A BIOSYSTEMATIC STUDY OF *BETULA* SPECIES IN CANADA

ABSTRACT

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A BIOSYSTEMATIC STUDY OF SOME SPECIES OF BIRCH (BETULA) IN EASTERN CANADA

A study of several birch (Betula) species growing in eastern Canada was conducted. This included morphological, cytological, and cytophotometrical investigations, supplemented by a study of the chromatographic patterns of visible and fluorescent compounds in leaf extracts. Betula caerulea, B. caerulea-grandis, B. populifolia, B. cordifolia and B. papyrifera were studied in order to clarify the origin and taxonomic status of B. caerulea and B. caerulea-grandis. The morphological measurements for B. caerulea-grandis were shown to be intermediate between those for B. populifolia and and B. cordifolia. Betula caerulea-grandis did not appear to be directly related to B. papyrifera. Betula caerulea-grandis was considered to be a hybrid of B. populifolia and B. cordifolia. Cytophotometric measurements and chromatography supported the morphological observations.

A BIOSYSTEMATIC STUDY OF SOME SPECIES OF BIRCH (BETULA) IN EASTERN CANADA

by

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INTRODUCTION

There has been considerable disagreement among systematists as to the taxonomic status and the origin of the blue birches Betula caerulea Blanchard and Betula caerulea-grandis Blanchard. Blanchard (1904a) first described these white-stemmed birches as blue birches due to a bluish tint in the foliage and treated them as separate species. In a second publication in the same year, Blanchard (1904b) referred to Betula caerulea as a good species but referred to Betula caerulea-grandis as a variety of Betula caerulea, that is, "Betula caerulea variety grandis". Subsequently, there has ensued a great deal of controversy over the actual status of these two taxa.

Sargent (1922) considered both taxa to be hybrids. Later Fernald (1945) treated Betula caerulea-grandis as a good species and considered only Betula caerulea as a hybrid. On the other hand, Gleason (1952), in his flora, listed Betula caerulea as a good species, and in a footnote, stated that Betula caerulea-grandis was a hybrid. More recent workers (Erskine, 1960; Brayshaw, 1966; Brittain and Grant, 1967) agreed that the origins of Betula caerulea and Betula caerulea-grandis were probably complex involving interspecific hybridization but they did not agree on the parental species involved.

With disagreement over the origin and status of the blue birches

so evident in the literature, it was thought worthwhile to study the blue birches and the other birch species associated with these taxa in order to further examine and, if possible, clarify the status of Betula caerulea and Betula caerulea-grandis. Betula cordifolia and Betula populifolia have been considered as putative parents of the blue birches. Therefore, a comparative study of four species of birches, namely, Betula caerulea, Betula caerulea-grandis, Betula cordifolia, and Betula populifolia, from two localities in Quebec, one in New Brunswick, and one in Vermont, was carried out.

It was thought that a detailed analysis of these populations would reveal any possible effect of geographical location on the expression of variability in quantitatively measured characters. In addition to conventional morphological studies, the growing interest in chemotaxonomy prompted the use of thin-layer chromatography to provide biochemical characters for the recognition of differences between these taxa. The development of the thin-layer chromatographic technique for the analysis of secondary phenolics from leaf extracts in this laboratory for species of Lotus (Grant and Whetter, 1966; Grant and Zandstra, 1968) was used in order to see if these techniques which have been successfully developed for the identification of Lotus species could be equally applied to the analysis of species recognition in Betula. If successful, it was thought that this technique would be of use in resolving the difficulties associated with classifying

Betula taxa from herbarium specimens based on morphological and cytological information alone. In many cases, from data provided by the latter techniques, it has been impossible to provide decisive answers to the questions of relationships, and therefore, it is hoped that by providing additional characteristics in the form of biochemical markers, relationships between Betula taxa may be established.

LITERATURE REVIEW

A number of white birch species, so called because of their white bark, are found in eastern Canada. Those of particular interest are the following: Betula populifolia Marsh., grey birch, B. papyrifera Marsh., paper, canoe, or white birch, and its variety, B. papyrifera var. cordifolia (Regel) Fern., the heart-leaved or mountain white birch, B. caerulea Blanchard, the blue birch and B. caerulea-grandis Blanchard, the blueleaf or large blue birch (Fernald, 1950).

The discovery in 1904 by William H. Blanchard (1904a, 1904b) of the blue birches, in Vermont and Quebec, has touched off a debate on the origin of the blue birches which is most confusing due to the misunderstanding and misinterpretation of the recorded facts. Since the first description of these species was published (Blanchard, 1904), some of the subsequent published details concerning these species have been found to be incorrect, and this too has further complicated investigations to determine the origin of the blue birches.

On May 7, 1904, in a private publication called "Betula" by William H. Blanchard, he first reported the discovery of two new white birch species, which he called blue birches because of a bluish tint in the foliage. They were found growing near the townhouse in Stratton, Vermont. He called them Betula caerulea and Betula caerulea-grandis,

the blue and the large blue birch, respectively. He described B. caerulea as being a small tree, although it was larger than the grey birch with which it is sympatric. The leaves were thin, long pointed and somewhat cuneate at the base. They were quite regular in outline, glabrous on both sides and usually without tufts of tomentum. The petioles were long and slender. The catkins were one inch long by three-eights of an inch in width. Although he stated the fruiting bracts were very distinctive in shape he did not describe them.

Blanchard (1904a) described B. caerulea-grandis as being a tree larger than B. caerulea though in bark and foliage they resembled one another. The leaves of B. caerulea-grandis were described as being nearly truncate. The catkins were much larger than for B. caerulea. Again Blanchard did not describe the bracts although he stated they had a distinctive shape.

On May 13, 1904, in "Betula", Volume 1, No. 2, Blanchard once more reported B. caerulea and B. caerulea-grandis as new species of birch. He proposed Betula caerulea as the name for the small blue birch and for the large blue birch "B. caerulea variety grandis", that is, a variety of the smaller taxon. In his second publication it appeared that he no longer believed that the large blue birch was a good species but rather that it was a variety of B. caerulea and merely larger in morphology. Apparently this interpretation is not what he finally concluded since at the bottom of a copy of this article

which he sent to the Gray Herbarium of Harvard University, he wrote a note reaffirming his original conclusion. This stated, "I believe these are two good species", which is his original interpretation of these two blue birches as published in his first paper.

Before he published either article, Blanchard was examining herbarium specimens which he had made from these trees and on one sheet dated June 7, 1903, he stated that B. caerulea might be a hybrid of B. pendula Roth., the European white birch, and B. populifolia. Fernald (1922) interpreted B. pendula in this context as equivalent to B. caerulea-grandis and stated that Blanchard had considered B. caerulea to be a hybrid of B. caerulea-grandis and B. populifolia. On another specimen dated May 17, 1904, after publication of his second article, Blanchard wrote that B. caerulea-grandis is "not a variety of B. caerulea but a distinct species". It would appear certain that Blanchard did consider both B. caerulea and B. caerulea-grandis to be good species.

The first collection of B. caerulea-grandis in Canada was made by Blanchard in 1904 at Sherbrooke, Quebec, and he deposited the specimens in the Gray Herbarium. Fernald (1922) found B. caerulea-grandis growing in Prince Edward Island, Nova Scotia and Gaspé Peninsula of Quebec.

Sargent (1922) renamed B. caerulea-grandis as B. caerulea var.

Blanchardii Sarg. He considered B. caerulea to be a "natural hybrid between B. papyrifera and B. populifolia". At the same time he considered B. caerulea-grandis to be merely a larger form of B. caerulea. Therefore, it may be concluded from Sargent's statement that he considered B. caerulea-grandis also to be a hybrid of a cross between B. papyrifera and B. populifolia. Fernald (1922, 1945, 1950), on the other hand, believed that B. caerulea-grandis was a good species. In 1922, he suspected that B. caerulea was a hybrid between B. caerulea-grandis and B. populifolia but in 1945 he actually listed B. caerulea as x B. caerulea, a hybrid between B. caerulea-grandis and B. populifolia. Rehder (1940) concurred with this interpretation. Woodworth (1929, 1931) also agreed with Fernald's interpretation for cytological reasons. He determined the chromosome numbers for the white birches as follows:

Species	Haploid number (n)	Diploid number (2n)
B. caerulea	14	28
B. caerulea-grandis	14	28
B. populifolia	14	28
B. papyrifera var. cordifolia	28	56
B. papyrifera	35	70

He felt that neither B. caerulea, nor B. caerulea-grandis, could have arisen from a cross of B. papyrifera and B. populifolia because

"B. papyrifera is a pentaploid species and B. populifolia is a diploid. B. caerulea is a diploid". Since he reported meiosis to be normal in B. caerulea and B. caerulea-grandis, he listed B. caerulea as a hybrid of B. caerulea-grandis and B. populifolia. Johnson (1939), in listing birch hybrids, states that B. caerulea is a hybrid between B. caerulea-grandis and B. populifolia.

Little (1953), who also described B. caerulea as a hybrid between B. caerulea-grandis and B. populifolia, stated that Blanchard in giving B. caerulea-grandis full specific status "apparently (made) a typographical error for a large variety of B. caerulea". It was not clear from what Little stated what he considered as the true origin of B. caerulea. One parent could not be B. caerulea-grandis as Little stated, because later in this article he considered this species to be only a variety of B. caerulea (Little, 1953).

With the exception of Blanchard who considered both B. caerulea and B. caerulea-grandis to be good species, and Sargent who considered them both to be hybrids, the other authors (Fernald, Rehder, Johnson, Woodworth and Little) considered B. caerulea to be a hybrid of B. caerulea-grandis and B. populifolia.

As will be detailed below, treatment of these taxa by certain other authors failed to recognize B. caerulea-grandis as warranting specific status and recognized B. caerulea as a good species, whereas

others considered both taxa to be hybrids.

Gleason (1952), in his flora, listed B. caerulea as a good species. In a footnote, he stated that B. caerulea-grandis was a hybrid which is B. caerulea in part and B. pendula in part. In 1960, Erskine suggested that B. caerulea-grandis was of hybrid origin from a cross between B. papyrifera and B. populifolia. He did not mention B. caerulea. The specimen upon which he based his comments was found growing at the Experimental Farm at Charlottetown in Prince Edward Island. Brayshaw (1966), reported in a study of B. caerulea and B. caerulea-grandis "that the blue birches, in their diverse forms, are morphologically indistinguishable from a hybrid swarm between white and grey birches". He reaffirmed Sargent's opinion that both B. caerulea and B. caerulea-grandis were hybrids between B. papyrifera and B. populifolia. Brittain and Grant (1967) described B. caerulea and B. caerulea-grandis found in the three Maritime provinces, in Eastern Quebec, and in New Hampshire and Maine. They found no evidence to suggest that B. papyrifera was involved in the blue birch complex. They also reported (Brittain and Grant, 1967) that seedlings of artificial crosses which they had made between B. papyrifera and B. populifolia did not resemble seedlings of B. caerulea-grandis. They stated that the situation might be clarified if the relationship of the mountain white birch, B. cordifolia Regel, was taken into consideration.

While B. cordifolia Regel was originally given specific status, Fernald (1922) considered this species not to warrant specific rank, and accordingly, named it a variety of B. papyrifera. This taxon has been known as B. papyrifera var. cordifolia (Regel) Fern. Fernald (1945) reconsidering B. papyrifera var. cordifolia based his argument against specific status for this taxon on the discovery of B. papyrifera var macrostachya Fern. which "exactly bridges the gap between it (B. cordifolia) and typical B. papyrifera".

Evidence against Fernald's view for varietal status of "cordifolia" came from Brittain and Grant (1965b) who showed that B. papyrifera var. macrostachya was closer to B. papyrifera per se than to cordifolia both morphologically, and in somatic chromosome number. They re-instated cordifolia to specific status as B. cordifolia Regel. Evidence for specific status for cordifolia came from Woodworth's (1930) cytological studies. He noted that cordifolia is a tetraploid ($2n = 56$), whereas papyrifera is a hexaploid ($2n = 70$). But he stated that in spite of the fact that he treated cordifolia as a variety of papyrifera, that is B. papyrifera var. cordifolia, the "difference in chromosome number is certainly adequate evidence for considering the two plants of specific rank". Also in favor of specific status for cordifolia is Rosendahl (1928) who studied cordifolia in Ontario and concluded that it should be reinstated to specific status as B. cordifolia Regel.

Although Woodworth (1930) determined the somatic chromosome number of B. cordifolia to be 56, further investigations by Brittain and Grant (1965b, 1967b) have shown that nearly all of the individuals of this taxon which they have examined are diploid ($2n = 28$) and only a few possess the tetraploid number ($2n = 56$). These authors consider this information as further evidence to distinguish the diploid cordifolia ($2n = 28$) from the hexaploid papyrifera ($2n = 70$) by giving it specific status as B. cordifolia Regel.

As can be seen from the review of the literature the investigations into the origin and taxonomic status of B. caerulea and B. caerulea-grandis have passed through three successive phases. The first phase included the studies of Blanchard and Sargent who considered morphological characters to be the main criterion in their investigations. Blanchard concluded that B. caerulea and B. caerulea-grandis were good species, whereas Sargent considered them both to be hybrids. In the second phase, Woodworth basing his conclusions on cytological evidence in addition to morphological evidence, considered B. caerulea to be a hybrid in which B. caerulea-grandis, a good species, was involved. The third phase has employed morphological, cytological, chemical and ecological criteria and has subjected the resulting data to statistical analysis. Brayshaw, and Brittain and Grant, basing their conclusion on a number of the above criteria have considered B. caerulea-grandis to be a hybrid.

The present study was stimulated by the efforts of the latter investigators in their studies to further examine and if possible, to further clarify the origin of B. caerulea and B. caerulea-grandis. This investigation is an effort to clarify these taxa utilizing the methods of morphology, cytology, and chemotaxonomy, and subjecting the data to detailed analysis.

MATERIALS AND METHODS

In order to analyse different populations of birch, collections were made in late August 1966, at Valcartier, Quebec; in August, 1967, at Grand Manan Island, New Brunswick, and at Lac Carré, Quebec. All the specimens had twigs of the current years growth, mature leaves and mature female catkins. The specimens were given accession numbers 560 to 613 for Valcartier, 420 to 559, 660 and 801 to 804 for Grand Manan Island, and 700 to 762 for Lac Carré. Two specimens, accession numbers 394 and 398, collected at Valcartier in September 1964, were included in the Valcartier group.

Herbarium specimens of Eastern North American species of Betula were borrowed from the following herbaria: Arnold Arboretum, Harvard University (AAH), National Museum of Canada, Ottawa (CAN), Phanerogamic Herbarium, Department of Agriculture, Ottawa (DAO), and the Herbier Marie-Victorin, Institut Botanique, Université de Montréal (MT). Two specimens collected by Blanchard in 1904 in Vermont and borrowed from the Pringle Herbarium of the University of Vermont (VT) were also studied. These were Blanchard's accession numbers 6 and 7 which appeared to be isotypes of B. caerulea and B. caerulea-grandis, respectively (Blanchard, 1904a,b). Altogether, over three hundred specimens were examined.

Morphological characters.

Each specimen was identified as to species and measurements of the following characters were made: attenuation factor of the leaf tip, number of serrations per side of a leaf, number of veins per side of a leaf, length of the mature female catkin, length of the bract, length and width of the achene, length/width ratio of the achene and the length of the styles. The attenuation factor is generally referred to as the a/m ratio (Brayshaw, 1966) where a equals one-half the apical segment, that is, the distance from the widest part of the leaf blade to the leaf tip and m equals the width of the leaf blade at the midpoint of the apical segment (Figure 1). The length of the guard cells was determined by the method of Celarier and Mehra (1958) substituting collodion for the cellulose nitrate-acetate mixture. An eyepiece micrometer was used to measure the length of the guard cells imprinted on the collodion after the collodion had been peeled off the epidermis of the leaf. In addition, the form of the lateral lobes of the bracts was classified as being either ascending, spreading, or recurved (Figure 2).

Due to insect damage and disease on some specimens, it was found impossible to use leaves from a standard position on the specimens. Therefore, five mature leaves chosen at random for each individual were used in the calculation of the morphological characters of the

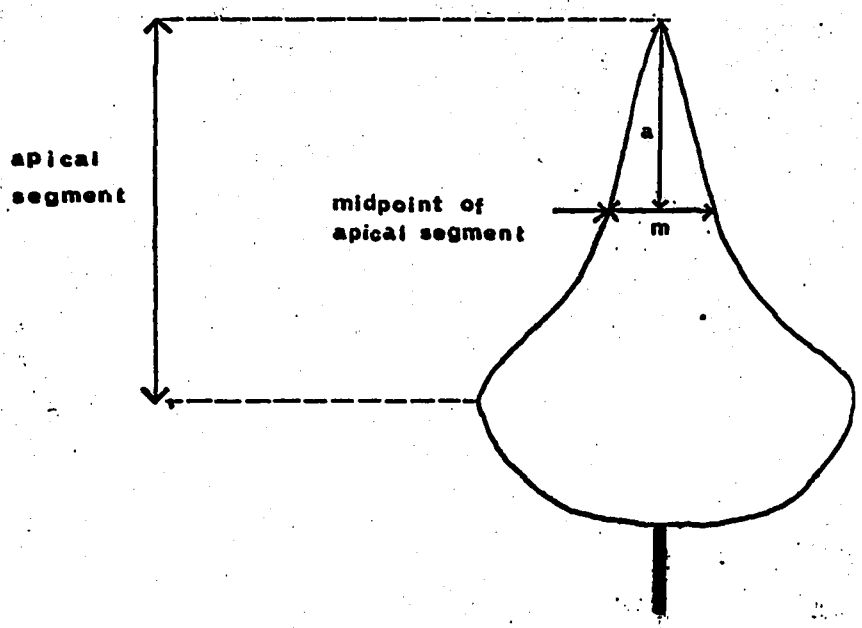
Figure 1. Key to the attenuation factor of the leaf tip.

Figure 2. Different bract shapes in Betula species.

Figure 2A. Bract with ascending lateral lobes.

Figure 2B. Bract with spreading lateral lobes.

Figure 2C. Bract with recurved lateral lobes.



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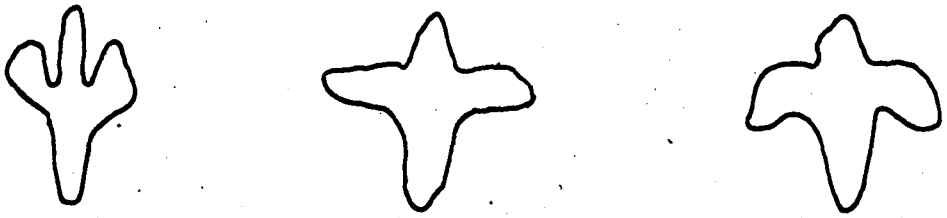


2 a b c

leaves. Ten guard cells were measured for each of five leaves making a total of fifty measurements for each individual. Five mature female catkins were also measured to obtain the mean catkin length for each accession number. For the determination of bract length, length and width of achene, length/width ratio, and also style length, ten individuals for each accession number were measured. A table was prepared comparing hypothetical hybrid mean values obtained when mean values of B. papyrifera and B. populifolia were added and averaged and mean values of B. cordifolia and B. populifolia were added and averaged (Table 4). Values of B. caerulea-grandis were also presented.

A comparison of the morphology between taxa was made by drawing to scale the leaves, bracts and achenes of each specimen with the aid of a camera lucida. They were grouped according to species and arranged according to the hybrid index value of the specimen.

Somatic chromosome number determination.

The chromosome number was determined using root tips obtained from progeny grown from seed of each accession number, where possible. Seeds from specimens collected the previous August were germinated from December 1966, to July 1967, on moist filter paper in Petri dishes and transplanted to pots filled with sand. The plants were grown in cold frames during the summer and where possible with artificial lighting to lengthen the photoperiod to twenty-four hours

per day. A better growth rate was found when long days were maintained rather than under normal day length.

The root tips were pretreated prior to fixation, by washing them thoroughly in tap water to remove particles of sand and then placing them in 0.002M 8-hydroxyquinoline for one hour. Fixation was by means of Carnoy's fluid (6:3:1 ethanol-chloroform-glacial acetic acid). Staining was carried out by the Feulgen technique according to the schedule given by Darlington and La Cour (1962). After staining, the root tips were placed in 4% pectinase for three to three and a half hours to break down the pectic salt in the middle lamella of the cell walls so that the cells would become well separated. Slides were prepared for cytological examination by squashing the root tip meristems in 45% acetic acid after which they were made semi-permanent by sealing the coverslips with paraffin wax. Photographs of chromosome complements were taken using phase contrast optics.

In some cases, in order to determine the chromosome number for plants when good metaphases were not available, the density of nuclear deoxyribonucleic acid (DNA) was calculated and compared with the density obtained for plants of known chromosome number. A Barr and Stroud Integrating Microdensitometer was used in this procedure. Root tips were collected and washed as usual, but the pretreatment with 8-hydroxyquinoline was omitted as it was not necessary to arrest

mitosis at metaphase. The root tips were then fixed in 10% formalin at a pH of 7.1 for twelve hours.

