1977

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Recommended Citation

Wittler, George Henry, "An investigation of putative hybridization between Calochortus selwayensis and Calochortus apiculatus (Liliaceae)" (1977). *Graduate Student Theses, Dissertations, & Professional Papers*. 6749.
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AN INVESTIGATION OF PUTATIVE HYBRIDIZATION BETWEEN CALOCHORTUS SELWAYENSIS AND CALOCHORTUS APICULATUS

(LILIACEAE)

By

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B.A., Carleton College, 1971

Presented in partial fulfillment of the
requirements for the degree of
Master of Arts
UNIVERSITY OF MONTANA
1977

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Date 10/28/77

Wittler, George H., M.A., 1977 Botany

Director: Thomas J. Watson

A biosystematic study was carried out to determine the origin and status of populations intermediate in exomorphology to *Calochortus selwayensis* and *C. apiculatus*. Such populations were first collected in the mid sixties northeast of Missoula, Montana.

In the present study, population samples were taken from 115 sites throughout a large portion of the distributional ranges of *C. selwayensis* and *C. apiculatus*. Sixty-eight population samples were chosen for morphological analysis. Measurements were made of 13 characters per individual. Hybrid index scores were calculated for each individual and averaged to determine the mean hybrid index score for each of the measured populations. Mean hybrid numbers were also calculated. Scatter diagrams based on two relative measurements and four multistate qualitative characters were also constructed. Pollen viability studies were carried out on three individuals from each of twelve populations using aniline-blue lacto-phenol. Chromosome counts were made of three individuals from three of the intermediate populations and on one individual from *C. apiculatus*. Chromatographic analysis to determine flavonoid profiles were carried out on two individuals from three populations, one each of *C. selwayensis*, *C. apiculatus*, and the intermediates.

The restricted range, intermediate habitat preference, morphological intermediacy, large intrapopulational variability, and reduced pollen viability suggested that the intermediate populations were of hybrid origin between *C. selwayensis* and *C. apiculatus*. Widespread introgression was evident in the populations occupying the western half of the distributional range of *C. apiculatus*. The widespread introgression found in the western populations of *C. apiculatus* appears to be somewhat unique and shows the possible significance of introgressive hybridization in evolution.

The hybrid populations appeared to be stabilized either by introgression or selection for adaptive recombinant types. Polyploidy was not evident in any of the intermediate populations sampled.

The parental species were shown to be very closely related as indicated by nearly perfect meiotic figures and similar flavonoid profiles. However, the hybrid zone was sufficiently small so as not to blurr the delimitations of the morphologically distinct parental species. For the sake of tradition and without loss of clarity taxonomic changes were not considered to be necessary.

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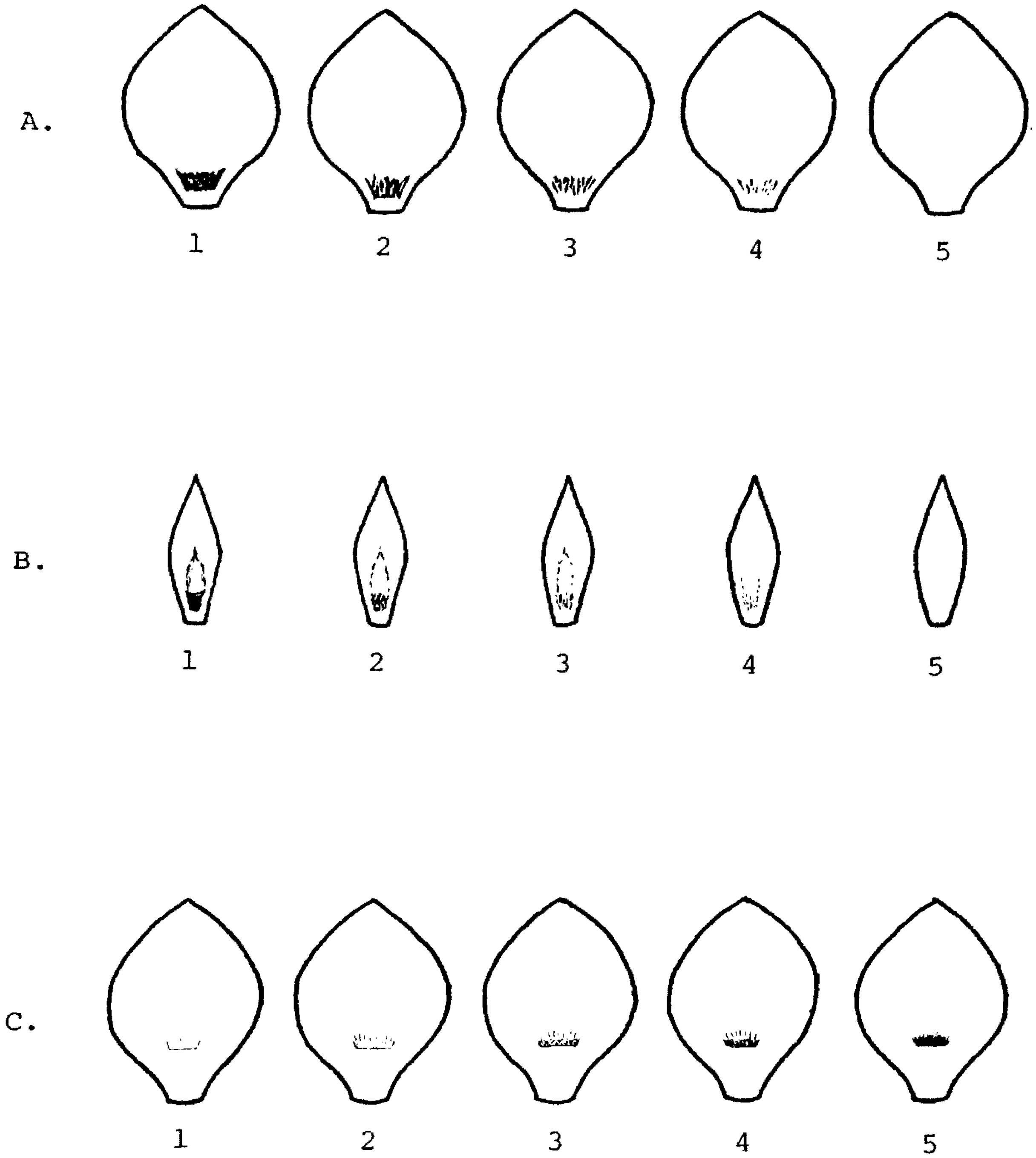
Figure 4

States of the selected characters.

A. petal blotch

B. sepal blotch

C. gland color


A.
1
2
3
4
5
B.
1
2
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C.
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Chapter 1

INTRODUCTION

The genus *Calochortus* Pursh, commonly known as Mariposa or Sego Lily, is comprised of approximately fifty-seven species and thirteen varieties which are located in western North America from British Columbia to Guatemala (Ownbey, 1940). All members of the genus are showy scapose perennials from deep seated, scaly bulbs. Most species occur in xeric to mesic habitats from lowland deserts to alpine meadows.

Calochortus selwayensis St. John =*C. elegans* Pursh var. *selwayensis* (St. John) Ownbey and *C. apiculatus* Baker are two closely related species of the Section *Calochortus* Lemaire, subsection *Eleganti* Ownbey. Both *C. selwayensis* and *C. apiculatus* occur in western Montana and northern Idaho but are allopatric throughout their respective ranges (Fig. 1).

Calochortus selwayensis occurs in low to middle elevations associated with *Pinus ponderosa* Dougl. ex. Laws. It is a shade intolerant species preferring open to semi-open south facing slopes. The plants are small with a scape approximately one half the length of the slender

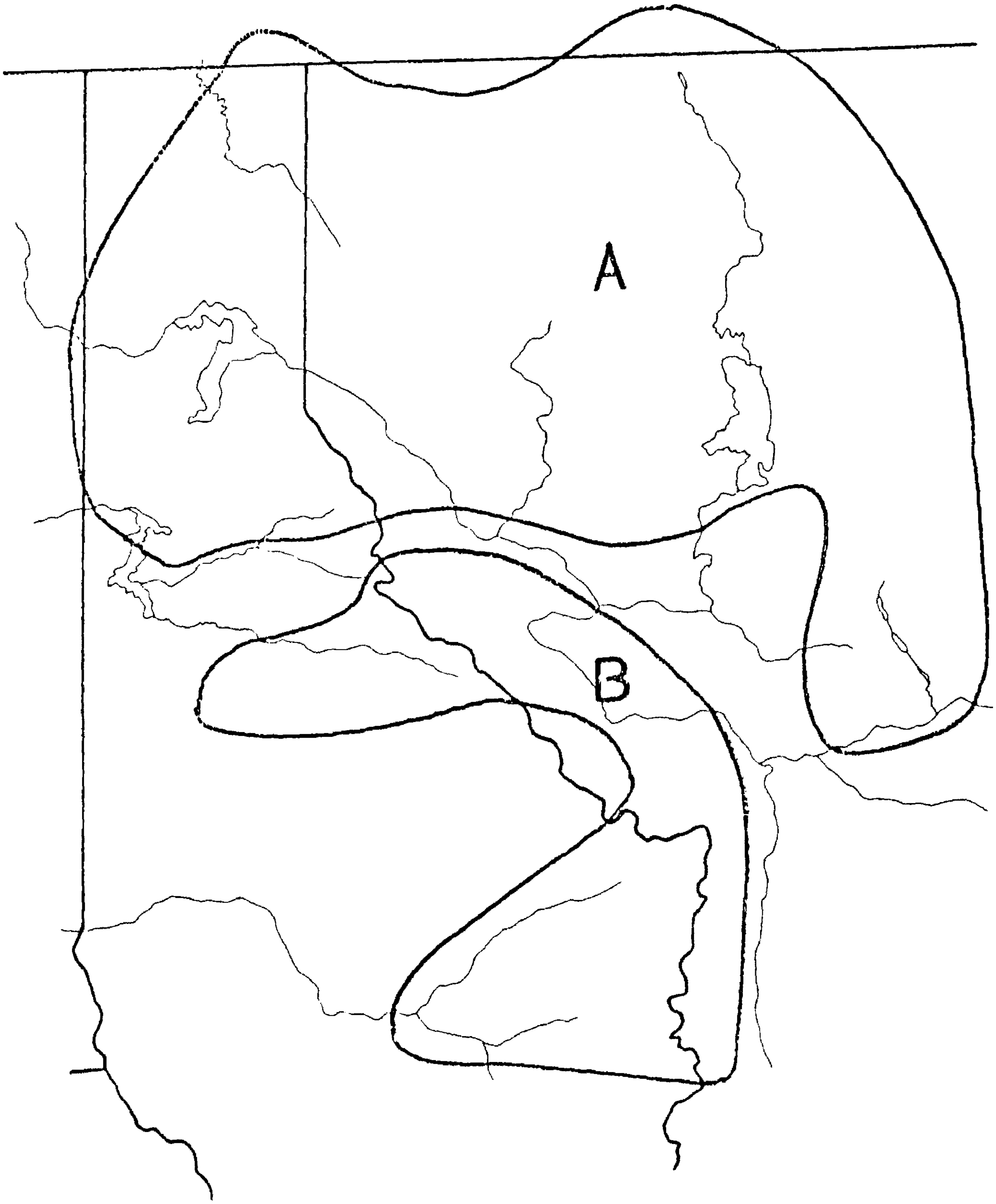
Figure 1

Distribution of *Calochortus selwayensis* and *C. apiculatus*

(Ownbey, 1940; present investigation)

A. *C. apiculatus*

B. *C. selwayensis*


A
B

basal leaf. The flowers are small yet showy. The petals and sepals have a small purple blotch near the base. The petals are pilose on the inner face, many of the hairs having a purple tip. A colorless, linear gland (approximately three millimeters long) is present near the base of the petal (Ownbey, 1940; Ownbey in Hitchcock, et. al., 1969; present investigation).

Calochortus apiculatus occurs in more mesic sites typically associated with *Pseudotsuga menziesii* (Mirbel) Franco at middle to high elevations. A more shade tolerant species, *C. apiculatus* often grows in semi-open forests, preferring level areas to slopes. The individuals are usually much larger than those of *C. selwayensis* with a scape nearly as long as the basal leaf. The flowers tend to be large without purple blotching on the petals and sepals. The petals are pilose on the inner face with hairs that are yellow to colorless. An oval, black gland (approximately one millimeter long) is present on the claw (Ownbey, 1940; Ownbey in Hitchcock, et. al., 1969; present investigation).

In 1966 individuals intermediate in exomorphology to *C. selwayensis* and *C. apiculatus* were collected at Mitouer Gulch northeast of Missoula, Montana (Ownbey, 1966; Ownbey in Hitchcock et. al., 1969). These individuals had scapes approximately three fourths the length of the basal leaf and petals and sepals with mottled purple blotching. The petal pubescence was brown in color. Due to their apparent morphological intermediacy and restricted distribution, these individuals were considered hybrids and/or introgressants (Ownbey, 1966; Ownbey in Hitchcock, et. al., 1969). This interpretation was inconsistent with observations discussed below.

The putative parents were known to be allopatric throughout their distributional ranges (Ownbey, 1940). There was no immediate evidence that the putative parents were at any time even partially sympatric. Such would seemingly preclude interspecific cross-pollination. Evidence of hybridization between currently allopatric species, though rare, has been reported (see Davis and Heywood, 1963 and Remington, 1968 for reviews). These situations required prior contact between the parents (Muller, 1961) or long range pollen dispersal mechanisms. Both putative parental taxa considered here have as pollen vectors beetles, flies, and bees that are short distance pollinators.

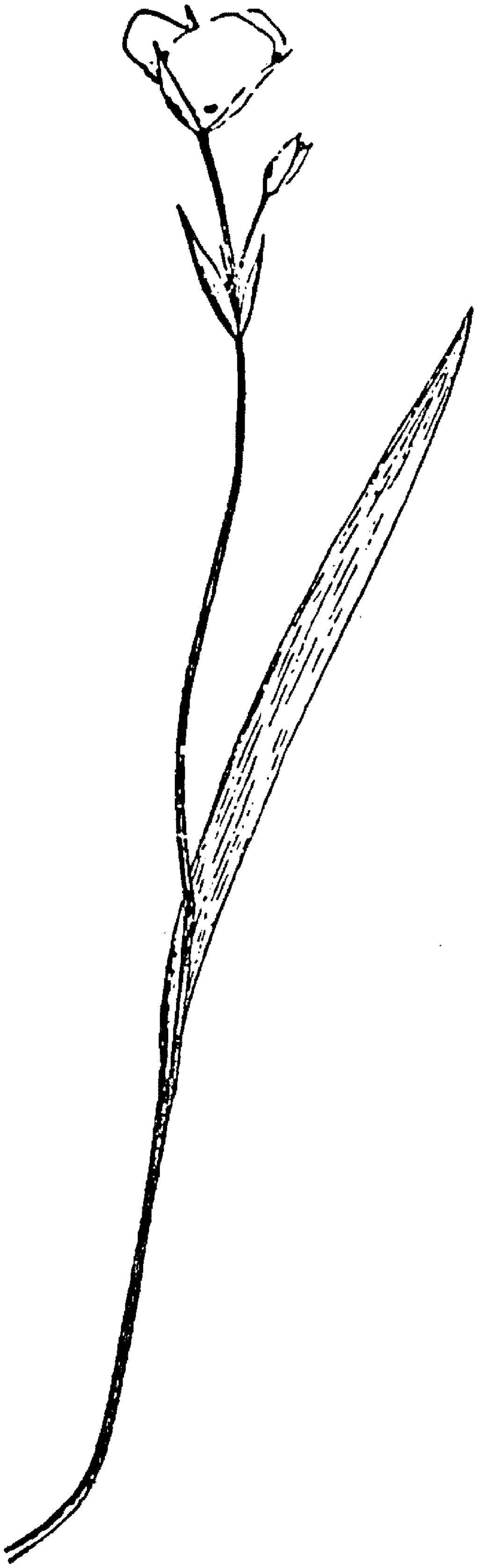
Wagner (1969, p. 785) cautions that "one must distinguish the intermediacy of hybrids and the kind of intermediacy to be found in diverging species." For example, a widely cited study by Hall (1952) apparently documented hybridization between *Juniperus ashei* Buchholz

Figure 2

A. Gross morphology of *C. selwayensis*

B. Gross morphology of *C. apiculatus*

A. actual size



B. ½ X

and *J. virginiana* L. A subsequent statistical study using terpenoid data revealed that hybridization between *J. virginiana* and *J. ashei* had not occurred (Flake, von Rudloff, and Turner, 1969). Using an Adansonian clustering technique with weighted characters, they were able to show that there existed a northeast to southwest cline within *J. virginiana*. This cline was interpreted as being an early stage in the divergence of certain populations within this species. In this case, the divergence resulted in the establishment of populations of individuals that superficially appeared intermediate to *J. ashei* and *J. virginiana*, but were actually within the range of variation of *J. virginiana* (Flake, von Rudloff, and Turner, 1969).

Also a relictual ancestral population that has given rise to two divergent species may be intermediate to those divergent species but not be of hybrid origin (Dobzhansky, 1941). An example of this is provided by Turrill (1934) in which intermediates between *Ajuga chamaepitys* Schreb. and *A. Chia* Schreb. were found to represent remnants of an ancestral complex.

Thus, it is clear that mere intermediacy does not necessarily imply hybridity. Ownbey (in Hitchcock, et. al., 1969) based his assessment of hybridity in the Mitouer Gulch populations on the results of a prior taxonomic study (Ownbey, 1940). This study revealed no clinal variation either between or within the putative parental taxa, nor did it show the presence of patristic intermediates or remnants of an ancestral complex. The study was based solely on herbarium specimens and cannot be considered as substantial evidence of hybridization between *C. selwayensis* and *C. apiculatus*.

Purdy (1901) reported that hybridization between species of *Calochortus* is encountered quite often in the field. However, all interspecific cross pollinations yielded completely sterile offspring. This would tend to preclude introgression between *C. selwayensis* and *C. apiculatus* as indicated by Ownbey (1966), if they are indeed valid species.

The primary objectives of this study are twofold: (1) to locate, describe, and determine the source and extent of the intermediate plants over the distributional ranges of *C. selwayensis* and *C. apiculatus* and (2) to assess the taxonomic status of the population systems involved, based on the results of the above studies.

Definitions of Terms

In order to discuss the concept of "hybridization," it is first necessary to construct a working definition. This is fundamental since the term has been used in different ways by various authors.

For example, hybridization may mean the crossing of different genotypes or the grafting of different species (Grant, 1971). The products of such interbreedings then are referred to as "hybrids," as in "hybrid corn." To the plant taxonomist dealing with natural systems, such definitions constructed to encompass artificial phenomena, become useless. The crossing of different genotypes is a common occurrence in all outcrossing populations, while grafting is almost non-existent in the field.

The taxonomist who envisions the "population" as the unit of evolutionary change, normally refers to hybridization as a phenomenon of the population and not of individuals. For example, Sibley (1957) defines hybridization "as the interbreeding between populations in secondary contact, regardless of taxonomic rank." This definition might be interpreted to mean interbreeding between two slightly differing populations of the same variety. This is to be expected in natural populations of sexual organisms.

A more useful definition for natural phenomena might be that of Bigelow (1965) who feels that the term hybridization should only include interbreeding between those populations in secondary contact that have developed a certain amount of genetic incompatability. A similar definition has been proposed by Mayr (1963) which is generally accepted as a good working definition (Hieser, 1973). Mayr (in Hieser, 1973, p. 349) states, "hybridization is the crossing of individuals belonging to two unlike natural populations that have secondarily come into contact." Both of these definitions require that a certain amount of divergence must occur before hybridization can take place. However, the extent of the divergence is not specified.

The term hybridization as used here will be that of Bigelow or Mayr with an additional qualification. That is the extent of divergence should be enough to produce populations of two separate species.

Hybridization may often result in the formation of "hybrid swarms." A hybrid swarm is a complex mixture of species, hybrids, backcrosses, and later generation recombinants (Grant, 1971) or any combination thereof. Hybrid swarms tend to be quite variable if the F_1 individuals are at least partially fertile. This variability is largely due to recombinations in later filial generations and to backcrossing. The hybrid swarms tend to occupy habitats intermediate to those preferred by the parental population systems (Anderson, 1948). The intermediate habitat is often referred to as the "hybrid habitat."

