of pappus parts plotted against percentage of achenes in the various hybrid crosses studied.



D.

Fig. 2. Continued

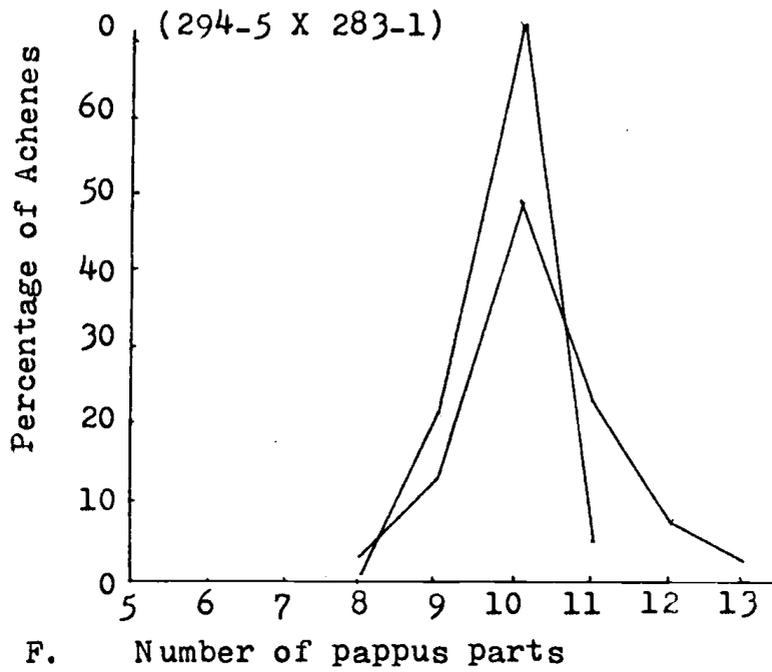
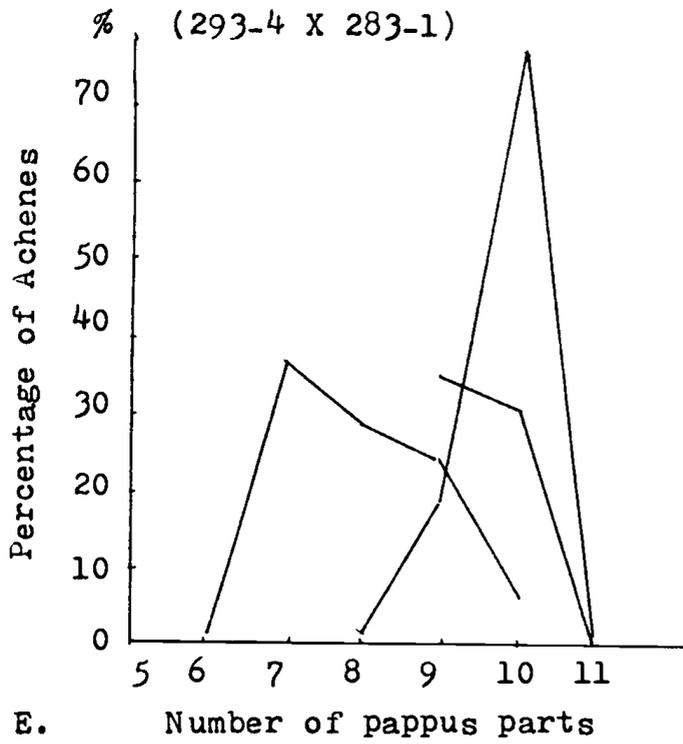


Fig. 2. Continued

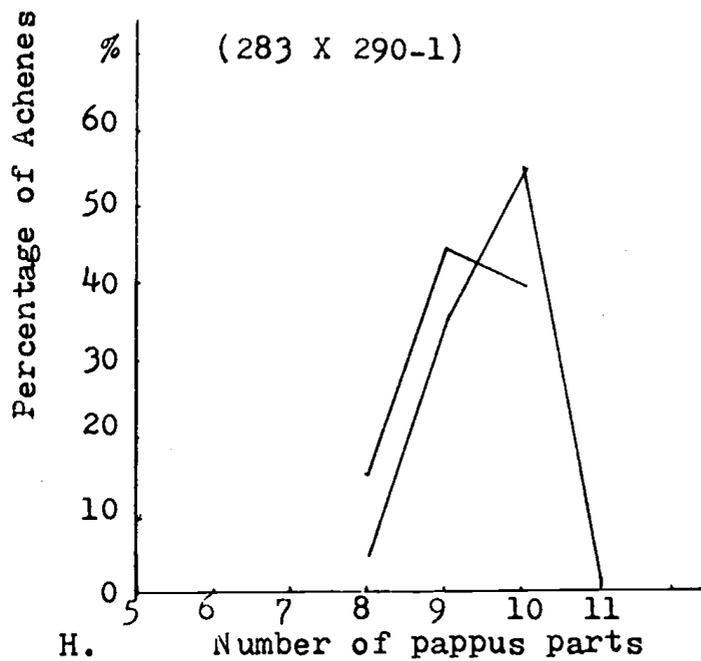
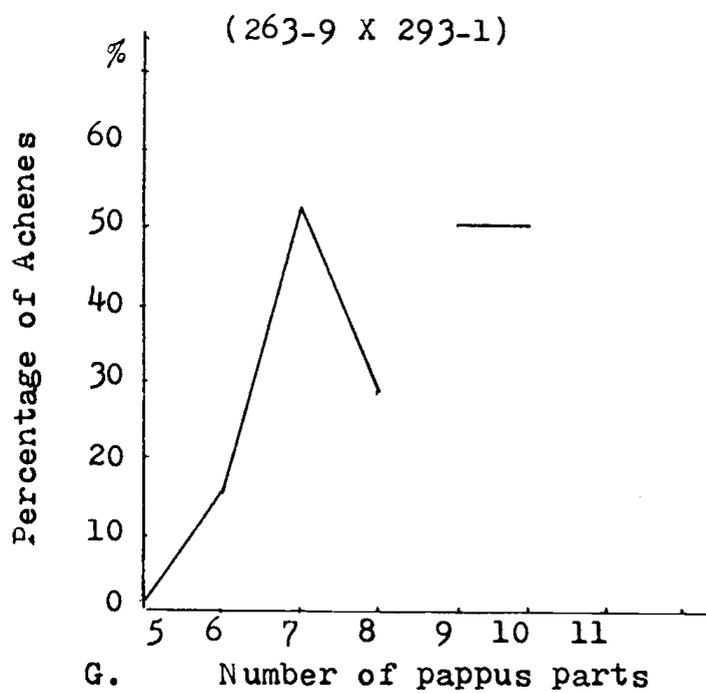


Fig. 2. Continued

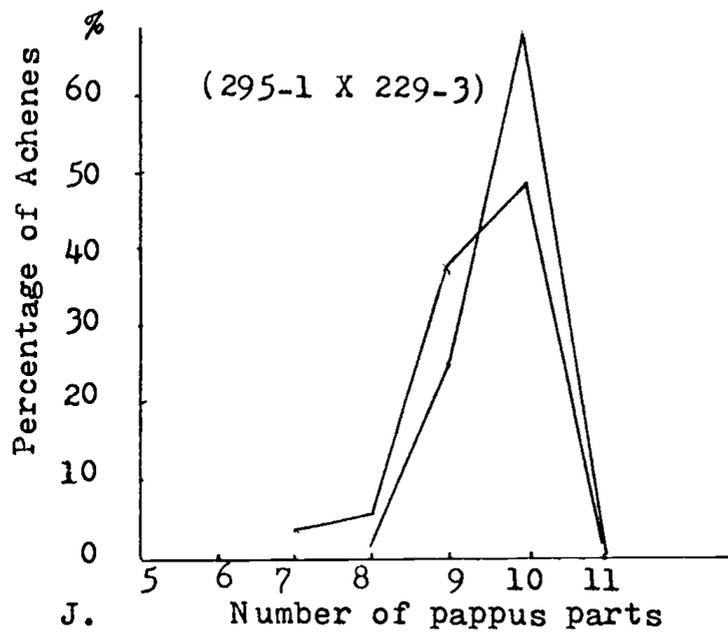
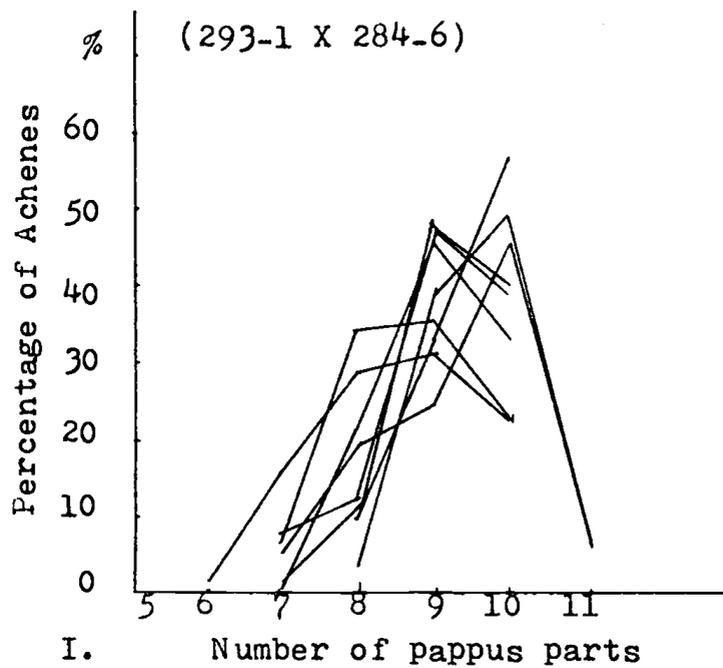


Fig. 2. Continued

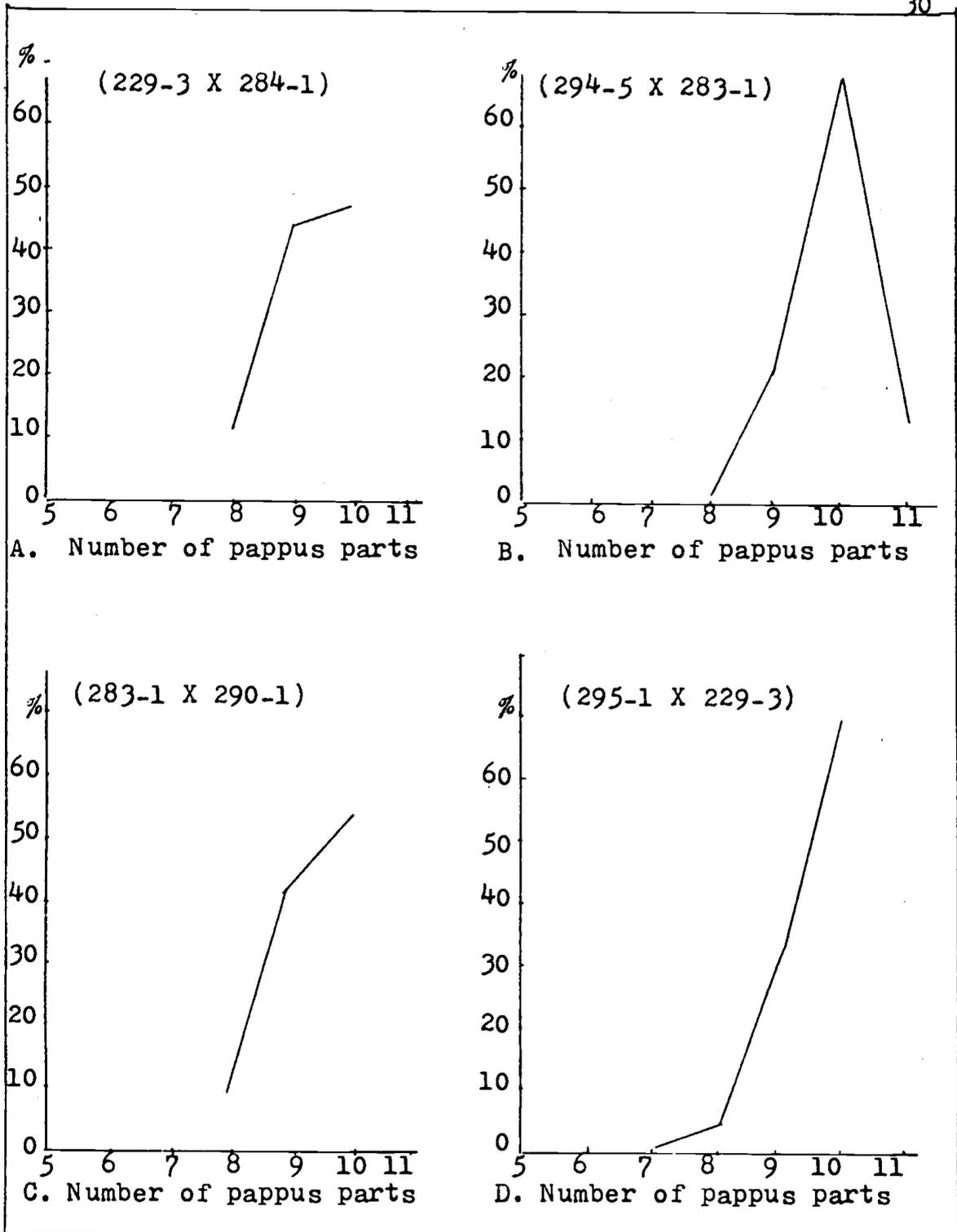


Fig. 3. Pappus-parts distribution in four hybrid crosses where sibling data, being equal, were combined.

accidental cannot be ruled out. It is worthwhile to note that in all four hybrid families the number of pappus-parts per achene is skewed toward the values 9 and 10, and a "high determining" genotype (Bachmann and Price 1978) is therefore indicated.

We now take a closer look at those six crosses in which the pappus-part distribution among siblings was significantly different.

1. (293-1 X 284-6)

Ssp. leptosepala from Trinity Co. X ssp. laciniata from Humboldt Co.

Table 1 shows that the Chi-square value for this cross with 21 degrees of freedom is 100. Since the critical value is 32.67, the null hypothesis that the distribution of pappus-part numbers among the eight siblings is the same was rejected. Figure 4, comparing the siblings, suggests a similarity between siblings 1 and 6. Despite the variation in distribution of pappus parts among the siblings, their means were remarkably close and ranged from 8.59 to 9.59 (Table 2A).

2. (229-3 X 290-1)

Ssp. laciniata from Jackson Co. X ssp. laciniata from Humboldt Co.

For this hybrid, Table 1 also shows a high Chi-square for the test of equal distribution in the number of

pappus parts per achene. Although the siblings were found to be different in pappus distribution, the mean values for this characteristic were close and ranged from 9 to 10 (Table 2B). Figure 5 shows the individual patterns of pappus parts of the separate siblings.

Table 2. Means of pappus parts in siblings of the two best surveyed hybrids, (293-1 X 284-6) and (229-3 X 290-1).

A: (293-1 X 284-6)

Siblings	Mean	N(Achenes)
1	8.75	97
2	9.25	71
3	9.59	69
4	9.47	123
5	9.09	126
6	8.59	126
7	9.08	60
8	9.27	159

B: (229-3 X 290-1)

Siblings	Mean	N(Achenes)
1	9.43	112
2	9.40	70
3	8.74	57
4	8.61	68
5	8.88	124

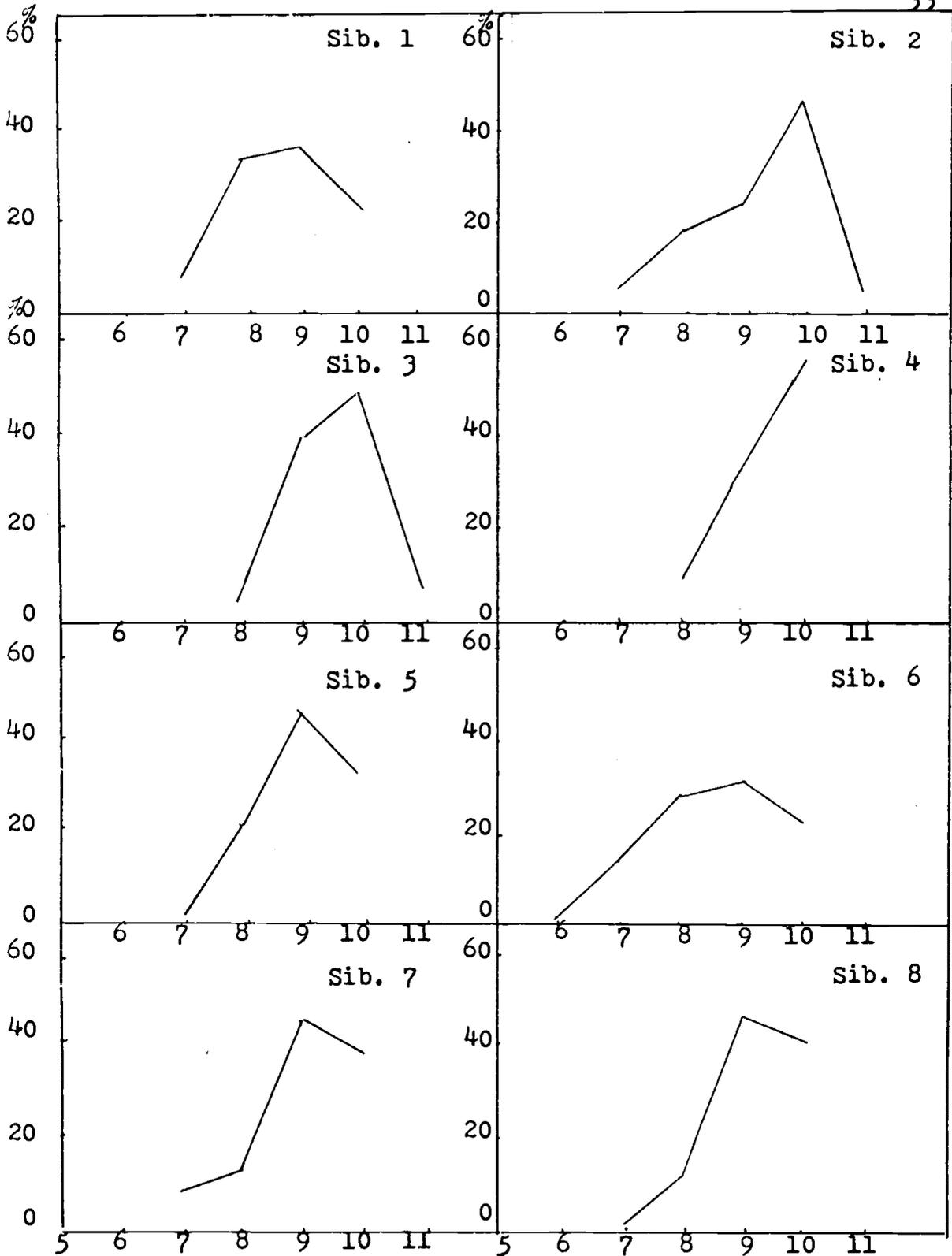


Fig. 5. Distribution of pappus parts in fruits of eight siblings of cross (293-1 X 284-6). On the vertical axis are percentages of achenes and on the horizontal axis the number of pappus parts.