In preparation for staining, the root tips were washed thoroughly in distilled water for forty-five minutes, and hydrolysed with 1N HCl for thirteen minutes at 60°C. Hydrolysis was arrested with cold (4-6°C) distilled water. The root tips were stained in basic fuchsin for two hours and partially decolorized with potassium metabisulphite for seven minutes. After being washed thoroughly the root tips were placed in 4% pectinase for twenty-four hours, washed and stored in 70% alcohol. Root tips from a plant with the known chromosome number were processed in the same vial with root tips for which the chromosome number was to be determined. Individual birch trees belonging to the same species presumably should have the same genetic makeup and preliminary experiments have shown that the density of DNA is similar for such individuals. Considerable differences in DNA densities have been shown for closely related species which differ in ploidy (Hughes-Schrader, 1958) and preliminary experiments carried out in this laboratory between individuals of birch which differ in ploidy also give differences in DNA densities which are, in general, proportional to the number of chromosomes possessed by the species. Root tips of plants belonging to the same or closely related species were stained together. Root tip meristems from the plants of known and unknown chromosome number were squashed on the same slide at

different locations, the length of the root tip distinguishing known from unknown. Late interphase 4C nuclei contain twice the DNA as late telophase or early interphase 2C nuclei due to chromosome duplication prior to mitotic division. Accordingly, nuclei of cells in late telophase or early interphase were measured. Three readings per nucleus and three corresponding background readings were taken and the readings averaged. Ten nuclei were examined per root tip. The difference between the background reading and that obtained for the nucleus is the estimated density of the DNA. The density of the DNA from the plant of unknown chromosome number should be the same as that obtained for the plant with the known chromosome number, that is, if the chromosome number is the same for both individuals and providing they are members of the same species. Species or individuals which are triploid, tetraploid or of higher ploidy, that is, with a greater number of chromosomes, have greater densities, and hence, the different levels of ploidy could be distinguished from one another.

Karyotypes.

Karyotypes of metaphases for each species were drawn from root tip cells with the aid of a camera lucida.

Polygonal graphs.

Davidson (1947) and Löve and Nadeau (1961) used the polygonal graph first devised by Hutchison (1940) to simultaneously portray

several variables in population analysis. Froiland (1952) applied this method in an analysis of a hybrid birch population.

The polygonal graph is a circle with numerous radii, the units on each radius representing the measurements of one character. Each accession number is represented by the mean measurements of the characters being plotted. Since each measurement is plotted along a different radius, when all the points representing the measurements of one individual are plotted and joined together, a polygon is formed. This permits the visual comparison of members of a population on the basis of several characters at the same time.

Hybrid index.

Anderson (1949) described the hybrid index as a means of illustrating visually the presence or absence of hybridization in a natural population. A character which varies in the population studied, is assigned values of "zero", "one", or "two" or more, depending on the number of states the character displays. Characters associated with one parent are rated as "zero", whereas those associated with the other parent are rated as "two". Intermediate situations are assigned the value of "one". When this type of rating is carried out for a number of characters then the members of one of the two parental populations would have a total or hybrid index value of "zero" even if we assume eight characters were used, as in the

the populations studied. The other parental population, with a value of "two" for each character, would have a total or hybrid index value of "sixteen" if we assume eight characters were used. Total values which are intermediate indicate that hybridization might have occurred or be expected. Bar graphs have been constructed plotting hybrid index values against the frequency with which they occur in the population. A visual indication of the absence, or presence of hybridization, the degree of hybridization, the direction in which hybridization and backcrossing is occurring between species, may be obtained from this procedure.

Pictorialized scatter diagrams.

This method of population analysis (Anderson, 1949) has been used to illustrate character-association and the effects of hybridization in a natural population. It has been used to illustrate the effects in a mixed parental and hybrid population of birch. The relationship between two characters was plotted for each specimen in a two-dimensional field. Each spot in the field represents one specimen. The spots were then modified to include the analysis of additional characters simultaneously. For example, when the length of the catkin is less than 2.5 cm it was represented by "O", between 2.5 and 3.49 cm by "O-", that is a line at three o'clock, and over 3.49 cm by a triangle "O◁". The association of several characters may be observed and the effect of hybridization on this association if hybridization

has occurred, may also be observed.

Thin-layer chromatography.

Secondary phenolic compounds have been useful as a basis for species identification in some plant genera (Alston and Turner, 1962; Harney and Grant, 1964). In order to extend the use of thin-layer chromatography of fluorescent compounds to Betula systematics, the procedure of Grant and Whetter, (1966) was followed with some modifications as described below.

An analysis of individual specimens from the Valcartier collection was carried out as follows: 0.05 grams of fresh leaf material which had been rinsed in distilled water and dried at 80°C for 1.5 hours, and which had been chosen at random from the progeny of the accessioned trees, was placed in a vial with 1.0 ml of freshly prepared 1% hydrochloric acid in methanol. Grant and Whetter (1966) in their study of the secondary phenolics of different Lotus species found no difference if leaves were chosen from different positions on the plant. Extraction was allowed to take place for 96 hours. Chromatographic plates were coated with silica gel G, 25 microns thick, and spotted with 20 lambda aliquots. The plates were run in different two-component solvents. The first two-component solvent consisting of cyclohexane and ethyl-acetate (1:1 v/v) was run up the plate to a distance of 15.0 cm, twice, to remove a number of larger spots away

from the more numerous definitive smaller spots between R_f 0.0 and 0.5. The second two-component solvent consisting of methanol and chloroform (30:70 v/v) was allowed to run up the plate to a distance of 7.0 cm. The plate was kept in a tank with methanol and chloroform while it was viewed under long-wave ultraviolet light. At other times the plate was dried with a cool air jet and sprayed with concentrated sulphuric acid. More spots became visible under long-wave ultraviolet light and visible light after spraying with sulphuric acid. A comparison of spots was undertaken in an attempt to differentiate the species according to the presence, or absence, of the fluorescent compounds. Photographs were taken of the plates to aid in the comparison of the spots. Drawings were made of the plates while they were viewed under visible and long-wave ultraviolet light.

Pollen grain viability test.

Branches of different species of birch with immature male catkins were collected in early May. The branches were kept in water at 20°C until the pollen grains were shed. Pollen was placed on a slide, mixed with one drop of fast green in lactophenol, covered with a cover slip and then allowed to stand for eight hours before being examined. At least five hundred pollen grains were examined for each slide before the percent stainable pollen was determined. Although this test gives an estimate of the percentage viable pollen, no actual germination tests were performed.

Statistical analyses.

The standard deviation of the individual mean to the mean of the species was calculated for the following measurements: attenuation factor (a/m), number of serrations on one side of a leaf, number of veins on one side of a leaf, guard cell length, length of the catkin, length of the bract, length and width of the achene, length/width ratio of the achene, and style length. The means of the species were subjected to a t test employing an IBM computer to determine if the means were significantly different at the 5% and 1% levels.

Geographical distribution of the species.

A distribution map was prepared by mapping the localities given by the herbarium specimens examined.

RESULTS

Morphological comparison of the species.

Measurements of ten phenotypic traits of B. cordifolia, B. caerulea-grandis and B. populifolia from Valcartier are presented in Table 1. These measurements are the mean measurements for each accession number and are based on herbarium material.

From the leaf tracings which were made of leaves from each tree studied in the Valcartier population, morphological differences were evident between B. cordifolia, B. caerulea-grandis and B. populifolia (Figure 3). A similar comparison of shapes and dimensions in bracts and achenes are shown in Figures 4 and 5.

For all the characters measured, the mean values for B. caerulea-grandis may be seen to be intermediate between those of B. populifolia and B. cordifolia. The means for the ten traits in B. populifolia are usually smaller than the means for B. caerulea-grandis whereas the means for B. cordifolia are usually larger than the means of B. caerulea-grandis. For example, the mean length of the styles in B. populifolia is 0.606 mm whereas in B. cordifolia the mean style length is 1.440 mm. The mean style length for B. caerulea-grandis is intermediate at 1.170 mm.

Table 1. Results of measurements of herbarium material for several characters of Betula species.

B. populifolia	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
561	2.31	35.6	6.4	1.9	3.12	39.24	1.43	0.82	1.74	0.51
562	1.79	28.4	5.6	2.1	2.80	39.59	1.34	0.78	1.72	0.65
570	1.64	43.0	7.4	1.8	1.50	36.25	0.89	0.52	1.67	0.32
589	1.39	33.8	7.6	2.4	4.45	30.03	1.70	0.96	1.77	0.81
590	1.41	46.0	8.0	1.9	3.61	30.18	1.59	0.81	1.96	0.52
596	1.64	41.0	8.0	2.2	2.97	33.45	1.69	0.85	1.99	0.60
604	1.33	42.8	8.8	2.3	3.15	32.45	1.57	1.14	1.37	0.83

Table 1 - Cont'd.

Table 1 (Cont'd)

B. caerulea-grandis	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
560	0.93	34.2	8.2	3.1	4.33	36.75	2.32	1.26	1.84	0.91
563	1.07	51.8	8.0	3.0	4.99	28.60	2.02	1.03	1.95	0.98
564	1.16	42.2	8.2	3.0	5.07	29.68	1.84	1.14	1.61	1.04
565	1.02	44.4	7.6	3.3	5.13	35.10	2.40	1.12	2.14	1.21
566	1.01	49.4	8.4	2.9	5.55	30.69	2.28	0.92	2.37	1.20
567	1.22	46.8	9.0	2.9	4.65	38.90	2.11	1.12	1.88	1.12
568	1.08	51.6	8.2	3.0	5.42	28.90	1.98	1.05	1.88	1.04
569	1.20	39.6	9.6	3.6	5.47	39.78	3.38	1.21	1.88	1.28
571	1.16	37.2	10.2	3.2	6.01	36.36	2.56	1.36	1.88	1.43
572	1.57	49.4	9.8	3.3	4.90	32.79	2.16	1.23	1.76	0.87
574	1.49	39.2	6.8	2.1	4.46	38.78	2.00	0.83	2.05	1.06
575	1.00	41.6	9.6	2.5	4.38	38.27	2.26	1.26	1.79	1.28

Table 1 - Cont'd.

Table 1 (Cont'd)

B. caerulea-grandis	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
578	1.20	50.4	9.0	3.4	4.90	29.91	2.01	0.99	2.03	1.15
579	1.06	45.2	8.4	4.4	6.97	29.30	2.25	1.26	1.79	1.34
580	1.18	34.4	10.0	3.4	6.15	37.90	2.43	1.42	1.71	1.35
582	1.11	46.8	9.2	3.0	5.26	39.40	2.02	1.16	1.74	1.23
585	1.66	49.0	8.8	3.2	5.85	39.44	2.49	1.21	2.06	1.12
586	1.26	47.0	9.2	3.2	6.11	39.97	2.58	1.17	2.21	1.29
591	1.45	50.4	8.4	2.7	5.22	29.49	1.53	1.02	1.50	0.94
593	1.13	52.8	8.6	3.3	6.00	33.06	1.87	1.09	1.72	1.13
597	1.02	45.0	9.4	3.1	7.14	32.79	2.19	1.07	2.05	1.18
598	1.10	38.0	7.4	2.3	4.88	30.33	2.17	1.07	2.03	1.09
599	1.05	42.2	10.0	2.8	4.87	29.20	2.29	1.26	1.82	0.98
600	1.05	43.0	7.6	2.1	5.66	30.19	2.38	1.26	1.88	0.98

Table 1 - Cont'd.

Table 1 (Cont'd)

B. caerulea-grandis	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
601	1.08	36.6	8.6	2.3	5.71	30.50	2.68	0.95	2.84	1.60
606	1.14	40.0	8.2	3.1	5.31	31.82	2.43	1.27	1.91	1.08
607	1.01	44.2	7.6	-	6.01	35.21	2.13	1.21	1.77	1.85
608	1.09	51.0	9.6	2.5	3.48	32.79	1.56	0.90	1.73	1.16
609	1.43	58.0	10.4	2.9	4.77	33.71	1.78	1.05	1.69	1.06

Table 1 - Cont'd.

Table 1 (Cont'd)

B. cordifolia	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
394	0.85	46.0	10.4	4.68	5.37	39.10	2.56	1.57	1.62	1.37
398	0.80	69.0	11.2	5.30	7.47	30.62	2.97	1.96	1.51	2.13
576	0.82	49.8	10.0	4.20	7.29	34.41	2.51	1.50	1.67	1.15
577	0.95	49.6	9.4	3.90	7.41	39.63	2.89	1.61	1.80	1.74
581	0.99	45.6	8.6	3.90	6.03	37.70	2.08	1.26	1.65	1.25
583	1.00	59.4	9.6	3.50	5.49	36.00	2.31	1.41	1.64	1.21
584	1.20	34.0	9.6	3.50	6.22	37.63	2.98	1.31	1.88	1.63
587	0.85	53.0	9.8	3.60	5.53	34.90	2.46	1.50	1.60	1.48
588	0.75	54.4	9.0	3.50	5.43	40.44	2.34	1.22	1.92	1.40
592	0.89	53.6	12.2	3.10	5.59	33.41	2.34	1.50	1.56	1.26
594	1.25	42.6	8.2	3.80	5.40	34.59	2.28	1.43	1.59	1.24
595	0.91	45.9	8.6	3.30	6.82	32.06	2.50	1.62	1.54	1.64

Table 1 - Cont'd.

Table 1 (Cont'd)

B. cordifolia	a/m	Serrations	Veins	Catkin (cm)	Bract (mm)	Guard Cell	Achene length (mm)	Achene width (mm)	Achene L/W ratio	Style length (mm)
605	0.84	41.8	9.0	3.40	7.20	31.18	2.65	1.70	1.56	1.36
611	0.83	49.6	9.4	4.30	7.33	32.91	2.90	1.81	1.60	1.37
612	0.92	38.0	8.4	3.30	7.09	30.97	2.31	1.41	1.64	1.40
613	0.80	53.0	8.4	-	-	29.08	-	-	-	-

Figure 3. Representative leaf tracings of B. populifolia, B. caerulea-grandis and B. cordifolia, drawn actual size.

Figure 3A. ..... B. populifolia

Figures 3B, C, D, E ..... B. caerulea-grandis

Figures 3F, G, H ..... B. cordifolia

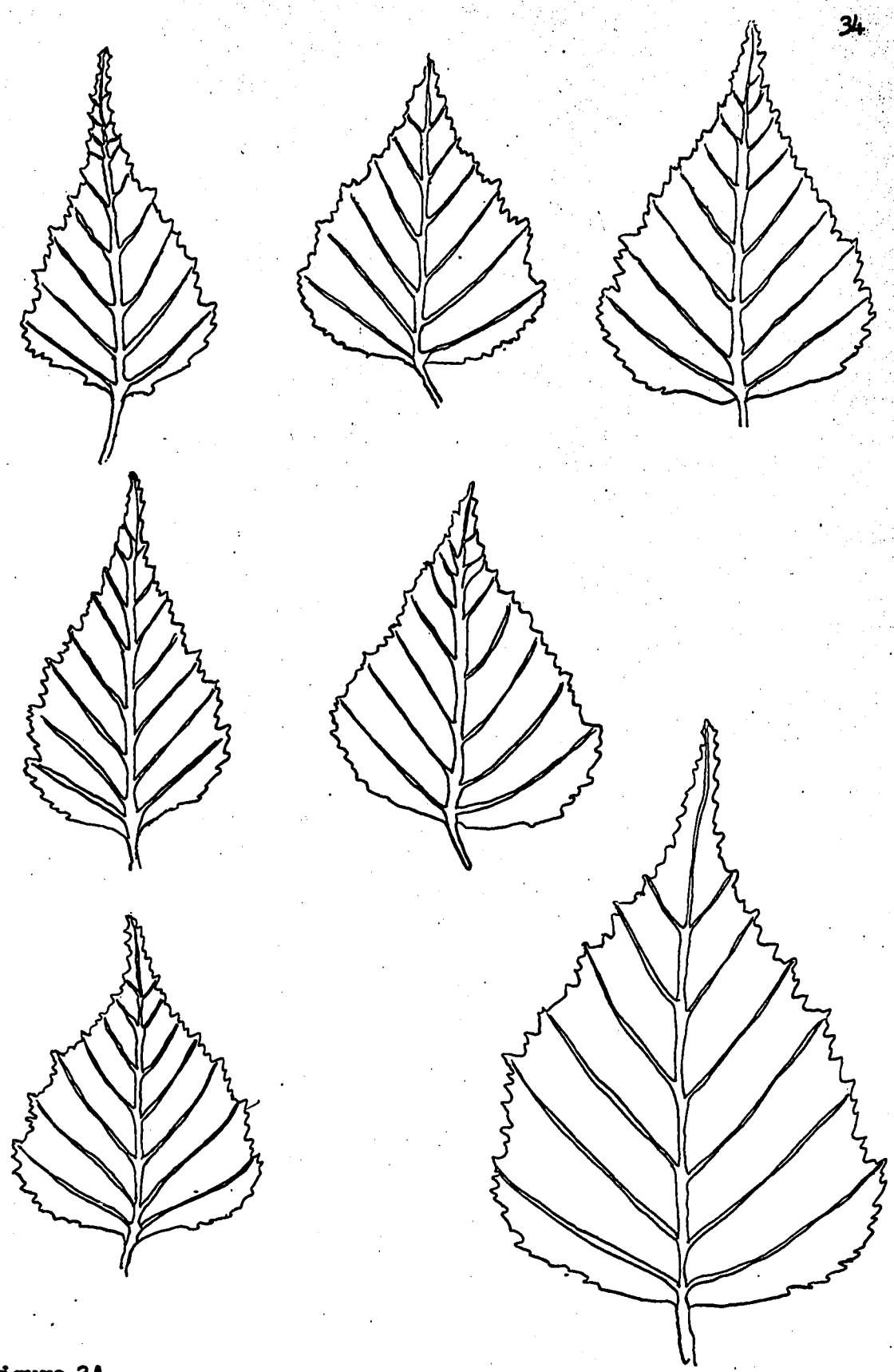


Figure 3A.