The distributional range of hybrid swarms is called the "hybrid zone" which may be geographically restricted or quite extensive. A restricted hybrid zone is quite obvious in nature since it is usually flanked by well defined parental populations. However, if the hybrid zone is spread over a large area, it becomes difficult to identify, since the morphological-geographical delimitations of the parental population systems may become blurred (Anderson and Sax, 1936). In the latter situation, hybridization may resemble clinal variation which is merely a morphologic gradient throughout the range of a population system still in primary contact.

Often hybridization is followed by repeated backcrossing to either one or both of the parental populations. This phenomenon is called "introgressive hybridization" or simply "introgression" (Anderson, 1949). Introgression results in the flow of discrete blocks of genetic information between divergent populations which if adaptive can increase the fitness of the recurrent parent (Anderson and Stebbins, 1954). Populations made up largely of introgressants are indistinguishable from hybrid swarms that have become stabilized through recombinant adaptation to environments resembling that of one of the parents.

Stabilization (= self perpetuation) of hybrid swarms is quite a common occurrence in the plant kingdom (Anderson and Stebbins, 1954) and can occur through a number of mechanisms. Sterile hybrids may become fertile through allopolyploidy, or reproduce apomicticly or vegetatively to create population systems readily recognized as discrete entities in the flora. Stabilization can also occur without such mechanisms if diploid hybrids are not at least partially fertile. Such situations usually involve backcrossing (introgression) or selection for certain recombinant types (Anderson and Stebbins, 1954; Davis and Heywood, 1963).

Chapter 2

MATERIALS AND METHODS

Morphology

Sampling. Population samples were collected during the months of May through August in 1975 and 1976. One hundred fifteen collections were taken from areas of putative hybridization and a large portion of the distributional ranges of the putative parents (Fig. 3, Appendix I). Of these, 68 populations were chosen for morphological analysis. Whenever possible 20 individuals were collected from each population. However, some populations were sufficiently small to warrant collections of as few as five individuals.

The individuals were selected from each population in a random fashion. This was accomplished by walking in an erratic manner through a population while collecting individuals at intervals during two of three traverses of the population. An attempt was made to avoid choosing individuals solely because they were in some way unusual in size or morphology.

Since *Calochortus* reproduces vegetatively by bulbs,

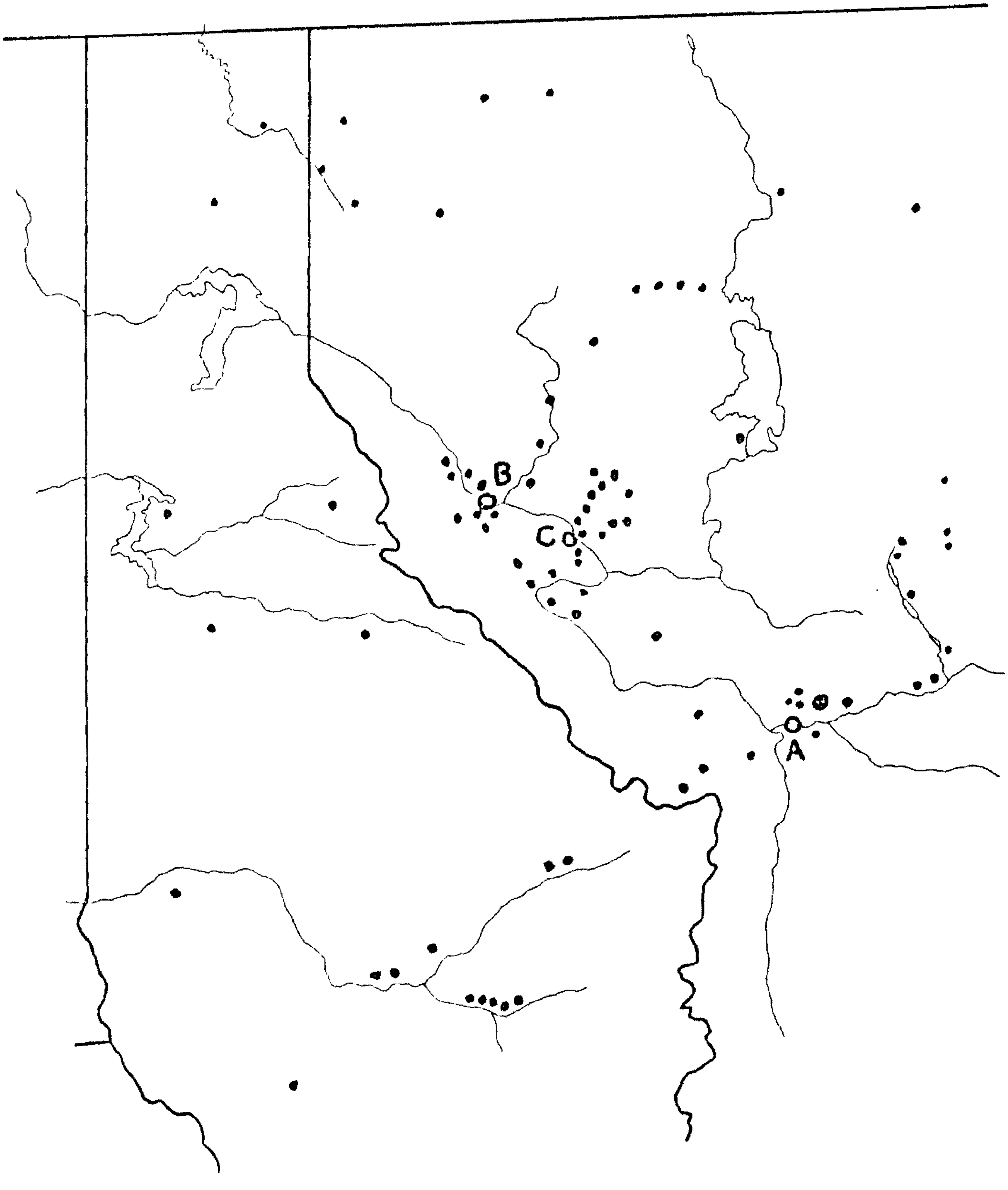
Figure 3

Locations of all Populations

A. Missoula

B. Thompson Falls

C. Plains


B
C
A

an effort was made to avoid collecting two individuals growing within a few inches of one another. Individuals without fully expanded corollas and those with extensive insect damage to the basal leaf were likewise not collected.

The above ground portions of the plants were removed by gently pulling at the base of the stem. The point of articulation between stem and bulb seemed too consistent from plant to plant so no effort was made to remove the bulb. The materials were pressed and dried before morphological measurements were made.

Selection of Characters. Based on descriptions of the putative parental taxa, fourteen characters were chosen for morphological analysis. After an initial examination of 95 herbarium specimens from the Ownbey Herbarium (WS) and the U. S. Forest Service Herbarium (FSH), four of these characters were eliminated. These four were either too variable or did not consistently delimit the two parental taxa. The final character set is given in Table 1.

In the subsequent analysis, relative measurements were employed as necessary. Field and herbarium studies suggested that absolute measurements such as scape height, basal leaf length, gland length and claw width were likely affected by environmental conditions. However, it is less probable that proportional measurements would be affected. Thus, the following ratios were used in the evaluation:

Table 1

Final Character Set

Character	Character States
1. Scape height	continuous
2. Basal leaf length	continuous
3. Petal pubescence color	1. purple 2. purplish-brown 3. brown 4. brownish-yellow 5. yellow to colorless
4. Petal blotch	1. no mottling 2. slight mottling 3. mottled 4. trace of color 5. no color
5. Petal length	continuous
6. Sepal blotch	1. no mottling 2. slight mottling 3. mottled 4. trace of color 5. no color
7. Sepal length	continuous
8. Gland length	continuous
9. Claw width	continuous
10. Gland color	1. colorless 2. light blue-black 3. blue-black 4. dark blue-black 5. black

Scape/leaf lengths and gland length/claw width.

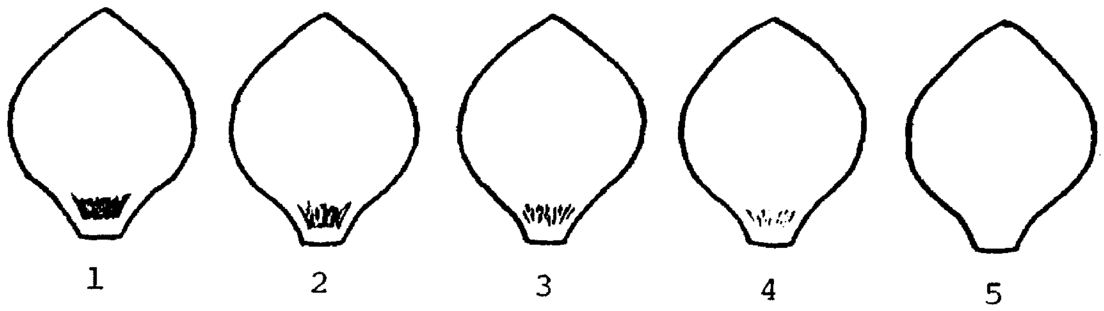
Measurement of Characters. Scape height, basal leaf length, petal length, and sepal length were measured with a standard millimeter rule to an accuracy of ± 2 mm. Gland length and claw width were both measured with an ocular micrometer to an accuracy of ± 0.2 mm.

Color of the petal pubescence was arbitrarily divided into five states (see Table 1) as were petal blotch, sepal blotch, and gland color. Character state (1) represents that exhibited by pure *C. selwayensis*, character state (2) that of pure *C. apiculatus*, and character states (3), (4), and (5) indicate intermediate plants. The character states are given in Figure 4.

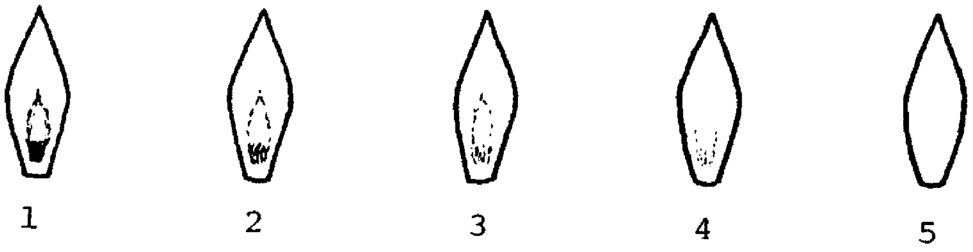
After the measurements were completed, each character state was given an index score based on its position between the two extreme values of that character. The extreme *C. selwayensis* character state was always designated as lowest while the *C. apiculatus* state was the high extreme. Thus, the numerical value of the character index would be lower if approaching *C. selwayensis* and higher if resembling *C. apiculatus*. In order to avoid weighting certain characters, all character indices were transformed to a 0 to 1 scale using the following equation:

$$CI_{ij} = (X_{ij}-ES_i)/EA_i-ES_i)$$

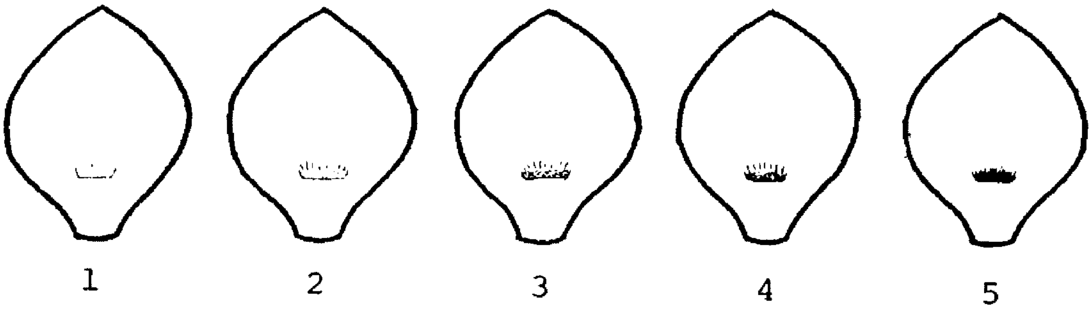
A.



B.



C.



where CI_{ij} is the ith character index of the jth individual, X_{ij} is the ith character state of the jth individual, and ES_i and EA_i are the extreme values of the ith character exhibited by *C. selwayensis* and *C. apiculatus* respectively.

A hybrid index score was then calculated for each individual by simply adding the character index scores:

$$HI_j = \sum_{8}^{i=1} CI_{ij}$$

where HI_j is the hybrid index score of the jth individual. Since eight of the characters and the character index values range from zero to one, the individual hybrid index scores could possibly range from zero to eight.

The hybrid index is a way of evaluating each individual on multiple characters such that each individual can be judged as to its affinities to one of the putative parents. In this case a low hybrid index score would indicate *C. selwayensis* affinities while a high score would indicate *C. apiculatus* affinities.

The hybrid number is similar to the hybrid index but rather indicates the degree of intermediacy. The hybrid number is calculated by determining the distance between the hybrid index score and nearer of the two extremes of the hybrid index scale (Gay, 1960). The range of hybrid number scores would thus be one half the range of the hybrid index scores, in this case that range would be between zero and four.

The amount of intrapopulational variability can provide information on the status of a putative hybrid population. Anderson (1949) has pointed out that hybrid populations, especially those containing many filial generations, have a tremendous variability. The variability of each population was determined by calculating the sample standard deviation of hybrid index scores. The following equation for the sample standard deviation was used for all calculations:

$$HISD_k = \sum_{n}^{j=1} (HI_j-MHI_k)^2/(n-1)$$

where $HISD_k$ is the standard deviation of the _kth_ population, MHI_k is the mean hybrid index of the _kth_ population, and n is the sample size of the _kth_ population.

Another useful tool that can be of value in visualizing the status of intermediate populations is the hybrid scatter diagram of Anderson (1949). The scatter diagram can be used to describe in some detail the composition of populations. The disadvantage of these scatter diagrams is that they are tedious to construct and require considerable space for display.

Thus, only six representative populations were chosen and scatter diagrams were constructed for each. Quantitative characters were chosen as the abscissa and ordinate, while four qualitative characters were displayed by flags on the symbol. The following six characters were used in the scatter diagramming: (1) scape/leaf length, (2) gland/claw width, (3) petal pubescence, (4) petal blotch, (5) sepal blotch, and (6) gland color. A list of symbols used and their corresponding character states are given in Figure 5.

Ecology and Geography

For each population, information concerning canopy cover, dominant species, associated plants, slope direction and aspect, and elevation was recorded. The elevation was determined by use of a standard pocket altimeter with an approximate accuracy of ± 100 feet. Such information, aside from being good collecting procedure, should provide other, non-morphological characters for assessing putative hybridization. It has been pointed out that F_1 hybrids require habitats that are intermediate to those of the parental species (Anderson, 1948). In addition Anderson (1948, p. 4) states, "The second generation will be made up of individuals each of which will require its own peculiar habitat for optimum development." Therefore, evaluation of the various habitats would likely have significant value in the interpretation of the situation in question.

Since hybrid swarms are typically found growing in

Figure 5

Character state symbols for use in Scatter Diagrams

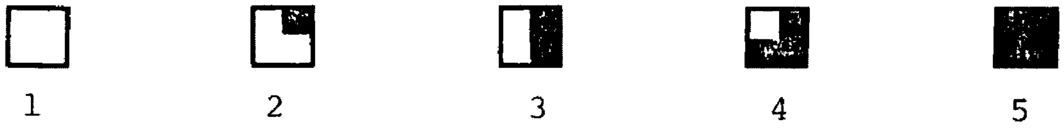
A. petal pubescence

B. petal blotch

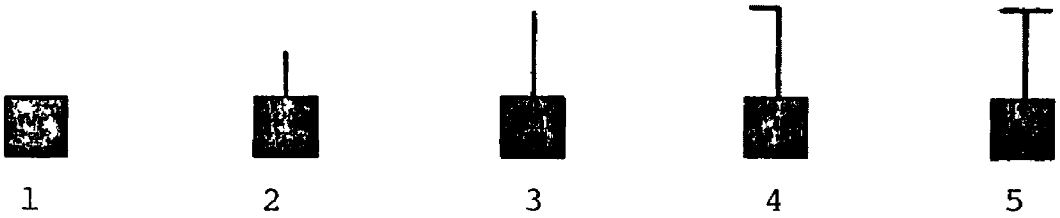
C. sepal blotch

D. gland color

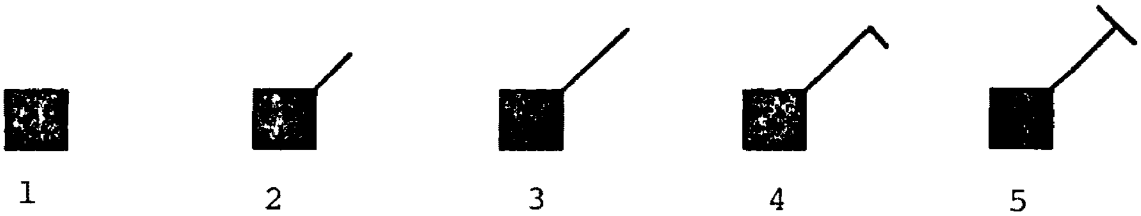
A.



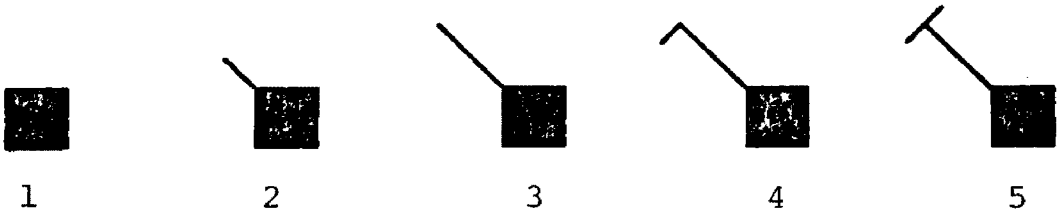
B.



C.



D.



regions of sympatry between the ranges of allopatric parents, the locations of the various populations used in the study must be determined. In most cases the locations were determined to the nearest mile using U. S. Forest Service Ranger district maps. Geographic data were then compared to mean hybrid index scores for the various populations in a manner similar to that of Sibley (1954).

Cytology

Chromosome numbers have been priorly determined for many of the Calchorti (Beal, 1939; Beal, 1941; Beal and Ownbey, 1943). Both *C*. *selwayensis* and *C*. *apiculatus* had meiotic counts of n=10 and somatic counts of 2n=20. All other species of the Subsection *Eleganti* have somatic numbers of 2n=20. Polyploidy, though apparently absent in the *Eleganti*, can be found in a few species of the section *Calochortus* (Beal, 1939).

It has been amply demonstrated that amphidiploidy is a common result of hybridization in plants (for examples see Clausen, et. al., 1945). Since both hybridization and polyploidy have been documented for the genus *Calochortus*, it was surmised that the intermediates encountered in this study might reveal their hybrid nature chromosomally. In order to determine this an aceto-carmine squash technique was used to determine the chromosome number(s) of individuals in some intermediate populations.

Buds of varying sizes were collected at four sites and immediately fixed in either ethanol and acetic acid (3:1) or chloroform, ethanol, and acetic acid (5:3:1). After remaining in the fixative for at least 48 hours, the anthers were removed from the buds. The anthers were macerated with a scalpel on a microscope slide onto which a single drop of aceto-carmine was added. All counts were made from microspore mother cells in either prophase I or metaphase I. Anaphase I chromosomes were also observed and any irregularities (such as bridges, fragments, etc.) noted. Voucher specimens are stored at the University of Montana Herbarium (MONTU).

Pollen Analysis

In most cases, hybrid individuals exhibit reduced fertility (Stebbins, 1958). As a measure of fertility, pollen viability has been used extensively, especially in studies of hybridization. Often pollen stainability is used as a measure of the viability. Though this relationship has been questioned recently by Jones (1976), pollen stainability remains a useful tool in estimating fertility.