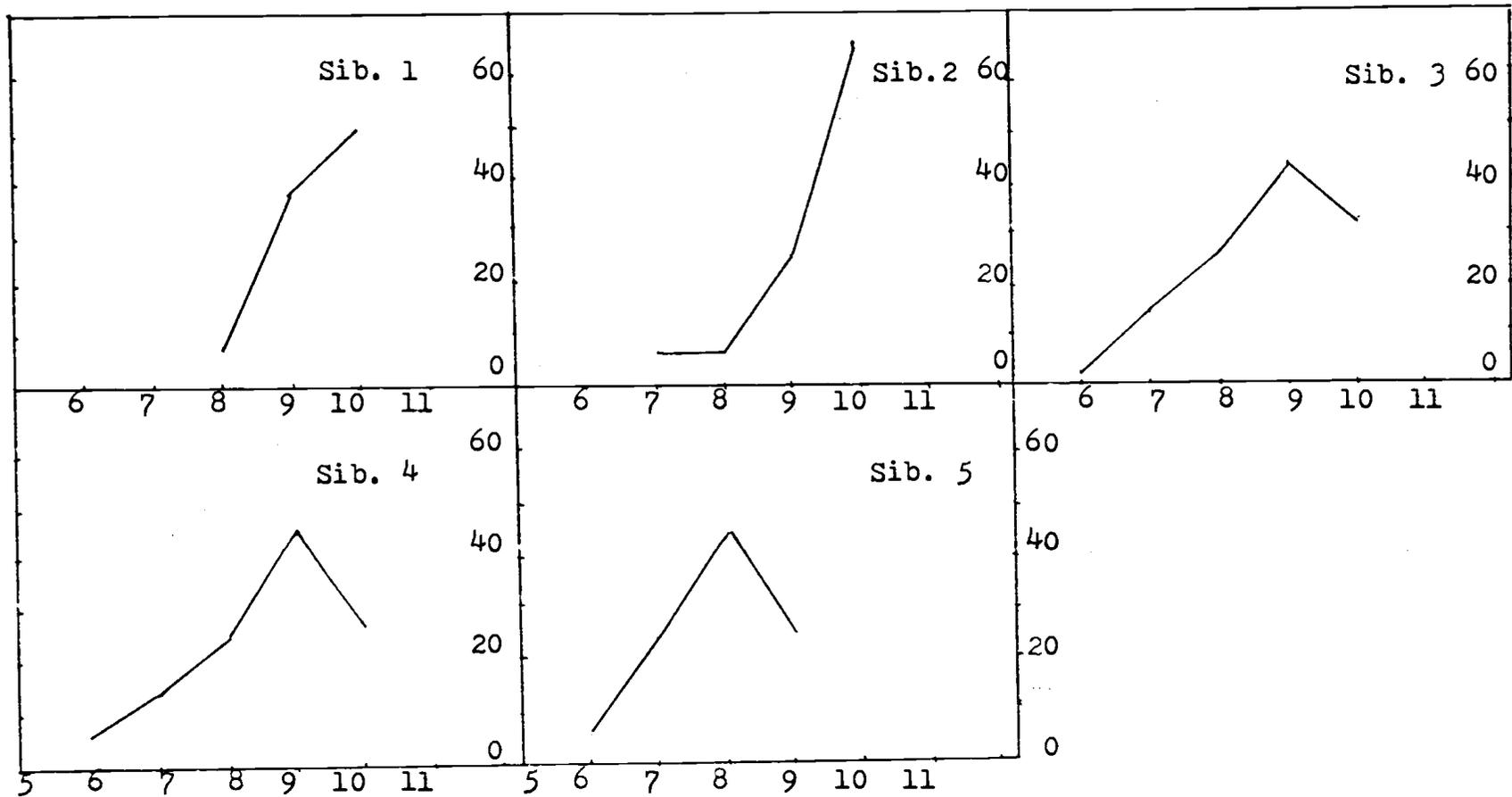
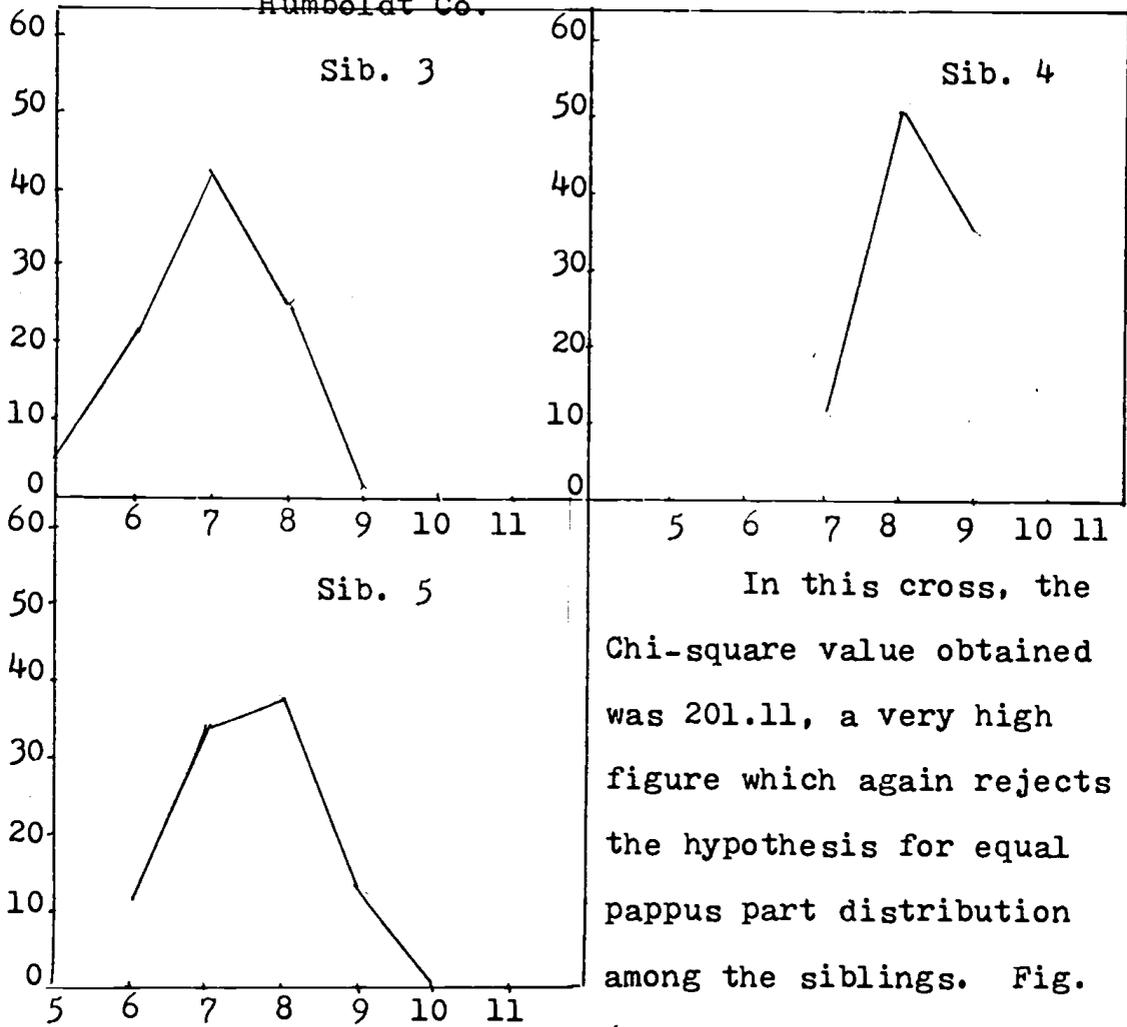


Fig. 5. Distribution of pappus part number in fruits of five siblings of cross (229-3 X 290-1). The vertical axis shows the percentages of achenes and the horizontal axis shows the number of pappus parts.

3. (284 X 290-1)
 ssp. laciniata from Humboldt Co. X ssp. laciniata from Humboldt Co.



In this cross, the Chi-square value obtained was 201.11, a very high figure which again rejects the hypothesis for equal pappus part distribution among the siblings. Fig. 6 seems to indicate either two or three patterns of

pappus-part distribution depending on how one characterizes the similarities of siblings 3 and 5.

Fig. 6. Distribution of pappus part number in fruits of three siblings of cross (284 X 290-1). The vertical axis show the percentage of achenes and the horizontal axis the number of pappus parts.

Ssp. laciniata from Humboldt Co. x ssp. laciniata from Humboldt Co.

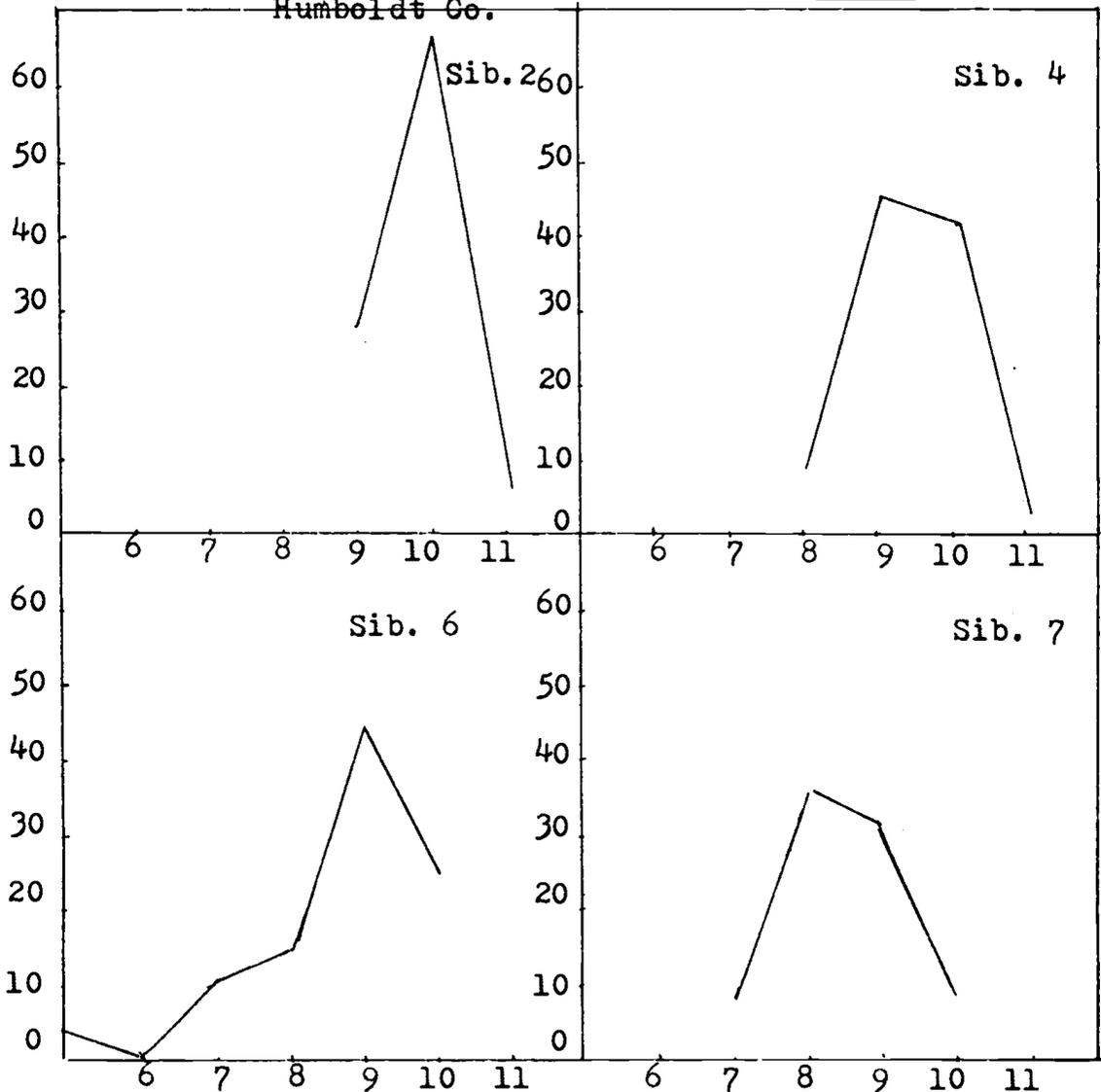


Fig. 7. Distribution in pappus part number in fruits of four siblings of cross (290-2 X 263-9). Horizontal axis shows numbers of pappus parts.

Here, all four individuals differ in their pappus part distribution. Sibling 2 in particular centers around 10 with a very small standard deviation. The other siblings have means of 8's and 9's but larger standard deviations. Sibling 6 is unique in having a distribution ranging from 6 to 10 (Figure 7).

5. (293-4 X 283-1)

Ssp. leptosepala from Trinity Co. X ssp. leptosepala from Del Norte Co.

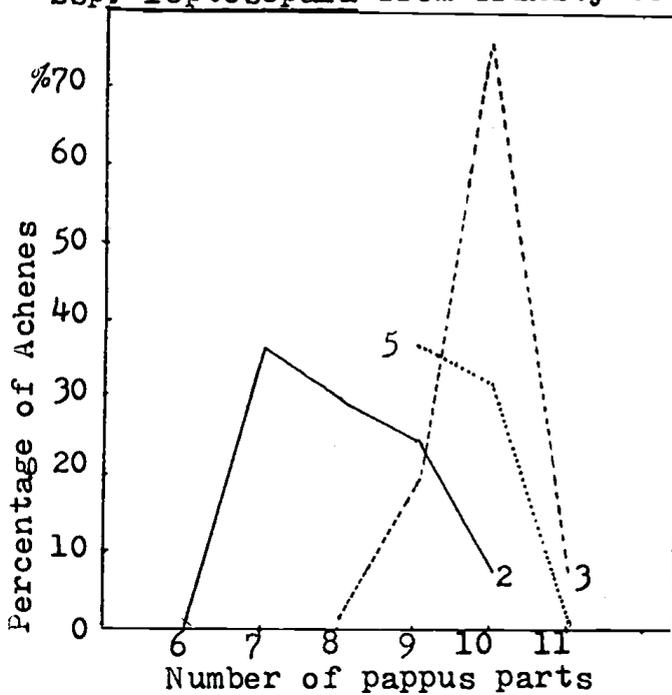


Fig. 8. Distribution in pappus part number in fruits of three siblings of cross (293-4 X 283-1).

Here, all 3 siblings show clear individual differences (Figure 8). Sibling 2 is centered between 7 and 9 while sibling 3 averages close to 10. Sibling 5 seems to fall between the other two.

6. (263-9 X 293-1)

Ssp. laciniata from Josephine Co. X ssp. leptosepala from Trinity Co.

This cross is illustrated in Figure 2C. The two siblings studied are clearly different from each other. Sibling 3 has equal proportions of 9-parted and 10-parted pappi. Sibling 1 is notable for having a pappus-part distribution falling in the lower range of the scale, that is, between 5 and 8. More siblings need to be counted for a better understanding of this cross.

Other crosses are shown here for illustration only, since they are each represented by only one individual (Fig. 9, A, B, C).

As an example of the mathematical analysis possible for pappus patterns found in M. laciniata hybrids, we can combine the data for those siblings in plants whose pappus-part distributions proved to be the same, and plot them next to their expected Poisson distributions. The data fit the Poisson values as was shown previously by Bachmann and Chambers (1977). Figure 10 compares observed distributions pappus parts for these four hybrids with their expected Poisson values.