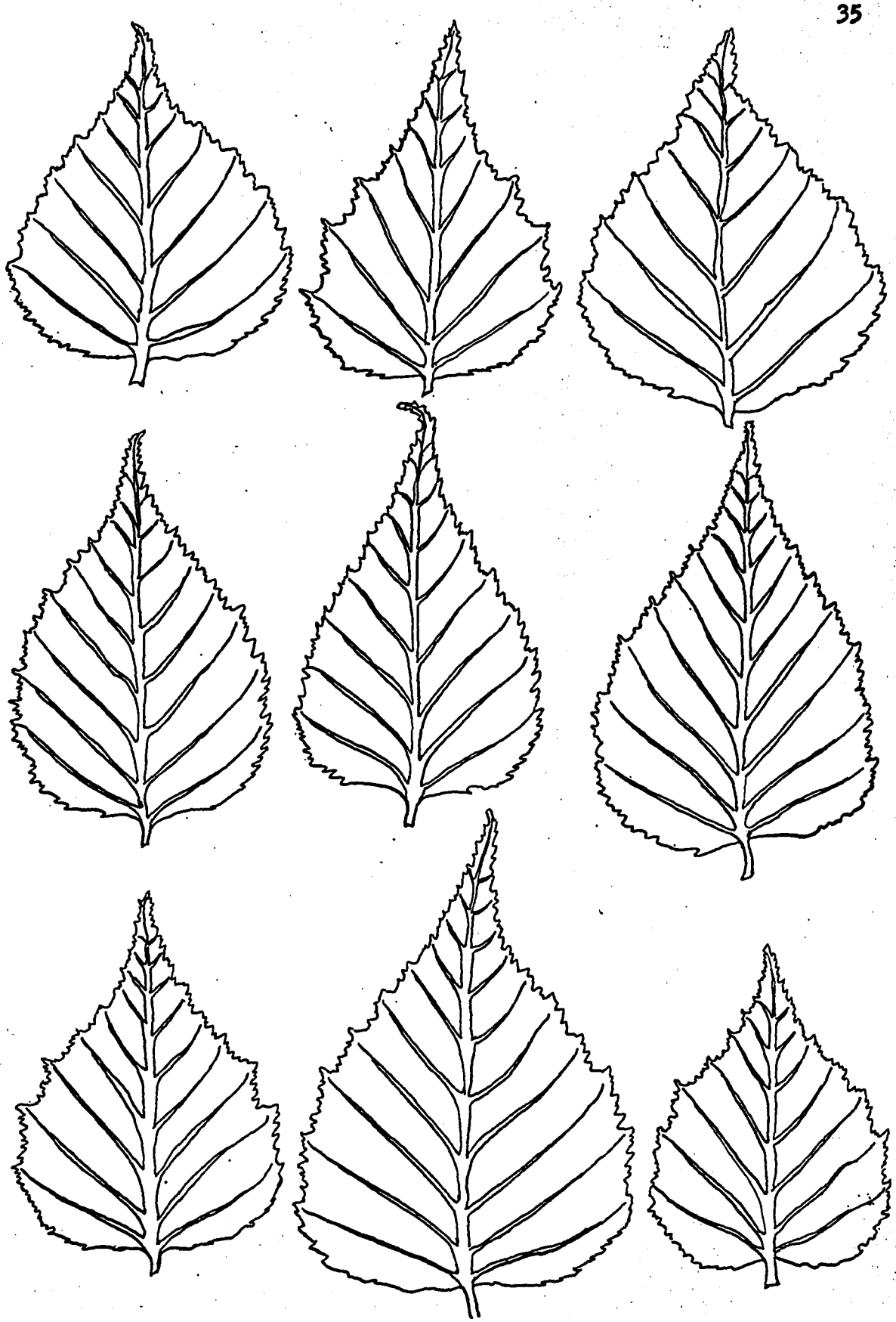
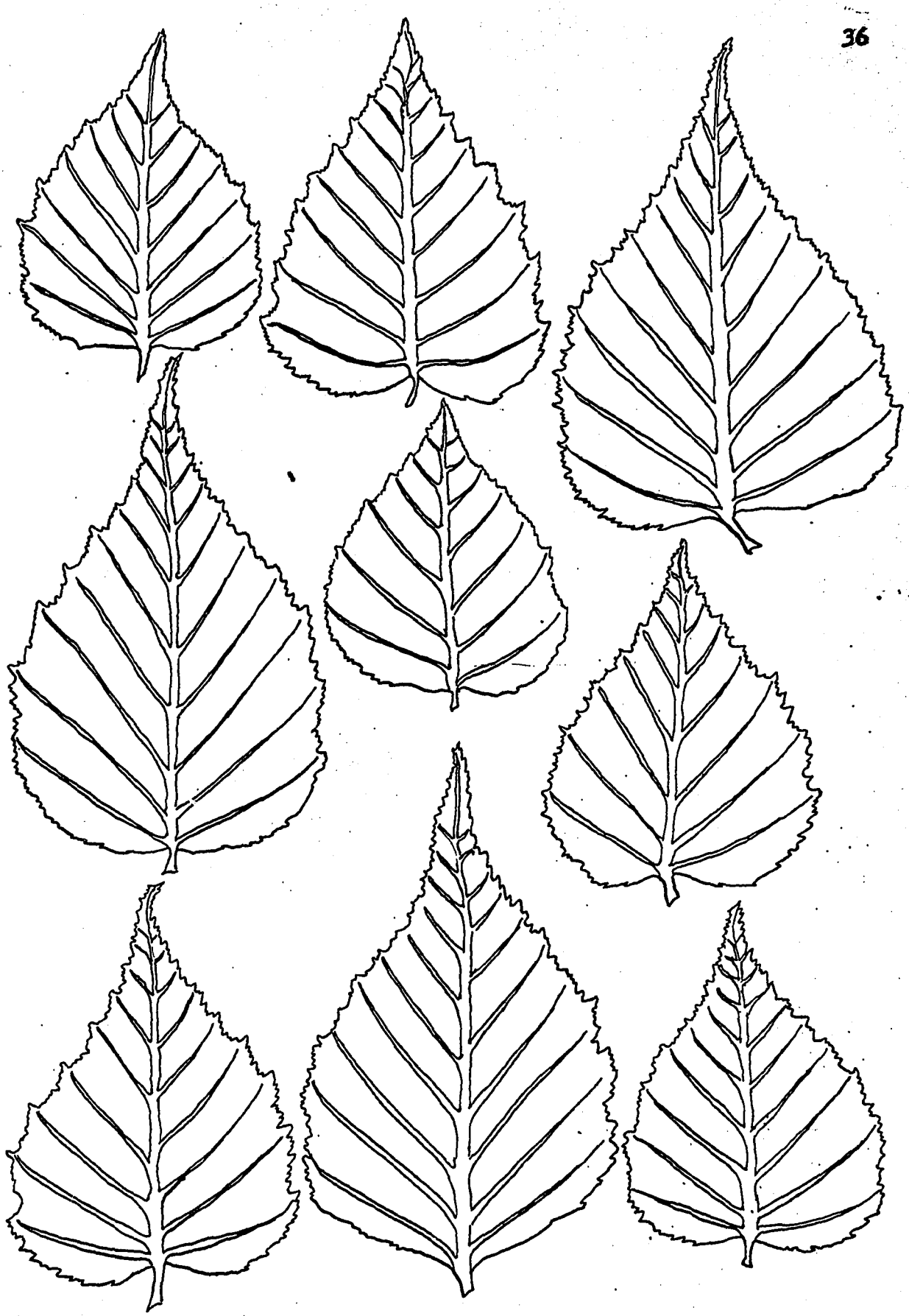


Figure 3B.



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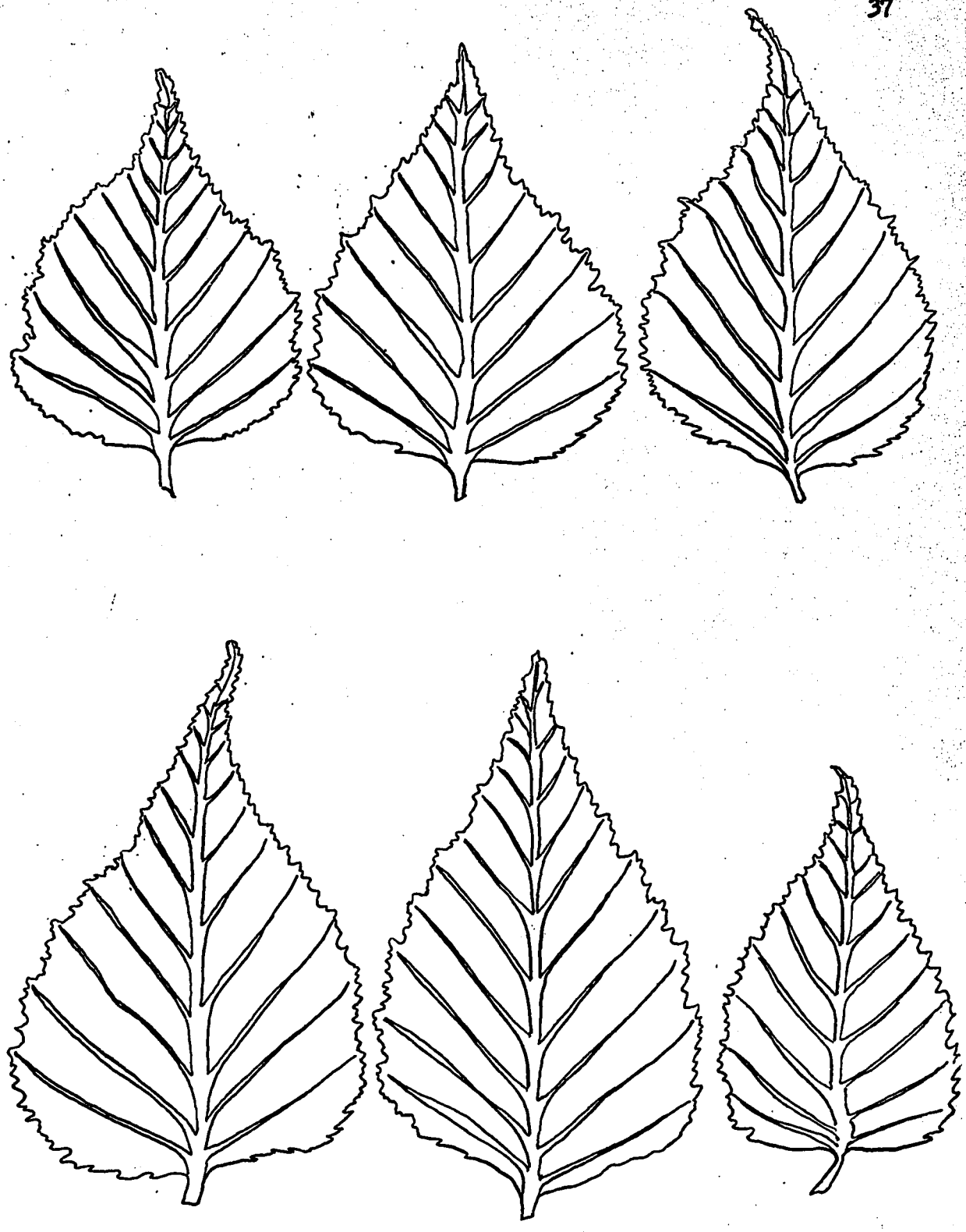


Figure 3D.

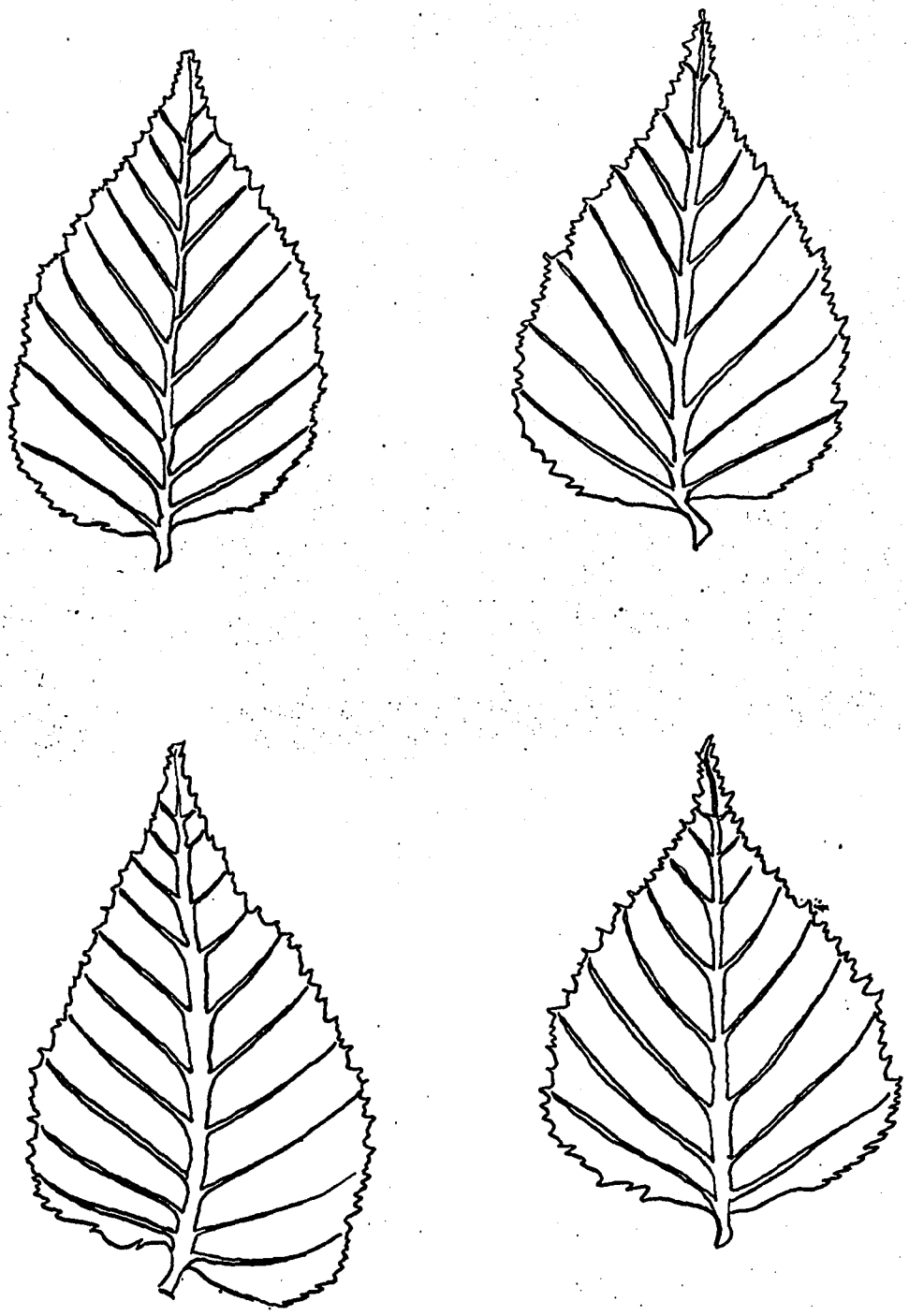


Figure 3E.

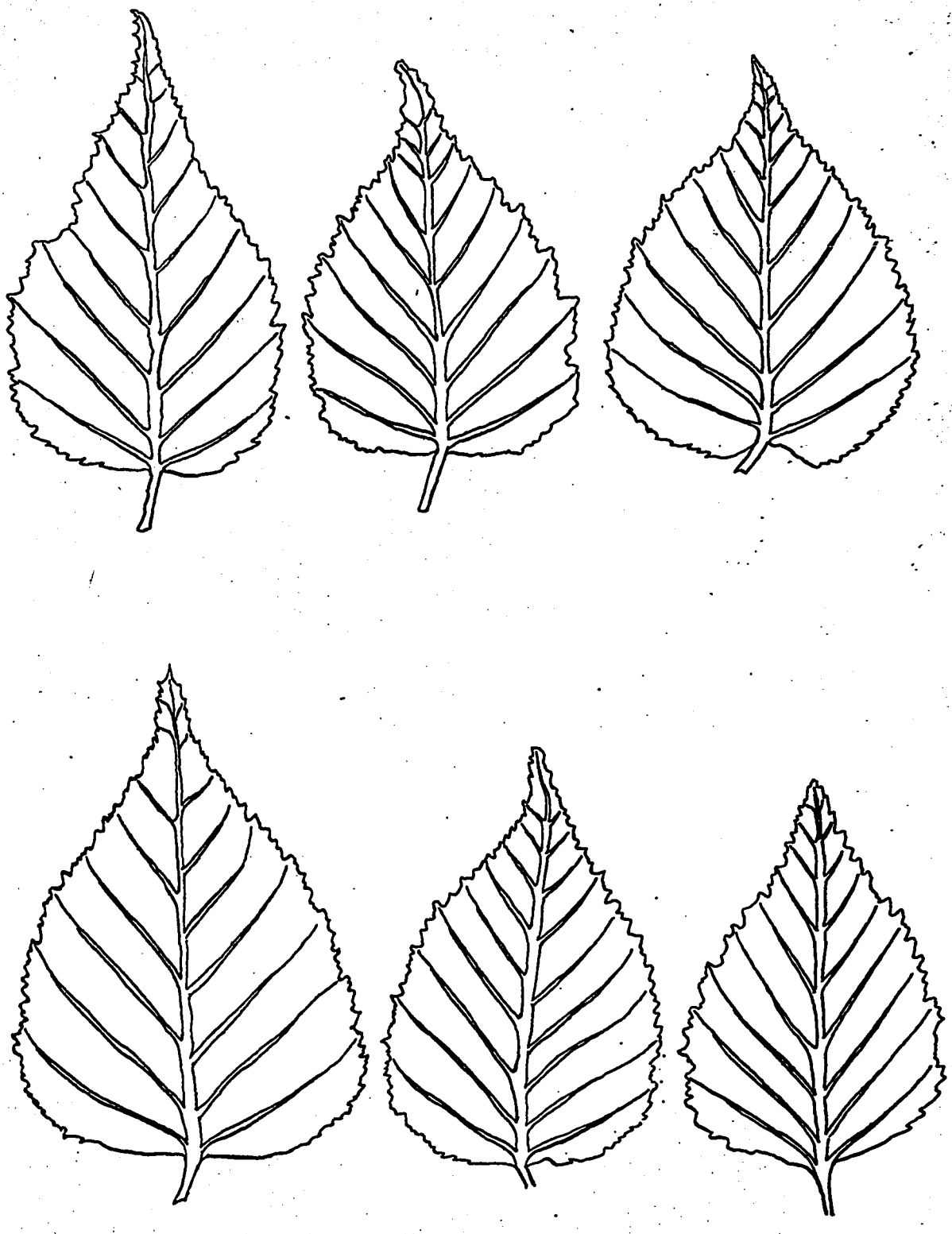


Figure 3F.

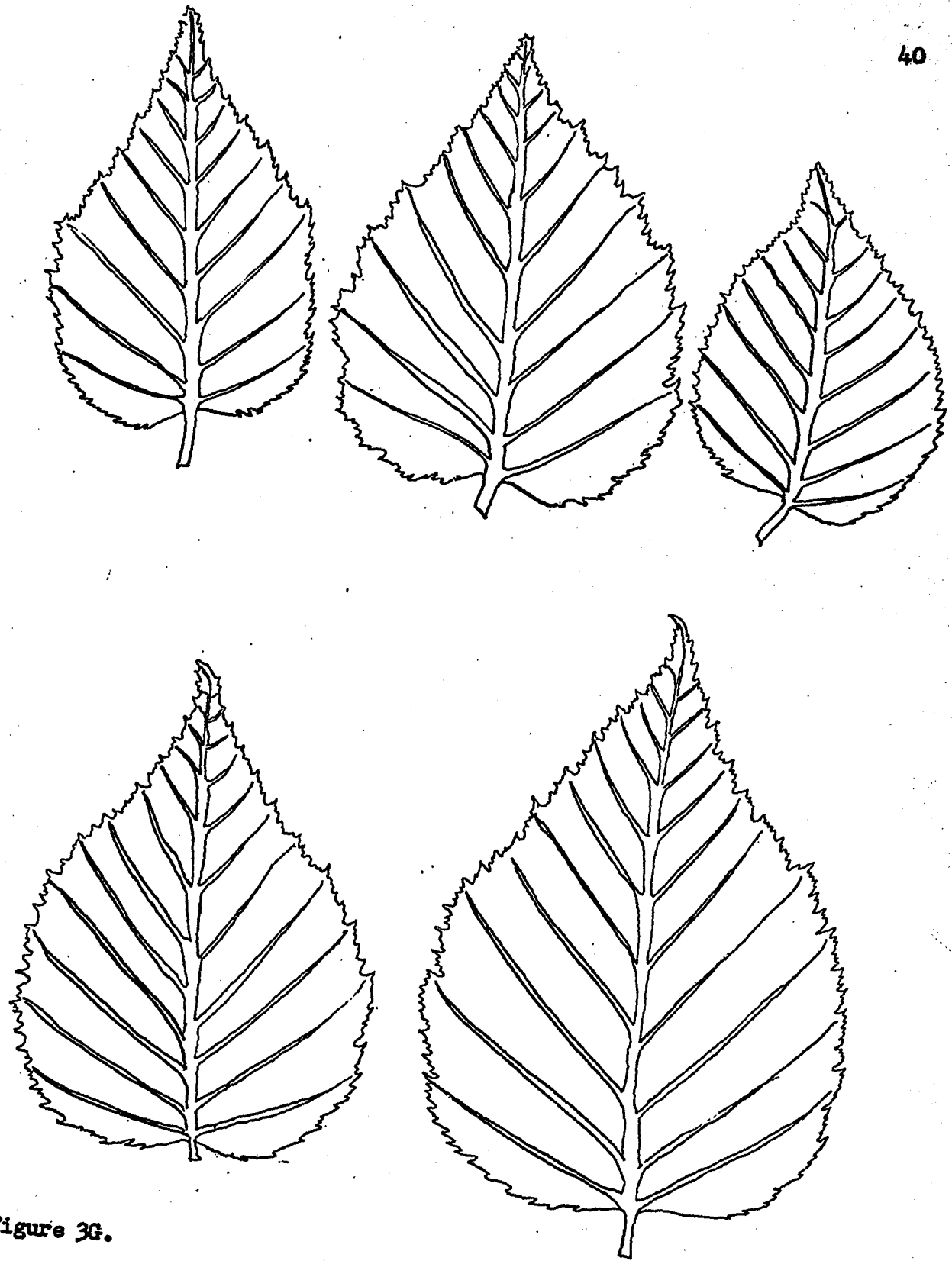


Figure 3G.

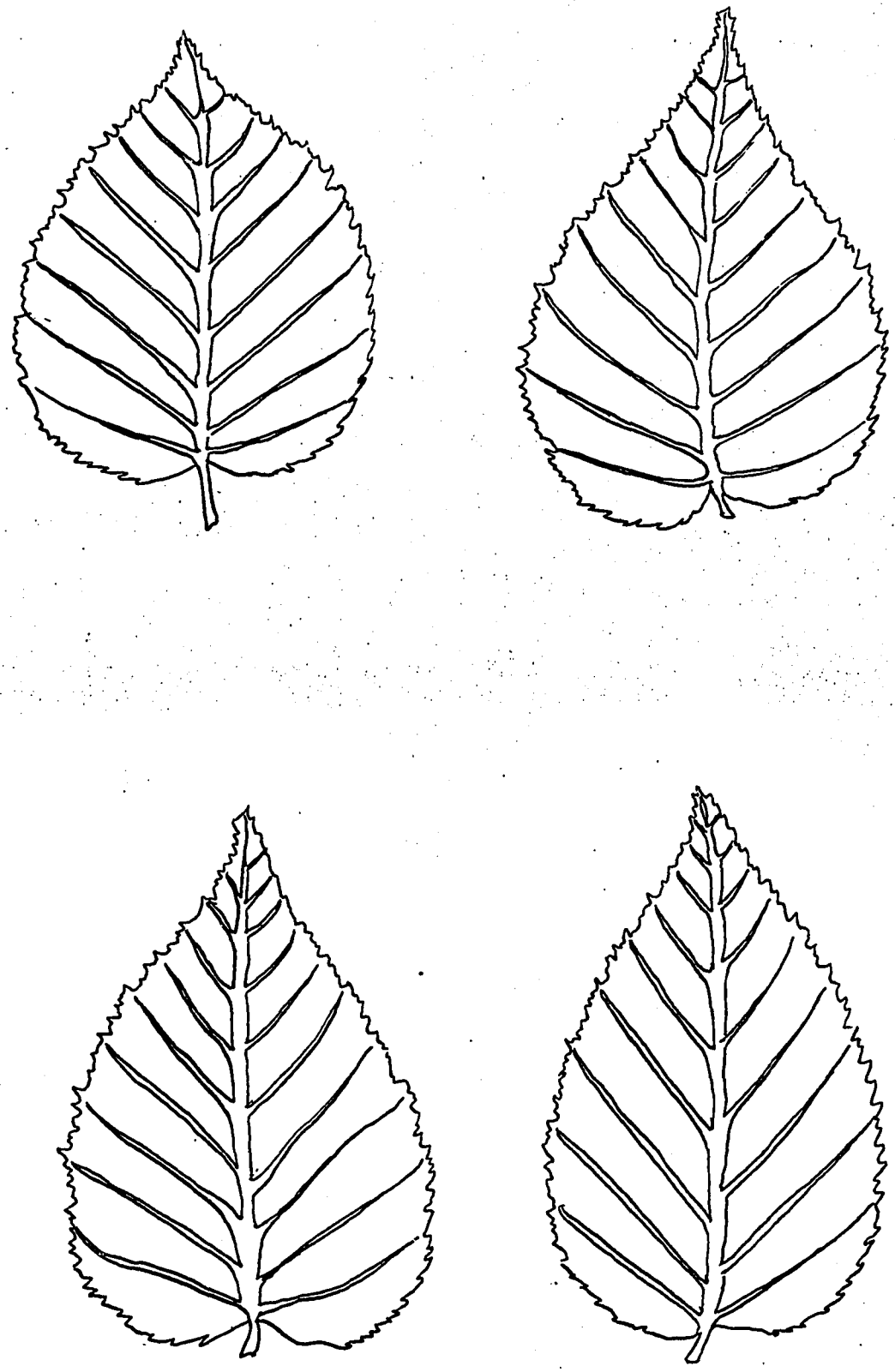


Figure 3H.