Of the different techniques for the study of pollen viability, aniline-blue lacto-phenol stainability is the most extensively used (Hauser and Morrison, 1964). A more recent technique, which was devised by Hauser and Morrison (1964) using nitro-blue tetrazolium, appears to be a better technique but requires living material. Since dried material was used for this phase of the study, the aniline-blue lacto-phenol technique was appropriate.

The aniline-blue lacto-phenol was prepared after Maneval (1936) as follows: ten grams of melted phenol crystals, ten ml. lactic acid, 20 ml. glycerine, and ten ml. distilled water were combined; to this mixture was added 2 gr. of aniline-blue (cotton-blue) dye.

Analysis was carried out using three plants from each of 12 populations that had been measured and scored for mean hybrid index. Of these 12 populations, eight were of putative parents, and four were putative hybrid populations.

Pollen was obtained from anthers of closed buds and allowed to stain in the aniline-blue lacto-phenol overnight before the counts were made. Approximately 300-400 grains were counted from each slide. Only those grains that stained well, had well formed pollen walls, contained a nucleus, and had uniform cytoplasm that extended to the pollen wall were counted as viable. All other grains were scored inviable.

Chromatography

Leaves of two plants from each of three populations previously measured and scored by mean hybrid index were used in the original chromatographic analysis. Of the three populations, two represented the putative parents and the third a putative hybrid population.

The dried leaves were crushed and the pigments extracted in 95% methanol for two days. The extracts were then spotted on Whatman 3mm chromatographic paper and run in two dimensions after the method of Mabry, et. al. (1970). Tertiary butyl alcohol, acetic acid, and water (3:1:1) and acetic acid and water (3:17) were the solvent systems utilized.

The chromatograms were observed under ultraviolet light as well as ultraviolet light and ammonia. In all cases, three purple spots were observed. Two spots were dark while the third was extremely faint. Since no differences were observed between the pure parents nor the hybrids, an analysis of more populations was considered unnecessary.

Chapter 3

RESULTS

Morphology

Field observations. Though not quantitative, field observations can be an important tool in assessing systematic problems. Often populations and population systems are far too complex to allow for the quantification of all biological and physical parameters. When no attempt is made to observe populations in the field, as with an herbarium study (e.g., Ownbey, 1940), valuable insights are often lost.

Pure populations of *C. selwayensis* and *C. apiculatus* were quite distinct in the field owing to the very different floral characteristics of the two species. However, there appeared to be quite a bit of intrapopulational variability which was largely due to differences in absolute size. It was also noted that *C. apiculatus* was found growing in much denser stands (some numbering into the thousands) than *C. selwayensis*. In no cases were *C. selwayensis* and *C. apiculatus* seen growing in the same locality.

Populations of intermediates were likewise easily identified as such. Individuals of these populations were much more varied than the populations of the putative parental forms. The variability was so great that no two individuals appeared closely similar. Often these intermediate populations were very dense; one was estimated to have over 50,000 individuals covering a hillside.

Occasionally a population was found that at first appeared to be either _C_. _selwayensis_ or _C_. _apiculatus_ but had characters normally found in the other species. These populations were always within a few miles of an obviously intermediate population and gave the appearance of introgression.

Quantitative analysis. The mean hybrid index and mean hybrid number scores of each population analyzed are given in tabular form (Table 2), as well as graphic form (Fig. 6).

Table 2

MEAN HYBRID INDICES AND MEAN HYBRID NUMBERS

Pop. no.	MHI	MHN	HISD	Pop. size
12	1.6	1.6	.37	9
14	1.3	1.3	.61	14

Table 2 (continued)

Pop. no.	MHI	MHN	HISD	Pop. size
22	4.9	2.9	.85	22
23	4.7	2.7	1.30	27
24	4.4	3.0	1.10	21
25	5.8	2.2	.82	20
26	5.6	2.4	.64	19
27	5.5	2.4	.90	19
28	5.6	2.4	.72	20
29	2.9	2.3	1.7	10
31	3.8	3.1	1.08	5
33	4.3	3.0	1.17	20
34	4.1	3.4	.81	16
35	4.2	3.1	1.03	18
37	5.8	2.2	.59	19
38	6.1	1.9	.79	21
39	6.3	1.7	.64	12
40	2.0	2.0	.40	24
41	2.1	2.0	.73	16
42	1.5	1.5	.46	18

Table 2 (continued)

Pop. no.	MHI	MHN	HISD	Pop. size
45	1.3	1.3	.41	30
46	1.6	1.6	.52	21
47	1.2	1.2	.37	19
48	3.5	3.1	1.03	24
49	4.1	2.9	1.31	14
50	4.3	2.9	1.24	41
51	3.3	2.9	1.07	6
52	3.9	3.0	1.10	31
53	1.3	1.3	.52	15
54	1.5	1.4	.73	9
55	1.2	1.2	.33	13
56	2.3	2.3	.57	11
57	1.6	1.6	.43	7
59	4.6	3.0	1.10	16
60	4.4	2.9	1.15	18
61	4.0	3.4	.77	9
62	4.2	3.2	1.04	13
63	1.4	1.4	.45	27

Table 2 (continued)

Pop. no.	MHI	MHN	HISD	Pop. size
64	6.5	1.5	.27	27
65	6.5	1.5	.25	14
66	5.4	2.6	.70	13
67	5.8	2.2	.54	12
68	6.4	1.6	.27	13
69	6.3	1.7	.25	24
70	6.0	2.0	.35	18
71	6.6	1.4	.35	27
72	6.6	1.4	.27	11
73	6.6	1.4	.34	14
74	6.6	1.4	.21	27
75	6.6	1.4	.29	17
98	6.5	1.5	.44	9
99	4.1	3.2	.93	16
101	4.9	2.8	1.13	18
102	1.1	1.1	.56	11
103	1.3	1.3	.45	15
104	6.2	1.8	.35	12
105	5.8	2.1	1.15	15

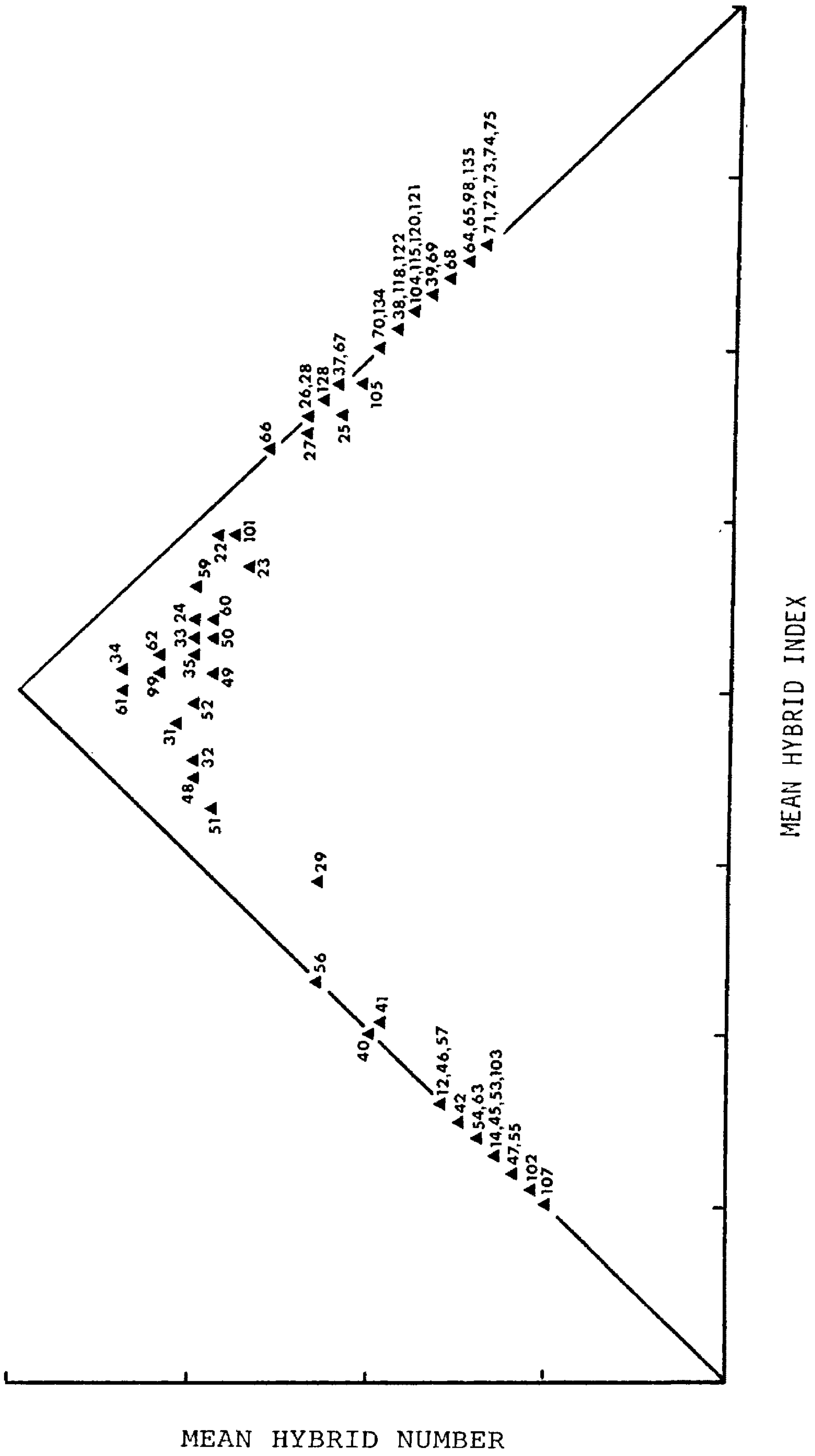
Table 2 (continued)

Pop. no.	MHI	MHN	HISD	Pop. size
107	1.0	1.0	.27	14
115	6.2	1.8	.41	14
118	6.1	1.9	.29	12
120	6.2	1.8	.46	10
121	6.2	1.8	.48	15
122	6.1	1.9	.36	9
127	6.2	1.8	.34	16
128	5.7	2.3	.34	16
134	6.0	2.0	.66	10
135	6.5	1.5	.27	15

The graph of mean hybrid index -vs- mean hybrid number allows for the visual comparison of these two population parameters. From this graph it is clear that all populations lie either on the left or right hand legs or scattered near the apex of the limiting triangle. This is indicative of a situation in which an interpopulational morphological gradient exists between the two extremes. The graph also shows that very few populations were com-

Figure 6

Mean Hybrid index -vs- Mean Hybrid Number


MEAN HYBRID INDEX
MEAN HYBRID NUMBER
61
34
99
62
31
52
35
33
24
49
50
60
59
22
101
23
48
32
51
29
56
40
41
12,46,57
42
54,63
14,45,53,103
47,55
102
107
66
27
26,28
128
25
37,67
105
70,134
38,118,122
104,115,120,121
39,69
68
64,65,98,135
71,72,73,74,75

posed of individuals of both extremes. Such populations would have plots closer to the center and nearer the baseline of the limiting triangle. Only population 29 shows evidence of this.

The morphological gradient is not continuous but appears to have some more or less distinct breaks. These breaks delimit five population classes which can be characterized on the basis of the two parameters (i.e., MHI and MHN). Since many populations were considered here, it facilitates the discussion to group the populations for reference. The subsequent discussion will refer to population classes 1, 2, 3, 4, and 5 based on the mean hybrid index and mean hybrid number described below.

The populations designated class one consist of those with mean hybrid index scores less than 1.6 and a mean hybrid index score that is equal to the mean hybrid number score for that population. (The populations in this category are 12, 14, 42, 45, 46, 47, 53, 54, 55, 57, 103, and 107.) These characteristics suggest that the populations are pure _C_. _selwayensis_.

The populations designated class two consist of four populations (29, 40, 41, and 56) with mean hybrid index scores between 2.0 and 2.9. The higher mean hybrid index scores indicate that these populations have individuals with _C_. _apiculatus_ characteristics. In fact, populations 41 and 29 do not fall on the edge of the limiting triangle so thus contain some individuals that more closely resemble *C. apiculatus* than *C. selwayensis* (i.e., have a hybrid index score greater than 4.0). Populations 40 and 56 have plots that lie on the edge of the limiting triangle and are considered to be populations that contain individuals more similar to *C. selwayensis* than to *C. apiculatus* (i.e., have hybrid index scores less than 4.0) but are polarized in the direction of *C. apiculatus*. These populations are thus considered to be intermediate but favoring *C. selwayensis*.

The populations designated class three consist of those with mean hybrid index scores between 3.3 and 4.9. (The populations in this category are 22, 23, 24, 31, 32, 33, 34, 35, 48, 49, 50, 51, 52, 59, 60, 61, 62, 99, and 101.) Since the mean hybrid number scores are quite high (greater than 2.7), it can be assumed that these populations consist almost entirely of hybrid individuals. No other explanation can be given for such high mean hybrid number scores. No populations have plots at the apex of the limiting triangle, thus no populations observed were made up of solely F_1 hybrids. Only populations 34 and 61 are close to this point. By in large the populations in class 3 are the intermediates, some of which were taken from the Mitouer Gulch area.

The populations designated class four is another intermediate type but tends to favor C. apiculatus. Mean hybrid index scores for the populations in this group (25, 26, 27, 28, 37, 66, 67, 105, and 128) were between 5.4 and 5.8.

The populations designated class five consist of populations with high mean hybrid index scores (greater than 6.0) and plots of mean hybrid number -vs- mean hybrid index that fall on the right leg of the limiting triangle. (The populations in this category are 38, 39, 64, 65, 68, 69, 70, 71, 72, 74, 75, 98, 104, 115, 118, 120, 121, 122, 134, and 135.) They are interpreted as populations containing only individuals of C. apiculatus.

A comparison of the variability (standard deviations are given in Table 2) and the mean hybrid index scores (Fig. 7) shows the intermediate populations are far more heterogeneous than either of the parental types. The graph of mean hybrid number -vs- standard deviation of the hybrid index (Fig. 8) shows that there is a positive correlation between intermediacy (as measured by mean hybrid number) and variability (as measured by the standard deviation of hybrid index scores).

Thus, on the basis of mean hybrid index, mean hybrid number, and hybrid index variability the 68 measured populations can be divided into five population classes as

Figure 7

Mean Hybrid Index -vs- Hybrid Index

Standard Deviation

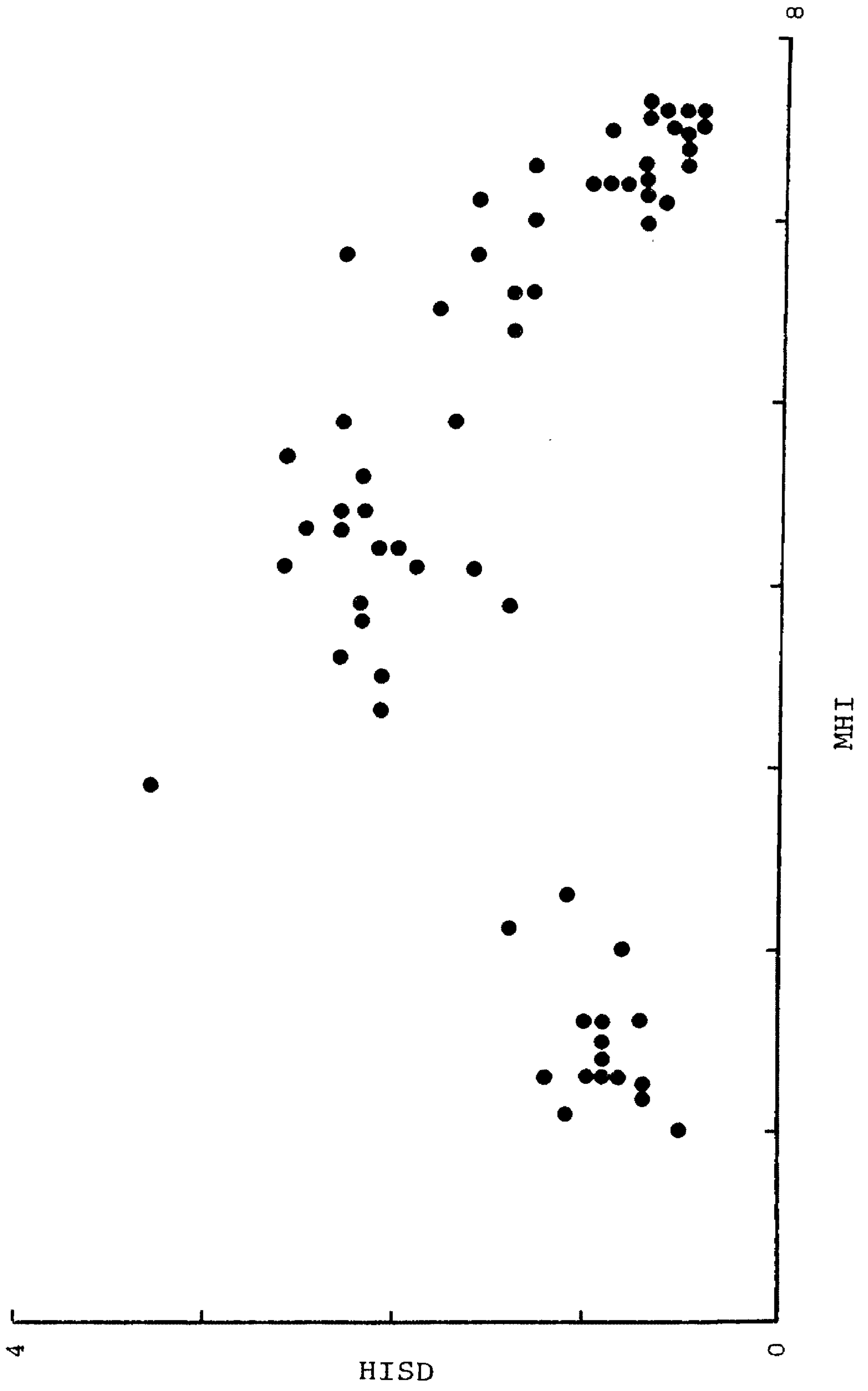
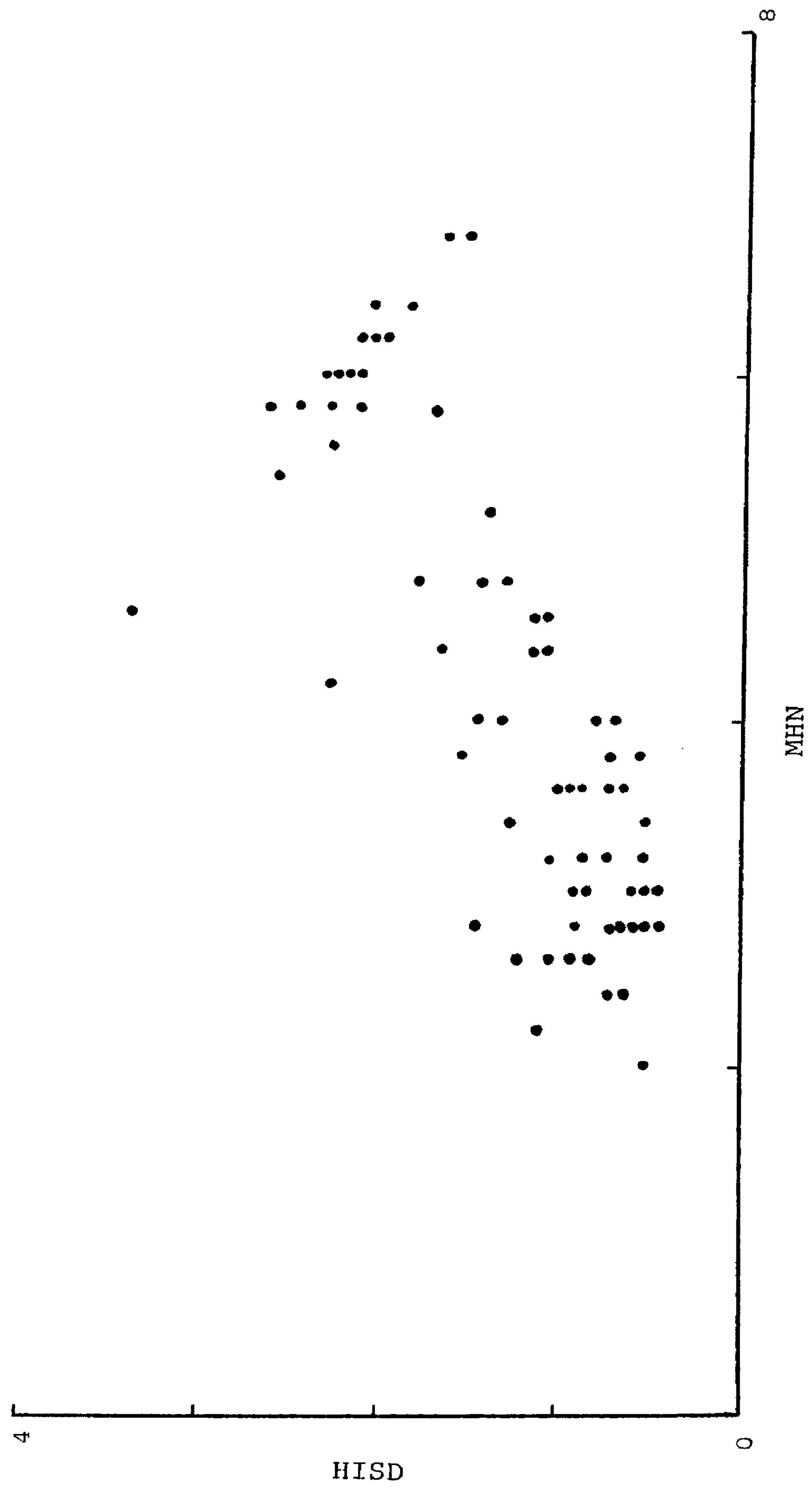

HISD
4
0
MHI
8

Figure 8

Mean Hybrid Number -vs- Hybrid Index

Standard Deviation


8
MHN
0
4
HISD

summarized in Table 3.