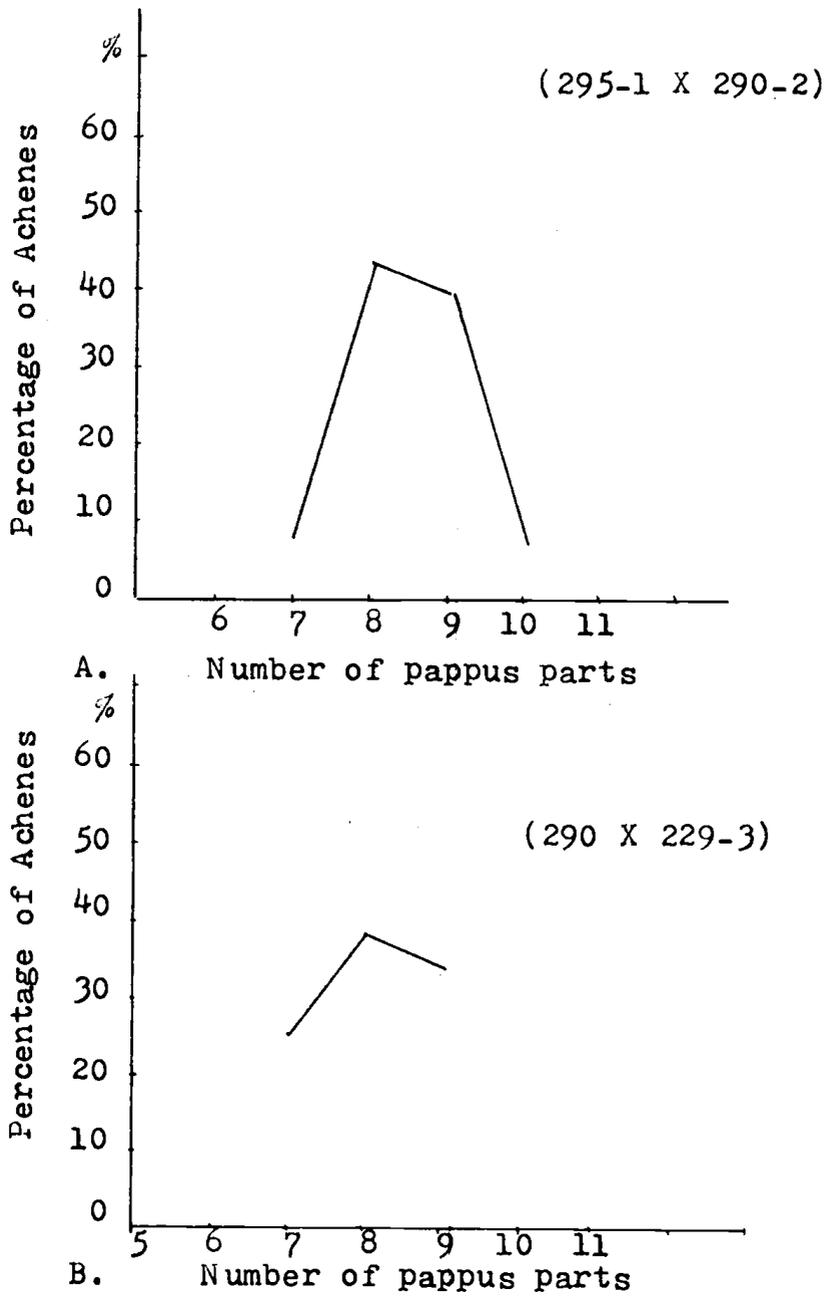


Fig. 9. Distribution in pappus part numbers in three crosses each represented by one offspring.

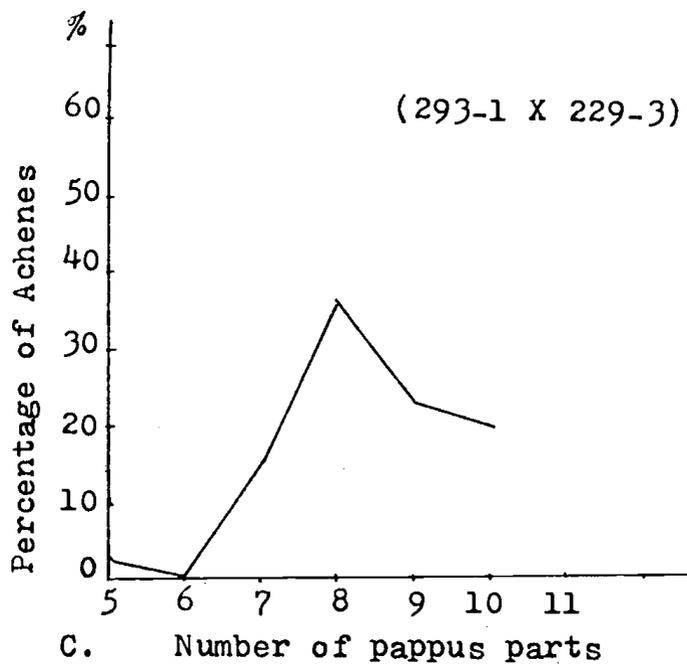


Fig. 9, Continued.

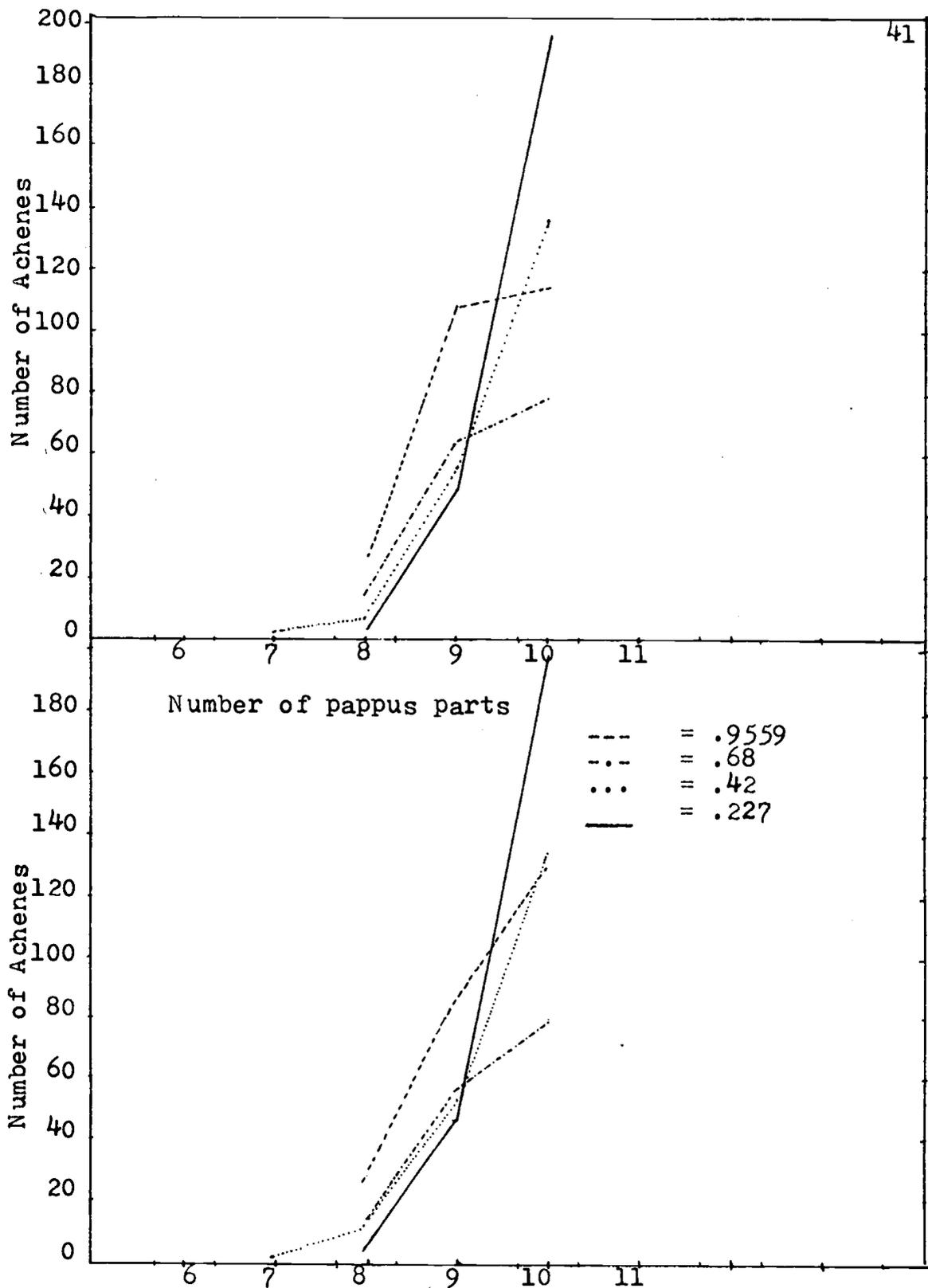


Fig. 10. (A) Observed frequencies of achenes in four hybrid genotypes of *M. laciniata*. Data from siblings were combined since they were found to be the same. (B) Respective observed Poisson distributions for these four hybrids.

Number of Phyllaries per Head

The spirally arranged phyllaries of M. laciniata comprise two more or less distinct series surrounding the florets of the capitulum. The phyllaries of the outer series are separable from the inner ones in being shorter and relatively much wider than the latter. The number of outer phyllaries in a head was found to be less than the number of inner phyllaries. The proportion of outer versus inner phyllaries was tested statistically to determine whether there were significant differences among the plants of each family of hybrids (Table 3). In all crosses studied there were no significant differences between siblings for the ratio of outer versus inner phyllaries.

Three crosses were not tested because the number of outer phyllaries could not be determined exactly (Table 3, crosses 6, 7, 9). These hybrids had an indefinite number of very tiny leaflets that extended from the capitulum downward onto the stem, forming perhaps a third layer of phyllaries. However, the number of inner phyllaries was very much the same for all siblings in each cross. Assuming that the various individuals of the same cross had the same phyllary distribution, we combined the data collected for separate siblings and used it for comparison with the combined data for other crosses. Table 4 shows the number of involucreal phyllaries per capitulum in the

Table 3. Chi-square test to determine whether the ratio of outer to inner phyllaries is the same for the various siblings of each cross. Null hypothesis: ratio sib 1 = ratio sib 2 = ratio sib 3, = etc.

alpha = .95

Crosses	Mean Ratio	χ^2	D.F.	Crit. Val.	N(heads)	N(sibs)
1. (229-3 X 284-1)	.610	2.193	2	5.99	7	3
2. (229-3 X 290-1)	.701	1.440	4	9.49	8	5
3. (284-1 X 290-1)	.516	0.323	2	5.99	6	3
4. (290 X 229-3)	.440	-	-	-	2	1
5. (290-2 X 263-9)	.650	0.571	3	7.91	8	4
6. (293-4 X 283-1)	-	-	-	-	5	3
7. (294-5 X 283-1)	-	-	-	-	4	2
8. (263-9 X 293-1)	.666	0.105	1	3.84	6	2
9. (283-1 X 290-1)	-	-	-	-	3	2
10. (293-1 X 229-3)	.500	-	-	-	2	1
11. (293-1 X 284-6)	.594	0.886	7	14.04	18	8
12. (295-1 X 229-3)	.692	1.00	1	3.84	2	2

Table 4. Means and standard deviations of the total number of phyllaries in ten hybrid crosses of M. laciniata.

Hybrid	Mean	S.D.	N(phyllaries)
1. (229-3 X 284-1)	16.570	2.15	116
2. (229-3 X 290-1)	20.625	2.97	165
3. (284-1 X 290-1)	15.66	1.97	94
4. (290 X 229-3)	19.50	0.71	39
5. (290-2 X 263-9)	20.00	3.55	160
6. (293-4 X 283-1)	-	-	-
7. (294-5 X 283-1)	-	-	-
8. (263-9 X 293-1)	16.66	1.86	100
9. (283-1 X 290-1)	-	-	59
10. (293-1 X 229-3)	18.00	-	18
11. (293-1 X 284-6)	15.06	1.55	271
12. (295-1 X 229-3)	22.00	1.00	44

twelve crosses studied, presented as the means and standard deviations. The numbers of phyllaries form two modes, one with means around 15 and 16 and the other with means ranging from 18 to 22.

Figure 11 gives the phyllary distributions in all the crosses, including means and one standard deviation on each side of the mean. Again, the two trends are easily recognizable. The lower mode comprises crosses 1, 3, 8 and 11. The other hybrids fall into the upper mode. Figure 12 gives the distribution of phyllary numbers in the head of different individuals of a single cross:

(293-1 X 284-6). The very sharp peak at 14 and 15 is evident, showing a strong tendency for morphogenetic canalization of involucre development.

Previous studies in Microseris laciniata subsp. laciniata have indicated a predominance of 21 phyllaries per head, with plants ranging from 18 to 33 (Bachmann and Price 1978). The lower mode of 15-16 in some of the crosses given here may be due in part to environmental effects. Heads of M. laciniata are largest (with the most numerous florets and phyllaries) on robust plants growing under optimum conditions. Our test plants were raised in six-inch diameter clay pots; those reported by Bachmann and Price (1978) were from an outdoor garden. The two experiments are therefore not comparable for the trait of phyllary number. Nonetheless, our data support the hypothesis that M. laciniata is genetically variable with respect to phyllary number, which in turn is an expression of the size of the capitulum.

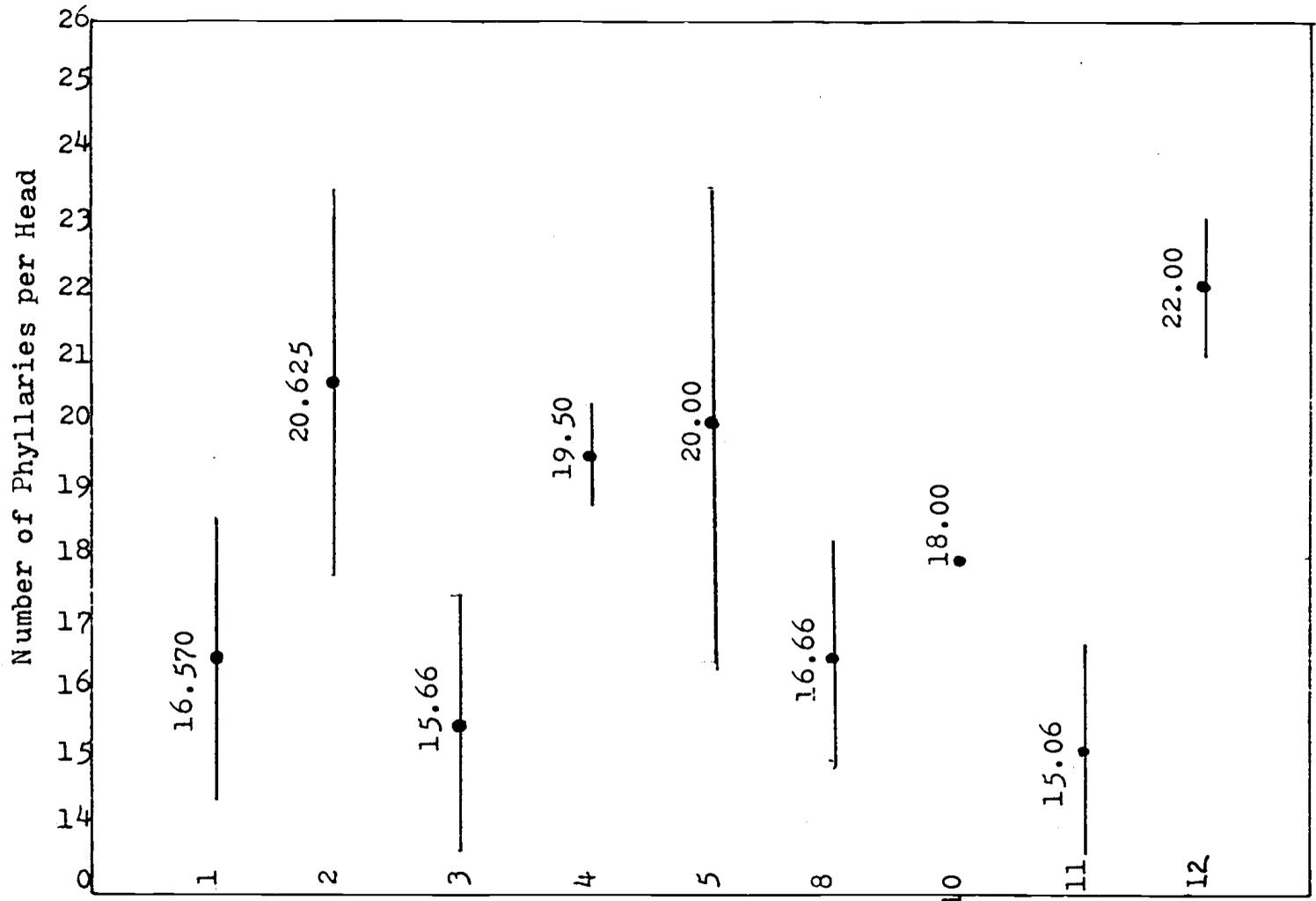


Fig. 11. Diagram of the phyllary distributions of the hybrid crosses studied. The dots represent their respective means and the lines represent one standard deviation to each side of the mean.

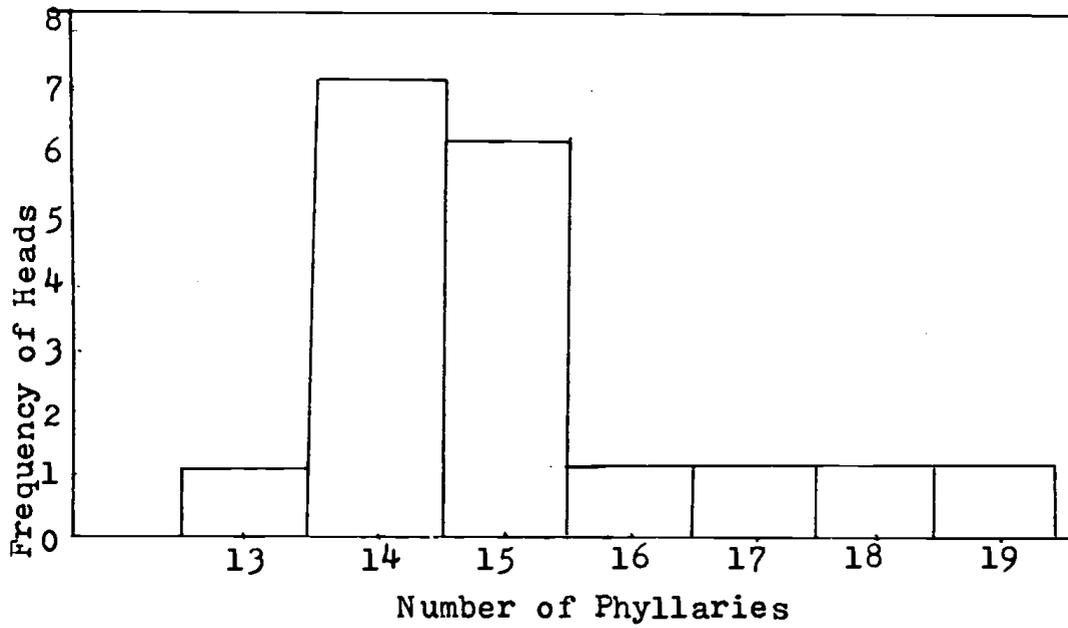


Fig. 12. Histogram showing frequency of phyllaries in cross (293-1 X 284-6).