Figure 4. Drawings of representative bracts of *B. populifolia*, *B. caerulea-grandis*, and *B. cordifolia* illustrating bract shape, x 10.

Figure 4A. ..... *B. populifolia*

Figures 4B, C, D, E, F, G ..... *B. caerulea-grandis*

Figures 4H, I, J, K ..... *B. cordifolia*

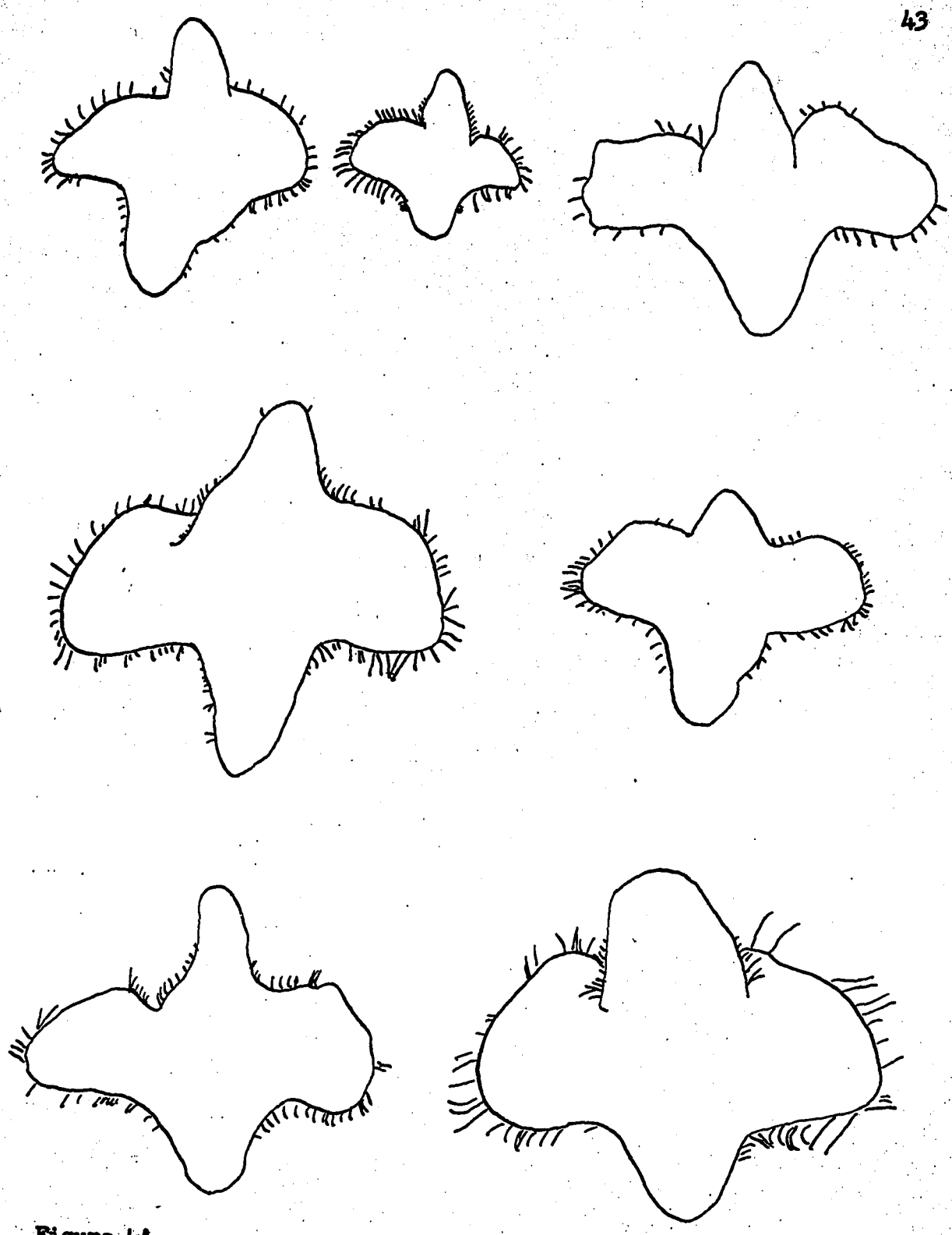


Figure 4A.

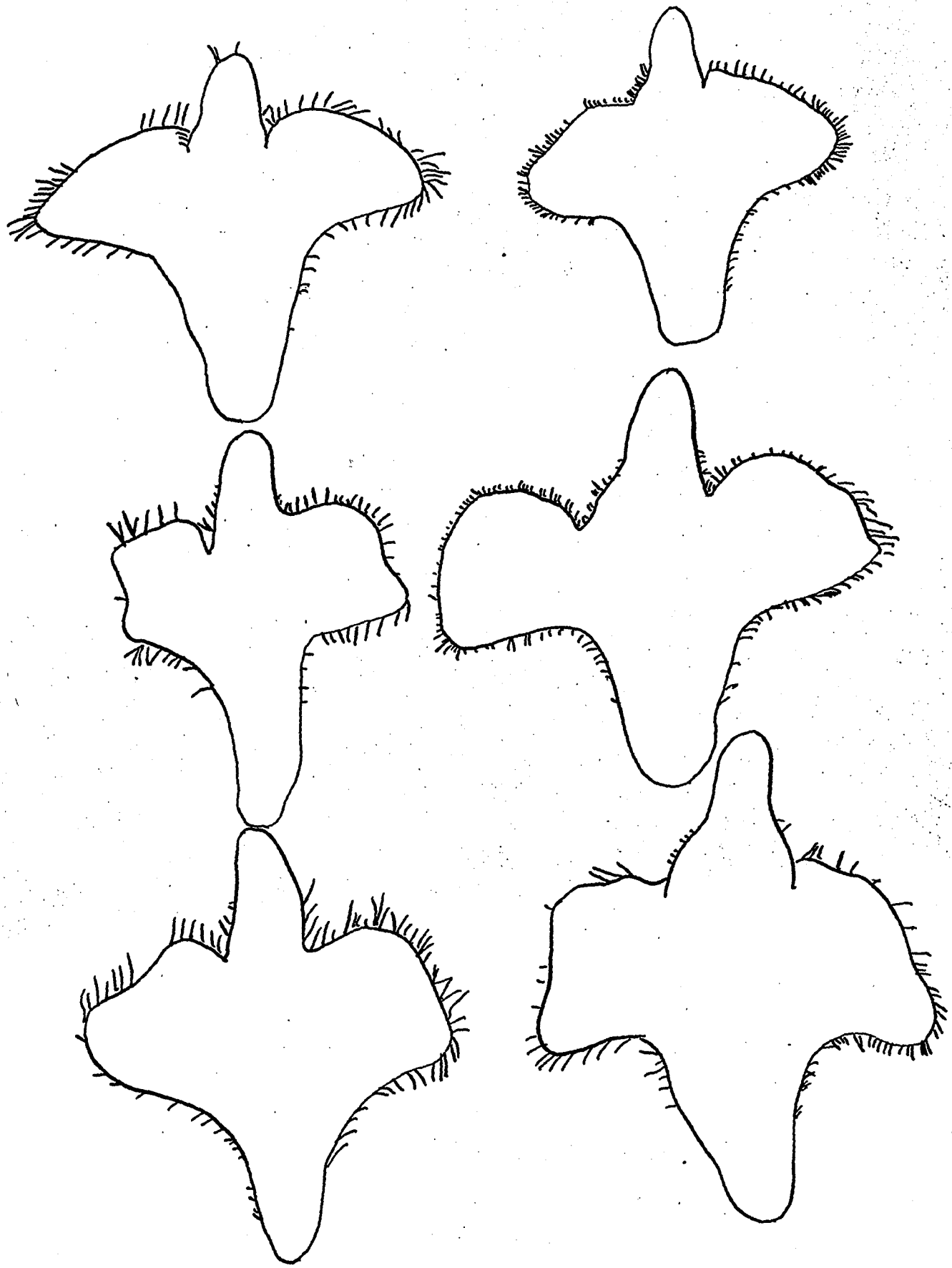


Figure 4B.

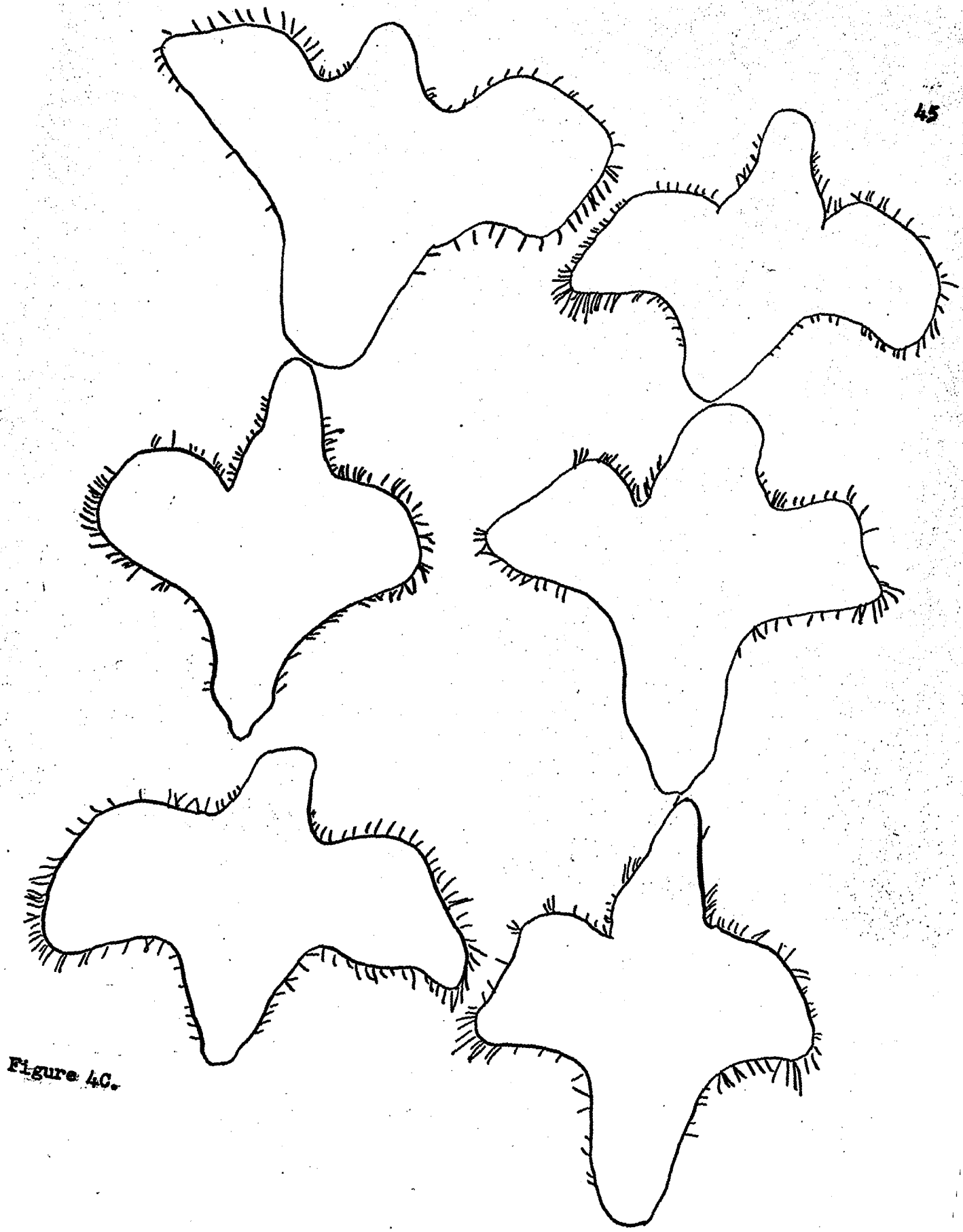


Figure 4C.

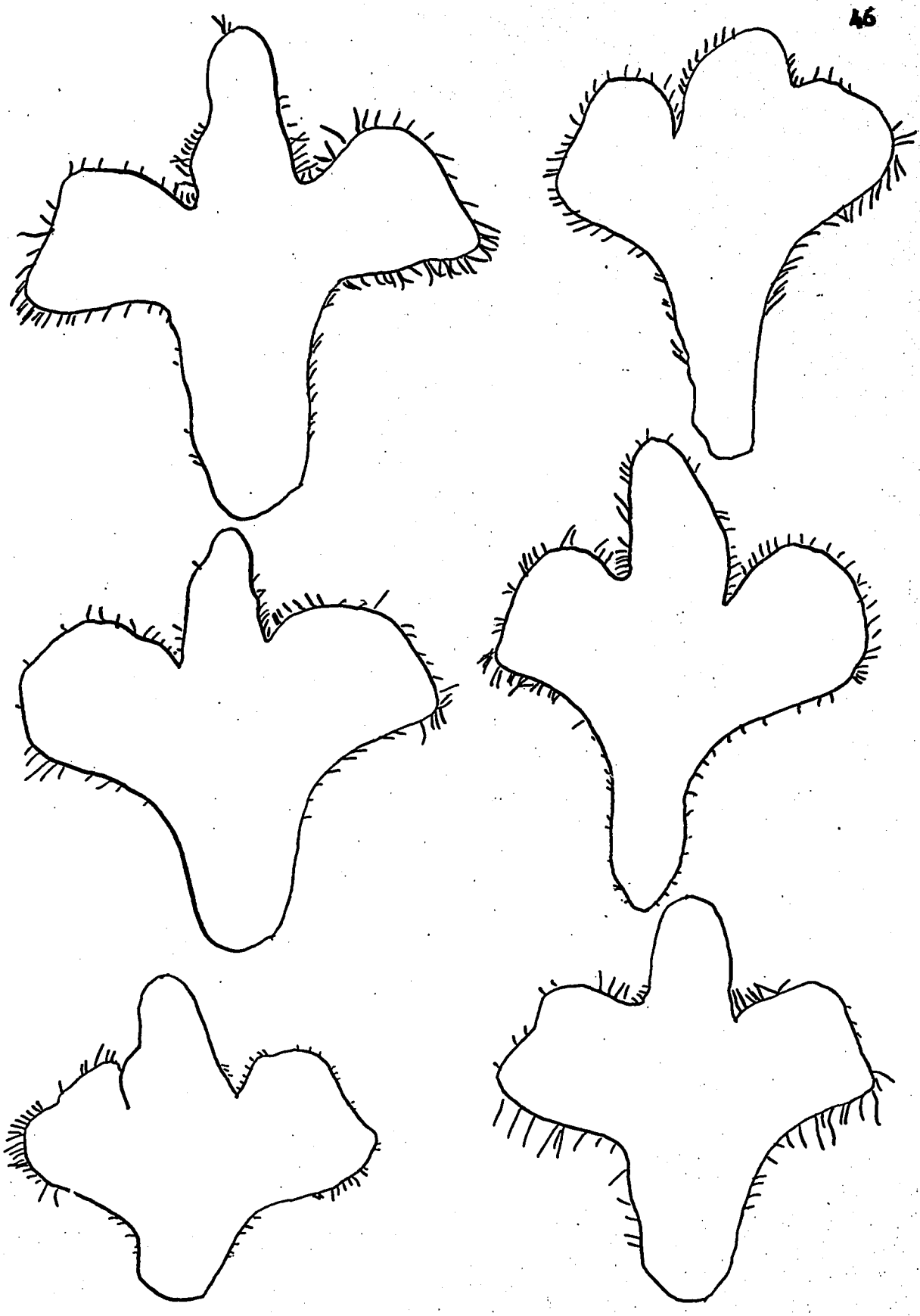
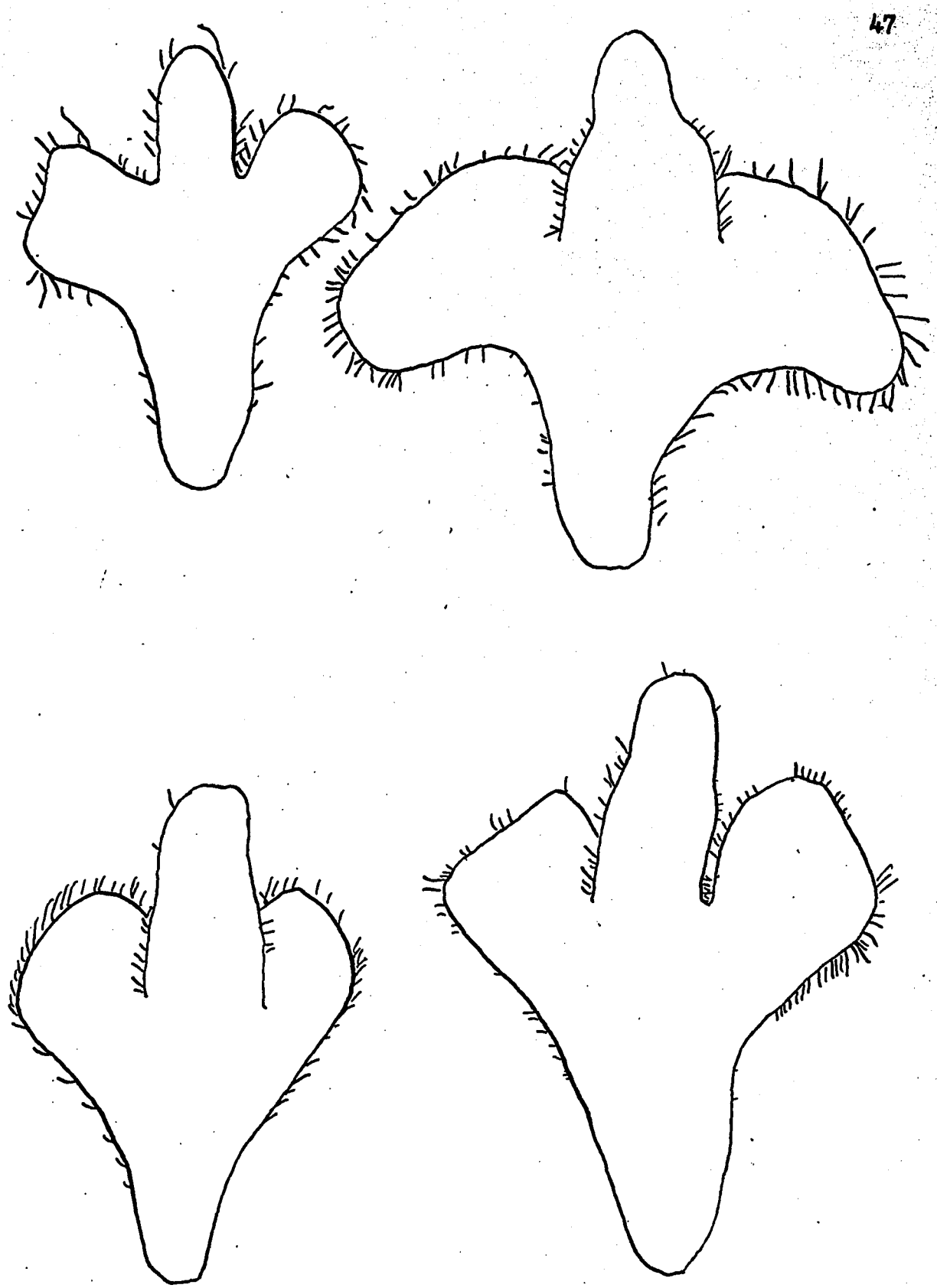


Figure 4D.