Table 3

Population Classes

Population Class	Description	Variability
1	*C. selwayensis*	Low
2	Intermediate resembling *C. selwayensis*	Intermediate
3	Intermediate	High
4	Intermediate resembling *C. apiculatus*	Intermediate
5.	*C. apiculatus*	Low

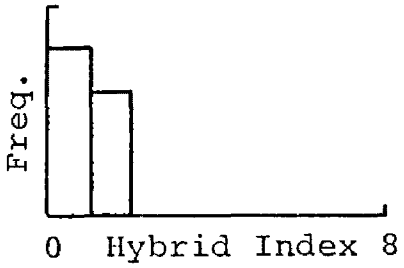
A more detailed picture of each population can be gained by looking at the individual hybrid index scores. Individual hybrid index scores for each plant measured are given in Appendix II. Frequency histograms of nine selected populations are provided here to illustrate the approximate composition of those populations (Fig. 9).

Population class one is represented by populations 57 and 107. The histograms concur with the previous data showing low hybrid index and little variability. Population class two is represented by populations 40 and 56 which show a slightly greater variability. Populations 26 and 38

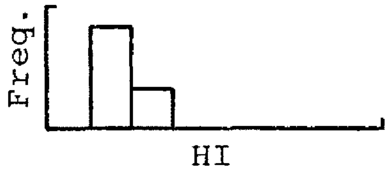
Figure 9

Hybrid Index Frequency Histograms of Representative Populations

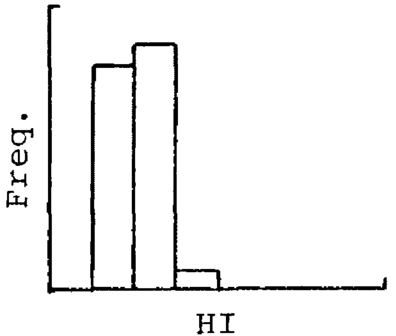
A. Population 107
B. Population 57
C. Population 40
D. Population 56
E. Population 48
F. Population 24
G. Population 26
H. Population 38
I. Population 70


Freq.
0 Hybrid Index 8

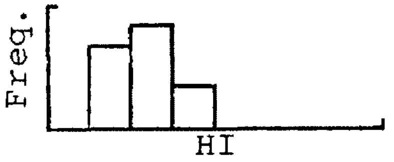
A


Freq.
HI

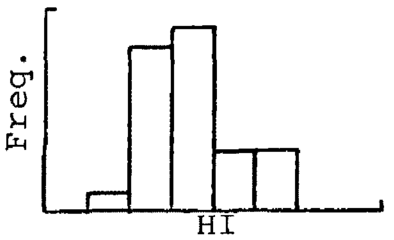
B


Freq.
HI

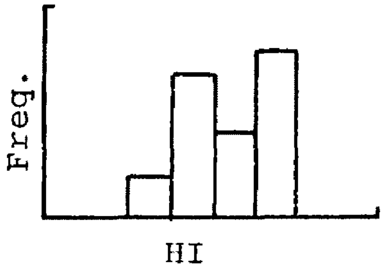
C


Freq.
HI

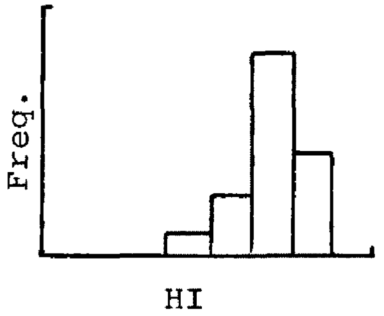
D


Freq.
HI

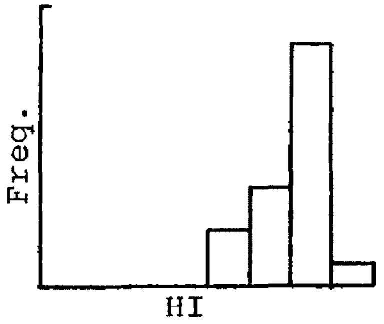
E


Freq.
HI

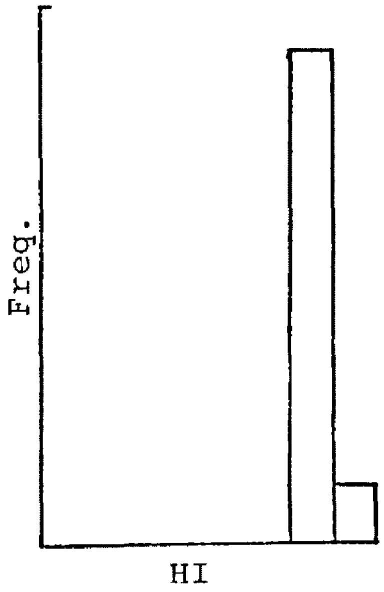
F


Freq.
HI

G


Freq.
HI

H


Freq.
HI

I

(class four) and population 70 (class five) show similar variability but on the *C. apiculatus* side of the hybrid index scale. Populations 48 and 24 (class three) show the most variability of all populations depicted in this manner. Population 70 (class five) illustrates a population of pure *C. apiculatus* with high hybrid index scores and low variability.

The histograms demonstrate that in no population were *C. selwayensis* and *C. apiculatus* found growing together. Even in the intermediate populations (class three) pure parental forms were rare or non-existent.

Scatter diagrams provide more detailed descriptions of the composition of populations than the frequency histograms. The scatter diagrams of a few representative populations of all five population classes are given in Figures 10-15.

Population 45 is a typical *C. selwayensis* (class one) population (Fig. 10). All individuals cluster near the upper left hand corner of the diagram. However, a few individuals exhibit qualitative characters which are suggestive of *C. apiculatus*. Introgression may not be the cause of such character states since the deviations from typical *C. selwayensis* are slight and the color loss may be due to fading or environmental influences.