Fertility of the F 1 Hybrids of *Microseris laciniata*

Seed Production

Cross pollinations involving the F 1 hybrids were carried out in the greenhouse during the months of March and April, when flowering in *Microseris* reaches its peak. Whenever possible these crosses were done with sister plants, although some were done with half-sib plants. The fruits produced were examined for the presence of a normal embryo, and the percentage of pollinations that yielded such fertile fruits was calculated. The results are given in Figure 13, where the various F 1 hybrids are listed in order of increasing seed fertility.

The hand pollinations of these intraspecific hybrids in a greenhouse environment gave widely varying success in seed formation. Sometimes a particular sibling plant would yield one capitulum with no fertile fruits and a second capitulum with mostly normal fruits, for example. The success of sibling crosses ranged as widely as 0-100% in a single family of F 1's (e.g., 293-4 X 283-1). There was no tendency for F 1's within the same subspecies to be more fertile than F 1's between species. One of the least fertile crosses (229-3 X 284-1) was between populations of ssp. laciniata, and some of the most fertile hybrids (283-1 X 290-1 and 295-1 X 229-3) were between ssp. laciniata and ssp. leptosepala. Another interesting result

was that sibling crosses were sometimes less productive of fertile fruits than half-sib crosses (pollinations where the F 1's being tested had one parent in common). This is seen in (294-5 X 283-1) and (290-2 X 229-3). The low seed productivity of many sibling crosses, despite their high pollen fertility (see next section) is suggestive of shared self-incompatibility factors among sister plants. A high percentage of cross-incompatibility would be expected in sib crosses. Plants of M. laciniata are nearly always found to be highly self-incompatible. Some F 1 individuals would have inherited identical self-incompatibility alleles, therefore, while plants from different F 1 families are most likely to possess different alleles and be cross-compatible.

It was observed at the time of pollination that the stigma was sometimes too dry for pollen grains to stick to it, even during its most receptive period. Plants with sporophytic incompatibility are expected to have dry stigmas. When hand pollination was attempted for these plants, it was much more difficult and time-consuming than for other plants in which the applied pollen would easily stick to the stigmatic surface. A small experiment was set up to find out whether there was a stigma factor involved that would either promote or prevent pollen germination. This experiment was not carried enough times for final conclusions to be drawn. It consisted of washing the

stigmatic surface in warm water prior to pollination, by dipping the entire flower head into a beaker with 50°C water for 45 seconds. In all three hybrids tested, pollinations performed in these clean stigmas were more successful than pollinations involving the same sibling plants without the warm water treatment. For example, without treatment, the cross (293-4 X 283-1) 5 X sib 4 resulted in zero percent seed fertility. With treatment the same cross resulted in 43% fertility. Other example is (294-5 X 283-1) 8 X (283-1 X 290-1) 2 that with treatment gave 46% fertility, while (294-5 X 283-1) 8 X sib 4 gave zero percent fertility. A more systematic experiment needs to be designed in order to investigate incompatibility system and pollen-stigma interaction in Microseris laciniata.

Figure 13. Seed fertility of the F 1 hybrid families.

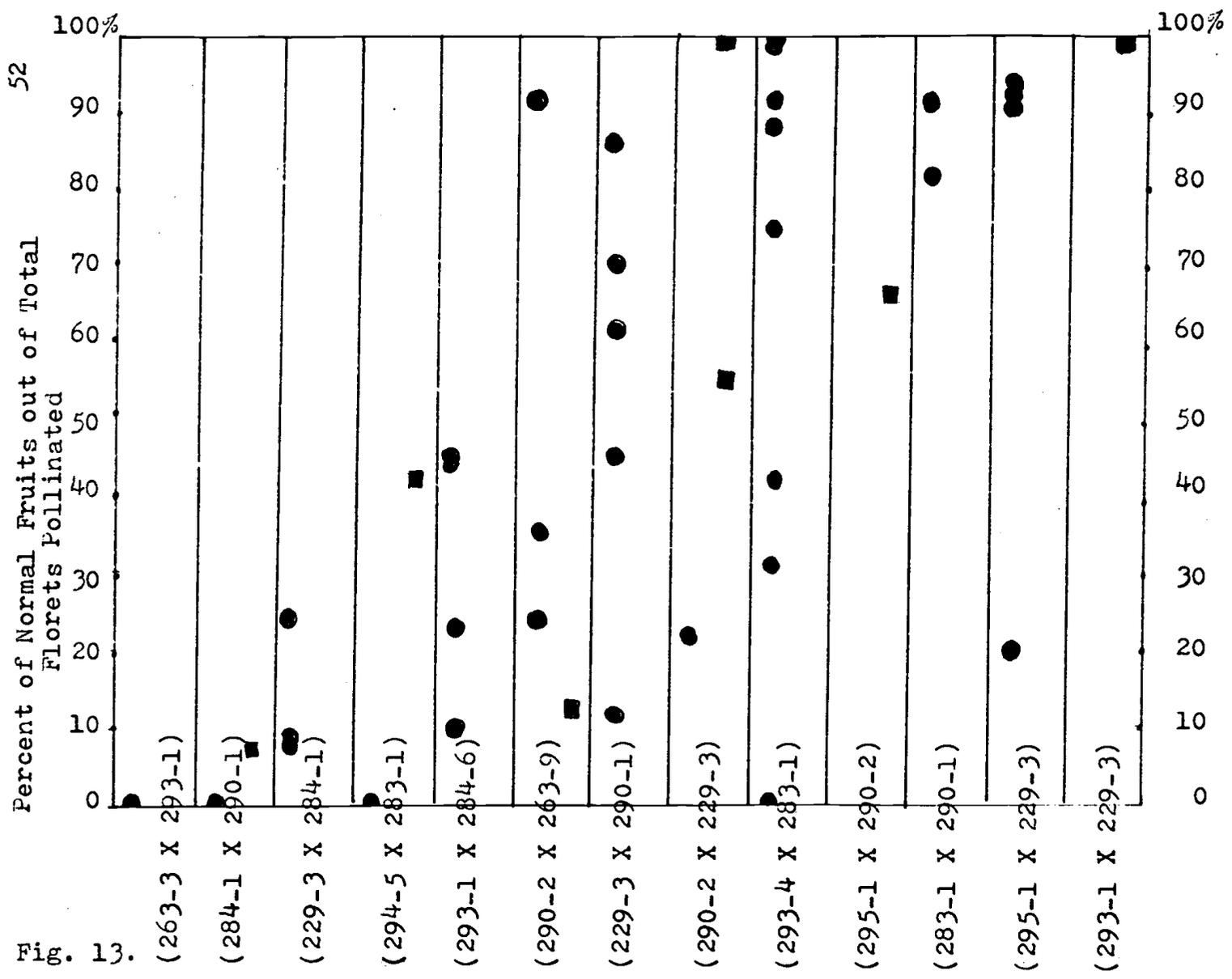
Each symbol represents a single capitulum on which a sample of florets was crossed to a particular pollen parent.

● = pollen parent a sibling of the seed plant;

■ = pollen parent a half-sib of the seed parent (i.e. F 1's having one parent in common).

Data derived from one or more plants of each F 1 family.

Fig. 13.



52

100%

0

Pollen Stainability

Pollen stainability was investigated for eight intraspecific and three interspecific hybrids of Microseris laciniata. One interspecific hybrid involving two other species of Microseris was also investigated.

Among the intraspecific hybrids sampled, only two had markedly reduced pollen stainability: (229-3 X 284-1) 1 with 68.6% stainable pollen and (293-1 X 284-6) 2 with 51%. All the other hybrids had more than 90% of their pollen grains stained. One interspecific hybrid involving Microseris laciniata ssp. leptosepala (294-5) times Microseris sylvatica (304) showed approximately sixty percent stainable pollen as did a hybrid between M. paludosa (168) times M. nutans (234-1). Two hybrids involving M. paludosa (261) times M. laciniata ssp. leptosepala showed a remarkably high amount of stainable pollen, exceeding ninety-five percent. Figure 14 illustrates the percentages of pollen stainability for the hybrids studied.

Pollen Germinability

Results of four attempts to observe in vivo pollen germination under the U.V. microscope are listed below:

<u>Crosses attempted</u>	<u>Observations</u>
1. (229-3 X 284-1) 1 X sib 1	fluorescent dots on the stigma
2. (229-3 X 290-1) 1 X self	fluorescent dots on the stigma
3. (229-3 X 290-1) 1 X sib 5	2 pollen tubes in the style

Crosses attemptedObservations

4. (290-2 X 263-9) 1 X sib 4 2 pollen tubes in the style

The presence of fluorescent dots observed on the stigmatic surface in the first two crosses is very much like the "rejection reaction" described by Heslop-Harrison (1975) for other members of the Compositae family. In the last two crosses, the occurrence of only two pollen tubes could have been due to a chance that most pollen grains present shared the same incompatibility alleles with the stigma. The results of these limited tests do support the suggestion, made elsewhere in the paper, that the seed-set was low in many sibling crosses because self-incompatibility genes inhibited pollen germination between closely related plants.

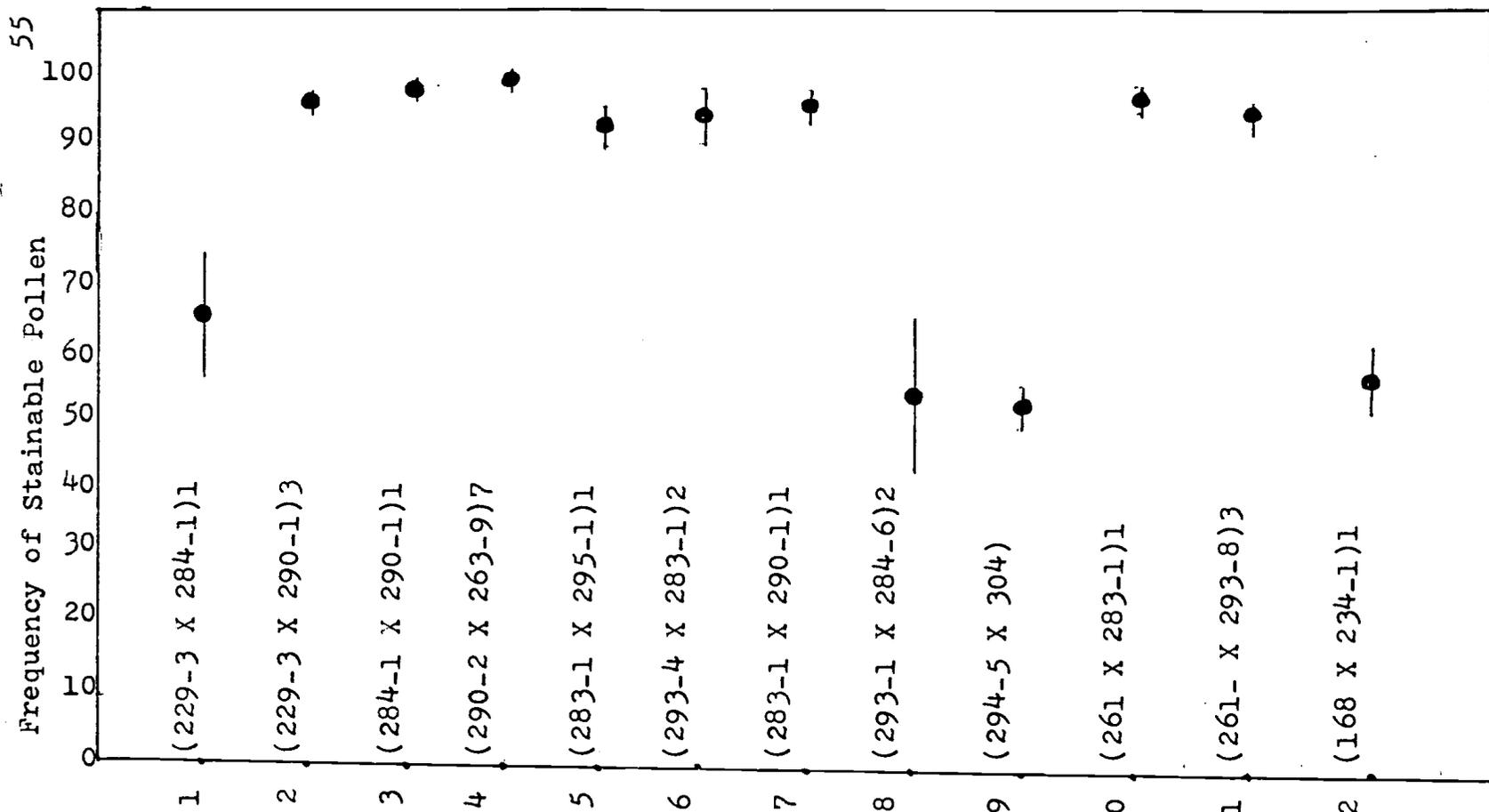


Fig. 14. Distribution of pollen stainability in both intraspecific (1-8) and interspecific (9-11) hybrids of Microseris laciniata and in one hybrid (12) between two related Microseris species. The dots indicate their respective means; the line through each mean represents one standard deviation in each direction.

Chromosomal Behavior at Meiosis

In order to determine the occurrence of chromosomal irregularities that could possibly account for the reduced fertility of some hybrids, permanent microslides were made showing dividing microspore-mother cells of seven intra-specific Microseris laciniata hybrids. For comparison, additional hybrids were studied from four interspecific crosses involving M. laciniata, M. borealis, M. nutans, and M. paludosa.

Table 5 shows the results of the meiotic studies, expressed as the percentage of apparently normal cells at each stage, for all the particular stages visible on each prepared slide.

The irregularities found in each hybrid are described as follows:

1. (229-3 X 290-1)

Loose association between bivalents was seen consistently during Prophase I (Fig. 15a). At Metaphase I two bivalents often appeared fused by a "sticky" contact (Fig. 15b). Anaphase I and Telophase I showed lagging chromosome arms but no bridges.

2. (284-1 X 290-1)

The visible stages of this slide showing first meiotic division were represented by very few cells (Fig. 15c, d; 16a). Anaphase I and Telophase I showed some

lagging (Fig. 16b).

3. (290 X 263-9)

During Prophase I and sometimes Metaphase I, two or three bivalents seemed associated with one another (Fig. 16c). Also, during Prophase I, a fragment was sometimes seen. Other stages were not available for study.

4. (283-1 X 295-1)

At Prophase I, including Diakinesis, two or three bivalents seemed associated with one another (Fig. 17a). Also, at these stages, two homologues sometimes did not pair, giving the appearance of ten pairs instead of the predictable nine (Fig. 16d). The unpaired chromosomes could be seen occasionally at Metaphase I (Fig. 17c, d). The few cells available at Anaphase I showed lagging but no bridges.

5. (283-1 X 290-1) 2

Prophase I showed association between two or three bivalents (Fig. 18a, b) and this association continued through Metaphase I (Fig. 19a, b). At this later stage, occasionally two homologues were unpaired (Fig. 19a). Fragments were observed at Metaphase I and Anaphase I (Fig. 19b, c). Lagging and bridges were also observed during Anaphase I (Fig. 20a, b, c, d; 21a, b).

6. (283-1 X 229-3)

Here, Diakinesis stages showed chromatid fragments associated with some bivalents (Fig. 22c, d). An early separating bivalent was seen during Metaphase I (Fig. 23b). Anaphase I showed lagging and an occasional fragment (Fig. 23c).

7. (283 X 291-5)

This hybrid involves a cross between M. laciniata and M. borealis, distantly related species that are classified in separate subgenera. Metaphase I showed bivalents misaligned on the spindle plate (Fig. 24d; 25b). Fragments and univalents were noted at this stage (Fig. 25b). Some of the bivalents could be seen to be heteromorphic (Fig. 25b).

8. (290-2 X 291-4)

This was also a hybrid of M. laciniata times M. borealis. Diakinesis and Metaphase I showed bivalents associated with one another or displaced from the spindle plate (Fig. 26b). Metaphase I showed 8 to 11 chromatic units, some of which could correspond to the fragments or the unpaired homologues seen outside the spindle plate in some other cells at Metaphase I (Fig. 26c). Heteromorphic bivalents were seen (Fig. 26d).

9. (261 X 293-8)2

This hybrid was from a cross between M. paludosa and M. laciniata. Association between bivalents was seen during Diakinesis and Metaphase I (Fig. 28a). Chromosomal fragments were observed at Prophase I and Metaphase I (Fig. 28d). At Metaphase I, bivalents were often not aligned in a straight row (Fig. 27d). Often one bivalent was seen completely off the spindle during Metaphase I and Anaphase I. Anaphase I showed lagging, and Telophase I seemed to have some irregular distribution of genetic material.

10. (294-5 X 304)

Association between two or three bivalents was seen at Prophase I and Metaphase I (Fig. 29a, b). Pairing at Metaphase I seemed normal, but the bivalents were not well aligned on the equatorial plate. Many of the cells studied had chromatic units numbering other than nine (Fig. 29c), ranging from eight to ten. Also at Metaphase I, fragments as well as unpaired chromosomes were regularly present (Fig. 29b). Anaphase I showed a considerable amount of delayed separation and bridges (Fig. 29d, 30a).