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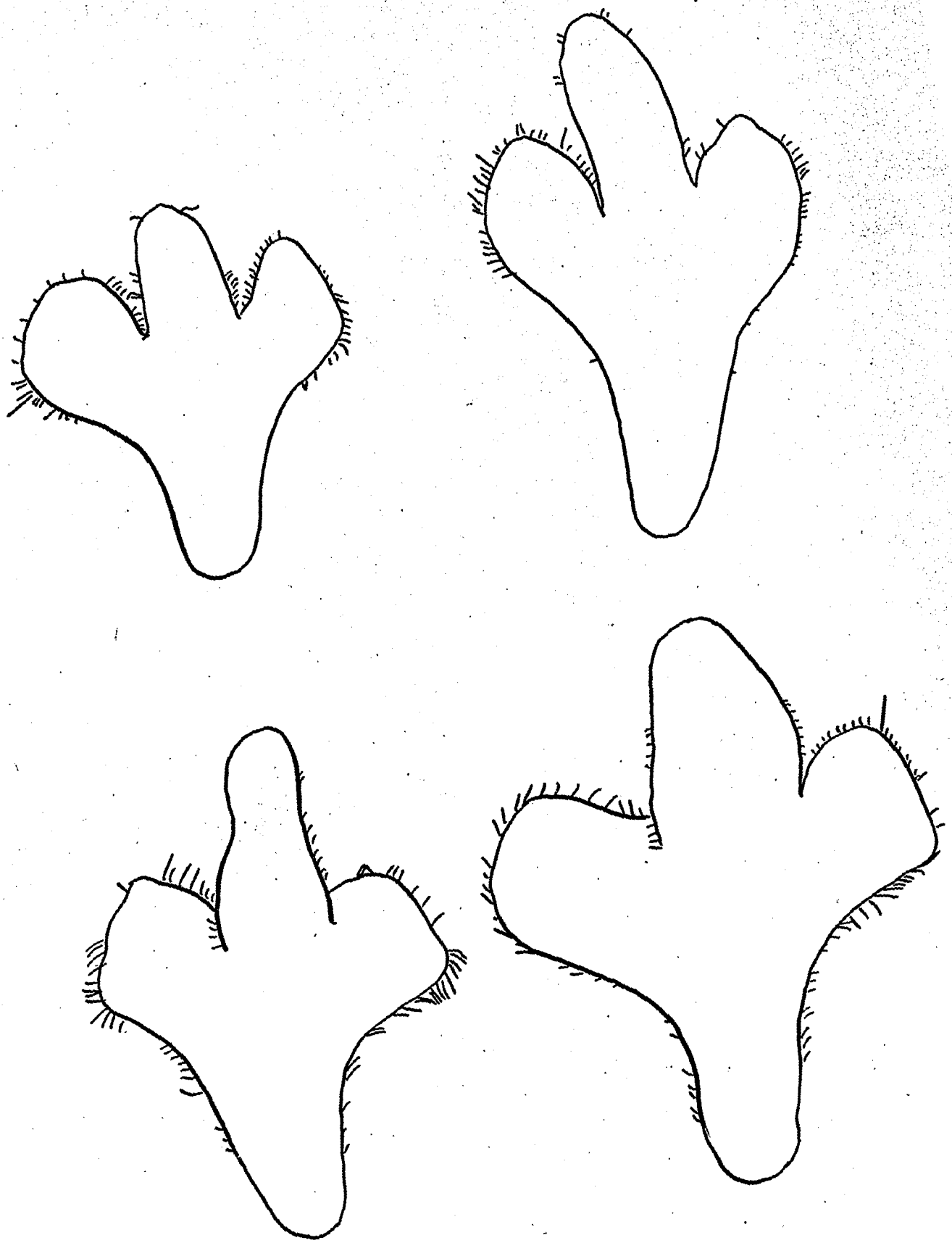

Figure 4F.

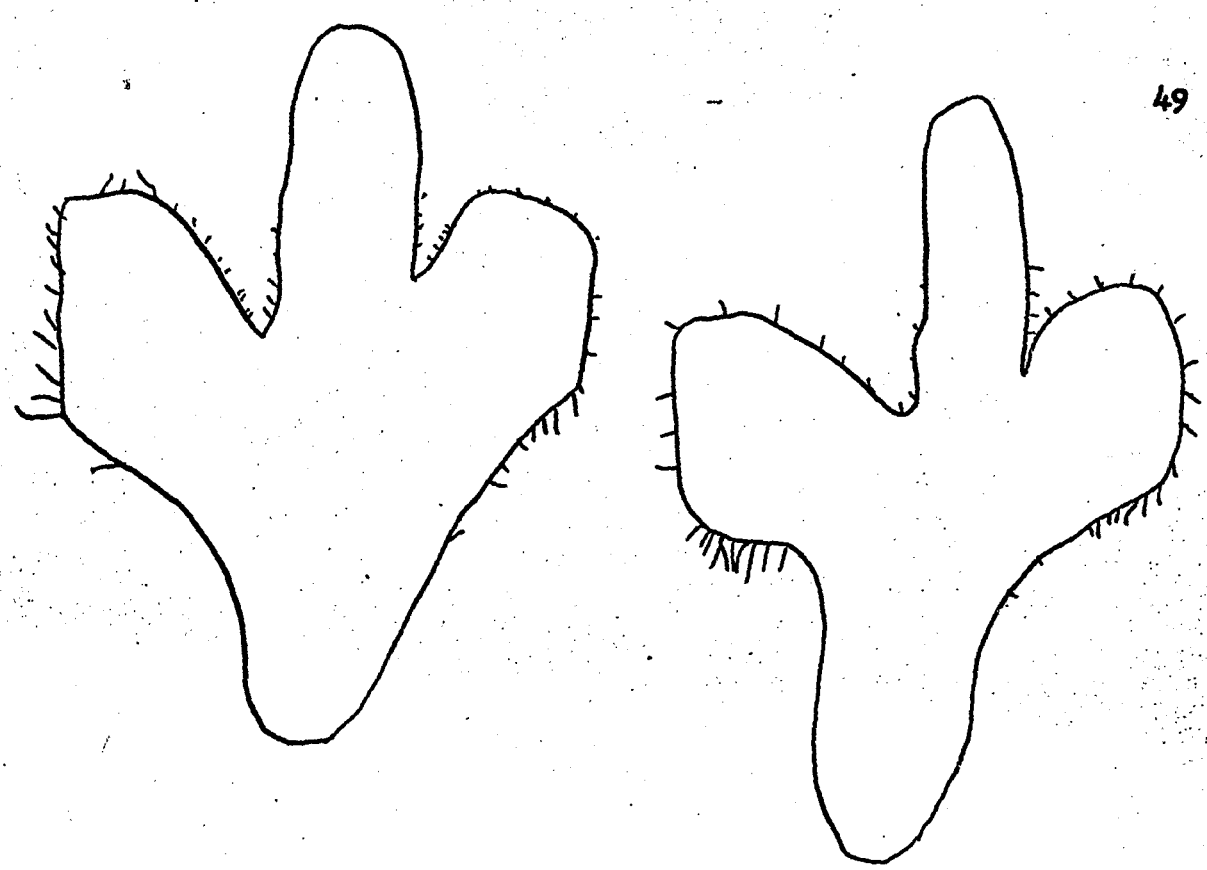

Figure 4G.

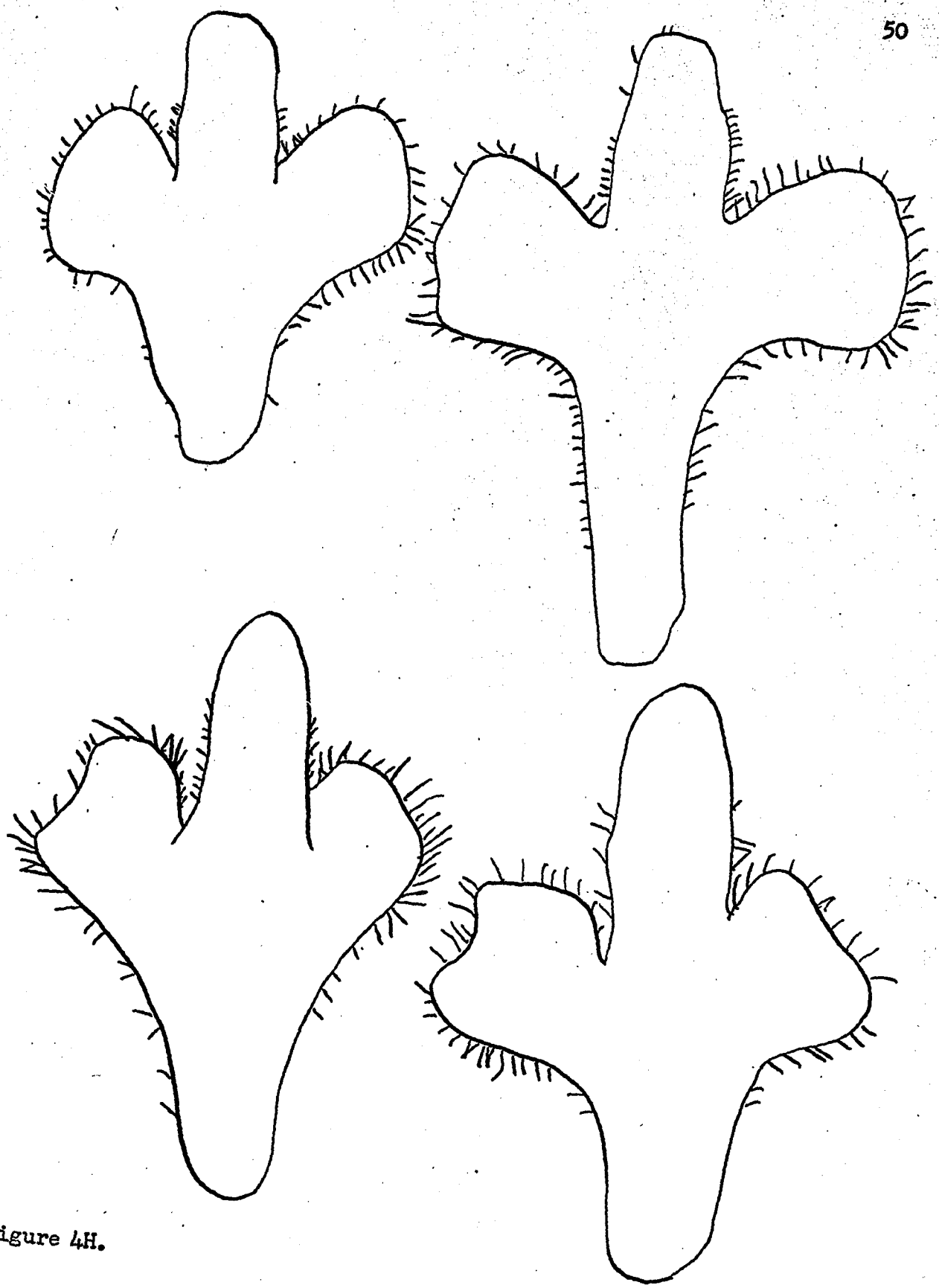

Figure 4H.

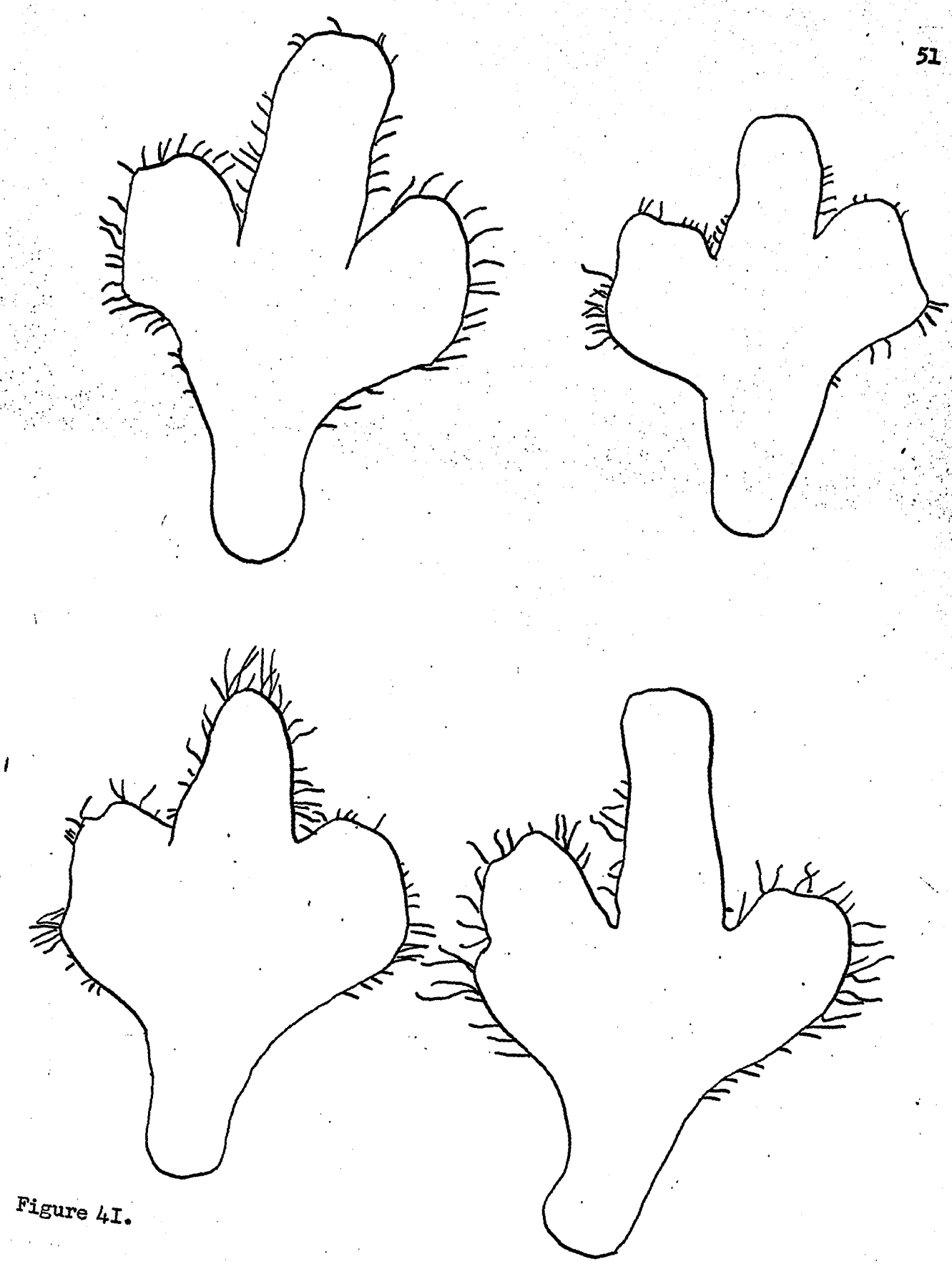

Figure 4I.

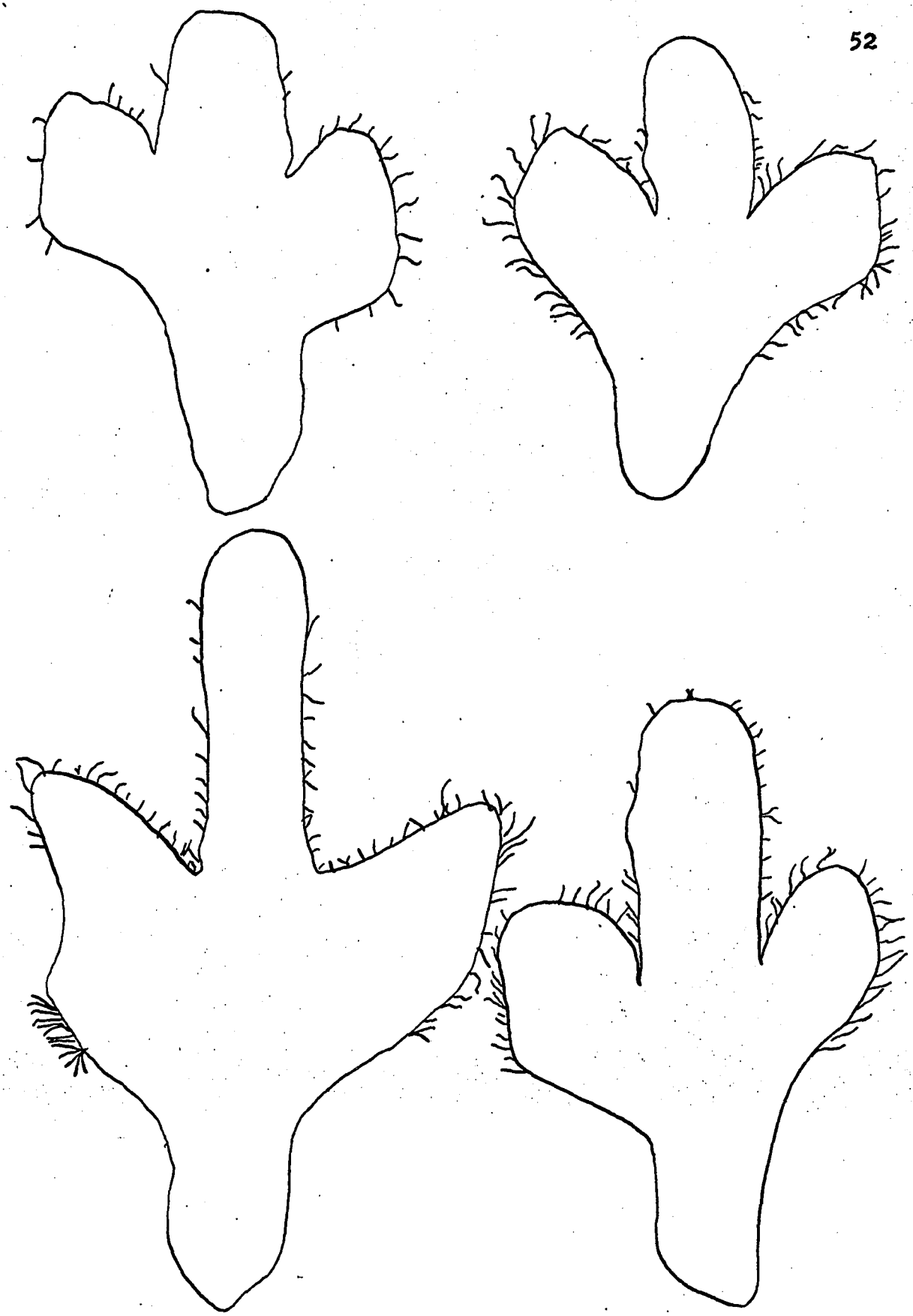

Figure 4J.

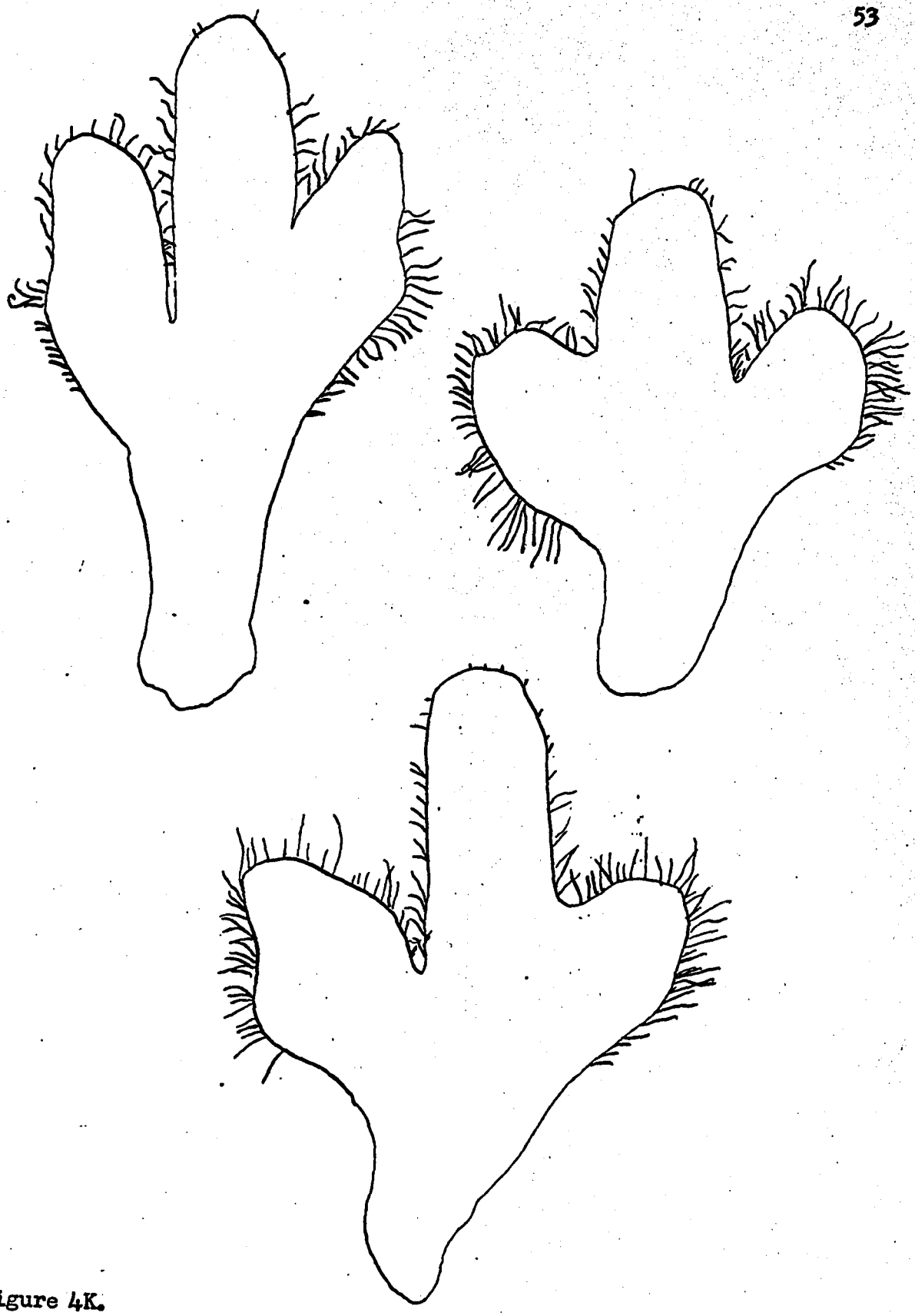

Figure 4K.