Figure 10

Scatter Diagram of Population 45

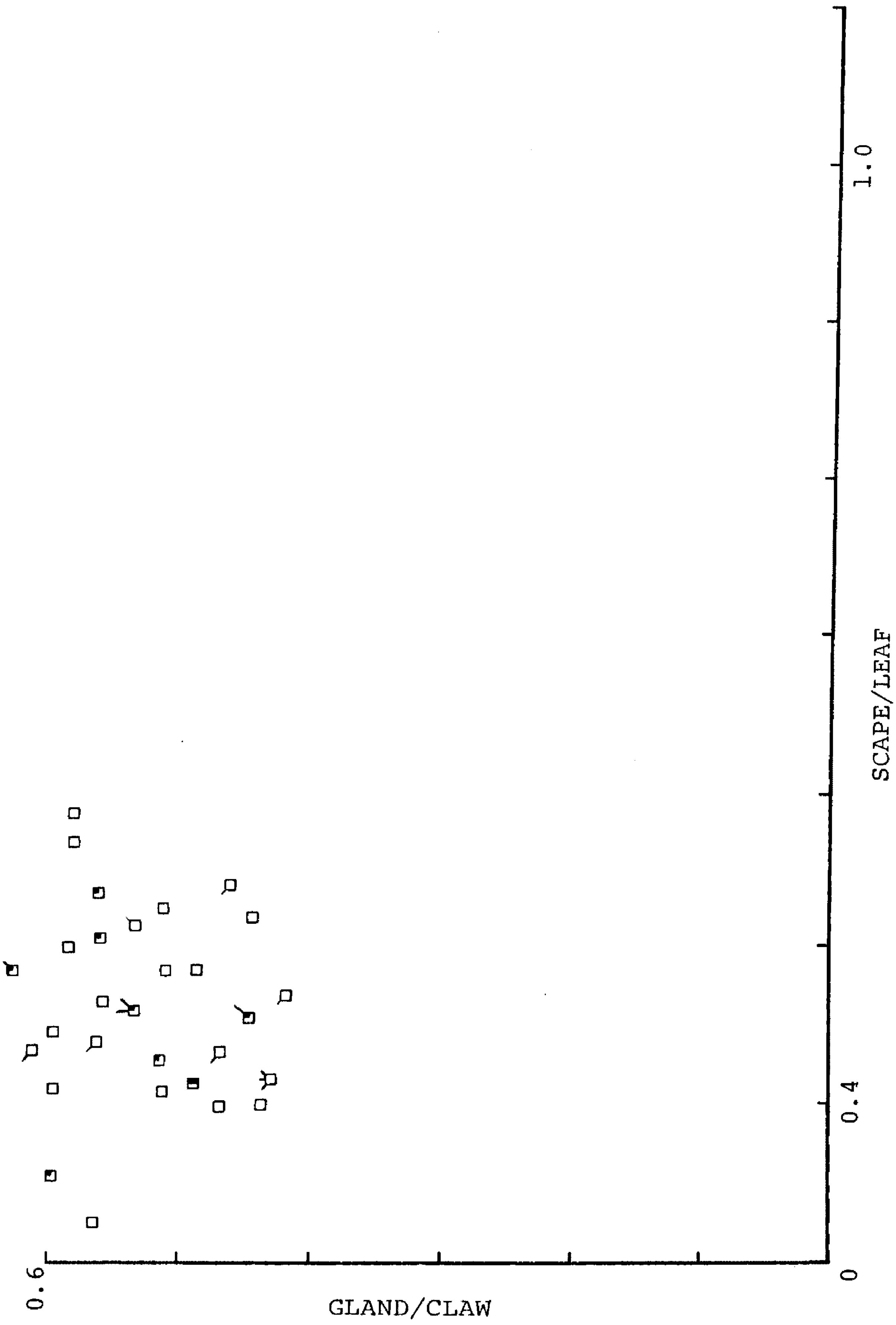

SCAPE/LEAF
GLAND/CLAW
0
0.4
1.0
0.6

Figure 11

Scatter Diagram of Population 74

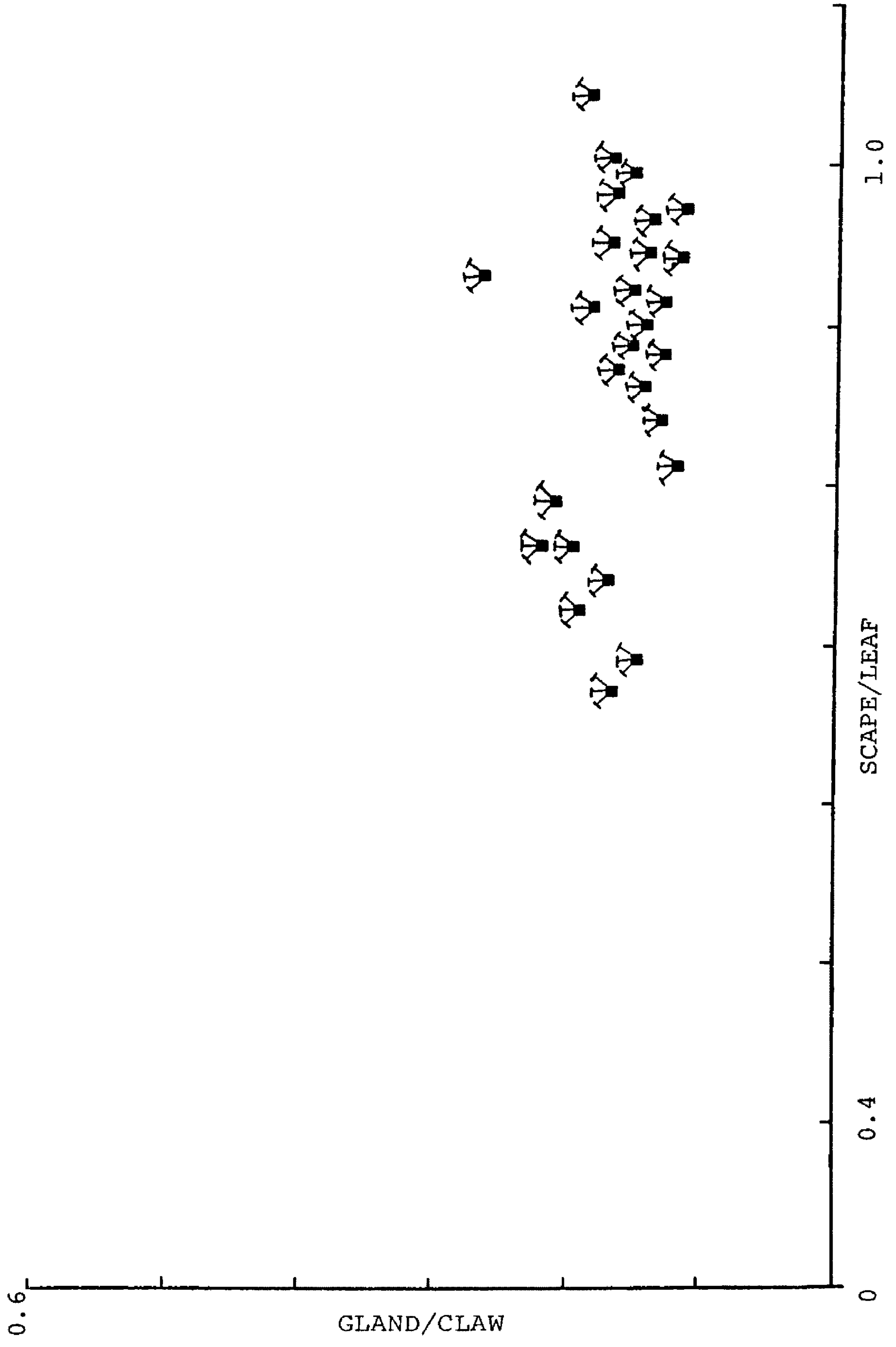

0.6
GLAND/CLAW
0
0.4
1.0
SCAPE/LEAF

Figure 12

Scatter Diagram of Population 23

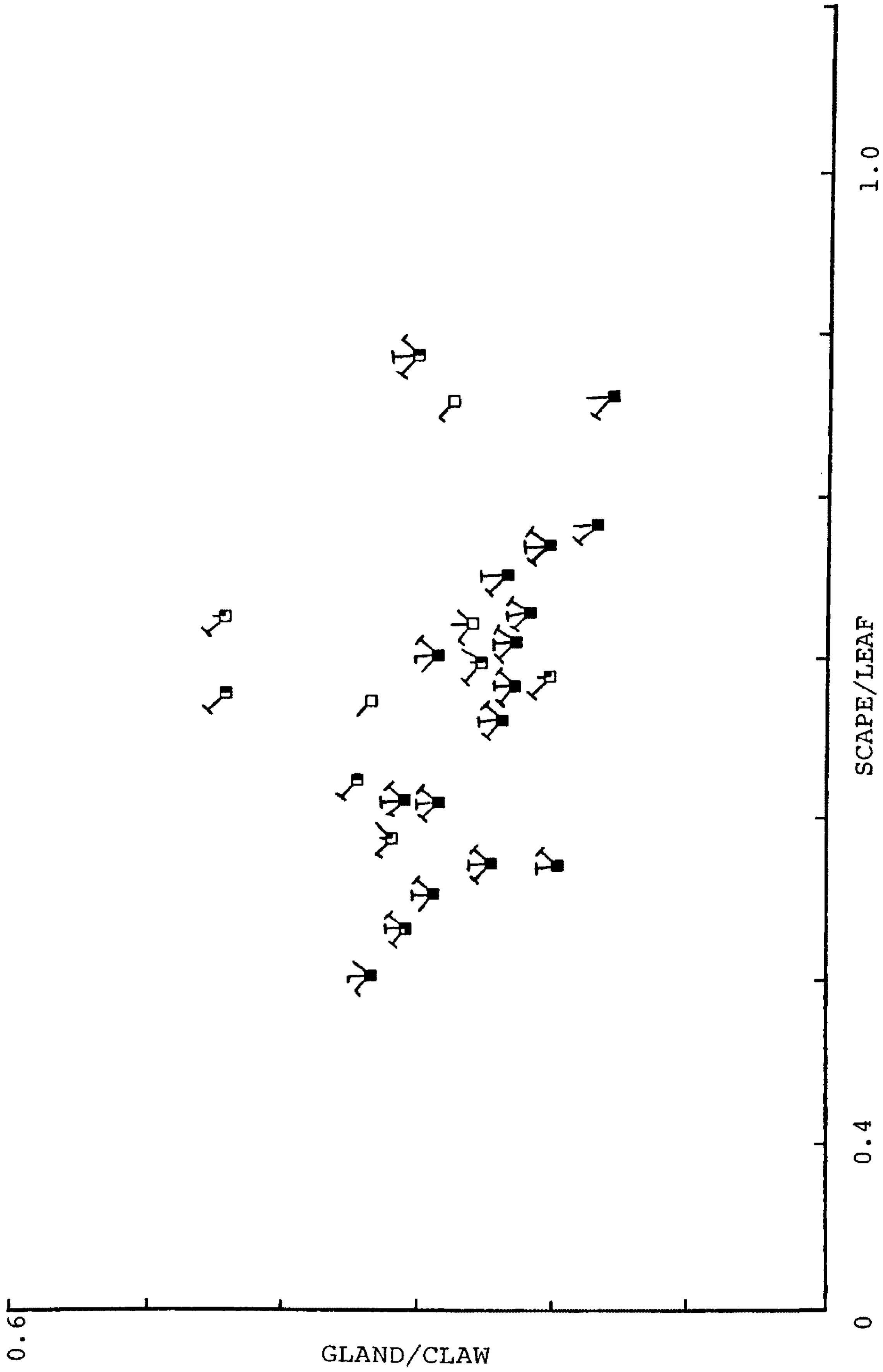

SCAPE/LEAF
0
0.4
1.0
GLAND/CLAW
0.6

Figure 13

Scatter Diagram of Population 50

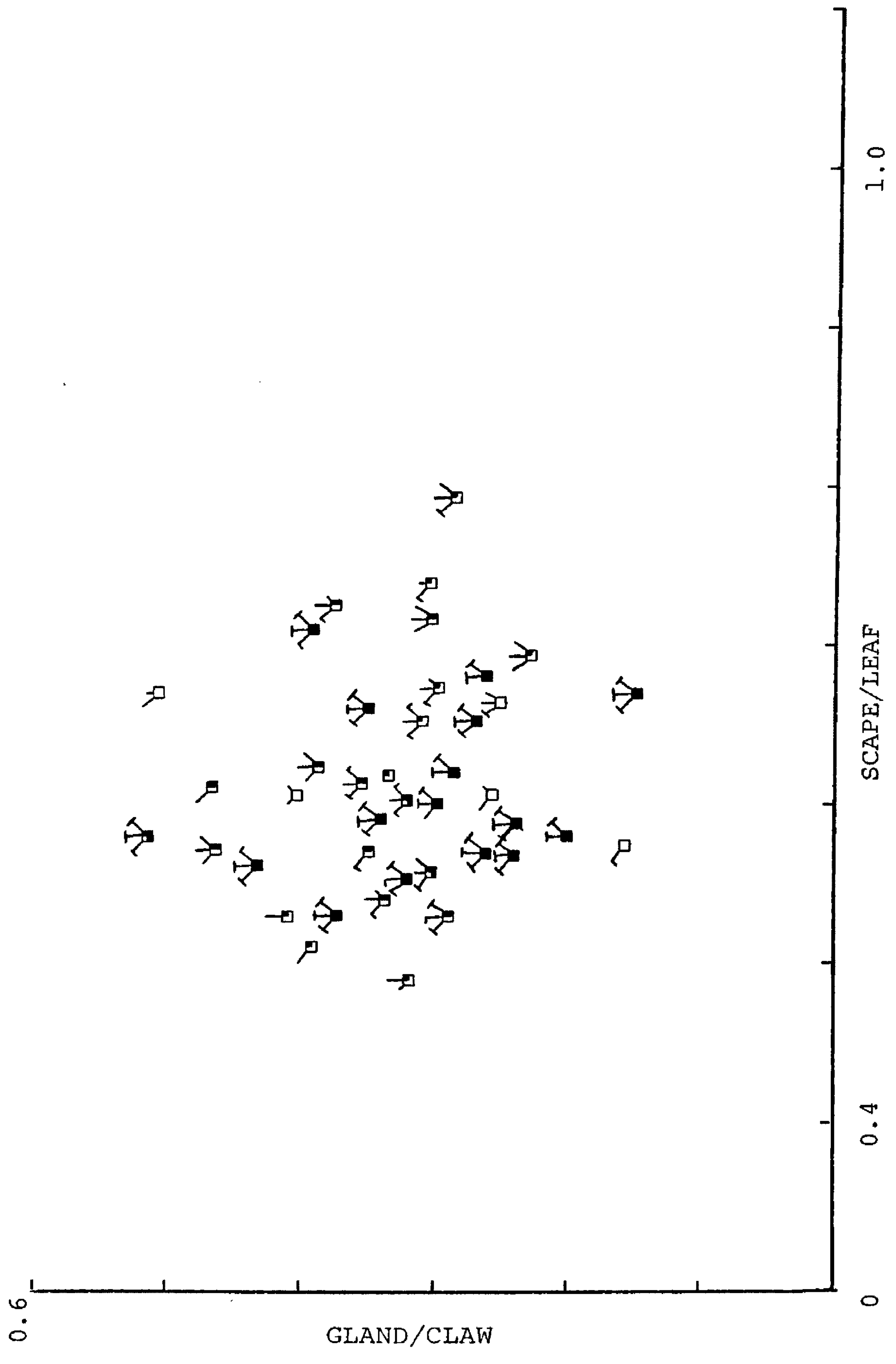

0.6
GLAND/CLAW
0
0.4
1.0
SCAPE/LEAF

Figure 14

Scatter Diagram of Population 29

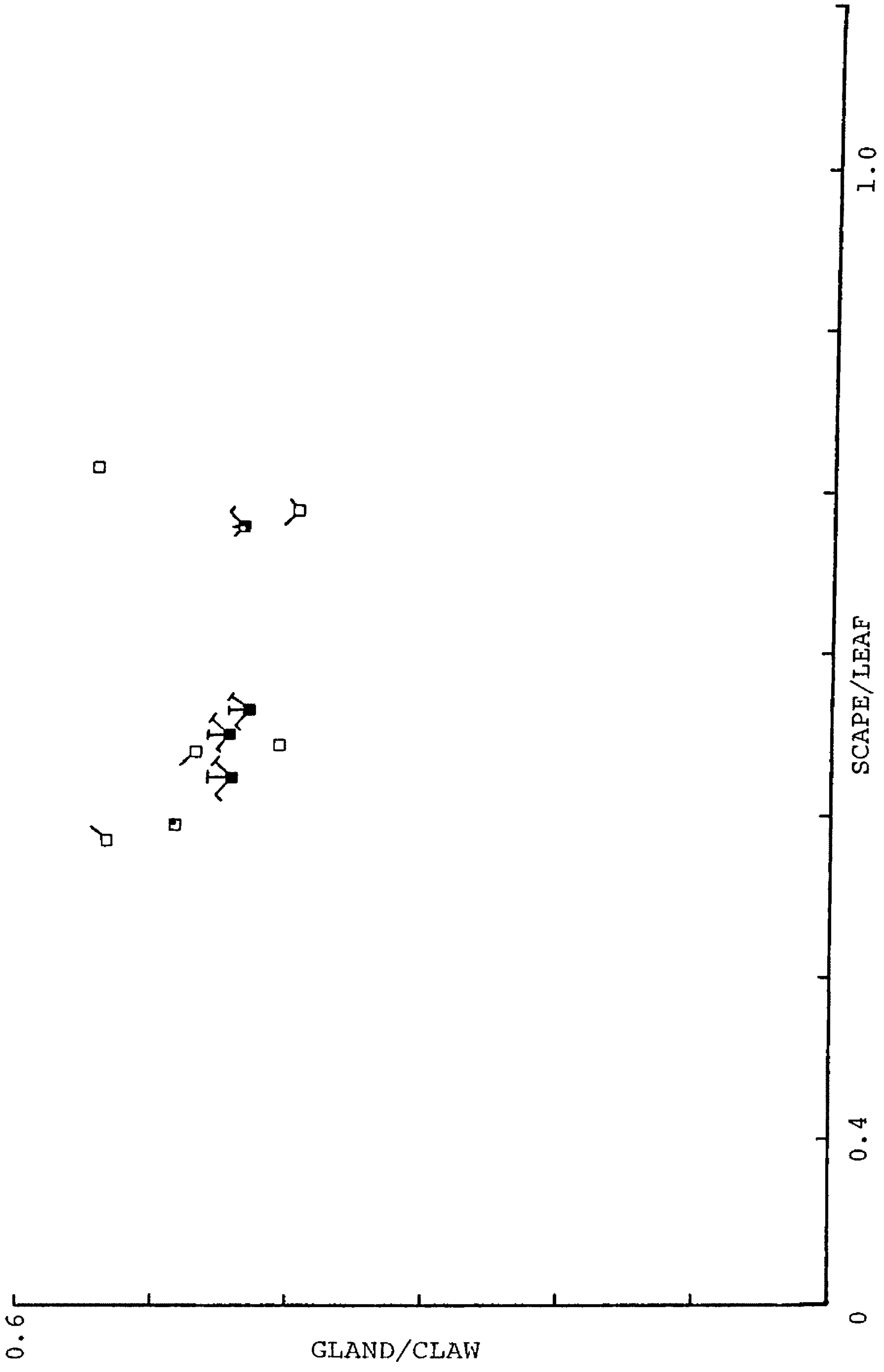
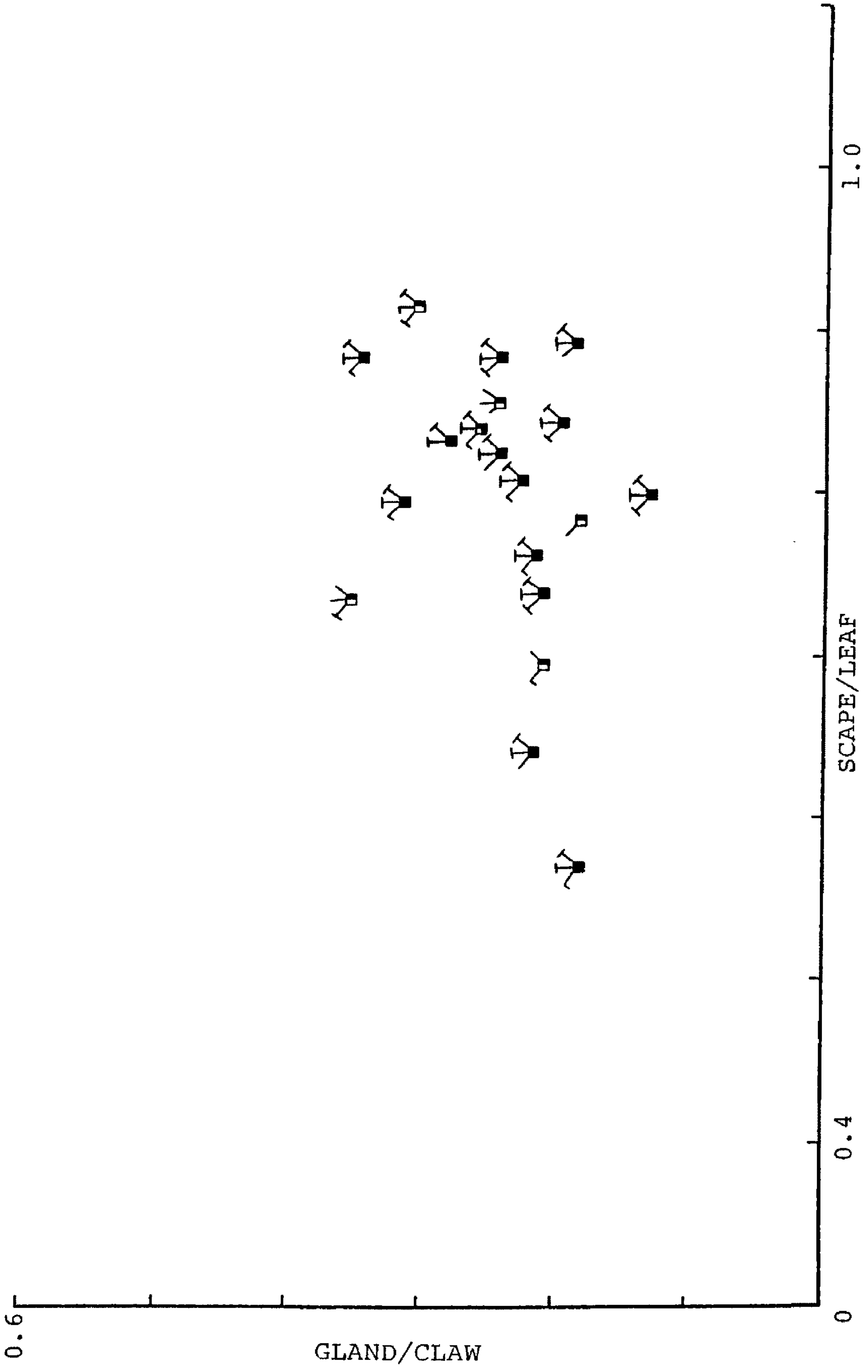

SCAPE/LEAF
0
0.4
1.0
GLAND/CLAW
0.6

Figure 15

Scatter Diagram of Population 27


0.6
GLAND/CLAW
0
0.4
1.0
SCAPE/LEAF

The scatter diagram of population 74 (class five) (Fig. 11) shows a distribution of individuals at the lower right hand corner of the diagram. This represents a population of pure _C_. _apiculatus_. In contrast to population 45, all individuals exhibit _C_. _apiculatus_ character states of those characters depicted in the scatter diagram.

Scatter diagrams of populations 23 and 50 (type 3), (Figs. 12 and 13) show the composition of two intermediate populations. In each case the points are widely scattered near the center of the diagram. This illustrates the mean hybrid index data which also indicates a great deal of variability. In both these populations there exist no individuals that could be considered "pure" _C_. _selwayensis_ or _C_. _apiculatus_. These diagrams also show that there seems to be very little or no correlation between any two characters with the possible exception of sepal blotch and petal blotch. If these populations represent hybrid swarms then much recombination is evident. This in turn indicates that the individuals in these populations were at least partially fertile.

Population 29 (population class two) has a very unusual scatter diagram (Fig. 14). This population sample though small has individuals that exhibit combinations of character states. This may indicate strong introgression in the direction of _C_. _selwayensis_ or a hybrid swarm in which certain recombinant types have undergone selection which favored C. selwayensis genotypes.

Population 27 (class four) (Fig. 15) is a population in which most individuals can be referred to C. apiculatus. Certain individuals have characteristics of C. selwayensis. The results here suggest introgression has taken place.

Geography

Intermediate populations (class three) were collected at Mitouer Gulch northeast of Missoula, Montana. In addition other populations were collected in the Marshall Valley approximately one mile west of Mitouer Gulch. These populations are located between the distributional ranges of C. selwayensis and C. apiculatus at a point where they are separated by ten miles or less. The intermediate populations in this area are more or less contiguous with the distributional range of pure C. apiculatus (class five), (Fig. 16). The Mitouer-Marshall populations are separated from the distributional range of C. selwayensis (class one) by a distance of approximately 10 miles and such physical barriers as the dry Missoula Valley and the city of Missoula.

Two class four populations occur near the Mitouer-Marshall area. These (#66 and #67 in Fig. 17) are located along Deer Creek and the Blackfoot River. This suggests that there is some gene flow from the Mitouer-Marshall

Figure 16

Geographical Distribution of the Five Population Types

A. Class 1
B. Class 2
C. Class 3
D. Class 4
E. Class 5

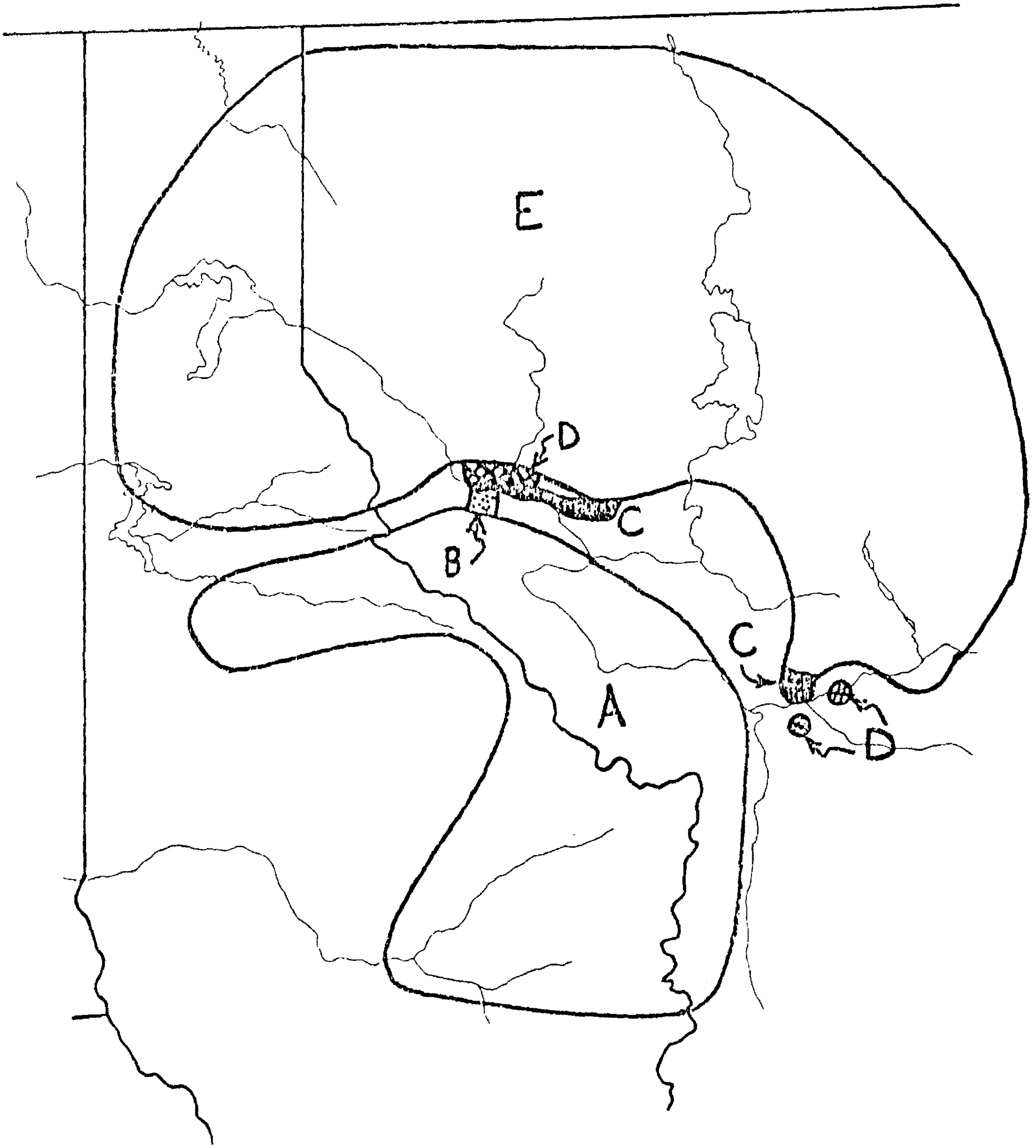
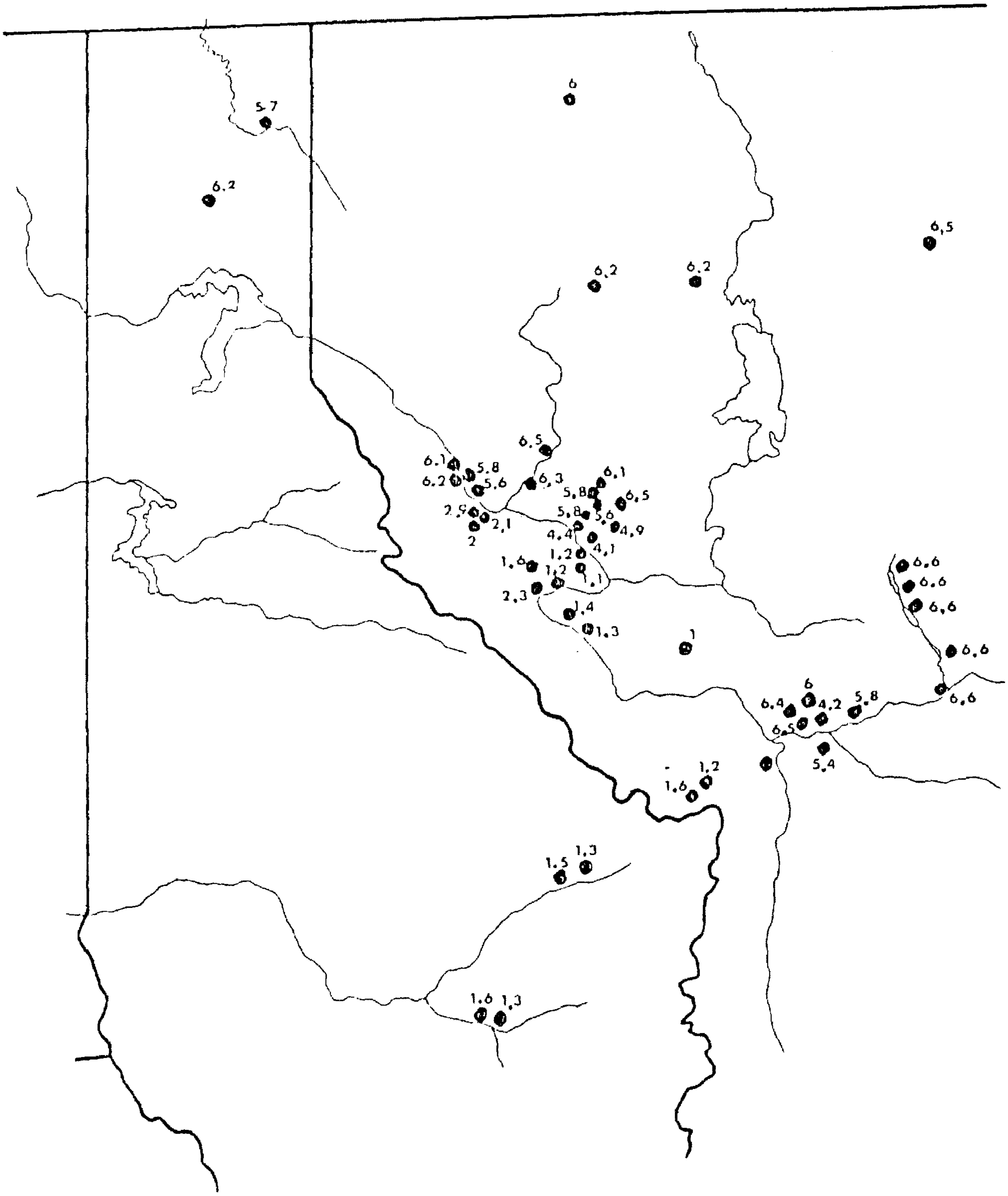

E
D
C
B
C
A
D

Figure 17

Mean Hybrid Index Score of Measured Populations as a Function of Location


5.7
6
6.2
6,5
6.2
6.2
6.5
6.1
5.8
6.2
5.6
6.3
6.1
5.8
6.5
2.9
2,1
2
5.8
5.6
4.4
4.9
1.2
4.1
1.6
1.2
1.1
2.3
1.4
1.3
1
6.6
6.6
6.6
6.6
6.6
6
6.4
4.2
5.8
6.5
5.4
1.2
1.6
1.5
1.3
1.6
1.3

populations up the Deer Creek and Blackfoot drainages.