11. (295-1 X 290-2)

The late meiotic stages available for this hybrid showed a high percentage of normal cells (Fig. 30, b, c, d).

Table 5. Summary of meiotic studies in seven intraspecific hybrids of *M. laciniata* and four interspecific hybrids involving *M. laciniata* times *M. borealis* (291-5, 291-4), *M. sylvatica* (304), and *M. paludosa* (261). T = total number of cells; %N = percentage of normal cells.

Hybrid Plants		Prophase I		Metaphase I		Anaphase I		Telophase I		Telophase II		Pollen Tetrad		
		T	%N	T	%N	T	%N	T	%N	T	%N	T	%N	
		Diff. Sub-Species	Same Sub-Species	(229-3 X 290-1)3	5	100	107	93	10	65	45	100	0	-
(284-1 X 290-1)5	5			80	6	100	0	-	0	-	50	100	100	100
(290 X 263-9)	55			90	19	100	0	-	0	-	0	-	0	-
(283 X 295-1)	10			100	45	89	0	66	8	79	0	-	80	100
Diff. Sub-Species	Diff. Species	(283 X 290-1)	11	90	132	87	10	50	21	100	0	-	0	-
		(283 X 229-3)	30	83	19	97	4	50	8	100	0	-	3	100
		(295 X 290-2)	0	-	0	-	0	-	1	0	50	96	95	100
		(283 X 291-5)	10	70	45	80	6	50	80	97	35	95	0	-
Diff. Species	Diff. Species	(290-2 X 291-4)	5	10	10	50	15	33	5	100	15	15	0	-
		(261 X 293-8)	15	62	30	80	0	-	15	33	0	-	0	-
		(294 X 304)	20	75	88	84	19	55	30	94	35	100	90	100

Figure 15:

Fig. 15a: (229-3 X 290-1)3

Late Prophase I showing an association between two of nine bivalents (lower right hand corner).

Fig. 15b: Same

An apparent normal Metaphase I with overlapping of two bivalents. In the upper left-hand corner there appears to be a chromosomal fragment.

Fig. 15c: (284-1 X 290-1)

Prometaphase showing what seems to be eight bivalents, one univalent, and possible trivalent.

Fig. 15d: Same

Normal Metaphase I.

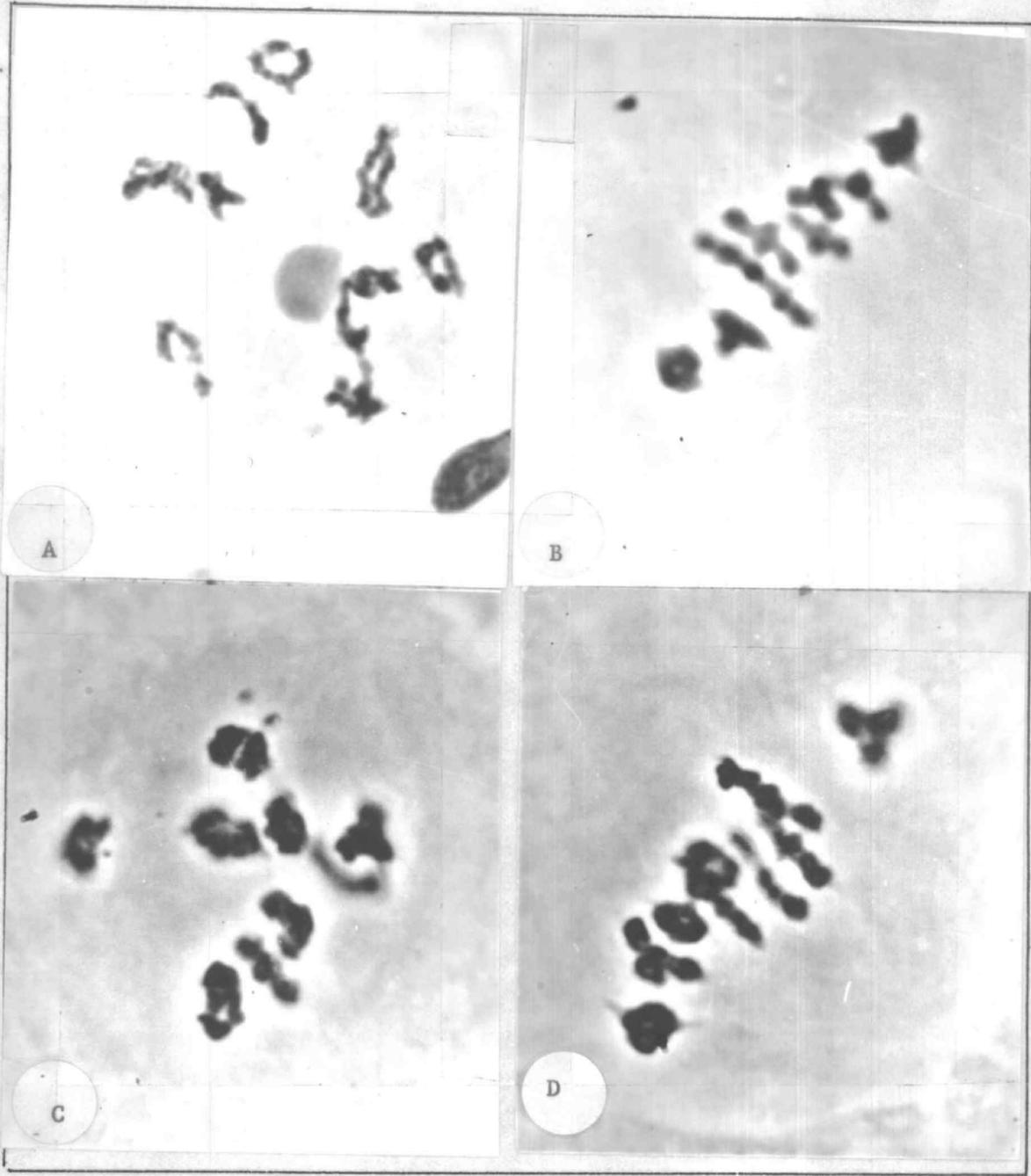


Fig. 15

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Figure 16:

Fig. 16a: (284-1 X 290-1)5

Metaphase with apparently eight bivalents and one univalent. The univalent on the right hand corner seems to be associated with a bivalent.

Fig. 16b: Same

Anaphase I.

Fig. 16c: (290 X 263-9)

Stage just prior to Metaphase I showing association between four or five bivalents.

Fig. 16d: (283-1 X 295-1)

Prometaphase with eight bivalents and two loosely associated univalents.

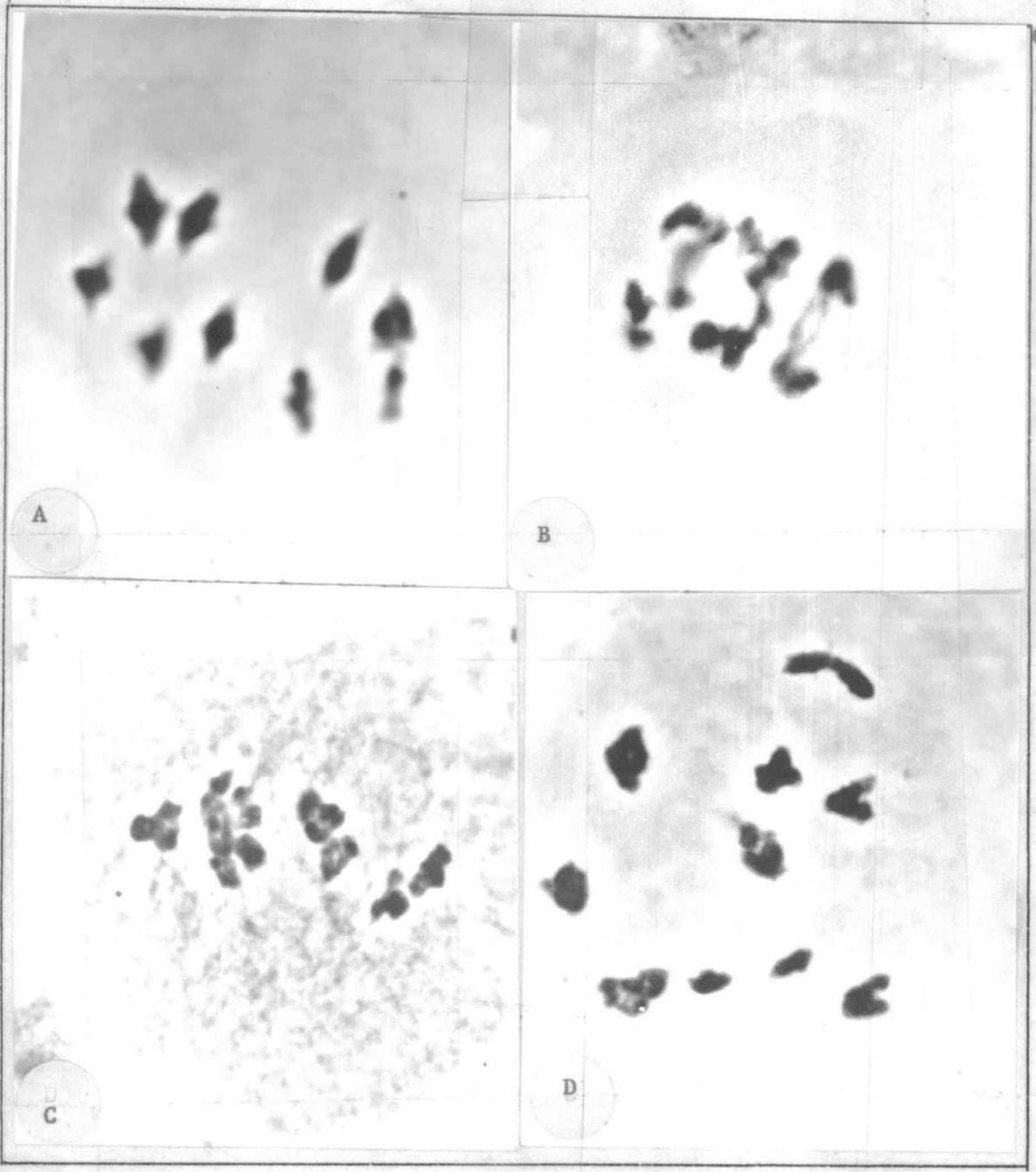


Fig. 16

Figure 17:

Fig. 17a: (293-1 X 295-1)2

Prometaphase showing an association or stickiness between three bivalents.

Fig. 17b: Same

Fig. 17c: Same

Metaphase I. This cell resembles that in Figure 16d. Here two univalents are on the left side of the plate and are faintly connected as though a pair had separated precociously.

Fig. 17d: Same.

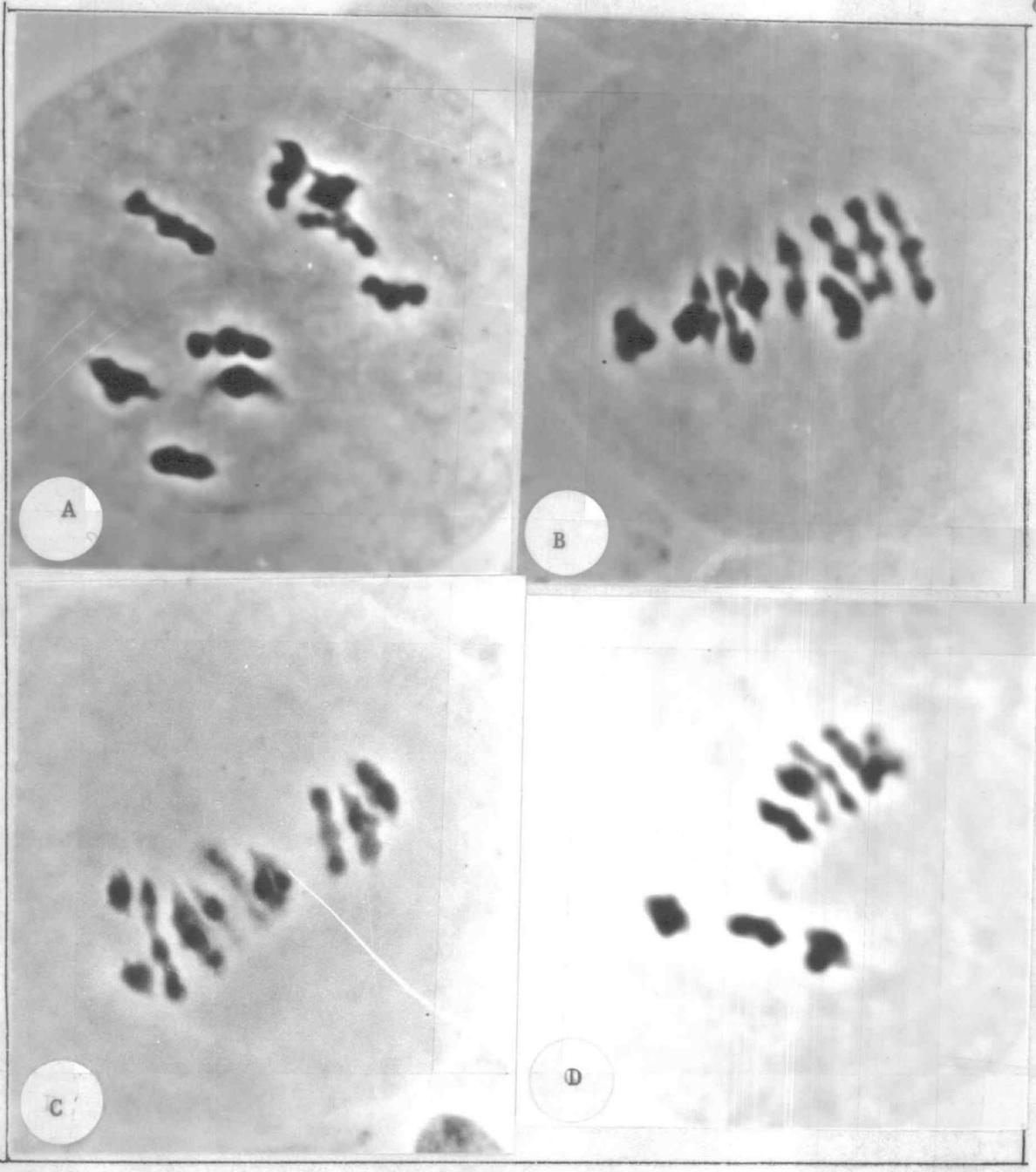


Fig. 17

Figure 18:

Fig. 18a: (283-1 X 290-1)2

Prophase I in a Diplotene stage showing paired chromosomes with chiasmata.

Fig. 18b: Same

Prophase I at Diakinesis stage, showing associations between some of the bivalents.

Fig. 18c: Same

A normal Prometaphase.

Fig. 18d: Same

Prometaphase showing possible association between bivalents.

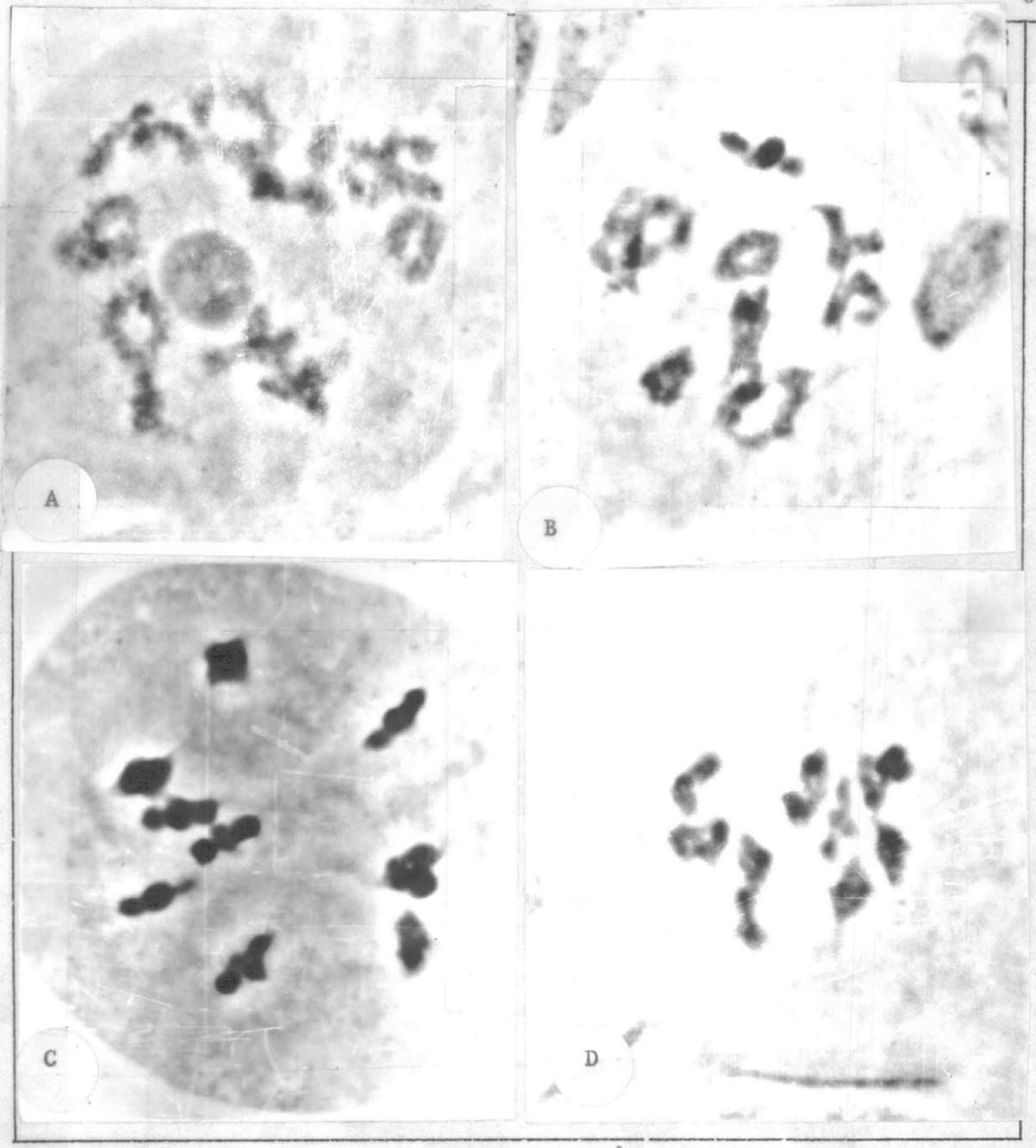


Fig. 18

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Figure 19:

Fig. 19a: (283-1 X 290-1)2

Metaphase I showing two univalents or a precociously separated pair.