Figure 5. Drawings of representative achenes of B. populifolia, B. caerulea-grandis and B. cordifolia illustrating achene shape, x 10.

Figure 5A. ..... B. populifolia

Figures 5B, C, D ..... B. caerulea-grandis

Figures 5E, F ..... B. cordifolia

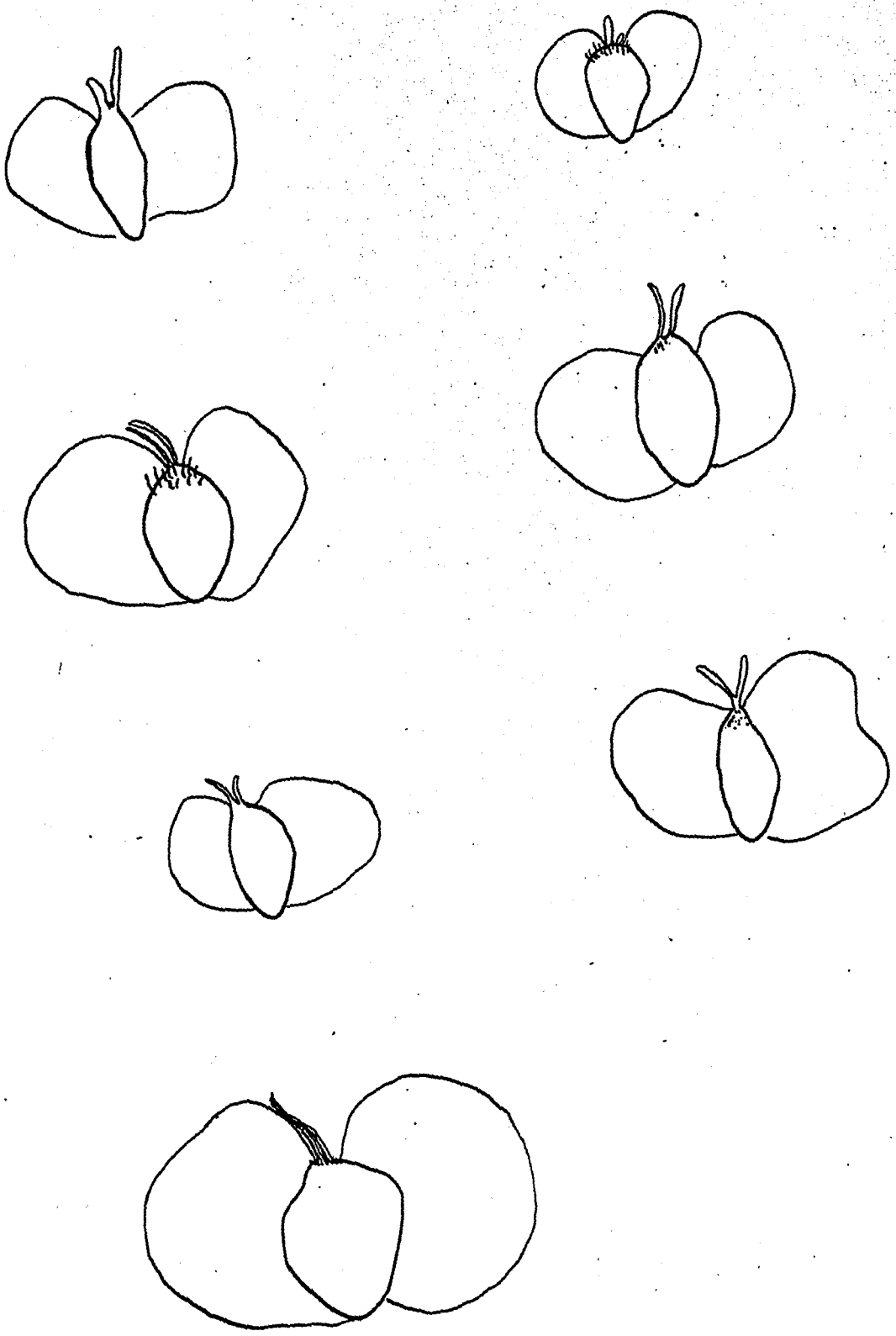

Figure 5A.

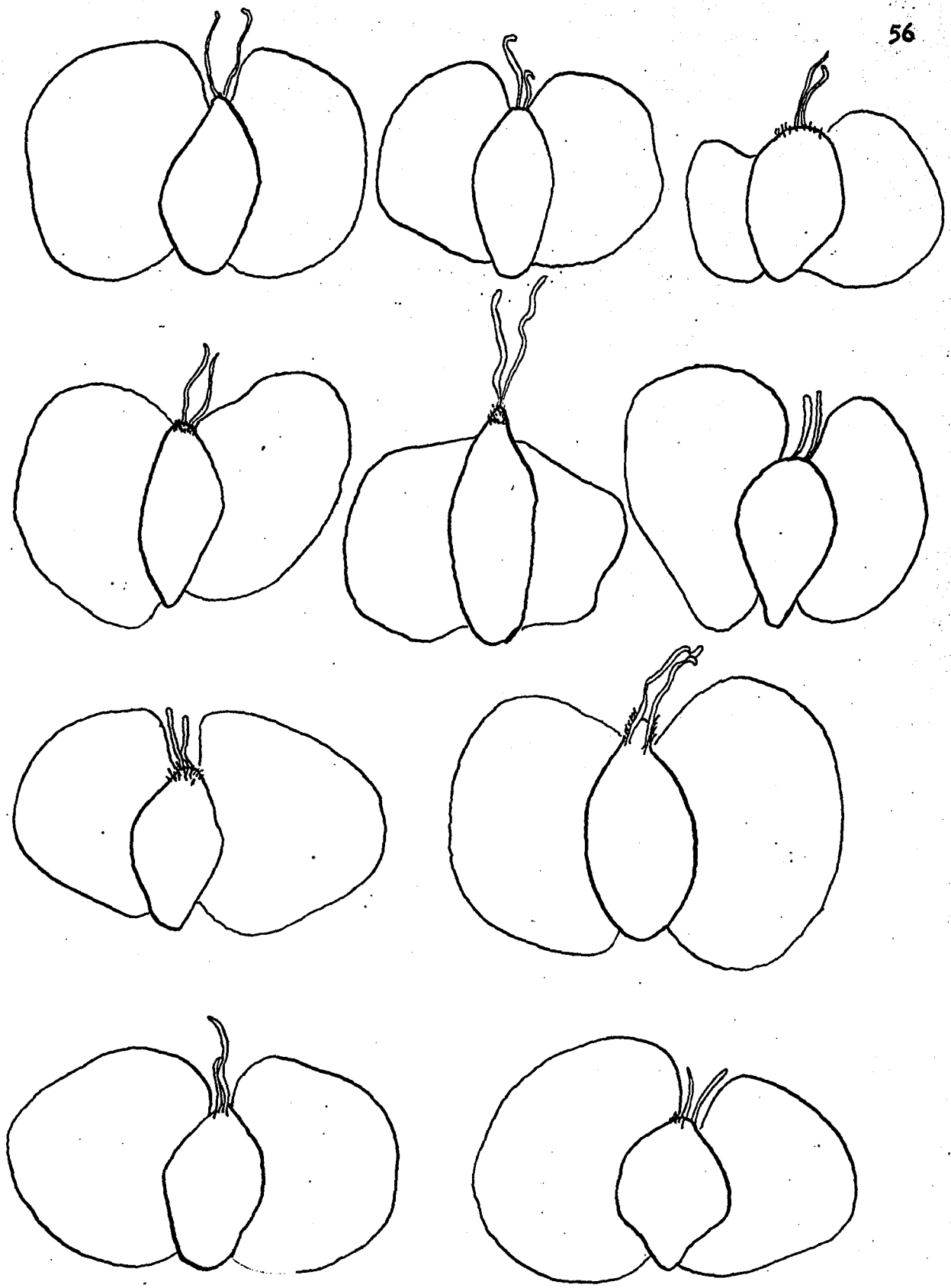

Figure 5B.

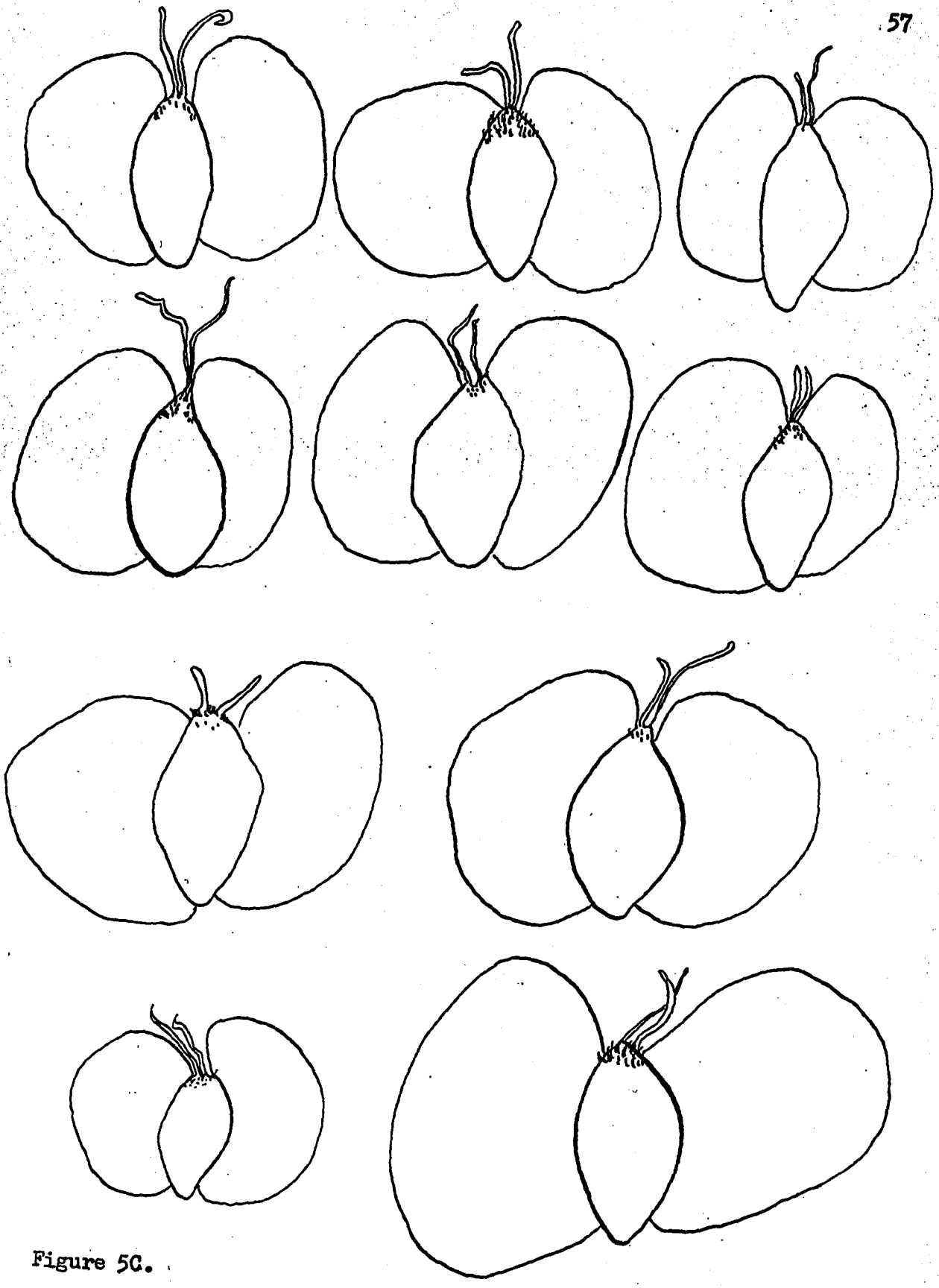

Figure 5C.

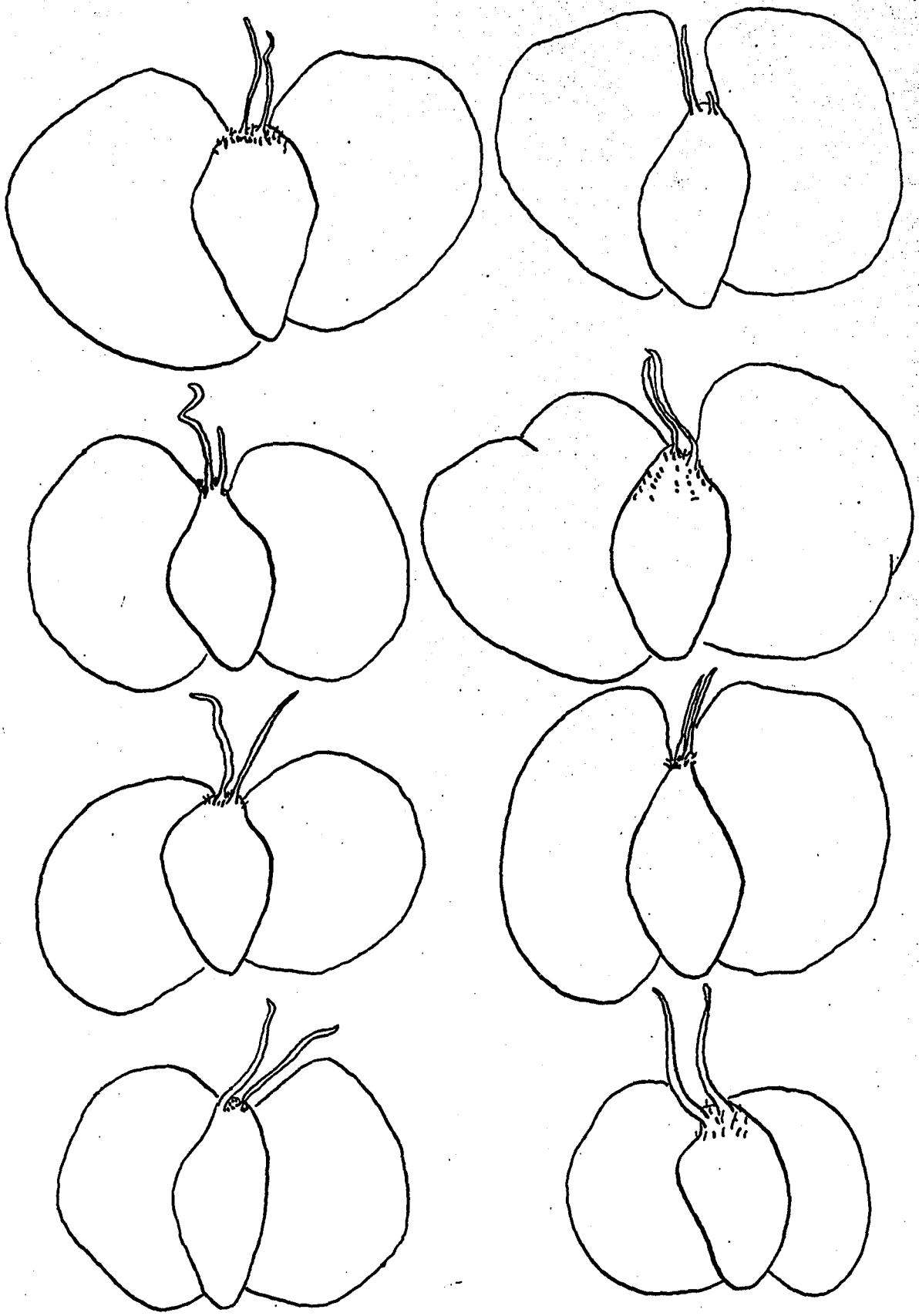

Figure 5D.

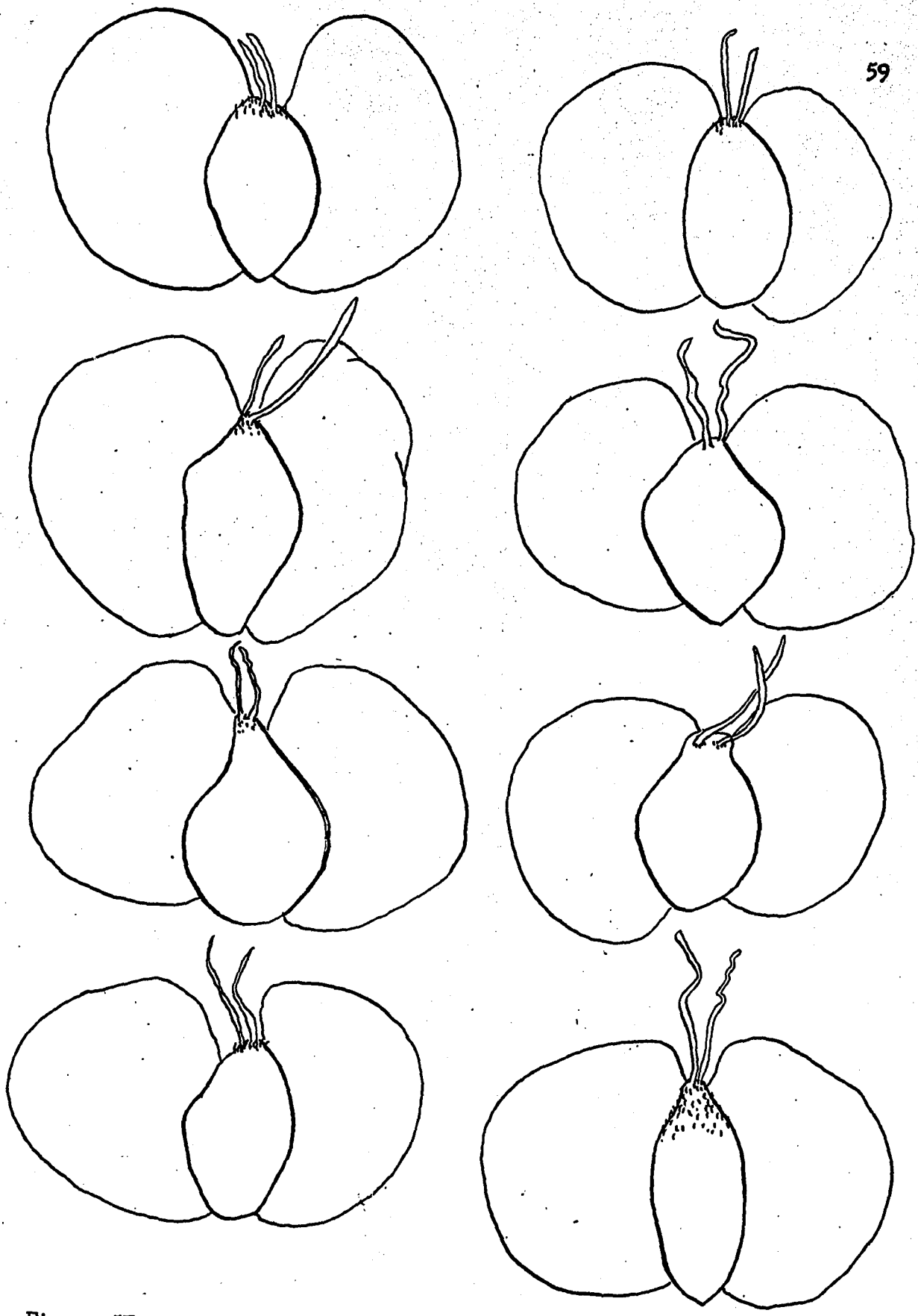

Figure 5E.