In contrast there are no class two populations near the Mitouer-Marshall area. The closest class one population (#63) was found on Blue Mountain south of Missoula. The barriers previously described would possibly preclude any gene flow between the *C. selwayensis* and intermediate populations in this area.

Additional intermediate populations (class three) were collected at Plains and Thompson Falls, Montana. These populations were confined to a small narrow band that seemed to follow U. S. highway 200 between Plains and Thompson Falls and U. S. highway 28 from Plains northeast approximately 20 miles. These populations also occur in an area between the distributional ranges of *C. selwayensis* and *C. apiculatus* (Fig. 16). Here the gap between putative parents is between ten and 20 miles. As with the Mitouer-Marshall populations, the Plains-Thompson Falls populations are contiguous with the range of *C. apiculatus* and ten or more miles from the nearest *C. selwayensis* populations, with the exception of the intermediate populations *in* the small town of Thompson Falls which seem to be within a few miles of both *C. apiculatus* and *C. selwayensis* populations. In *all* cases the intermediate populations in this area were separated from *C. selwayensis* populations by the Clark Fork or Jocko Rivers.

Class two and class four populations were found abundantly in this area. The class two populations were found near and on the south side of the Clark Fork River, while the class four populations were found only to the north and northwest of the intermediate populations. In most cases class two and class four populations were found within five miles of intermediate populations. (See Fig. 16.) Two exceptions to this generalization were found: population 56, near St. Regis, Montana, and population 128, near Bonner's Ferry, Idaho. (See Fig. 17.) A possible explanation for the discrepency here might be that the populations were not collected in a truly random fashion or that the sample size was too small; only five plants were collected from the Bonner's Ferry site and 11 from the St. Regis site. Other collections made in these areas seem to indicate that there might have been some sampling error.

The mean hybrid index scores of the C. apiculatus populations seem to indicate that there may be two different populations systems within that species. The mean hybrid indices of the western populations were consistently lower than the indices of the eastern populations. At first glance it appears that introgression is occurring in the west but not in the east. If this is introgression, the populations of C. apiculatus thus affected cover an extensive area from Plains-Thompson Falls to the Canadian border.

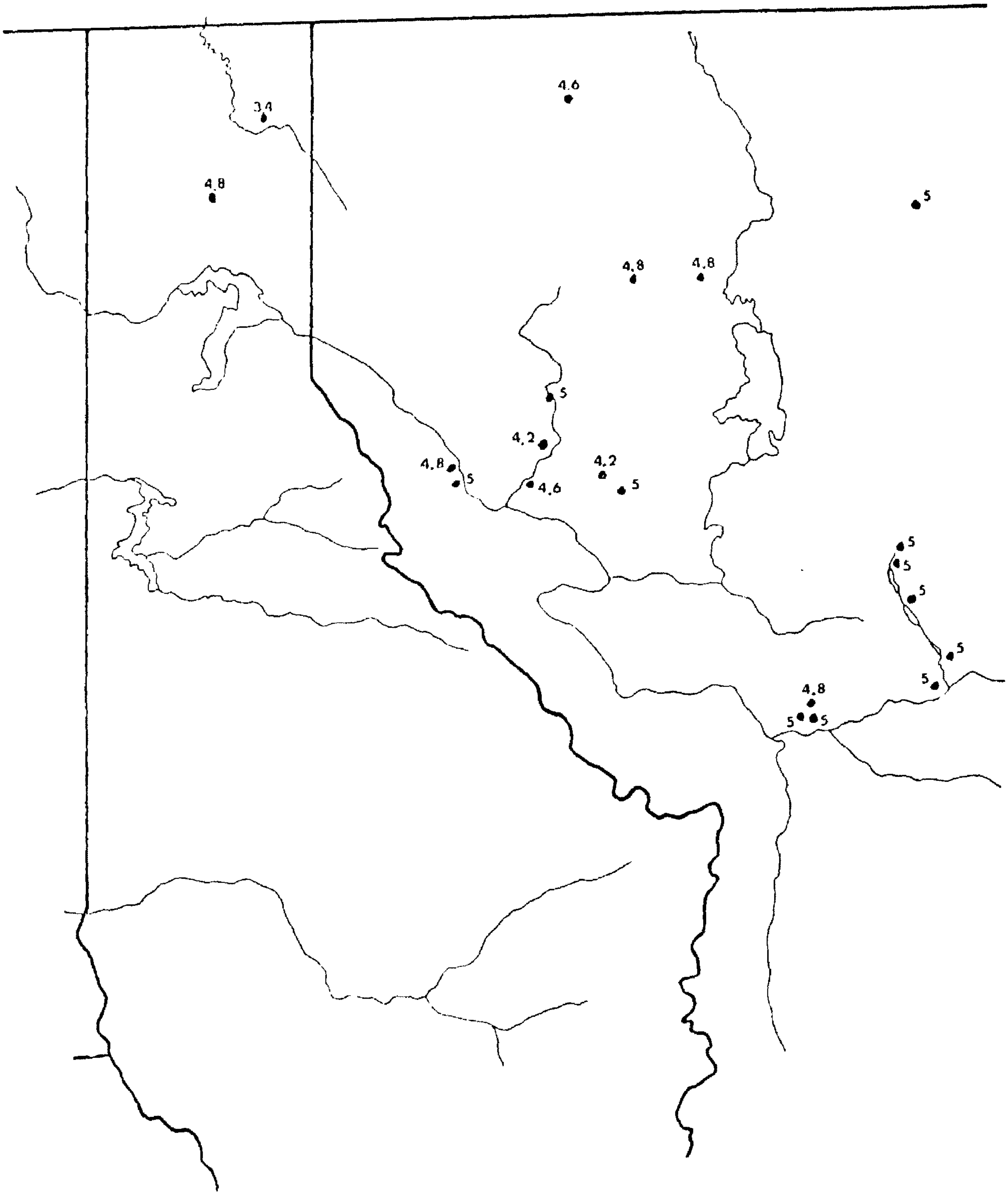
The lower mean hybrid index scores of the western populations were in part due to the fact that a few individuals in almost all those populations had at least some purple blotching on the sepal (Fig. 18). Any population with a mean sepal blotch less than 5.0 contains at least one individual with purple blotching on the sepal. Only two western populations (#120 and #104) had a mean sepal blotch of 5.0. The other somewhat western population (#98) near Hot Springs, Montana, also had a mean sepal blotch of 5.0 but is considered an eastern type. Only one eastern population (#70) had a mean sepal blotch less than 5.0 but is located very near the Mitouer-Marshall intermediates. The appearance of the purple blotch on the sepal in most of the western populations seems to suggest introgression throughout half the range of *C. apiculatus*.

Ecology

Calochortus selwayensis populations were typically found at elevations between 1300 and 4900 feet. Only two populations were collected at elevations above 4000 feet; one (#46) at 4000 feet near Lolo Hot Springs and the other (#63) at 4900 feet near the top of Blue Mountain south of Missoula. All other *C. selwayensis* populations were found at lower elevations.

Figure 18

Mean Sepal Blotch as a Function of Location


4.6
3.4
4.8
5
4.8
4.8
5
4.2
4.8
5
4.2
4.6
5
5
5
5
5
5
4.8
5
5

In nearly all instances, populations of *C. selwayensis* were associated with *Pinus ponderosa* or a mixture of *P. ponderosa* and *Pseudotsuga menziesii*. A few populations, especially those in the St. Regis area (populations 53, 54, 55, 84, and 88), were found growing under a pure *P. menziesii* canopy. Population 106 was the only *C. selwayensis* population found growing under *Larix occidentalis* Nutt.

Populations of *C. selwayensis* were always found on south to southwest facing slopes that were open to semi-open. These sites are typically quite dry during most of the summer months.

In areas where *C. selwayensis* was collected there seemed to be little or no evidence of either natural or man-made disturbance. Areas that otherwise appeared suitable, but were disturbed either by fire, logging, etc., typically did not support populations of *C. selwayensis*.

Calochortus apiculatus populations were found at elevations between 2400 and 8600 feet. The western populations were collected at 2000 to 3400 feet while the eastern populations were at elevations of 3000 to 8600 feet.

The predominant canopy cover in most *C. apiculatus* populations was *Pseudotsuga menziesii*, a mixture of *P. menziesii* and *Larix occidentalis*, or a mixure of *P. menziesii* and *Pinus ponderosa*. However, populations 69, 134, 135, and 136 were found under *P. contorta* Dougl., populations 75 and 124 under *Picea engelmannii* Parry, population 121 under *Abies grandis* (Dougl.) Forbes, and population 77 was found in a subalpine meadow above timberline. A few populations were found under pure *Pinus ponderosa*.

For the most part all populations were located on level to gradually sloping areas. The western populations preferred dry habitats while the eastern populations were found on wetter sites.

Evidence of disturbance was found at a number of *C. apiculatus* sites. The causes of disturbance ranged from logging and fire to highway building. Most of the populations that grew in disturbed areas were in the west while the eastern populations were found in more mesic, climax type habitats.

Intermediate populations were found at elevations between 4100 and 4300 feet in the Mitouer-Marshall area and between 2300 and 3500 feet in the Plains-Thompson Falls area. These populations were either growing on open ground or associated with *Pinus ponderosa*, with the exception of population 31 which was growing in a dense *Pseudotsuga menziesii* stand. The intermediate populations occupied dry sites with the exception of population 31.

Disturbance was very evident at all sites containing intermediate populations. The Mitouer-Marshall sites had obviously been logged; nearby areas were being clearcut at the time the collections were made. All Plains-Thompson Falls sites were on highway shoulders with the exception of population 31.

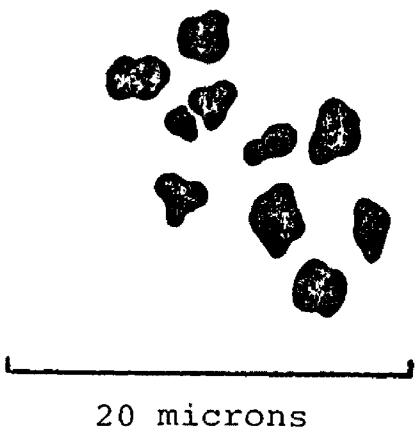
Cytology

Four collections yielded buds in the correct stage of development for the determination of chromosome number. Three collections (83, 92, and 94) were from intermediate populations. No less than three individuals from each population were counted. In each case ten bivalents were observed at metaphase I. Meiotic karyotypes of individuals from populations 83 and 92 are given in Figure 19. The individuals from populations 92 and 94 also contained abundant anaphase I figures. No irregularities such as rings or bridges were observed in any individual.

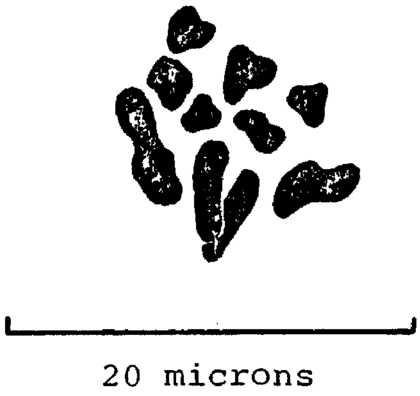
One count was obtained from a C. apiculatus population (#123) located in the Bob Marshall Wilderness near Shaw Creek. Ten bivalents were evident at metaphase I. (The karyotype of the individual from this population is given in Fig. 19.)

Reliable counts of C. selwayensis and C. apiculatus have been made by Beal (1939). In both species meiotic counts of $n=10$ and somatic counts of $2n=20$ were obtained. The data obtained in this study agree with that of Beal, demonstrating that all entities involved are diploid.

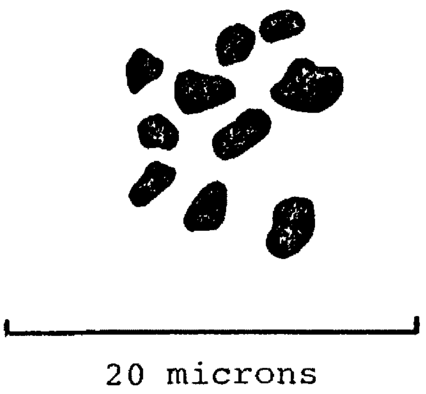
A.



B.



C.



Pollen Analysis

The results of the pollen analysis were somewhat inconclusive due to the variation encountered within the putative parental taxa. A list of percent pollen viability is given in Table 4 for each specimen analyzed.

Table 4

PERCENT POLLEN VIABILITY

Population	Individual	Taxon	Percent Viable Pollen
14	3	*C. selwayensis*	12
14	11	*C. selwayensis*	13
42	17	*C. selwayensis*	91
42	18	*C. selwayensis*	98
45	27	*C. selwayensis*	86
45	28	*C. selwayensis*	63
45	30	*C. selwayensis*	74
107	6	*C. selwayensis*	77
107	8	*C. selwayensis*	81
107	11	*C. selwayensis*	80
22	17	intermediate	06
22	18	intermediate	28
22	20	intermediate	00
99	1	intermediate	63
99	2	intermediate	98
99	3	intermediate	02
99	5	intermediate	40
99	6	intermediate	09
99	11	intermediate	02

Table 4 (continued)

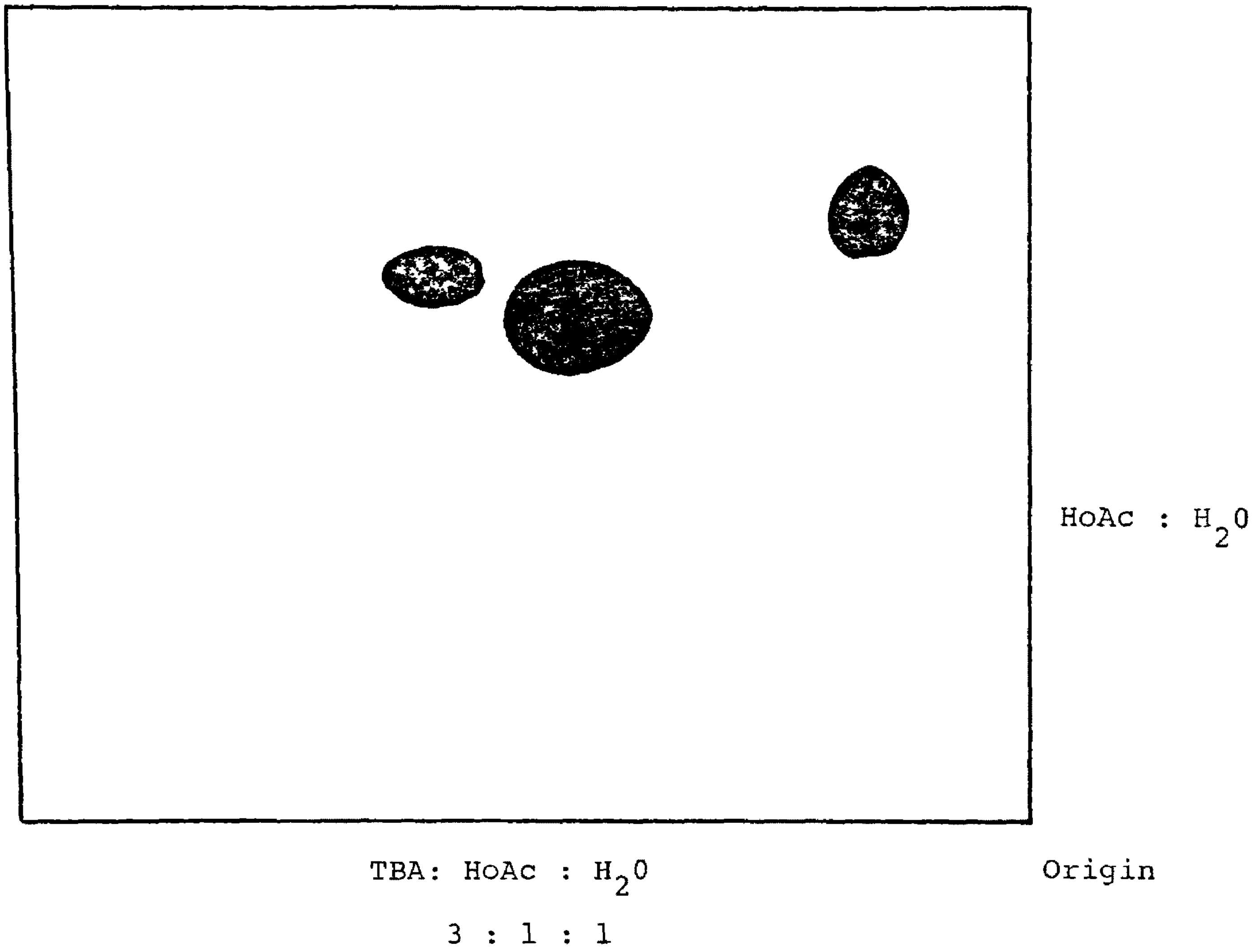
Population	Individual	Taxon	Percent Viable Pollen
101	5	intermediate	00
101	14	intermediate	03
101	15	intermediate	00
73	1	*C. apiculatus*	86
73	4	*C. apiculatus*	66
73	7	*C. apiculatus*	81
98	2	*C. apiculatus*	50
98	4	*C. apiculatus*	41
98	5	*C. apiculatus*	29
118	9	*C. apiculatus*	00
118	12	*C. apiculatus*	00
122	5	*C. apiculatus*	00
122	7	*C. apiculatus*	00
122	8	*C. apiculatus*	00

The *C. selwayensis* populations with the exception of population 14 have relatively high pollen viability counts. This is to be expected in any stabilized sexual taxon. The average percent viable pollen for all *C. selwayensis* populations analyzed was 67.5.

The intermediate populations on the other hand were considerably more variable with individual readings of between 0% and 98%. The overall average for the twelve plants analyzed was 20.9%. This is less than one third that observed in *C. selwayensis*.

The results obtained from *C. apiculatus* populations are somewhat difficult to interpret. Two populations (#73 and #98) have average percent viable pollen of 77.7 and 40. The remaining two populations produced no viable pollen. Interestingly the two populations of *C. apiculatus* that had high viable pollen counts were growing in the east while the two populations with counts of zero were of the western type.

Chromatography. The results of the chromatographic analysis are given in Figure 20. All chromatograms run had the same profile. There seemed to be no difference between *C. selwayensis*, the intermediates, and *C. apiculatus*.


HoAc : H_2O
TBA: HoAc : H_2O
3 : 1 : 1
Origin

Chapter 4

DISCUSSION

Present Status

As previously stated, intermediacy can be caused by a variety of natural phenomena. These would include hybridization, clinal variation, patristic intermediacy, and the presence of an ancestral complex. Theoretically, a cline is a gradual change in the composition of populations of a single species in response to a climatic gradient. Within a cline there would be only a slightly restricted gene flow between nearby populations. Also within a cline one would expect to find a variety of populations each with its own adaptive norm for a particular environmental situation.

Patristic intermediacy could arise when three (or more) species are derived from a single ancestral type. In this situation one of the three (or more) species may appear to be intermediate to the others. The probability that the patristic intermediate would be exactly intermediate to the other two is exceedingly small.