Fig. 19b: Same

Metaphase I showing association between four bivalents.

Fig. 19c: Same

Metaphase I showing a probable quadrivalent, below, and a fragment, above.

Fig. 19d: Same

Metaphase I with nine bivalents. A large fragment is seen off the spindle plate.

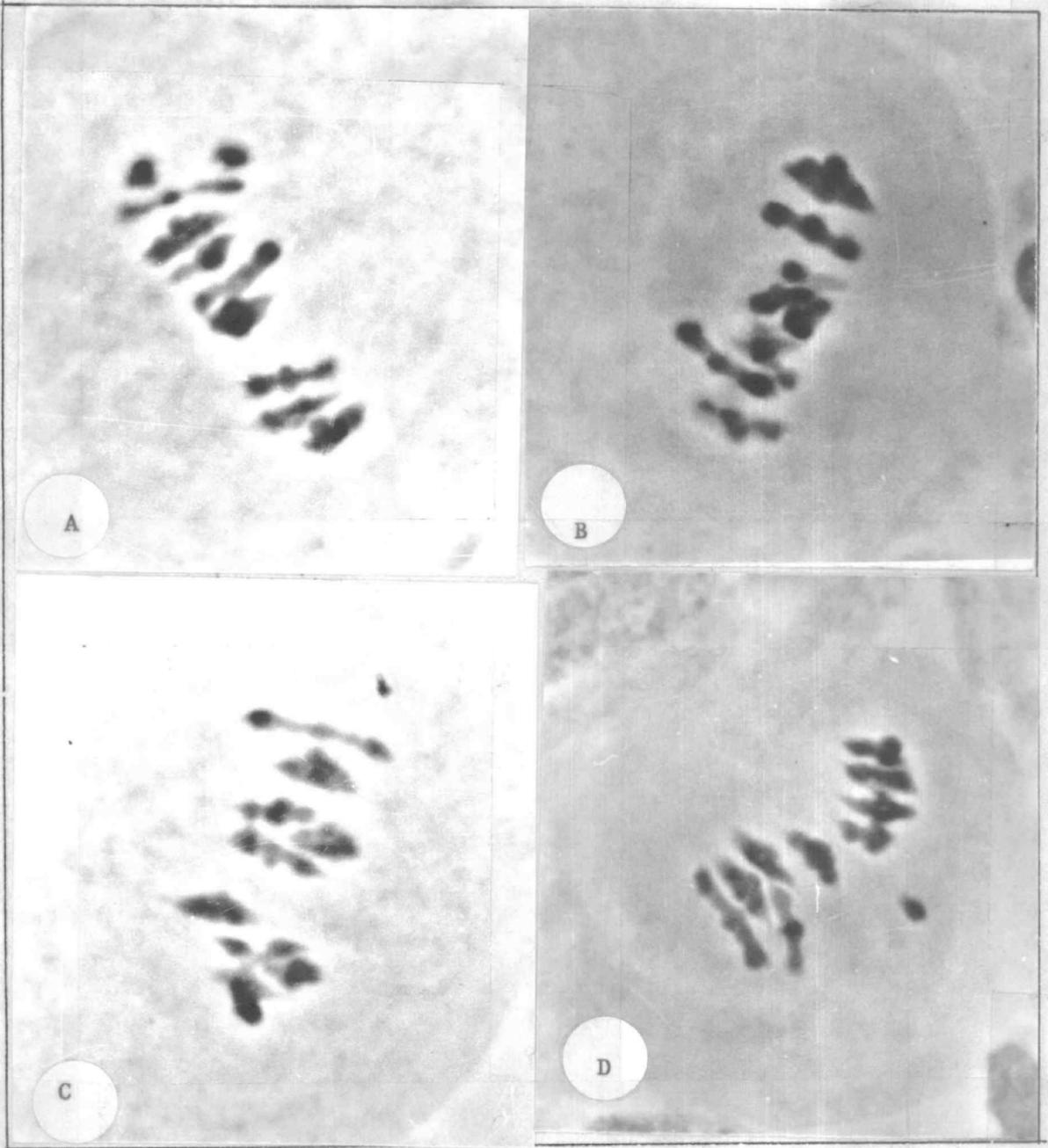


Fig. 19

Figure 20:

Fig. 20a: (283-1 X 290-1)2

Anaphase I, with late separation of two pairs in the center and a third pair at the top.

Fig. 20b: Same

Late Anaphase I showing late separation and possible bridging in the center. Nine chromosomes are counted at the upper pole.

Fig. 20c: Same

Telophase I with apparent bridge.

Fig. 20d: Same

Telophase I. Nine chromosomes can be counted at the upper pole. A delayed separation and possible breaking of chromosome arms is seen on the left and right sides. The piece on the right could be a fragment or a badly stretched chromatid.

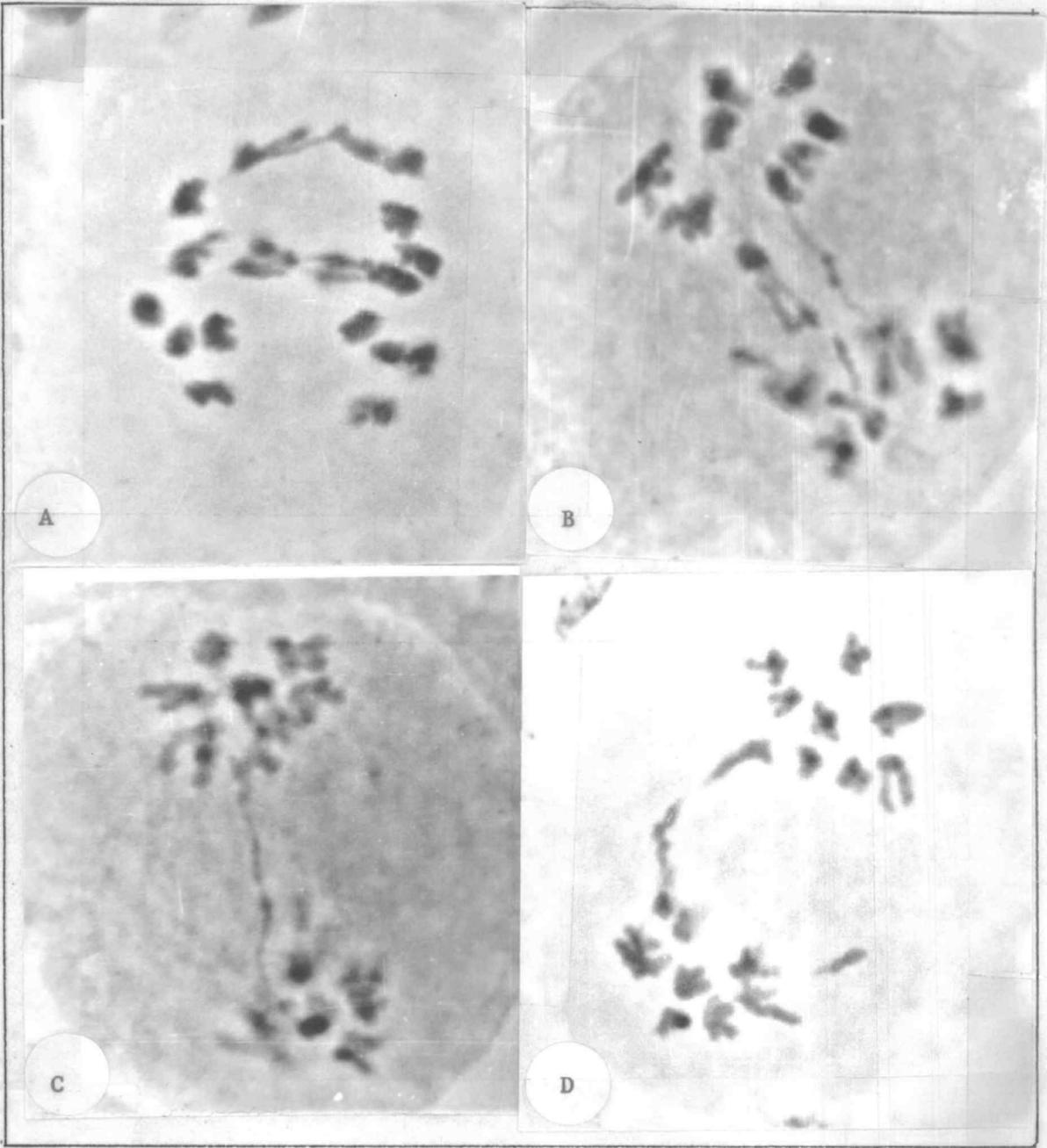


Fig. 20

Figure 21:

Fig. 21a: (283-1 X 290-1)2

Telophase I with bridge.

Fig. 21b: Same

The same cell as in Figure 21a. focused at a
different plane.

Fig. 21c: Same

Normal tetrads of haploid nuclei.

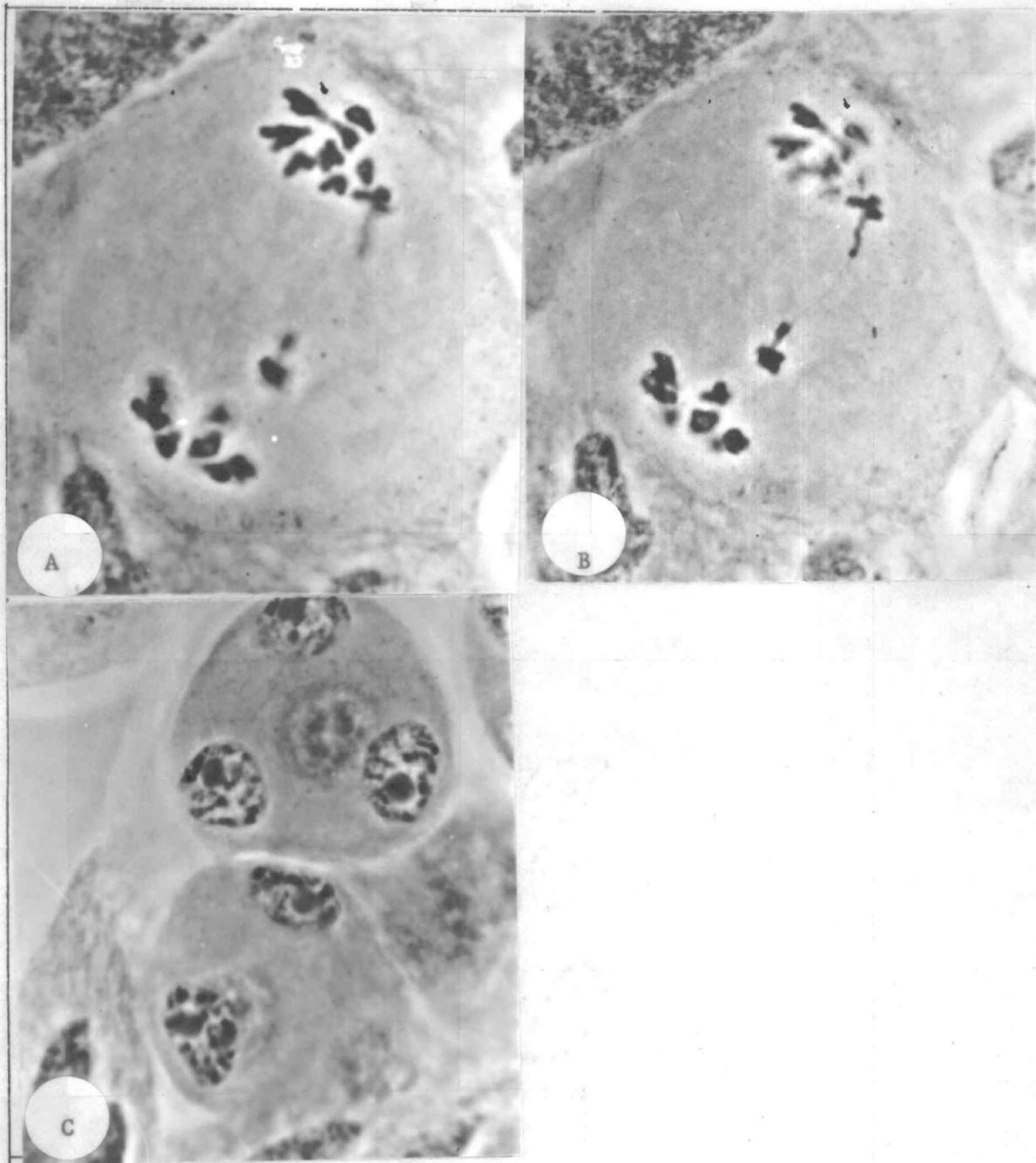


Fig. 21

Figure 22:

Fig. 22a: (283-1 X 229-3)1

Diakinesis showing association between two pairs.

Fig. 22b: Same

Normal Diakinesis.

Fig. 22c: Same

Diakinesis with ten chromatic units, a probable fragment at the left.

Fig. 22d: Same

Diakinesis with ten chromatic units, as above.

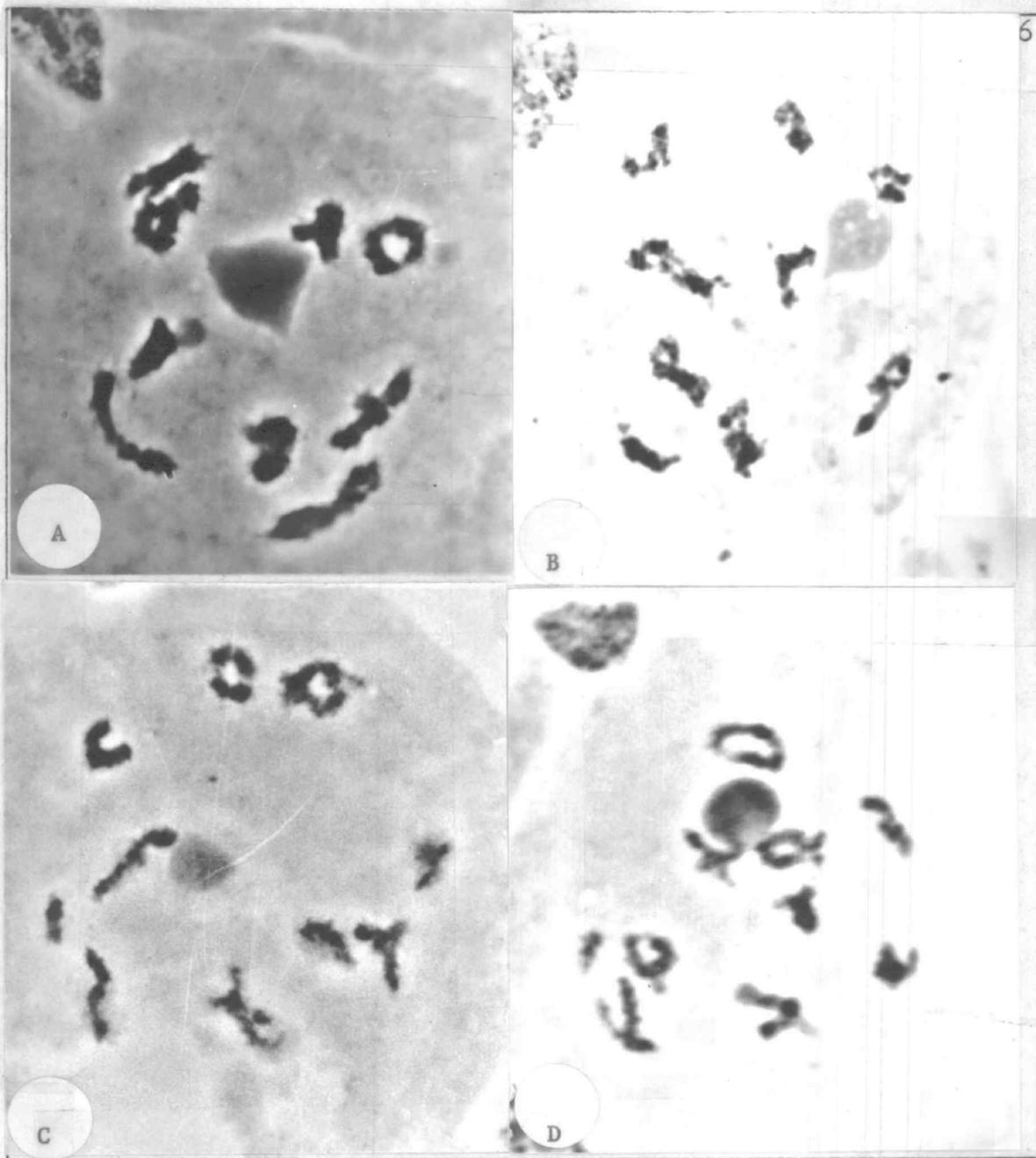


Fig. 22

Figure 23:

Fig. 23a: (283-1 X 229-3)1

Normal Diakinesis

Fig. 23b: Same

Metaphase I showing ten chromatic units.