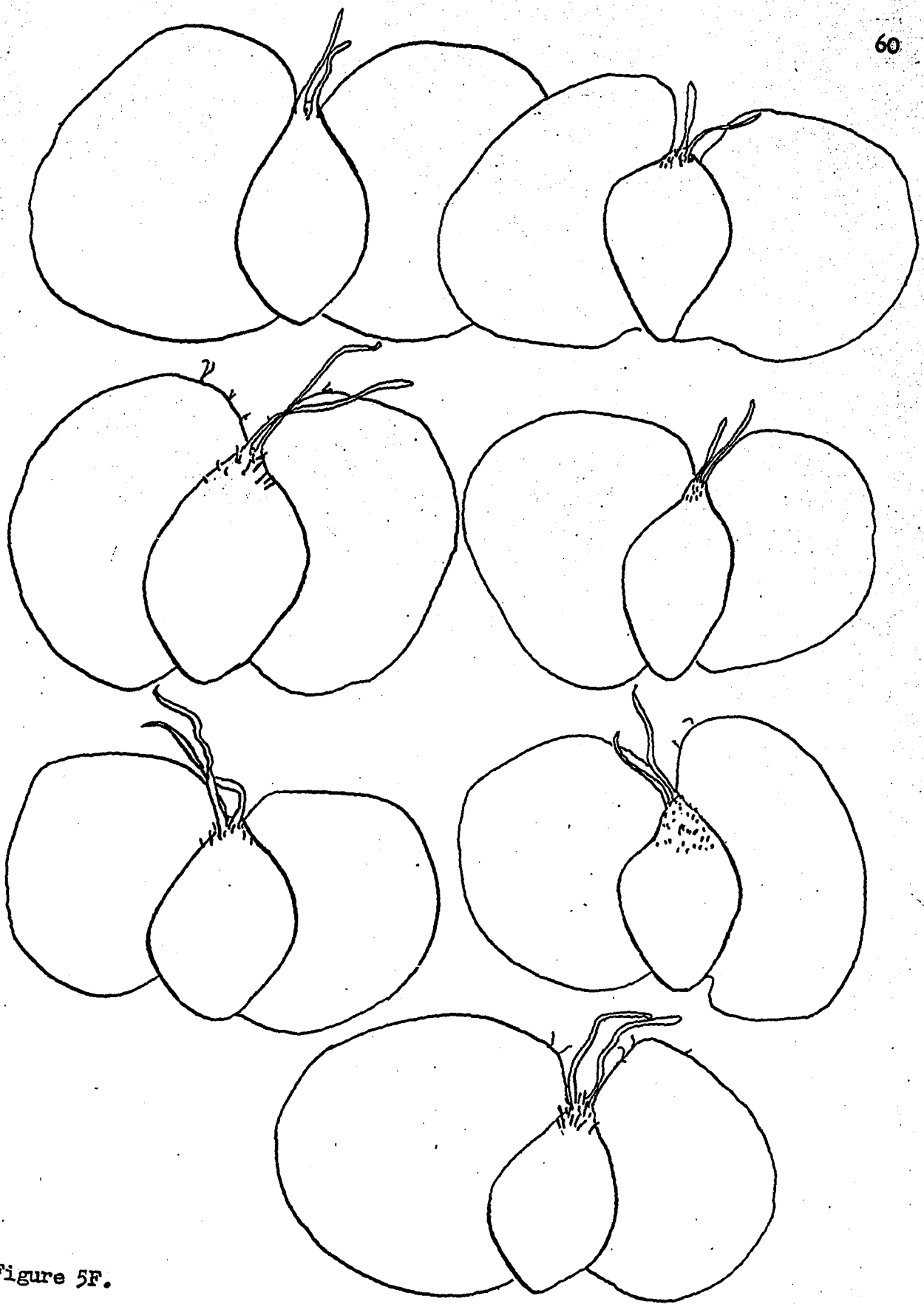

Figure 5F.

In order to see if differences such as those cited above concerning style length are significantly different when B. populifolia, B. cordifolia and B. caerulea-grandis are compared, a t test was conducted on the mean values. The results are shown in Table 2. Inspection of Table 2 shows that when B. cordifolia and B. caerulea-grandis are compared, in all characters except the length of the guard cells, the means of the two species are significantly different. Similarly, when B. populifolia and B. caerulea-grandis are compared, in eight characters the means are significantly different. In guard cell length and the length/width ratio of the achenes, the two species do not differ significantly.

In order to determine whether location affects the variability of one character in the same species, a comparison of means was undertaken for the same species in two different locations (Table 3). For example, the mean a/m ratios in B. caerulea-grandis from one location to another do not differ significantly. However, the mean number of veins per side of a leaf does differ significantly from Valcartier to Grand Manan Island.

The results of a comparison of hypothetical hybrid mean values computed for B. papyrifera and B. populifolia, and also for B. cordifolia and B. populifolia are shown in Table 4. The values of the hypothetical hybrid of a cross of B. cordifolia and B. populifolia

Table 2. Mean value, standard deviation, and t test for ten morphological characters in Betula populifolia, B. caerulea-grandis and B. cordifolia.

Characters	B. cordifolia	No. of plants	B. caerulea-grandis	No. of plants	t value[2]	df	Confidence 95% interval
a/m ratio[1]	0.92±0.1190	16	1.17±0.1816	29	5.020[3]	43	0.25±0.10
No. of serrations on one side of a leaf	49.11±7.6000	16	44.87±6.2160	29	2.061[4]	43	4.24±4.16
No. of veins on one side of a leaf	9.50±1.0900	16	8.75±0.8660	29	2.530[4]	43	0.75±0.60
Length of stomata (μ)	34.66±3.3500	16	33.78±3.9700	29	0.750	43	- not significant
Length of catkin (cm)	3.82±0.5907	15	2.99±0.4800	28	4.970[3]	41	0.83±0.34
Length of bract (mm)	6.38±0.8660	15	5.33±0.7815	29	4.071[3]	42	1.05±0.52
Length of achene (mm)	2.54±0.2723	15	2.21±0.3630	29	3.089[3]	42	0.33±0.22
Width of achene (mm)	1.52±0.2097	15	1.13±0.2008	29	4.468[3]	42	0.39±0.13
Achene length/achene width (ratio)	1.65±0.3769	15	1.91±0.2560	29	3.725[3]	42	0.26±0.14
Length of styles (mm)	1.44±0.2500	15	1.17±0.2100	29	3.772[3]	42	0.27±0.15

Table 2 - Cont'd.

Table 2 (Cont'd)

Characters	*B. populifolia*	No. of plants	*B. caerulea-grandis*	No. of plants	t value[2]	df	Confidence 95% interval
a/m ratio	1.65±0.1066	7	1.17±0.1816	29	5.315[3]	34	0.48±0.18
No. of serrations on one side of a leaf	38.66±6.2390	7	44.87±6.2160	29	2.440[4]	34	6.21±5.20
No. of veins on one side of a leaf	7.40±0.9700	7	8.75±0.8660	29	3.620[3]	34	1.35±0.76
Length of stomata (μ)	34.46±3.9800	7	33.78±3.9700	29	0.406	34	- not significant
Length of catkin (cm)	2.09±0.2190	7	2.99±0.4800	28	4.561[3]	33	0.86±0.38
Length of bract (mm)	3.09±0.0890	7	5.33±0.7815	29	6.653[3]	34	2.24±0.69
Length of achene (mm)	1.45±0.3000	7	2.21±0.3630	29	5.099[3]	34	0.76±0.30
Width of achene (mm)	0.84±0.1400	7	1.13±0.2008	29	3.593[3]	34	0.29±0.16
Achene length/achene width (ratio)	1.75±0.2100	7	1.91±0.2560	29	1.595	34	- not significant
Length of styles (mm)	0.61±0.1770	7	1.17±0.2085	29	6.577[3]	34	0.56±0.18

Table 2 - Cont'd.

Table 2 (Cont'd)

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[1] a/m ratio: a = half the distance from leaf tip to widest part of leaf; m = width of leaf at half the distance from leaf tip to widest part of leaf (see text).

[2] T-values:

df	T .05	T .01
33, 34	2.042	2.750
41, 42, 43	2.021	2.704

[3] Highly significant

[4] Significant

Table 3. Results of comparison of the same species in different locations.

Species	a/m ratio	No. of serrations	No. of Veins	Length of Catkin	Length of Bract	Length of Guard Cells	Length of Achene	Width of Achene	L/W ratio of Achene	Length of Style
B. caerulea-grandis	NS[1]	NS	DS[2,3]	NS	DS[3]	NS	NS	NS	NS	NS
B. cordifolia	NS	NS	DS[3]	NS	DS[4,5]	NS	NS	NS	NS	NS
B. populifolia	NS	NS	NS	NS	NS	DS[5]	NS	NS	NS	DS[3,6]

1. NS = no significant difference between locations.
2. DS = definite difference between locations.
3. Difference occurs when populations from Valcartier and Grand Manan Island are compared.
4. Difference occurs when populations from the Laurentians and Valcartier are compared.
5. Difference occurs when populations from the Laurentians and Grand Manan Island are compared.
6. Difference occurs when populations from Valcartier and Vermont are compared.

Table 4. Comparison of means among *B. papyrifera*, *B. populifolia*, *B. cordifolia*, and *B. caerulea-grandis*.

	B. papyrifera	*B. populifolia*	mean	*B. cordifolia*	*B. populifolia*	mean	*B. caerulea-grandis*
a/m ratio	0.77	1.65	1.21	0.92	1.65	1.28	1.16
No. of veins	8.08	7.40	7.70	9.50	7.40	8.50	8.70
No. of serrations	31.64	38.66	35.15	46.26	38.66	42.46	44.58
Guard cell length	40.13	34.45	37.30	34.48	34.45	34.47	33.81
Bract length	5.68	3.09	4.39	6.40	3.09	4.75	5.30
Catkin length	3.58	2.13	2.86	3.84	2.13	2.99	2.97
Achene length	2.05	1.45	1.75	2.52	1.45	1.99	2.17
Achene width	1.41	0.84	1.13	1.46	0.84	1.15	1.14
L/W ratio of achene	1.45	1.75	1.60	1.65	1.75	1.70	1.91
Style length	0.73	0.61	0.67	1.44	0.61	1.02	1.17

are seen to be very close to the values obtained for B. caerulea-grandis. For example, the mean catkin length of the hypothetical hybrid of B. papyrifera and B. populifolia is 2.86 cm. The mean catkin length for the hypothetical hybrid of B. cordifolia and B. populifolia is 2.99 cm. The actual value determined for B. caerulea-grandis is 2.97 cm.

Somatic chromosome number determinations.

The chromosome complements of B. cordifolia, B. caerulea-grandis and B. populifolia are shown in Figures 6-8 and photographs of complements including B. papyrifera, are shown in Figures 9-12. Betula cordifolia, B. caerulea-grandis and B. populifolia for all the populations studied had a somatic chromosome number of 28. Betula papyrifera had a somatic chromosome number of either 70 or 84.

Hybrid index.

The characters used to construct the index and the values assigned to the characters are given in Figure 13. The value of "0" is typical of one extreme and "2" is typical of the other extreme. With eight characters being analysed, the arbitrary range of 0-3 was selected for B. populifolia, and a range of 13-16 was selected for B. cordifolia. The index values for each character were added together to give the total hybrid inxex for each accession number. A bar graph was constructed plotting the index values against the

Figure 6. Camera lucida drawing of the somatic chromosome complement of *B. populifolia*, Acc. No. 561, $2n = 28$.

Figure 7. Camera lucida drawing of the somatic chromosome complement of *B. caerulea-grandis*, Acc. No. 578, $2n = 28$.

Figure 8. Camera lucida drawing of the somatic chromosome complement of *B. cordifolia*, Acc. No. 441, $2n = 28$.

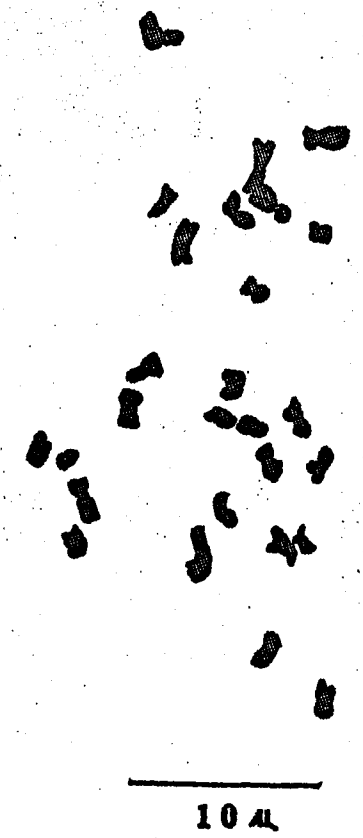


Figure 6.

Figure 7.

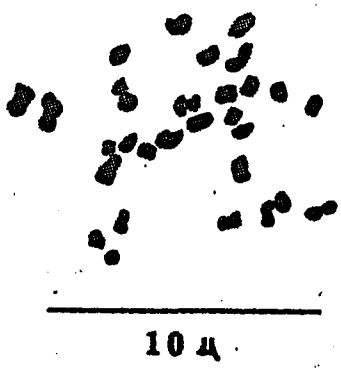


Figure 8.

Figure 9.
Somatic chromosome complement of
B. populifolia
Acc. No. 561, 2n = 28

Figure 10.
Somatic chromosome complement of
B. caerulea-grandis
Acc. No. 578, 2n = 28

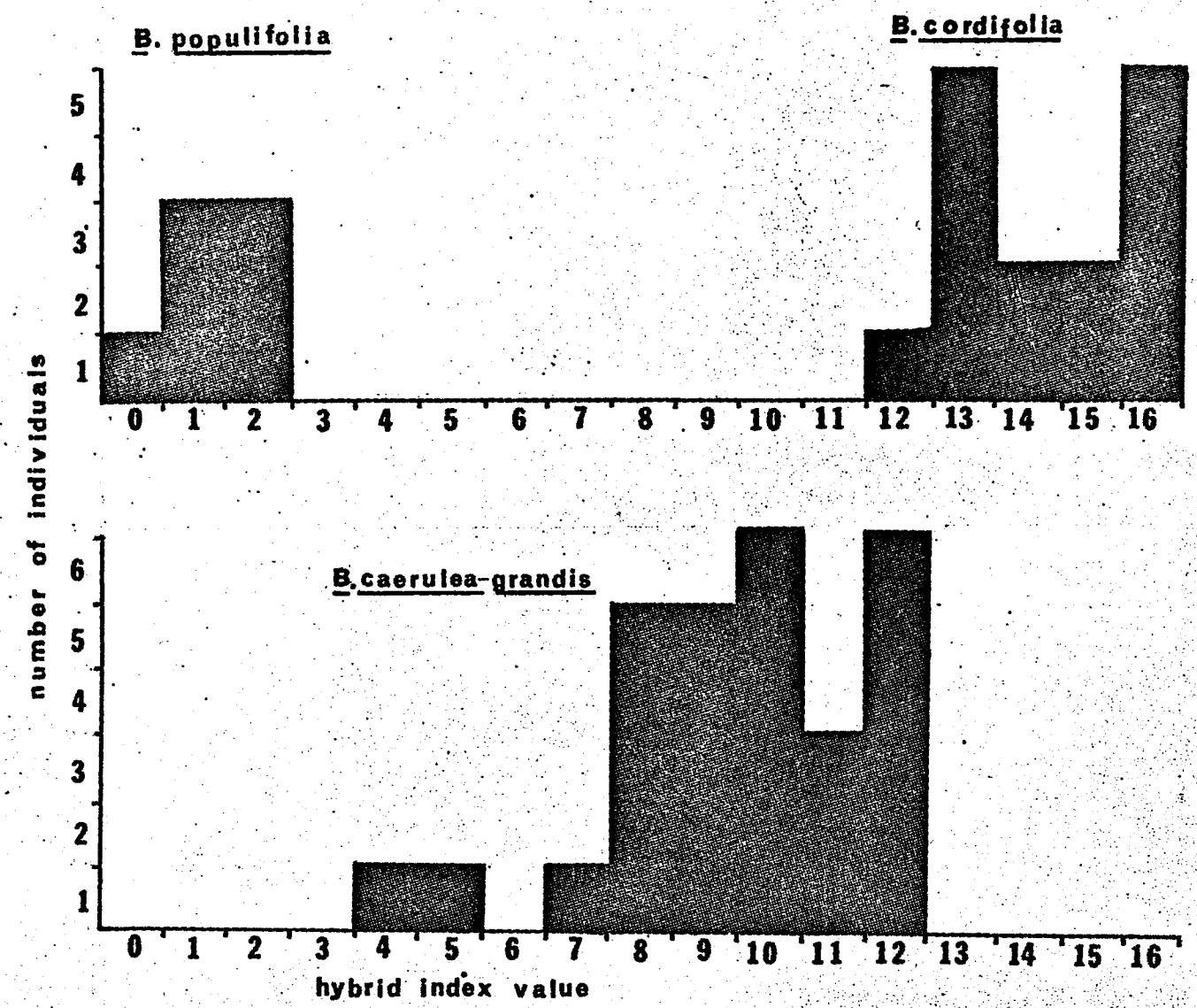
Figure 11.
Somatic chromosome complement of
B. cordifolia
Acc. No. 394, 2n = 28

Figure 12.
Somatic chromosome complement of
B. papyrifera
Acc. No. 610, 2n = 70

Figure 13. Frequency distribution of hybrid index values for B. populifolia, B. caerulea-grandis and B. cordifolia.

Key to hybrid index.

a/m ratio	value	Bract length (mm)	value
greater than 1.33	0	less than 3.6	0
1.00 - 1.33	1	3.6 - 5.49	1
less than 1.00	2	greater than 5.49	2
No. of serrations		**Bract form**	
less than 40.0	0	spreading or	0
40.0 - 47.0	1	reflexed (s)	
greater than 47.0	2	ascending (a)	2
No. of veins		**Achene length** (mm)	
less than 8.1	0	less than 1.7	0
8.1 - 9.0	1	1.7 - 2.3	1
greater than 9.0	2	greater than 2.3	2
Catkin length (cm)		**Style length** (mm)	
less than 2.5	0	less than 0.85	0
2.5 - 3.49	1	0.85 - 1.15	1
greater than 3.49	2	greater than 1.15	2


B. populifolia
B. cordifolia
B. caerulea-grandis
number of individuals
hybrid index value
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

frequency of its occurrence in the population. In Figure 13, it may be seen that three peaks are present; one within the B. populifolia range, one within the B. cordifolia range, and a group of intermediate individuals which have hybrid index values from 4 to 12. These latter individuals have all been morphologically identified as B. caerulea-grandis.

Polygonal graphs.

Polygonal graphs were constructed for each tree analysed in the Valcartier population. The values of characters, as seen in Figure 14, show the range of the measurements used to construct the polygonal graphs. In Figure 15, the graphs were grouped according to species. Within each species the graphs were arranged according to the hybrid index value of the individual accession number. Examination of Figure 15 shows that the measurements in B. populifolia are usually smaller than those for B. caerulea-grandis and that the measurements of B. cordifolia are usually larger than those of B. caerulea-grandis. Figure 16 illustrates the graphs of the overall mean measurements for B. cordifolia, B. caerulea-grandis and B. populifolia. Betula caerulea-grandis is shown to be intermediate in all characters measured between B. cordifolia and B. populifolia.

Pictorialized scatter diagrams.

Two scatter diagrams were prepared. The characters used as

Figure 14. Range of values for characters used in the polygonal graphs.

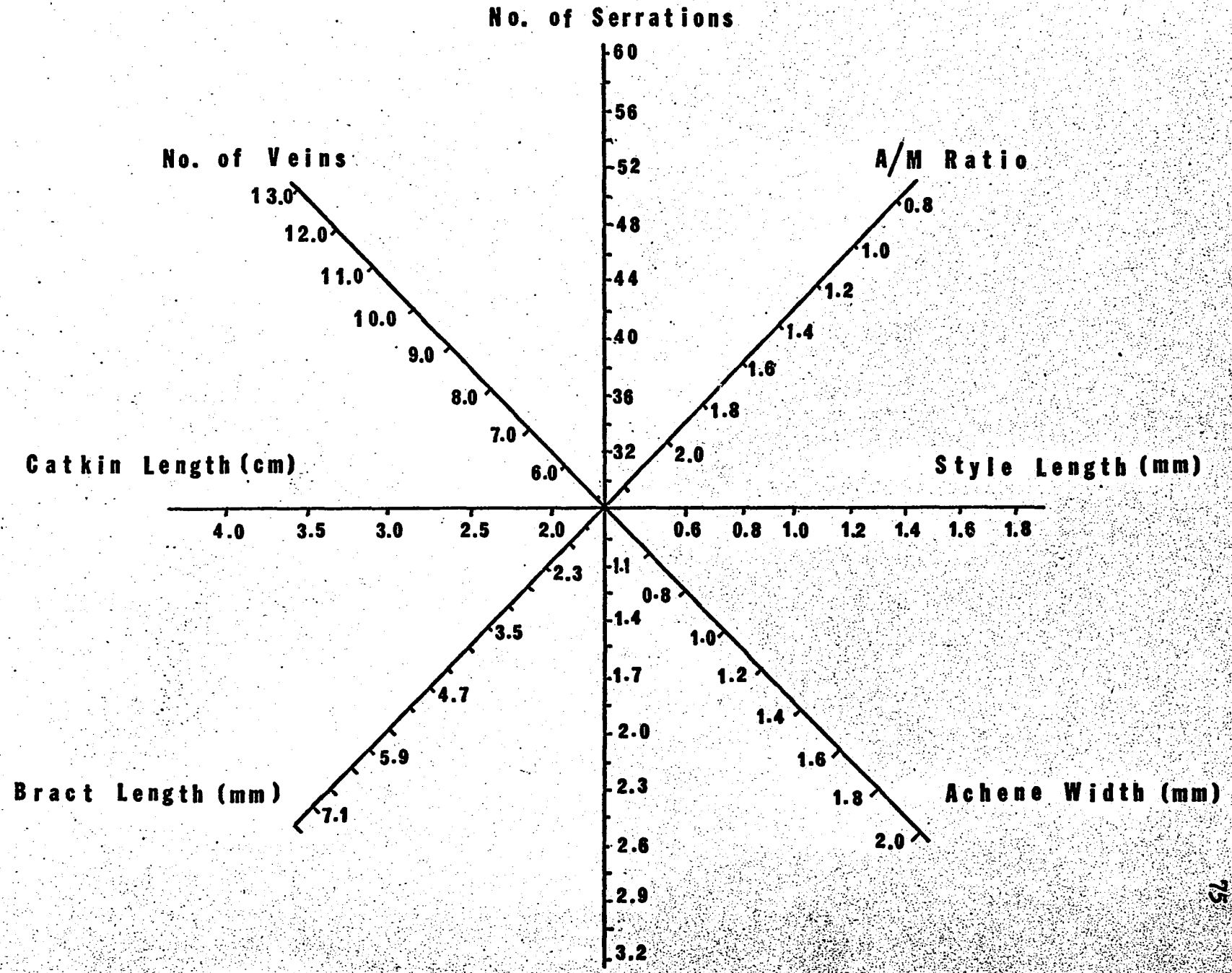

No. of Serrations
60
56
52
48
44
40
36
32
No. of Veins
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
A/M Ratio
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Catkin Length (cm)
4.0
3.5
3.0
2.5
2.0
Style Length (mm)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Bract Length (mm)
2.3
3.5
4.7
5.9
7.1
Achene Width (mm)
1.1
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Achene Length (mm)
1.4
1.7
2.0
2.3
2.6
2.9
3.2

Figure 15. Representative polygonal graphs among B. populifolia, B. caerulea-grandis and B. cordifolia, x 1/2.