Two species can have an ancestral complex that appears intermediate to them. In such situations the ancestral complex may not be exactly intermediate to the two divergent species.

The intermediacy caused by clinal variation, patristic intermediacy, and the presence of an ancestral complex is not necessarily restricted to a specific zone, these intermediates are not typically that much more variable than populations at the extremes, the distributional range of these intermediates may not be restricted to a zone exactly between the extreme populations, and the size of the distributional range of the intermediates tends to resemble those of the extreme populations.

Hybridization on the other hand requires that divergent populations be in secondary contact. Hybrid individuals will be found only at and near this zone of contact. The size of the hybrid zone will reflect the extent of secondary contact. If the F_1 hybrids are sterile very little variability will be evident in the hybrid swarms. This variability is due to the presence of F_2 and introgressant individuals.

The evidence obtained in this study seems to indicate that hybridization was the underlying cause of the intermediacy reported at Mitouer Gulch and subsequently discovered at Plains, Thompson Falls, and the Marshall Valley. In all cases conclusive proof cannot be obtained. However, the statistical probability of any other explanation is exceedingly small.

The extensive geographical study has shown the populations of *C. selwayensis* and *C. apiculatus*, although allopatric throughout their respective ranges, occur within close proximity in two regions. It is only in these two regions that plants exhibiting morphological characteristics intermediate to the aforementioned species are found. The spatial isolation barriers are weakest at these two points. Levin (1967) has shown that where spatial barriers between two closely related species in the genus *Liatris* have been disrupted, hybrid swarms are found. The fact that the intermediate populations found in the present investigation occur *only* in areas where the spatial barrier between the putative parental species is minimal suggests that the intermediates are hybrids, or of hybrid origin.

The fact remains that the spatial barriers are seemingly too great to permit interspecific cross-pollinations. Thus it is clear that hybridization between *C. selwayensis* and *C. apiculatus* is not occurring now nor has it occurred in the immediate past. This does not preclude more ancient hybridization, the derivatives of which are the intermediates of the present study. Many present "good" species are of hybrid origin yet are not totally sympatric with the parental species (Muller, 1961; Lewis and Epling, 1959; Stebbins and Ferlan, 1956).

Ophrys mubeckii Fleischmann (Stebbins and Ferlan,

1956) and *Lysimachia* X *producta* (Gray) Fernald (Cooperrider and Brockett, 1974) are both hybrid taxa which have become stabilized yet maintain a very limited distribution, at least in relation to the parental species. The intermediate populations in the present study also have an extremely restricted range in comparison to *C. selwayensis* and *C. apiculatus*. This suggests that the intermediate populations are of hybrid origin and not patristic intermediates which would be expected to have a distributional range similar to that of the related species (Stebbins and Ferlnan, 1956).

The intermediate individuals of the present study appear to occupy habitats that are in someway disturbed or otherwise unusual. This is in contrast to *C. selwayensis* and *C. apiculatus* which prefer more climax type habitats. In addition these two species occupy different niches: *C. selwayensis* occupies the drier sites and *C. apiculatus* is found in the more mesic sites. If *C. selwayensis* and *C. apiculatus* were to hybridize one would expect the hybrids to become established in a disturbed habitat that provides conditions intermediate to those preferred by the parents (Anderson, 1948). The disturbance encountered in this study could have produced such "hybrid habitats". The logging operations in the Marshall Valley (present investigation) could have resulted in the drying of the south facing sloped in an area that were formerly somewhat moist. In Mitouer Gulch the ravines run in many different directions providing a vast array of exposures each with different physical conditions. The Plains-Thompson Falls populations growing on roadsides are exposed to an unusual situation that may be intermediate to *C. selwayensis* and *C. apiculatus* habitats. Thus, it appears that the intermediates are not only growing in disturbed habitats but that the disturbances have created habitats which could be intermediate to the habitats of the putative parents.

Also, the "hybrid habitat" is somewhat varied. Many different habitats seem to support the intermediate populations. This wide habitat preference is characteristic of many hybrid taxa. Cooperrider and Brockett (1974) have shown that *Lysimachia* X *producta* has a very wide habitat tolerance. Of course, this does not necessarily imply that a particular genotype is adaptive in many situations but that the hybrid taxa contain a variety of genotypes each adaptive to a different habitat. This variability is characteristic of hybrid populations (Anderson, 1949).

Normally, the variability encountered in hybrid populations is due to recombinants since a population comprised of solely F_1 individuals is often uniform. However, in greenhouse studies, Lewis and Epling (1959) noted that F_1 hybrids between *Delphinium hesperium* and *D. recurvatum* are as variable as would be expected in F_2 crosses. Thus, the wide habitat preferences of the intermediates of the present study would suggest that they are either F_1 hybrids or recombinants and are not patristic intermediates nor an ancestral species.

The variability is evident not only in habitat preference but also in exomorphology. The hybrid index scores of the intermediate populations are quite variable in comparison to the scores of the parental populations. The hybrid index variability is supported by the character dispersion as illustrated in the scatter diagrams. The hybrid index and scatter diagrams though crude tools do display a tremendous recombination of characters and variety within the intermediate populations that could be expected only in hybrid populations with many filial generations.

It should be noted that variability in itself is not indicative of hybridization, but rather how that variability compares with closely related taxa. Intrapopulational variability merely indicates heterogeneity. However, if a certain taxon is much more variable than related taxa then it could be argued that the increased heterogeneity might be due to hybridization or greater outcrossing. Obligate inbreeders tend to store their variability between populations while outcrossers store variability within populations (Levin, 1975). In the present investigation it appeared that both *C. selwayensis* and *C. apiculatus* are outcrossers. The morphologic and ecologic variability of the intermediate populations is due to an increased heterozygosity originating through interspecific hybridization.

This variability is evident only in hybrid populations that are at least partially fertile. Sterile hybrid swarms formed through vegetative reproduction tend to be quite invariable as illustrated by the hybrid species *Streptopus oreopolus* Fern which is a sterile triploid (Love and Harries, 1963). The intermediate populations of the present study, however, are somewhat fertile as indicated by pollen viability counts. Thus recombination between the putative parents need have happened only a few times. The internediate populations could have become stabilized yet perpetuate the intrapopulational variability through amphiploidy, introgression, or selection of certain recombinant types (Davis and Heywood, 1963).

The intermediates exhibiting only those character states to be expected from an interspecific hybridization between *C. apiculatus* and *C. selwayensis*. In no cases in the intermediates were characters missing, added, or modified outside the realm of variability exhibited by the putative parents. This fact of course does not prove conclusively that hybridization has taken place but renders an alternative hypothesis statistically very unlikely (Clevenger and Heiser, 1963).

A perplexing problem is that the intermediate populations contain no parental individuals. Normally at least one or both parents are present or in the near vicinity of hybrid swarms as illustrated by Alston and Turner (1963) in Baptisia, Nelson (1963) in Prunella, Levin (1967) in Liatris, and many others. However, there are numerous examples in the literature in which hybrids are found in populations without parents present even when the parents are sympatric but ecologically separated. Some examples include Stebbins and Ferlan (1956) in Ophrys, Brophy and Parnell (1974) in Quercus, Stutz and Thomas (1964) in Cowania and Purshia, and others. This lack of parental types again indicates that the intermediate populations, if of hybrid origin, have become stabilized.

Often interspecific and intergeneric hybrids are stabilized through amphidiploidy as in Goodyera tesselata Lodd. (Kallunki, 1976), in many of the cereal grains (Stebbins, 1959), in Tragopogon (Ownbey, 1950), and in many others. However, the present investigation has shown that the hybrids are not polyploids and thus must have become stabilized either by selection for recombinant types or introgression (Davis and Heywood, 1963). Stabilized hybrid swarms that are not amphidiploids or segmental allopolyploids through uncommon have been reported in Delphinium (Lewis and Epling, 1959), in Lysimachia (Cooperrider and Brockett, 1976), and others. In these cases, it was difficult to document introgression. Specifically, it is difficult to determine whether an individual is an introgressant of extreme recombinant (Cooperrider and Brockett, 1976).

This is, in part, due to the fact that introgressed adaptive genes will "drag" other gene systems along with them to the recurrent parent. Thus, an introgressant will vary in many characters from the recurrent parent but in the direction of the non-recurrent parent (Grant, 1971). The same sort of character array can occur through recombination and the segregation of adaptive recombinants in habitats resembling those of one parent.

Within the hybrid zones between the ranges of *C. selwayensis* and *C. apiculatus*, the primary stabilizing force would be the selection of certain recombinant types. This is especially true in the Mitouer-Marshall area since here the nearest parent is many miles away. Introgression thus would be unlikely in the absence of long distance pollinators which do not frequent the flowers of *Calochortus* (present investigation).

However, introgression seems likely both north and south of the hybrid zones near Plains-Thompson Falls. Here both parents occur near the hybrid swarms. Populations resembling the recurrent parent were frequently encountered near the hybrid zone. This introgression is over a small area and does not result in the blurring of specific delimitations. This is, in part, due to the semi-sterility of the hybrids limiting gene flow in both directions. However, Anderson (1953) notes that introgression requires only that both parents be at least partially interfertile.

Thus, even a great (but not absolute) sterility barrier can permit widespread introgression.

Introgression appears to be of evolutionary significance to the western populations of C. apiculatus. The habitats occupied by these populations are drier and at lower elevations than those normally supporting populations of C. apiculatus. Thus it appears that the genes that allow these populations to inhabit drier, low elevation habitats have been incorporated into the gene pools of these populations through introgression. The evidence here is somewhat indirect, in that introgression in the direction of C. apiculatus from C. selwayensis is revealed by the presence of a sepal blotch on some individuals in these populations. Thus it is assumed that the genes adaptive in dry habitats have been donated to C. apiculatus through introgression and are linked to genes controlling sepal blotch. It is also interesting to note that genes controlling sepal blotch and petal blotch may also be linked since petal and sepal blotch seem to be inherited as a unit as indicated in scatter diagrams.

While there is evidence for widespread introgression in the western populations of C. apiculatus there is no such evidence for introgression in C. selwayensis. The reasons for this may be (1) that the C. apiculatus gene pool contains no genes that could increase the fitness of C. selwayensis even in marginal habitats, and (2) C. selwayensis is separated from the hybrid swarms by slight geographical barriers while C. apiculatus is not.

Thus it appears that hybridization and introgression in the direction of C. apiculatus have given rise to the intermediate stabilized populations at Mitouer-Marshall and Plains-Thompson Falls, and the western geographical race of C. apiculatus. This extensive mingling of gene pools between C. selwayensis and C. apiculatus implies a close phylogenetic relationship. Additional support for this conclusion was obtained from cytologic and chromatographic observations.

Almost perfect segregation at meiosis I of the hybrid individuals suggests very few if any chromosomal transformations occur between the parental species. This evidence is in slight disagreement with Beal (1939) who felt that C. apiculatus differs from C. selwayensis by perhaps a few translocations and/or inversions. In any case, these two species have exceedingly similar karyotypes. The chromatographic evidence also indicates that the parental species are very closely related. The flavonoid profiles of both C. selwayensis and C. apiculatus are identical.

The pollen analysis data seem to suggest some slight differences between the parental taxa. This is indicated by the fact the meiosis appeared normal yet many grains were malformed, unstained, or empty. Thus there might be some post meiotic breakdown in the pollen forming mechanism due to genetic differences or perhaps some environmental influences.

Thus it appears that C. selwayensis and C. apiculatus are closely related taxa and have undergone some genetic divergence. This divergence, however, was slight and allowed for some hybridization and introgression after a period of secondary contact.

Past Events

The present investigation has shown that C. selwayensis and C. apiculatus are two very closely related taxa. This divergence, however, is somewhat illusory in that genetically these two taxa are exceedingly similar. They are so similar that when geographical ioslating barriers become disrupted they hybridize to produce semi-fertile offspring.

A period of hybridization likely occurred sometime in the past between these two species resulting in hybrid swarms that have since become stabilized without an increase in chromosome number. Presently the derivatives of the original hybrid swarms have become established and are locally common but occur within a restricted distributional range.

Three major events must have taken place before this situation arose. They are in chronological order: (1) disruption of an ancestral population system by some geographical barrier allowing for divergent speciation, (2) a dissolution of the geographical barrier at a later time allowing divergent population systems to come into secondary contact, and (3) the retreat of the divergent population systems away from areas of secondary contact.

It seems clear from the evidence presented that C. selwayensis and C. apiculatus are sufficiently close as to warrant the assumption that they shared a common ancestor. Another possibility, however, is that C. selwayensis is actually of hybrid origin between C. apiculatus and C. elegans. This possibility has been suggested by Ownbey (1966) but has not been studied. The author feels that this is unlikely in view of the fact that C. selwayensis (1) has an extensive distribution, (2) is quite fertile, (3) shows some characteristics that are not intermediate to C. elegans and C. apiculatus such as gland color, petal pubescence color, plant size, etc., and (4) does not have an ecological preference intermediate to C. elegans and C. apiculatus (Wittler, unpublished).

The divergence of the hypothesized ancestral populations may have been caused by the intervention of a barrier to interpopulation pollen exchange. Adaptive radiation could not be a possible explanation here since few if any internal sterility barriers have been built up.

The Wisconsin glaciation could have provided the necessary isolating barrier. It was during this glaciation that most of northwestern Montana was covered by ice and glacial Lake Missoula (see Fig. 21), (Pardee, 1910; Flint, 1945).

Much of the area presently supporting *C. apiculatus* was ice covered while the area presently supporting *C. selwayensis* was not. The Bitterroot Valley was under water at this time. Hypothetically, an ancestral complex of central Idaho and western Montana could have been divided by the glacial activity in the Bitterroots.

During such periods of glaciation, cold weather may have caused a movement of the vegetational zones to lower elevations. In other words, during this glaciation mid-elevation species would have refugia at lower elevations. Two low elevation areas would have been in central Montana and eastern Washington state. It is then safe to assume that different climatic conditions existed in these two areas due to the wide spacial separation and different topographic conditions. In all probability the area east of the Bitterroot was wetter than the area to the west. If that ancestral complex were divided such that a group of populations inhabited eastern Washington and another group central Montana, then different selective pressures could

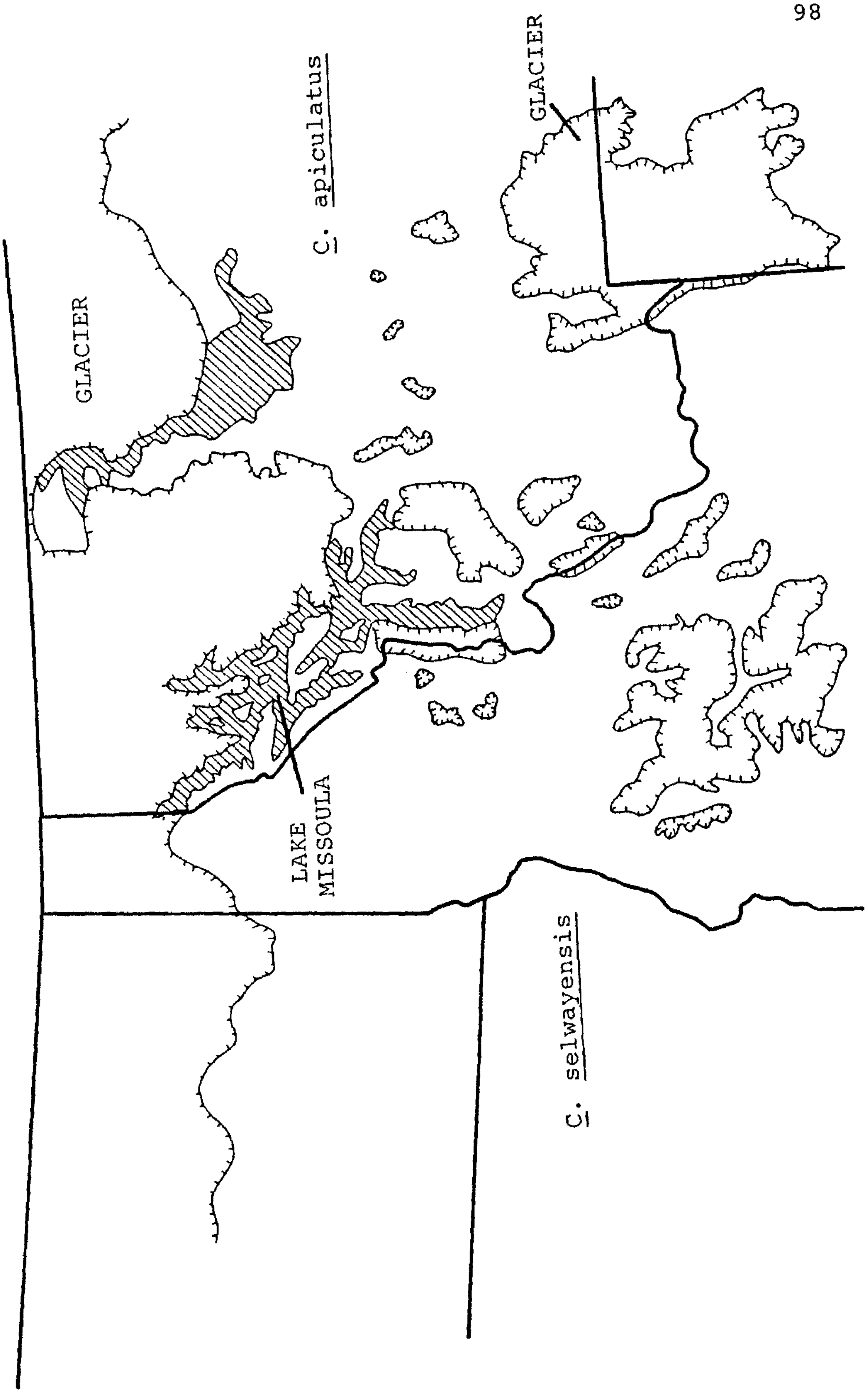
Figure 19

Calochortus Karyotypes

A. Population 83 (intermediate)

B. Population 92 (intermediate)

C. Population 123 (*C. apiculatus*)


GLACIER
C. apiculatus
GLACIER
LAKE MISSOULA
C. selwayensis

have resulted in divergence of the two systems. A divergence that could possibly have given rise to C. selwayensis in the drier Columbia River drainage and C. apiculatus in the moister central Montana area.

With the retreat of the glaciers and drainage of Lake Missoula, a drying trend most likely occurred throughout the area. Today both central Montana and eastern Washington are much too dry to support populations of either C. selwayensis or C. apiculatus. Thus during deglaciation the populations of C. selwayensis and C. apiculatus would have had to migrate to moister habitats which would be found at higher elevations. The most likely movement for C. selwayensis would have been from eastern Washington eastward into central Idaho, while C. apiculatus moved from central Montana northwest into the Swan range and the Flathead Lake area.

The migration of C. selwayensis according to this scheme would have probably stopped along the western side of the Bitterroot Valley and south of the Missoula Valley and the Clark Fork River drainages. These areas are too dry to support populations of C. selwayensis and would thus prevent further migration. This scheme explains why C. selwayensis does not occur east of the Bitterroot Valley today, even though the habitats there appear suitable. Alternative refugia and subsequent migration routes cannot explain this phenomenon. For example, if C. selwayensis occurred in the central Montana region during the Wisconsin Glaciation one would expect to find populations east of the Bitterroots and not to the west. This situation is not found today.

The migration of C. apiculatus from central Montana would have been in a northwesterly direction following the damper habitats north of the Blackfoot River. The migration would have stopped on the east face of the Mission Mountains but continued north toward Glacier Park and west near Flathead Lake north of the Mission range. The Mission crest would have been a formidable barrier to such a migration due to the craggy snowcovered peaks found there. This explains in part why today no C. apiculatus occurs on the west face of the Missions even though the habitats there appear similar to those found in the Swan Range which support stands of C. apiculatus. The migration would also have been thwarted by the dry, low elevations habitats found in northwestern Montana. Today C. apiculatus occurs in northwestern Montana but only in the form of a highly introgressed geographical race. This race could only have arisen after secondary contact with C. selwayensis.

It is possible that the migration of C. apiculatus continued south from the Flathead Lake region down toward Plains, Montana. (Support for this is the fact that non-introgressed populations of C. apiculatus were found near

Hotsprings and Polson, Montana.)