Fig. 23c: Same

Anaphase I. Nine chromosomes can be counted in the lower pole. Some pairs are late in separating.

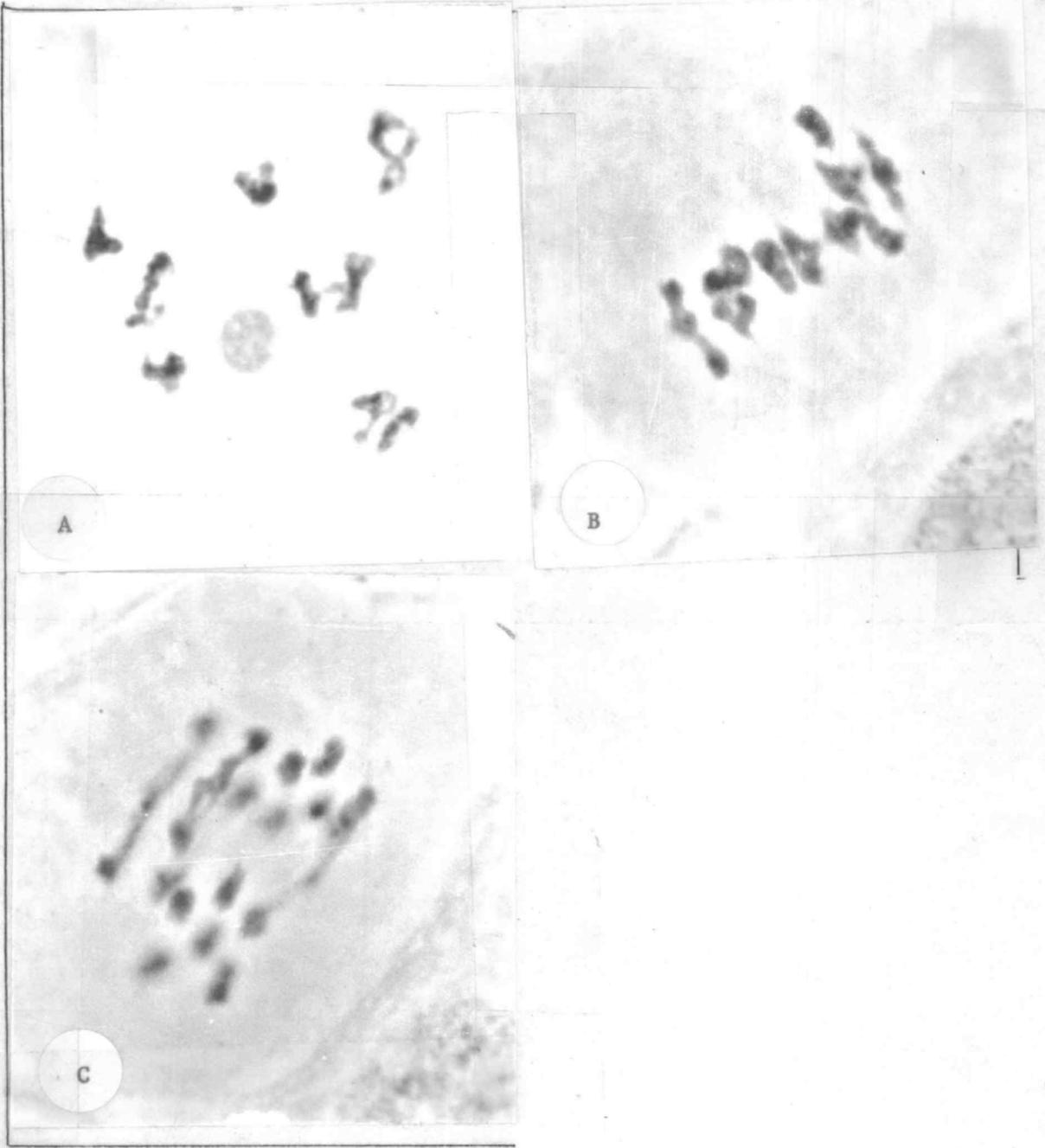


Fig. 23

Figure 24:

Fig. 24a: (283 X 291-5)

Normal Prometaphase.

Fig. 24b: Same

Prometaphase with one association between bivalents.

Fig. 24c: Same

Prometaphase showing heteromorphic pair and a multivalent.

Fig. 24d: Same

Metaphase I showing heteromorphic pairs and probable multivalent.

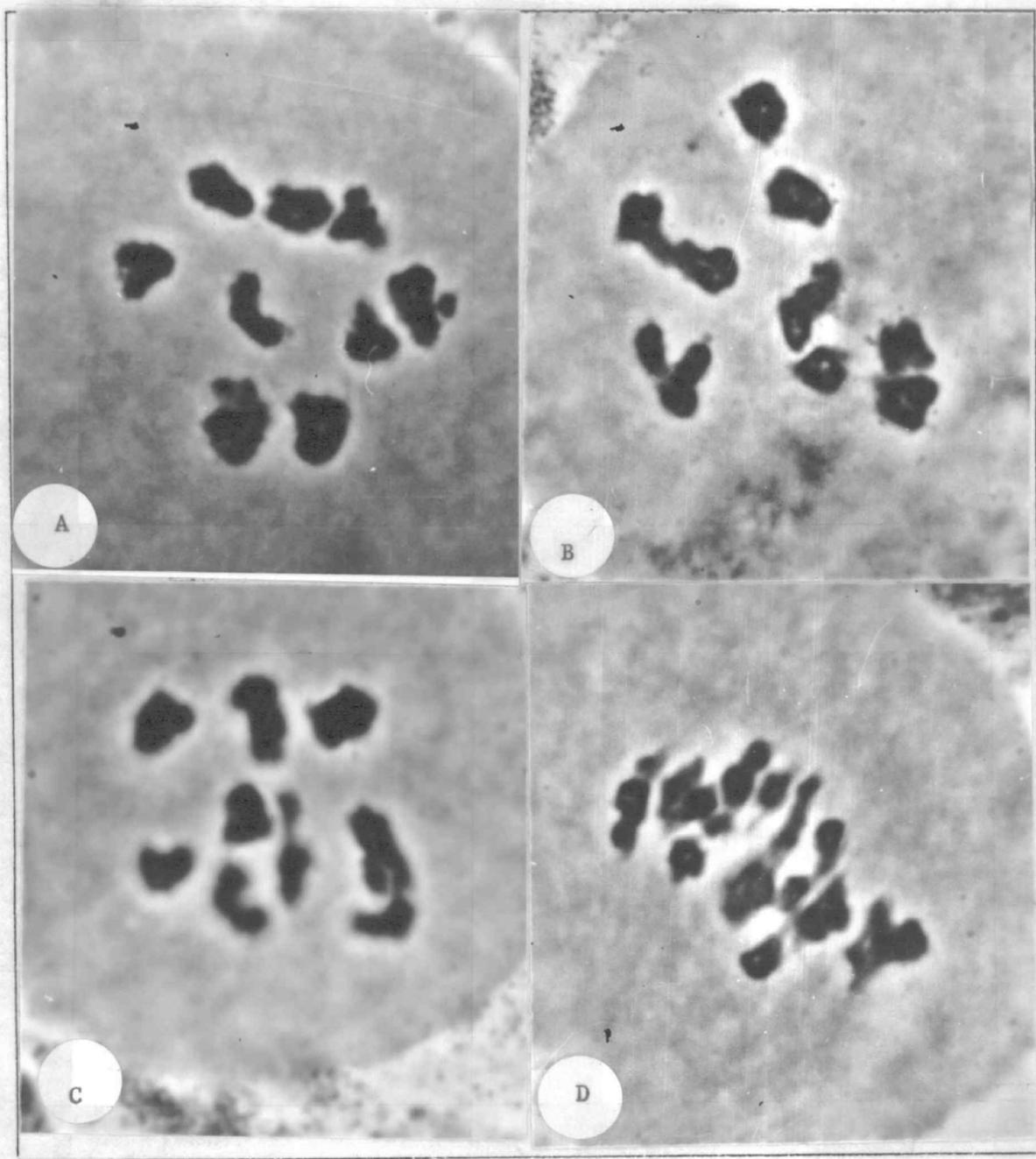


Fig. 24

Figure 25:

Fig. 25a: (283-1 X 291-5)

Squashed anther locule showing six dividing microspore-mother cells.

Fig. 25b: Same

Metaphase I. Some of the bivalents are irregularly formed and unequal.

Fig. 25c: Same

Metaphase with irregular multivalents above.

Fig. 25d: Same

Telophase I. In addition to four fragments, nine chromosomes can be counted at each pole.

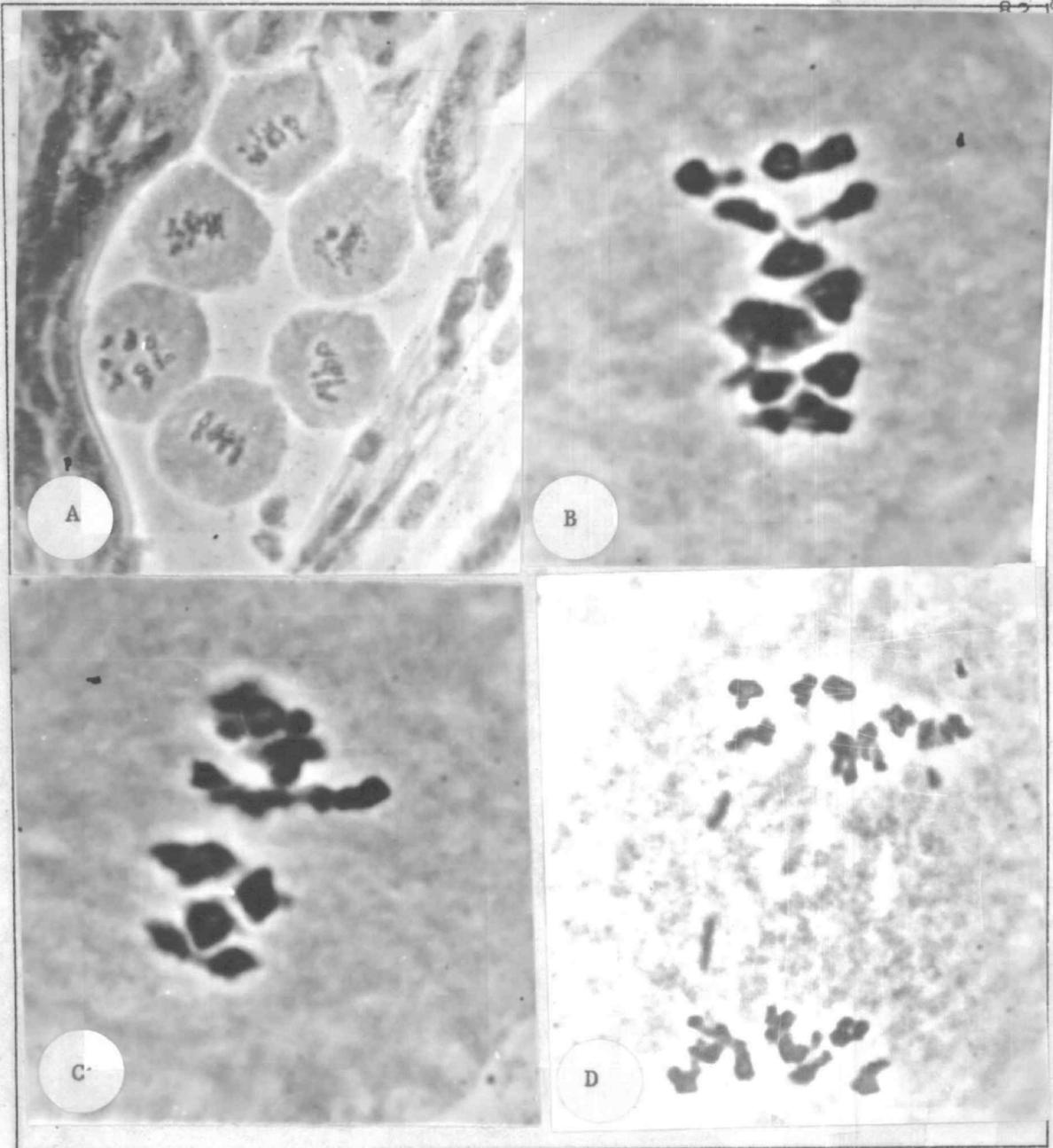


Fig. 29

Figure 26:

Fig. 26a: (290-2 X 291-4)

Prometaphase showing some associations between bivalents.

Fig. 26b: Same

Metaphase I. One bivalent is out of the spindle plate.

Fig. 26c: Same

Metaphase I showing two univalents or fragments outside the spindle plate.

Fig. 26d: Same

Metaphase I showing heteromorphic bivalents (center), fragmented bivalent (top), and possible multivalent (above center).

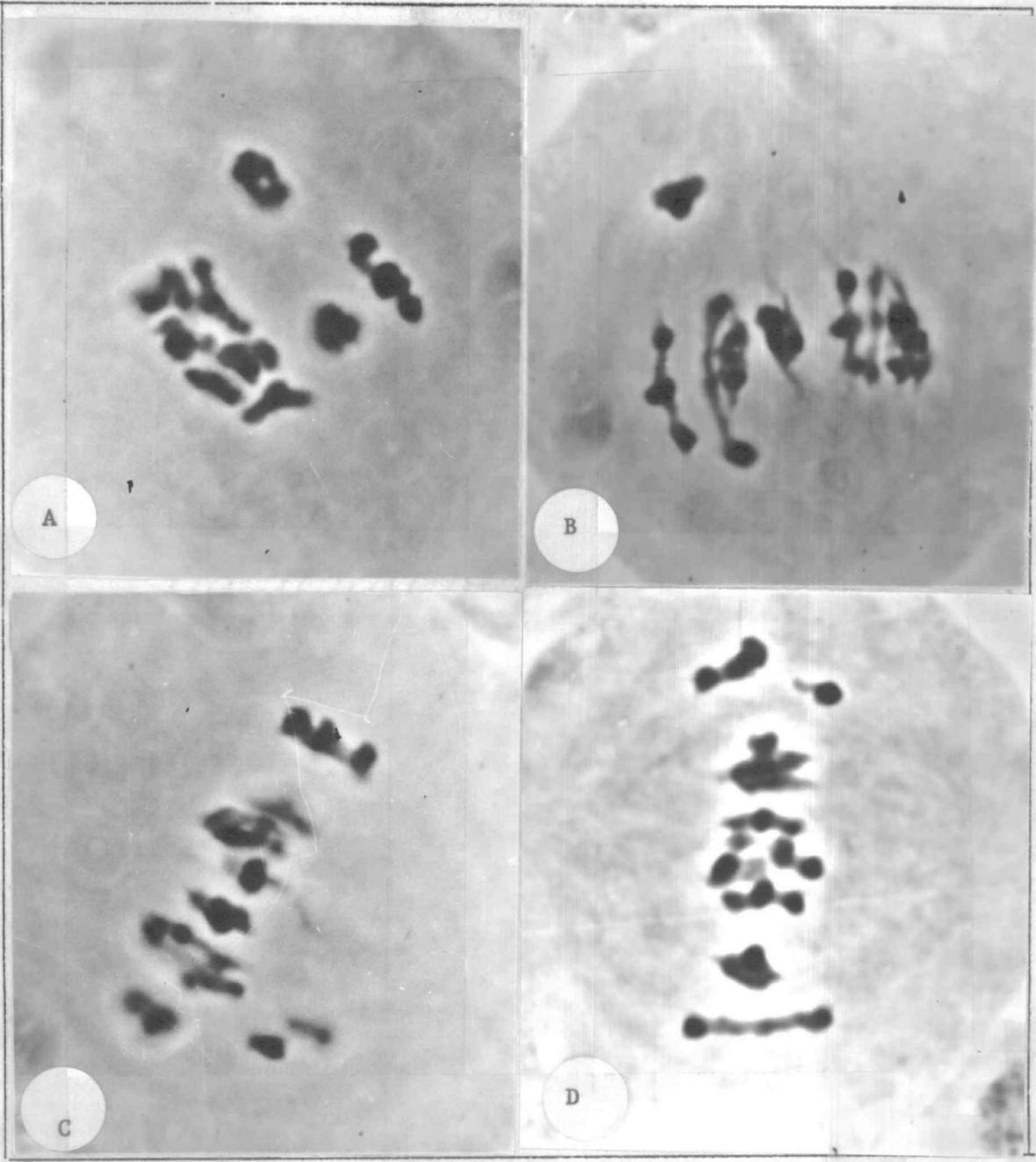


Fig. 26

Figure 27:

Fig. 27a: (261 X 293-8)2

Prometaphase.

Fig. 27b: Same

Early Metaphase I.

Fig. 27c: Same

Prometaphase showing eight bivalents and one univalent.

Fig. 27d: Same

Early Metaphase I with nine normal bivalents.

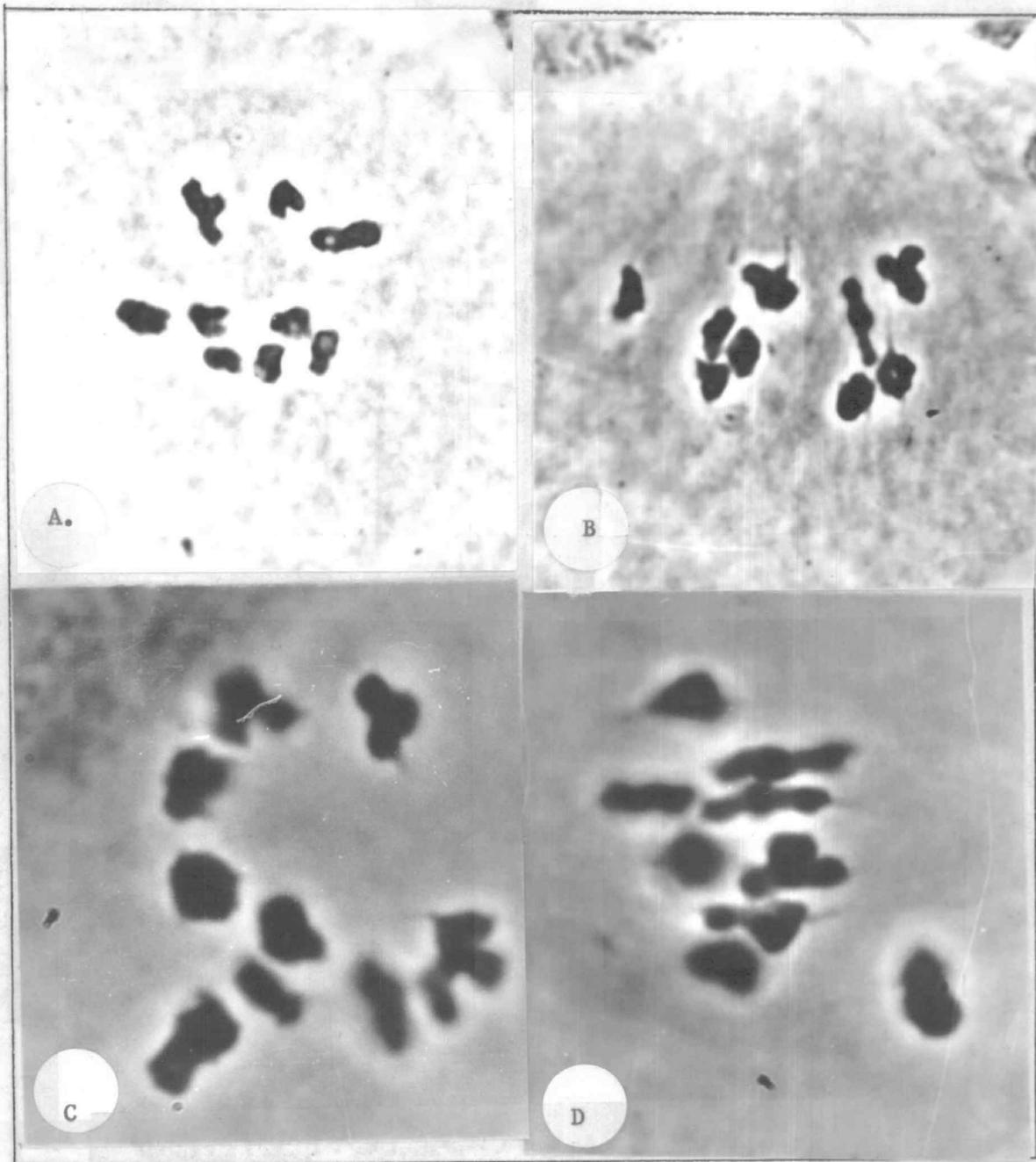


Fig. 27

Figure 28:

Fig. 28a: (261 X 293-8)2

Metaphase I showing possible multivalent associations between bivalents.

Fig. 28b: Same

Metaphase I, showing bivalents split into two groups.

Fig. 28c: Same

Metaphase I showing nine bivalents.

Fig. 28d: Same

Metaphase showing an irregularly shaped bivalent and fragment.

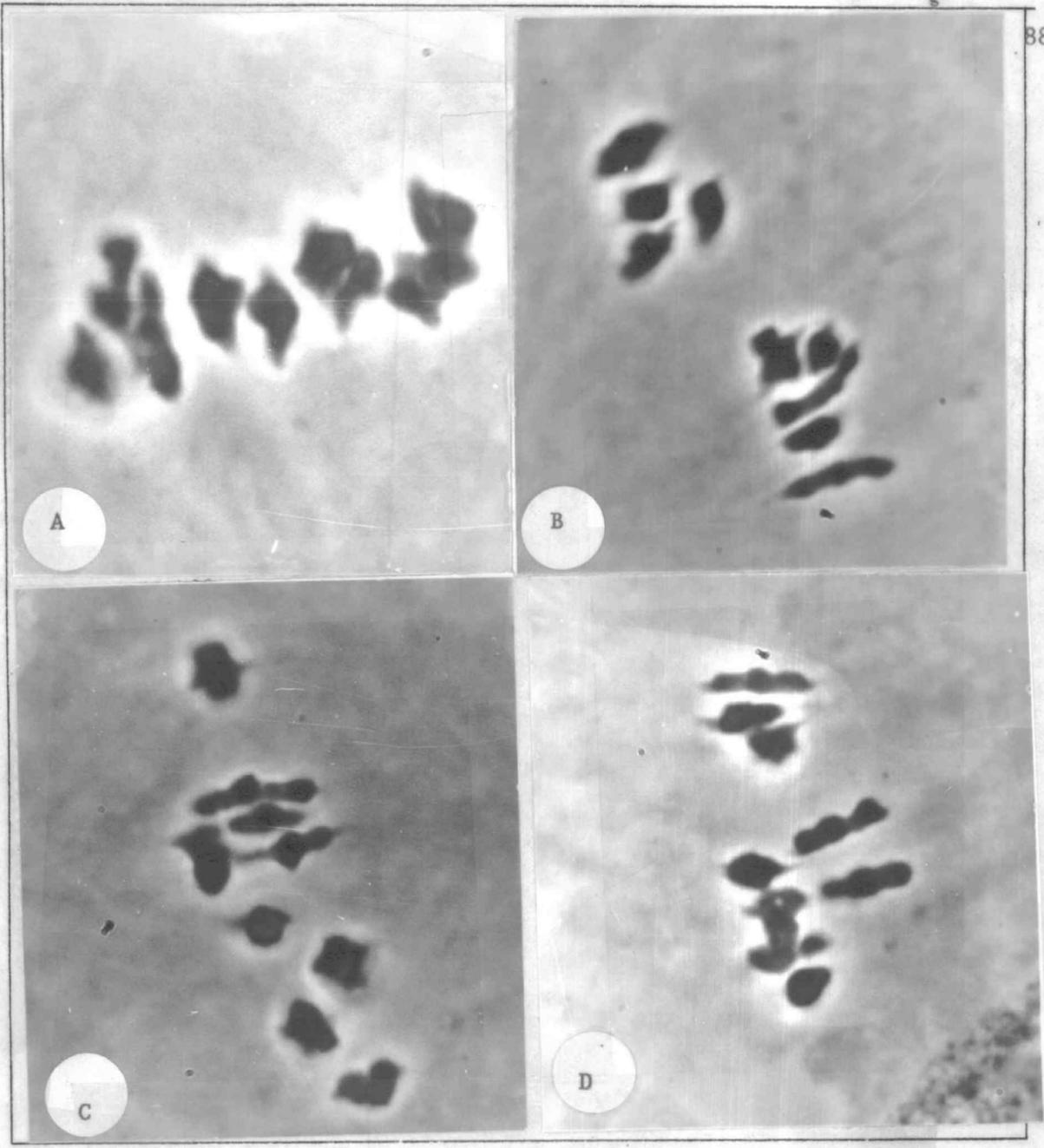


Fig. 28

Figure 29:

Fig. 29a: (294-5 X 304-5)

Metaphase I with apparently only eight bivalents
and stickiness.

Fig. 29b: Same

Metaphase I with two univalent chromosomes off
the spindle plate.

Fig. 29c: Same

Metaphase I with eight bivalents, or seven
bivalents and ring-of-four.

Fig. 29d: Same

Anaphase I showing bridging and breakage.

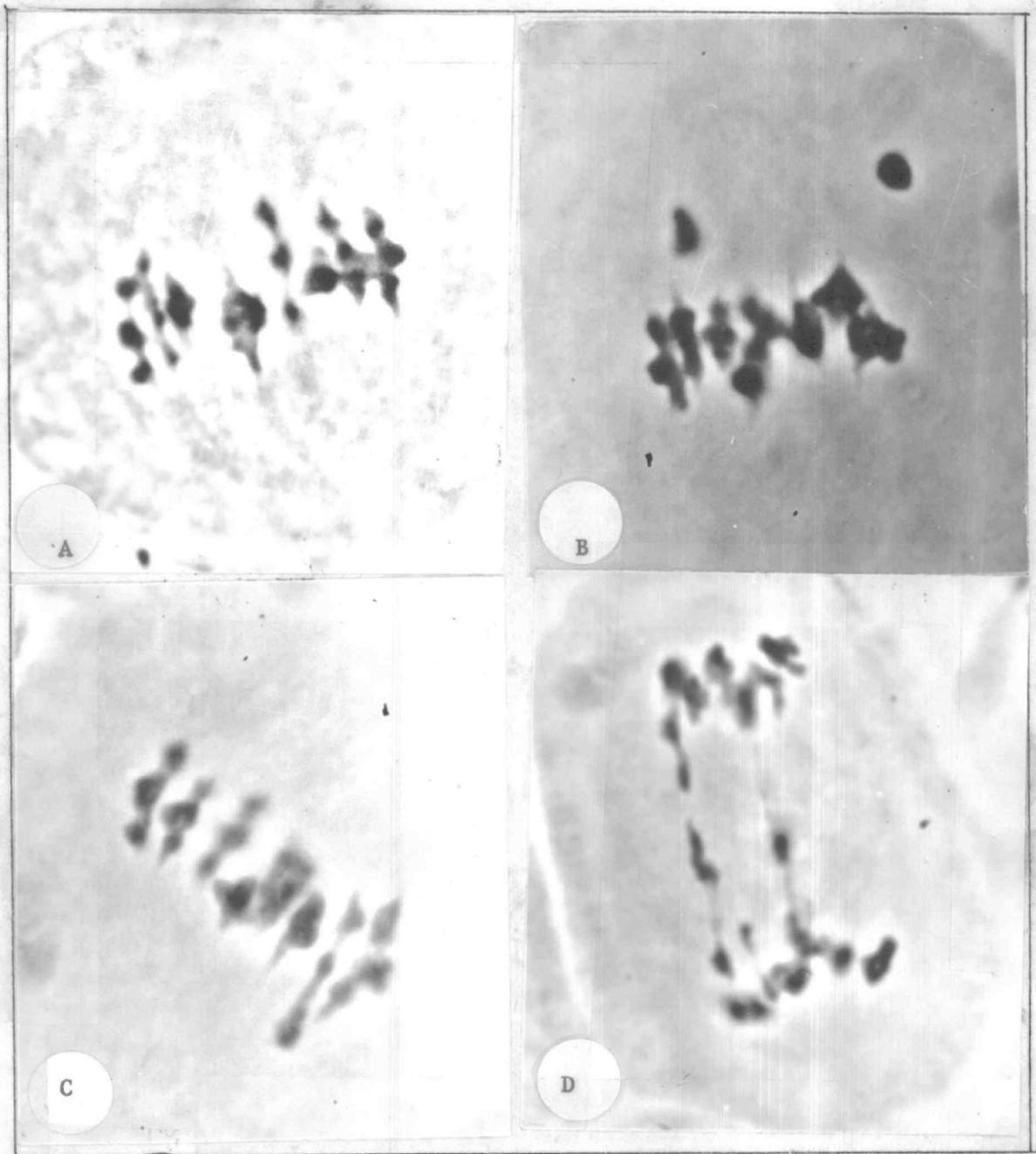


Fig. 29

Figure 30:

Fig. 30a: (294-5 X 304-5)

Telophase I and Anaphase I with lagging.

Fig. 30b: (295-1 X 290-2)

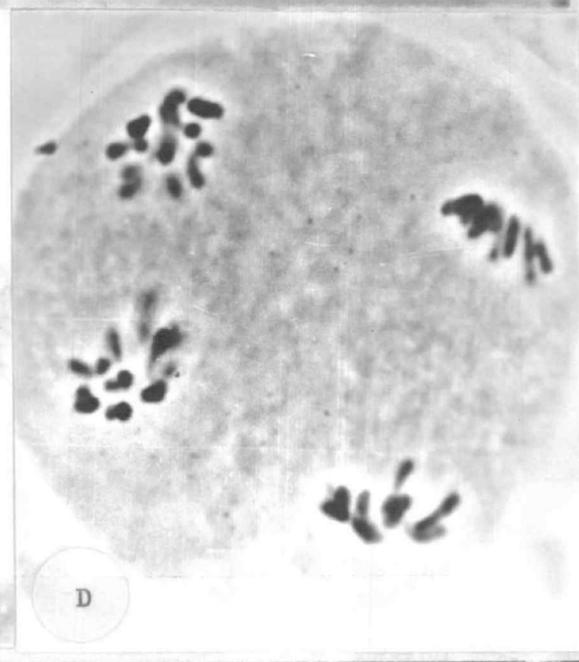
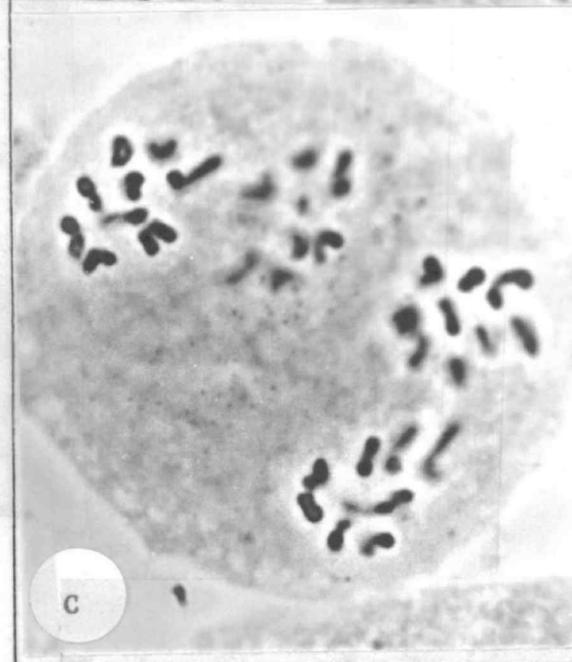
Early Metaphase I showing possible association
between two bivalents.

Fig. 30c: Same

Normal Telophase II. Nine chromosomes can be
counted at each pole.

Fig. 30c: Same

Normal Telophase II.



V. DISCUSSION

The hybrid crosses of the present study were made from plants representing eight populations of M. laciniata (Fig. 1). These populations differ from one another both geographically and in their ecological conditions. For instance, plant 290 of Humboldt County, California, comes from a mixed evergreen forest community in a mesic environment, while plant 229 from Jackson County near Medford, Oregon, represents a grassland community in an area of lower rainfall. Since such differences have existed for a long period of time, it is possible that genetic differentiation has occurred in the various populations which are now disjunct and isolated units.

Within any single population of Microseris laciniata, the individuals are necessarily outcrossing due to genetic self-incompatibility. A common gene pool will be maintained through continued interbreeding (Dobzhansky, 1950), and the plants will normally be heterozygous at many genetic loci. In the present study, there was segregation for pappus-part numbers in the families of interpopulation crosses, which is evidence for heterozygosity in the parental individuals. There are other factors affecting local populations, on the other hand, which may restrict variation and promote genetic uniformity. Lack of environmental change over an extended period of time will select for the best adapted

genotypes, and each population may come to consist of morphologically and physiologically similar individuals. Another powerful factor leading to differentiation between populations is genetic drift. If populations of M. laciniata fluctuate in size and sometimes pass through the bottleneck of low numbers of genotypes, then various alleles may be fixed at random. The overall result of natural selection, drift, and mutation should be gradual genetic divergence among the geographically isolated populations of M. laciniata; and this divergence can be expected to affect morphological traits as well as physiological ones that are important to local growth and survival.

Morphological divergence in M. laciniata has produced two taxonomically recognizable subspecies--ssp. laciniata and ssp. leptosepala. These subspecies are always allopatric (Chambers 1957), although some natural populations are intermediate with respect to their distinguishing traits of involucre and pubescence. The results of studies on interpopulation hybrid fertility given here demonstrate that geographical isolation has not produced significant genetic barriers to interbreeding between the two subspecies. Pollen stainability was 90-100% in all but two interpopulation crosses (Fig. 13), irrespective of whether the parents were members of the same or different subspecies. Chromosome behavior at meiosis was largely normal, as well, in all the intraspecific hybrids of

M. laciniata. Evidently, the factors of drift and local environmental selection, while important in maintaining adaptability of populations, have not led to the kinds of genetic changes that form internal barriers to free crossability between populations. As was pointed out earlier, there is reduced seed set in many attempted pollinations between siblings in some families of F 1 hybrids. However, the best explanation for the results of these experiments is that self-incompatibility genes were affecting the germination and growth of pollen in a number of such sibling crosses.

The two intraspecific hybrids that had reduced pollen stainability (55-70%, Fig. 14) involved population 284, ssp. laciniata from near Garberville, Humboldt County, as a parent. Although meiosis in one hybrid of 284-1 X 290-1 showed mostly normal Telophase II and pollen tetrad stages (Table 5), there were some suggestions of abnormalities earlier (Fig. 15a). Seed fertilities of the F 1 hybrids of 284 were also quite low (Fig. 13). Further studies of the hybrids from population 284 would help in understanding the nature of the incipient genetic barriers it displayed toward some other members of ssp. laciniata.

The success of the experimental hybridizations between the various populations of M. laciniata have shown that there is little or no selection for "genetic barriers" between these populations. In other words, any genetic

differentiation at the population level does not prevent gene exchange among the various types.

Additional studies of the inheritance of morphogenetic characters such as the pappus-part distribution will be possible from the hybridizations between various populations. These studies may elucidate such problem as the amount of genetic diversity as well as gene frequencies in these populations.

We may conclude that the ancient species Microseris laciniata is a dynamic entity whose many populations show a diversity of genetic adaptations, yet are held together as a biological species by their intercompatibility and reproductive continuity.

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