This proposed scheme also indicates that the migration of _C_. _selwayensis_ and _C_. _apiculatus_ would have resulted in two areas of secondary contact. One such area would be at the northeastern limits of _C_. _selwayensis_ migration and the southern limits of _C_. _apiculatus_ migration east of the Missions. This area is near Missoula, Montana, and could have been the area of hybridization resulting in the formation of hybrid swarms in the Marshall-Mitouer area. A second area of contact would be at the northernmost extent of the _C_. _selwayensis_ migration and the southernmost extent of _C_. _apiculatus_ migration west of the Missions. This contact could have been the area of hybridization giving rise to the hybrid swarms found in the Plains-Thompson Falls area.

The initial hybridizations most likely occurred in recent times after disruption of habitats. This disruption may have been caused by the advance of "civilization," the construction of roads, and logging operations. Hybrid habitats thus produced would have supported hybrid swarms in both areas previously described. The parental species, however, may have been slowly driven out by increased disruption of the habitat through the encrouchment of man in these two areas (especially in the Mitouer-Marshall area). Also the somewhat high fertility shown by the hybrids may have resulted in the repeated backcrossing to the parents and the selection of recombinants that out-competed both of the parental species. Thus the parents could have been "hybridized out of existence" which may have occurred in the Plains-Thompson Falls area.

After hybridization in the Plains-Thompson Falls area certain recombinants and/or introgressants formed populations that could survive in the drier areas of northwestern Montana. These populations began to inhabit such dry areas after the initial hybridization. The migration of this type was rapid along roads and drier creek bottoms resulting in the present distribution of the western race of _C_. _apiculatus_.

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Appendix I

Locations of All Collections

Pop. No.	Elev.	State	County
1	1350	Idaho	Idaho
2	1350	Idaho	Idaho
3	--	Idaho	Idaho
4	--	Idaho	Idaho
5	4200	Mont.	Powell
11	1150	Idaho	Idaho
12	1300	Idaho	Idaho
14	1700	Idaho	Idaho
22	2350	Mont.	Sanders
23	2350	Mont.	Sanders
25	2400	Mont.	Sanders
26	2500	Mont.	Sanders
27	2500	Mont.	Sanders
29	2450	Mont.	Sanders
31	2400	Mont.	Sanders
32	4200	Mont.	Missoula
33	4150	Mont.	Missoula
34	4250	Mont.	Missoula
35	4250	Mont.	Missoula
37	2800	Mont.	Sanders
38	3300	Mont.	Sanders
39	2500	Mont.	Sanders
40	2600	Mont.	Sanders
41	2200	Mont.	Sanders
42	2800	Idaho	Idaho
45	--	Idaho	Idaho
46	4000	Mont.	Missoula
47	3800	Mont.	Missoula
48	4000	Mont.	Missoula
49	4300	Mont.	Missoula
50	4200	Mont.	Missoula
51	4150	Mont.	Missoula
52	4150	Mont.	Missoula
53	2600	Mont.	Mineral
54	2400	Mont.	Mineral
55	2400	Mont.	Mineral
56	2550	Mont.	Mineral
57	3000	Mont.	Mineral
59	4200	Mont.	Missoula

Appendix I (cont.)

Pop. no.	Elev.	State	County
60	4200	Mont.	Missoula
61	4200	Mont.	Missoula
62	4200	Mont.	Missoula
63	4950	Mont.	Missoula
64	3450	Mont.	Missoula
65	3450	Mont.	Missoula
66	4000	Mont.	Missoula
67	3400	Mont.	Missoula
68	--	Mont.	Missoula
69	--	Mont.	Missoula
70	3900	Mont.	Missoula
71	3900	Mont.	Missoula
72	3900	Mont.	Missoula
73	4400	Mont.	Missoula
74	4350	Mont.	Missoula
75	4200	Mont.	Missoula
77	8600	Mont.	Missoula
78	7100	Mont.	Missoula
79	2400	Idaho	Idaho
80	3500	Idaho	Idaho
81	1600	Idaho	Clearwater
82	1500	Idaho	Idaho
83	4200	Mont.	Missoula
84	3100	Idaho	Shoshone
87	2950	Idaho	Shoshone
88	2800	Idaho	Benewah
89	2150	Idaho	Kootenai
90	2300	Idaho	Shoshone
91	2800	Mont.	Sanders
92	2400	Mont.	Sanders
93	2350	Mont.	Sanders
94	2350	Mont.	Sanders
95	2350	Mont.	Sanders
96	2350	Mont.	Sanders
97	4200	Mont.	Missoula
98	3250	Mont.	Sanders
99	3450	Mont.	Sanders
100	3500	Mont.	Sanders
101	3300	Mont.	Sanders
102	2500	Mont.	Sanders
103	2350	Mont.	Sanders
104	2150	Mont.	Sanders
106	2300	Mont.	Sanders
107	3400	Mont.	Missoula
108	3600	Mont.	Missoula
109	4600	Mont.	Missoula
110	4100	Mont.	Missoula

Appendix I (cont.)

Pop. no.	Elev.	State	County
111	4200	Mont.	Missoula
112	3400	Mont.	Missoula
113	4000	Mont.	Missoula
114	4300	Mont.	Missoula
115	3400	Mont.	Flathead
116	3400	Mont.	Flathead
117	--	Mont.	Flathead
118	3300	Mont.	Flathead
119	3100	Mont.	Flathead
120	--	Mont.	Sanders
121	--	Mont.	Sanders
122	2400	Mont.	Sanders
123	5200	Mont.	Missoula
124	5800	Mont.	Missoula
127	2100	Idaho	Bonner
128	2100	Idaho	Boundary
129	2300	Mont.	Lincoln
130	2200	Mont.	Lincoln
131	2900	Mont.	Lincoln
132	2100	Mont.	Lincoln
134	2800	Mont.	Lincoln
135	5200	Mont.	Flathead
136	3200	Mont.	Flathead
137	3000	Mont.	Lake
138	--	Mont.	Flathead

Appendix II

Individual Hybrid Index Scores

Population	Hybrid Indices
12	1.7, 1.2, 1.6, 1.7, 1.1, 1.2, 2.2, 1.8, 1.8
14	1.4, 1.5, 0.8, 1.2, 1.9, 1.3, 1.2, 1.1, 1.9, 1.9, 2.7, 0.7, 0.7, 0.4
22	4.3, 5.6, 4.2, 5.7, 5.6, 5.3, 5.4, 4.8, 6.0, 3.6, 4.1, 6.2, 5.0, 5.9, 4.9, 3.5, 3.2, 5.0, 4.1, 5.3, 4.8, 5.5
23	2.8, 4.8, 3.7, 4.7, 6.0, 2.5, 6.4, 6.1, 6.0, 3.5, 4.8, 5.7, 2.2, 6.2, 2.9, 5.8, 4.5, 3.0, 5.8, 5.7, 4.6, 3.3, 5.3, 5.3, 4.9, 5.0, 6.5
24	5.3, 3.6, 3.6, 5.8, 3.8, 5.7, 3.4, 5.7, 5.5, 5.6, 3.2, 4.6, 4.4, 5.0, 5.8, 5.4, 2.5, 4.0, 3.9, 2.4, 4.0
25	6.1, 6.5, 4.7, 4.9, 6.5, 4.0, 6.6, 6.1, 4.0, 6.2, 6.6, 5.2, 6.5, 6.5, 6.3, 5.8, 5.3, 5.8, 6.2, 6.0
26	3.9, 6.1, 5.9, 6.5, 6.0, 4.9, 5.3, 5.8, 5.7, 5.8, 6.0, 4.8, 5.3, 5.0, 5.4, 5.8, 6.1, 6.4, 5.8
27	6.5, 5.0, 5.8, 5.8, 6.1, 3.1, 6.4, 6.4, 5.7, 6.0, 5.7, 5.7, 4.1, 5.9, 6.2, 5.4, 5.1, 4.0, 5.8
28	6.9, 6.2, 5.8, 5.5, 4.5, 5.8, 5.9, 5.8, 5.5, 6.2, 5.9, 3.9, 5.3, 4.3, 5.5, 5.8, 6.2, 4.8, 6.2, 5.8
29	2.9, 1.7, 3.5, 5.1, 1.2, 5.0, 1.4, 5.3, 1.6, 1.6
31	4.3, 2.7, 2.5, 4.5, 4.8
32	2.7, 4.5, 4.5, 3.1, 2.1, 4.9, 2.4, 4.0, 3.4, 2.4, 0.8, 5.7, 3.7, 3.7, 4.5, 3.2, 3.0, 5.0, 2.9, 4.5, 2.9, 4.8

Appendix II (cont.)

Population	Hybrid Indices
33	5.5, 4.5, 5.4, 5.4, 4.5, 3.6, 2.8, 4.0, 4.7, 4.4, 2.0, 5.6, 3.6, 5.0, 2.9, 5.7, 3.8, 2.2, 5.5, 5.2
34	4.1, 3.5, 4.7, 4.7, 3.5, 4.0, 3.7, 5.7, 4.2, 5.5, 2.8, 4.5, 3.5, 2.9, 4.3, 4.3
35	2.8, 3.2, 4.1, 5.2, 3.5, 2.8, 4.9, 5.3, 4.6, 4.3, 5.0, 3.4, 5.9, 3.5, 5.4, 5.3, 2.7, 4.4
37	6.0, 6.0, 6.3, 5.5, 6.1, 6.4, 5.7, 5.4, 6.1, 6.7, 6.0, 5.9, 6.1, 5.4, 6.2, 5.9, 6.3, 5.1, 4.6
38	6.9, 6.6, 4.6, 6.5, 7.1, 6.7, 6.8, 6.8, 5.7, 6.9, 5.8, 5.2, 6.2, 4.6, 4.5, 5.7, 6.2, 5.9, 6.5, 6.1, 6.3
39	6.9, 6.8, 6.3, 5.8, 6.7, 6.7, 6.4, 6.8, 6.4, 4.6, 6.3, 6.0
40	2.2, 2.3, 3.2, 1.7, 1.7, 1.7, 1.5, 2.0, 2.1, 2.5, 2.0, 1.7, 2.3, 2.6, 1.5, 2.4, 1.7, 2.1, 1.7, 1.8, 2.2, 2.1, 2.2, 1.6
41	1.5, 2.4, 1.5, 1.8, 1.7, 1.8, 1.4, 1.7, 2.1, 1.8, 1.8, 4.3, 1.8, 3.1, 2.0, 2.3
42	1.5, 1.1, 0.7, 1.7, 1.9, 1.6, 2.6, 2.3, 1.0, 1.5, 1.4, 1.3, 1.7, 1.3, 1.6, 1.1, 1.3, 1.2
45	2.5, 1.5, 1.8, 0.9, 1.2, 1.0, 1.2, 1.3, 0.7, 1.5, 1.4, 1.5, 1.1, 0.5, 1.2, 1.0, 1.3, 0.7, 2.0, 0.8, 1.3, 1.3, 1.3, 1.4, 1.1, 1.5, 0.8, 1.0, 1.3, 1.5
46	1.6, 1.7, 1.3, 1.3, 1.7, 1.4, 1.8, 0.9, 2.2, 3.1, 1.0, 1.8, 1.0, 1.1, 2.3, 1.8, 1.0, 1.8, 1.7, 2.1, 1.5
47	0.9, 1.0, 1.9, 1.3, 1.9, 1.1, 1.2, 1.3, 0.8, 1.2, 1.3, 1.8, 1.5, 0.9, 1.4, 1.1, 1.0, 0.8, 0.6

Appendix II (cont.)

Population	Hybrid Indices
48	5.6, 2.4, 4.4, 3.8, 4.6, 3.7, 2.5, 2.4, 3.1, 4.3, 5.2, 3.8, 3.8, 5.5, 1.9, 3.1, 3.9, 3.0, 2.9, 2.9, 2.3, 2.8, 3.0, 3.6
49	4.8, 6.0, 2.7, 4.0, 4.7, 3.2, 3.1, 3.8, 2.0, 6.2, 2.6, 5.0, 3.3, 5.3
50	6.0, 5.1, 4.3, 2.8, 5.7, 5.9, 5.8, 4.8, 3.9, 4.5, 4.8, 4.7, 2.3, 5.1, 5.6, 5.4, 5.4, 6.1, 2.0, 2.4, 4.3, 3.9, 3.3, 2.4, 6.2, 3.4, 3.1, 2.5, 2.7, 2.9, 4.3, 3.8, 3.5, 5.2, 6.0, 4.1, 4.8, 4.5, 3.9, 5.4, 2.5
51	5.3, 2.3, 3.3, 2.6, 3.6, 2.8
52	3.6, 4.8, 5.7, 3.4, 4.0, 2.5, 3.4, 5.8, 4.5, 2.3, 3.1, 5.7, 4.7, 3.0, 2.8, 5.0, 2.5, 4.4, 2.6, 5.1, 3.3, 4.5, 2.8, 2.4, 3.8, 3.4, 5.0, 4.2, 2.7, 3.4, 5.6
53	1.3, 0.8, 2.0, 2.0, 1.9, 1.3, 1.0, 0.7, 1.1, 0.4, 0.9, 1.4, 1.5, 1.1, 2.1
54	2.3, 0.6, 2.6, 0.9, 2.0, 1.3, 1.7, 0.8, 0.8
55	1.6, 0.8, 1.2, 1.9, 1.2, 1.4, 1.3, 0.9, 1.2, 0.9, 0.8, 1.4, 1.1
56	2.6, 2.0, 3.2, 2.0, 2.7, 1.5, 2.1, 2.8, 3.1, 1.8, 1.9
57	1.3, 1.0, 2.0, 1.6, 1.2, 2.2, 1.7
59	5.4, 4.5, 4.1, 5.7, 5.3, 5.9, 5.2, 1.9, 6.2, 4.1, 4.0, 3.9, 3.7, 4.7, 3.6, 5.0
60	3.0, 5.6, 3.3, 3.4, 5.6, 5.8, 5.2, 5.3, 3.7, 6.0, 5.6, 3.1, 2.2, 3.5, 4.6, 4.8, 4.3, 4.8
61	3.7, 3.3, 5.2, 3.3, 3.1, 3.7, 4.6, 3.9, 5.0
62	3.6, 2.1, 4.7, 4.2, 5.8, 5.5, 4.3, 3.3, 3.1, 5.3, 5.0, 4.1, 3.8

Appendix II (cont.)

Population	Hybrid Indices
63	0.9, 0.8, 0.5, 0.8, 0.9, 1.4, 1.1, 1.3, 1.6, 2.6, 1.3, 1.6, 1.4, 1.4, 1.2, 1.2, 0.9, 1.5, 2.1, 1.1, 1.5, 1.7, 2.2, 2.0, 1.2, 0.7, 1.5
64	6.4, 6.9, 6.9, 6.3, 7.0, 6.4, 6.3, 6.1, 6.4, 6.0, 6.0, 6.7, 6.4, 6.8, 6.2, 6.3, 6.4, 6.6, 6.6, 6.6, 6.4, 6.2, 6.5, 6.6, 6.3, 6.4, 6.9
65	6.6, 5.8, 6.5, 6.4, 6.6, 6.7, 6.6, 6.5, 6.7, 6.4, 6.3, 6.7, 6.2, 6.6
66	5.5, 5.7, 6.6, 4.2, 5.7, 5.6, 6.2, 5.3, 5.4, 5.8, 4.9, 4.1, 5.2
67	6.7, 6.0, 5.3, 6.2, 5.9, 5.0, 5.8, 5.0, 6.1, 5.7, 5.2, 6.3
68	6.4, 6.6, 6.6, 6.4, 6.1, 6.8, 6.5, 6.4, 6.0, 6.5, 6.5, 5.9, 6.0
69	6.5, 5.9, 6.6, 6.3, 6.2, 6.4, 6.3, 6.2, 6.6, 5.8, 5.9, 6.8, 6.5, 6.0, 6.3, 6.5, 6.1, 6.2, 6.5, 6.3, 6.1, 6.3, 6.5, 6.5
70	6.6, 6.3, 6.2, 6.2, 5.7, 6.1, 6.1, 5.9, 5.7, 6.3, 5.7, 6.5, 6.0, 6.0, 6.3, 5.6, 5.2, 5.6
71	6.9, 6.3, 6.5, 6.4, 6.7, 6.9, 7.4, 6.9, 7.2, 6.6, 6.9, 6.4, 6.0, 6.5, 6.0, 6.7, 6.5, 6.5, 6.3, 7.2, 6.4, 6.5, 6.2, 6.9, 6.2, 6.7, 6.7
72	7.0, 6.4, 6.8, 6.6, 6.7, 6.1, 7.0, 6.5, 6.3, 6.5, 6.7
73	7.0, 6.7, 6.9, 7.1, 6.7, 5.9, 6.7, 6.5, 6.5, 6.8, 6.0, 6.4, 6.4, 6.5
74	6.5, 6.2, 6.9, 6.7, 6.9, 6.4, 6.6, 6.6, 6.7, 6.5, 6.8, 6.7, 6.9, 6.7, 6.5, 6.3, 6.6, 6.3, 6.2, 6.4, 6.4, 6.4, 6.9, 6.7, 6.6, 6.5, 6.9
75	7.3, 6.9, 6.7, 6.8, 6.6, 6.5, 6.4, 6.3, 6.5, 6.3, 6.1, 6.7, 6.4, 6.4, 6.6, 6.3, 6.8
98	6.5, 6.5, 6.9, 6.8, 6.3, 6.1, 5.4, 7.2, 6.3

Appendix II (cont.)

Population	Hybrid Indices
99	5.3, 2.9, 5.6, 5.2, 4.2, 3.8, 4.9, 3.5, 3.6, 5.2, 3.5, 4.3, 2.6, 3.3, 3.3, 4.6
101	6.4, 3.3, 5.3, 6.4, 6.0, 3.9, 5.8, 4.1, 3.2, 4.0, 6.2, 4.5, 6.1, 4.5, 4.1, 5.3, 3.6, 6.3
102	0.7, 0.7, 1.1, 1.3, 0.8, 1.4, 2.4, 1.5, 0.9, 0.4, 0.8
103	1.4, 2.3, 0.6, 1.2, 1.7, 1.3, 1.9, 1.2, 0.8, 0.8, 1.3, 1.2, 1.0, 0.9, 1.4
104	6.5, 5.6, 5.7, 6.0, 6.3, 6.0, 6.3, 6.0, 6.3, 6.8, 6.6, 6.3
105	6.7, 3.7, 6.5, 6.8, 7.2, 6.6, 6.0, 4.9, 4.1, 6.1, 6.3, 3.8, 6.2, 6.1, 6.7
107	0.9, 1.0, 0.9, 0.6, 1.3, 0.9, 0.5, 1.1, 1.0, 0.8, 1.2, 0.7, 1.1, 1.6
115	5.1, 6.3, 6.3, 5.9, 6.4, 6.8, 6.3, 6.0, 6.3, 6.2, 6.0, 6.6, 6.5, 6.3
118	5.6, 6.5, 6.3, 6.4, 6.0, 6.0, 5.7, 6.0, 6.4, 6.4, 6.3, 5.8
120	5.8, 6.7, 5.8, 6.5, 6.7, 5.7, 6.5, 6.3, 5.5, 6.1
121	6.4, 6.7, 6.0, 6.7, 6.9, 6.9, 6.4, 6.2, 6.1, 5.5, 5.2, 6.4, 6.4, 6.0, 6.1
122	5.9, 6.0, 6.2, 6.8, 6.2, 6.4, 6.3, 6.0, 5.5
127	6.0, 6.5, 6.1, 6.3, 5.7, 6.3, 6.8, 6.2, 6.1, 6.0, 6.4, 6.7, 5.9, 5.8, 6.5, 5.8
128	5.9, 4.8, 6.0, 5.6, 6.3
134	5.9, 5.3, 4.4, 6.3, 6.4, 6.3, 5.9, 6.5, 6.7, 6.1
135	6.8, 6.9, 6.5, 6.4, 6.7, 6.3, 6.6, 6.5, 6.4, 6.7, 6.2, 6.2, 5.9, 6.3, 6.4

Figure 20

Chromatographic Profile