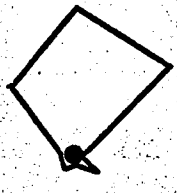
Figure 15A. ..... B. populifolia

Figures 15B, C, D, E ..... B. caerulea-grandis

Figures 15F, G, H ..... B. cordifolia

561

570



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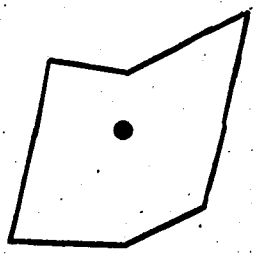

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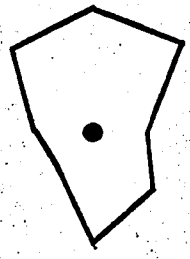

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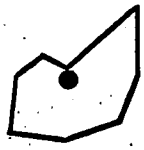

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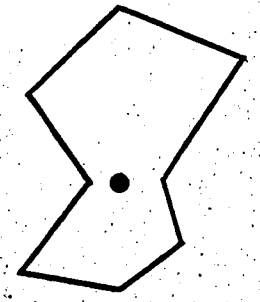

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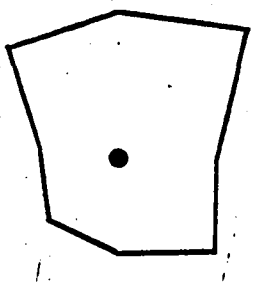
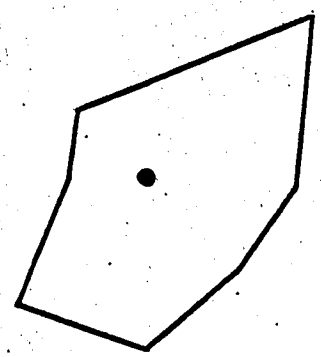


Figure 15A.

598



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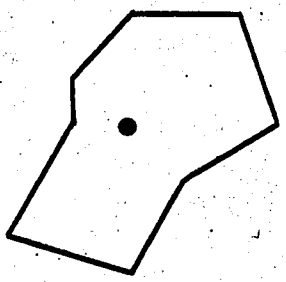

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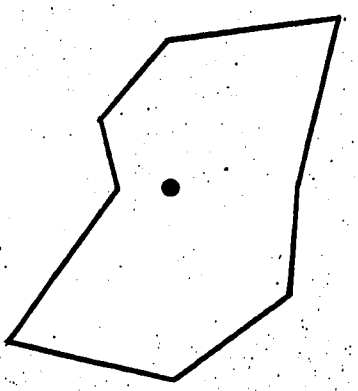

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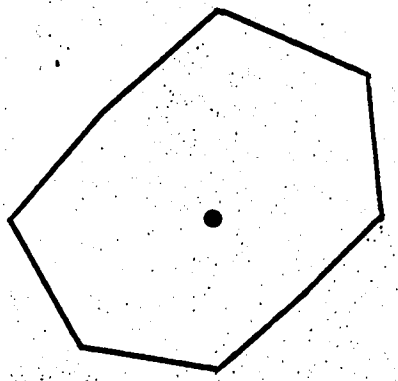

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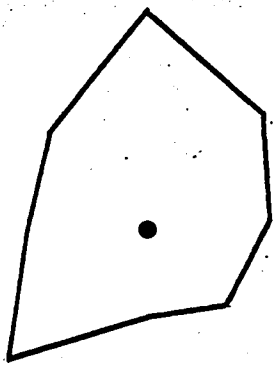

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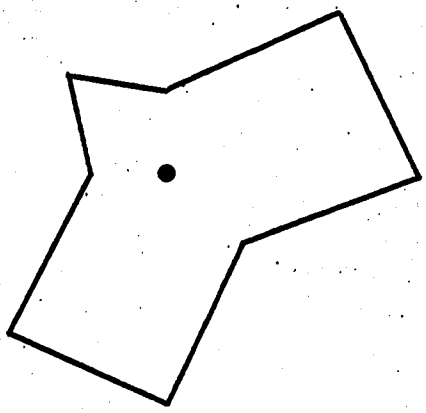

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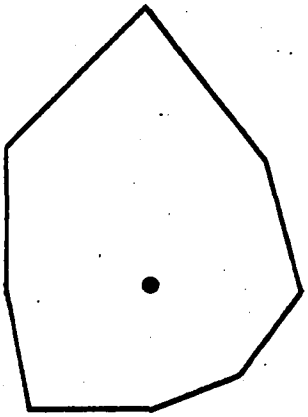

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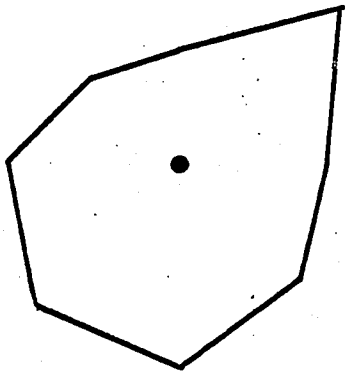
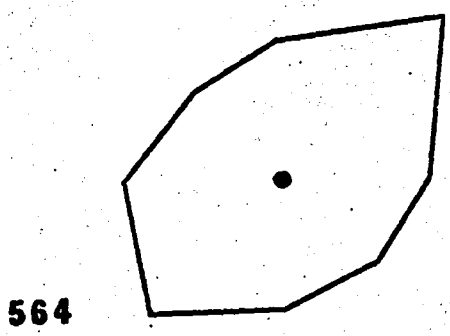


Figure 15B.



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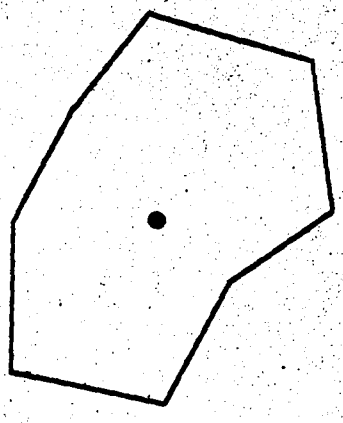

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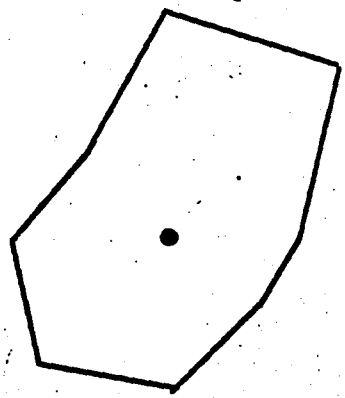

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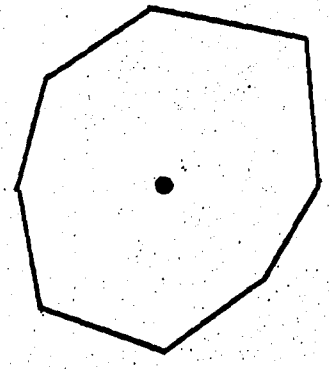

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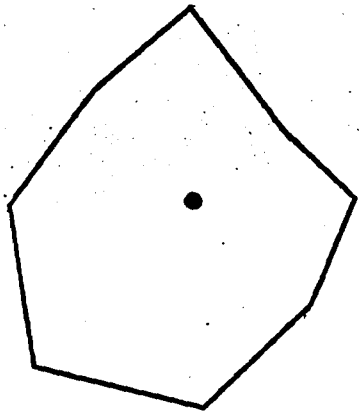

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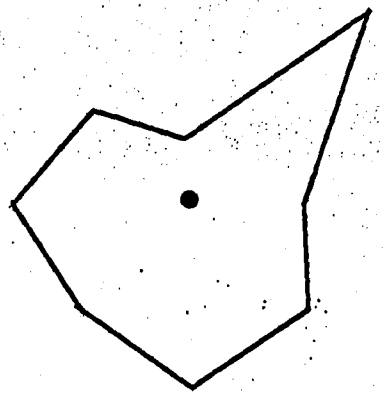

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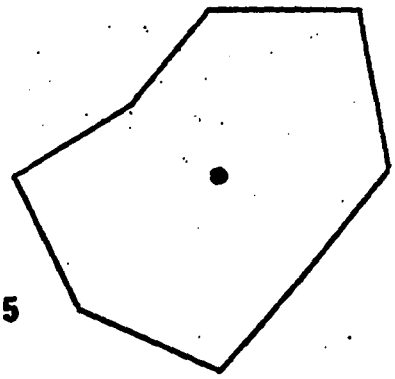

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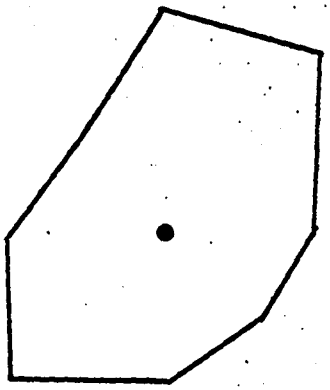

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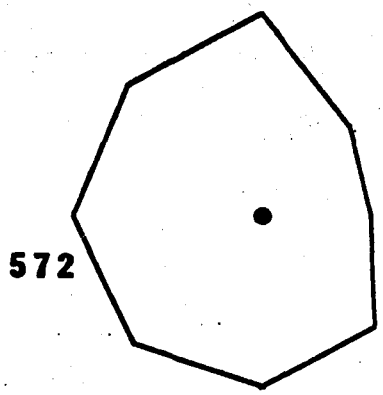
Figure 15C.



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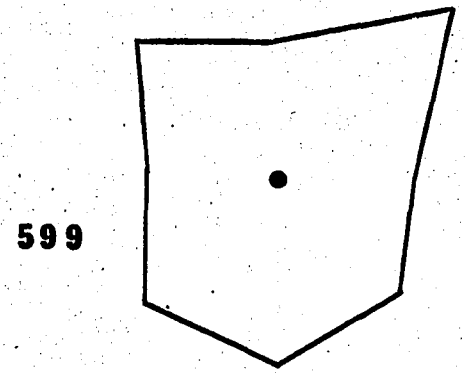

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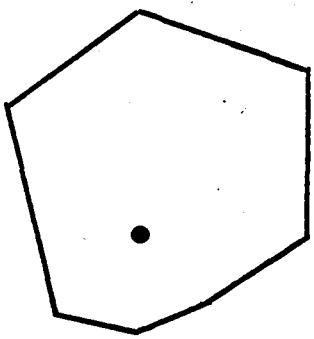

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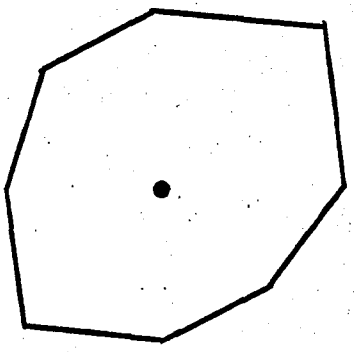

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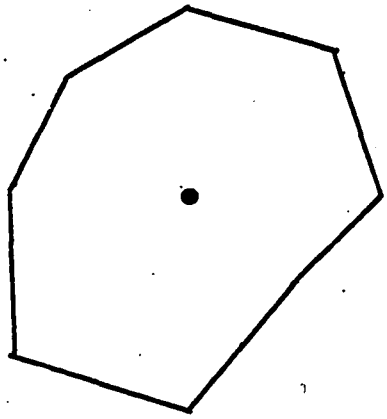

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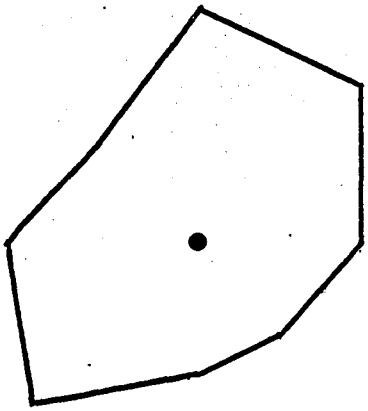

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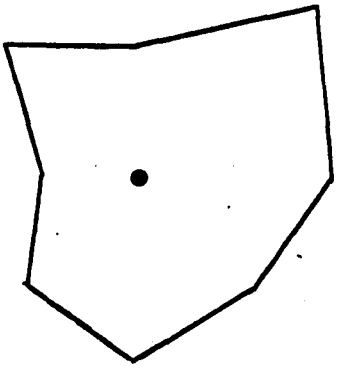

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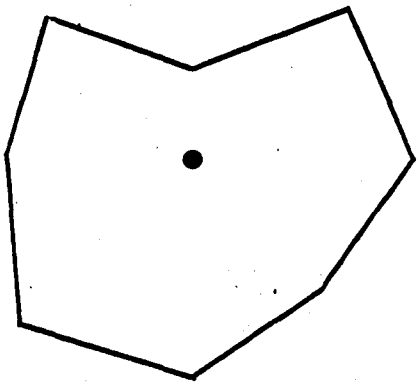


571

Figure 15D.

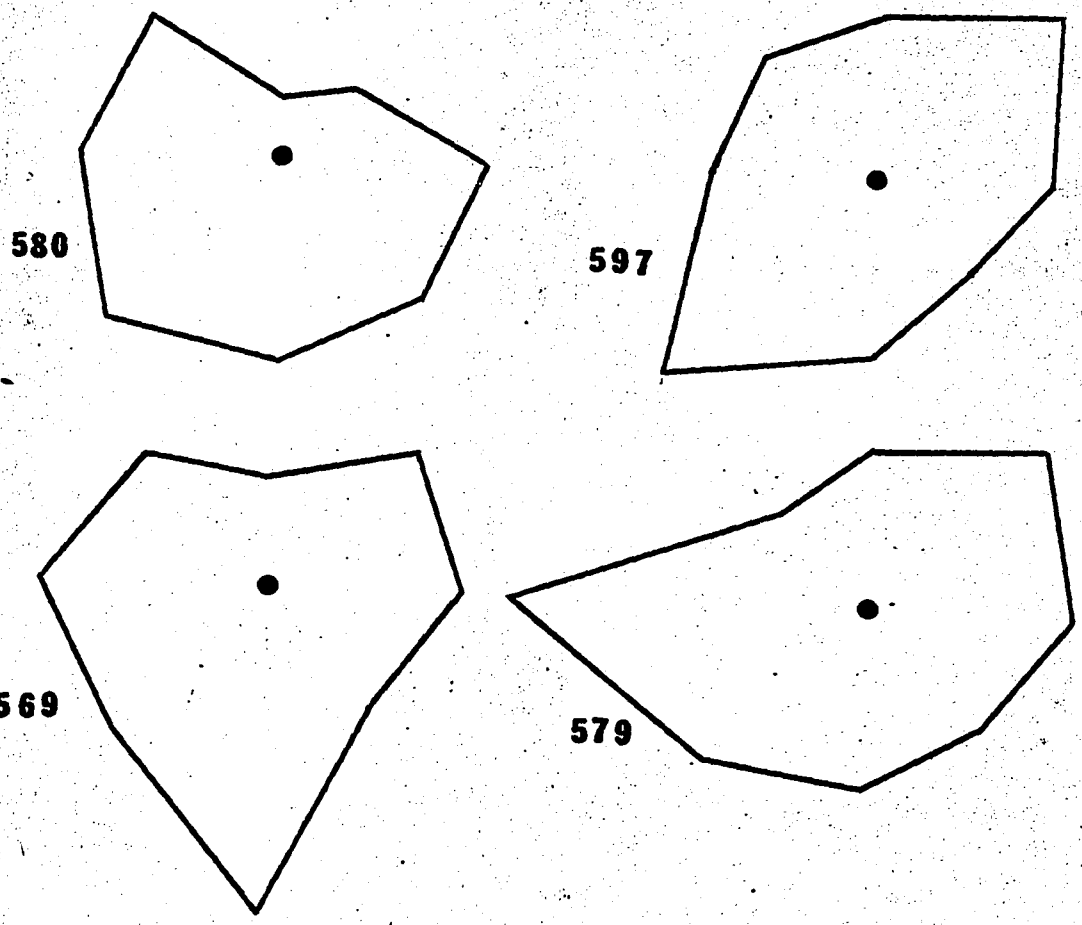
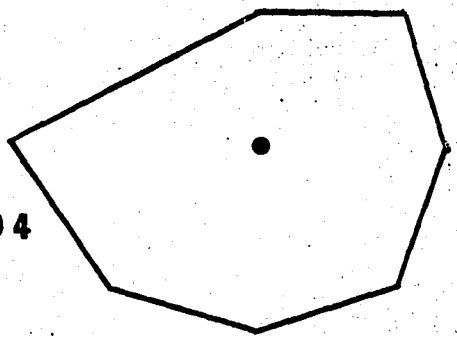
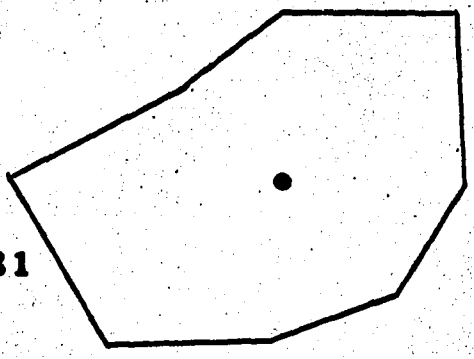
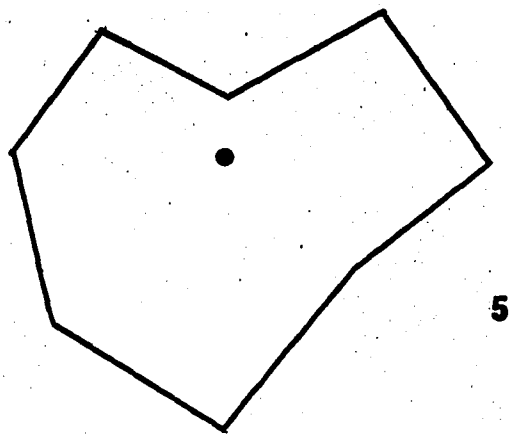
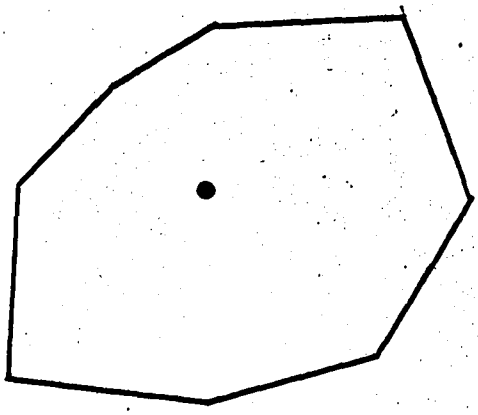


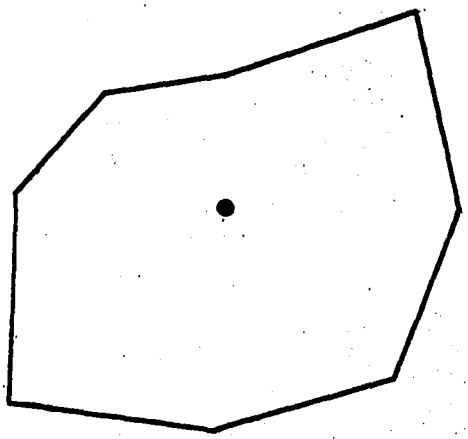
Figure 15E.











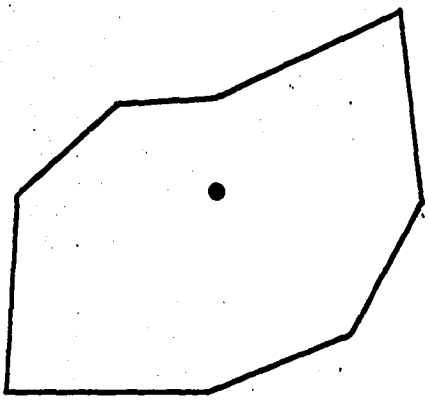
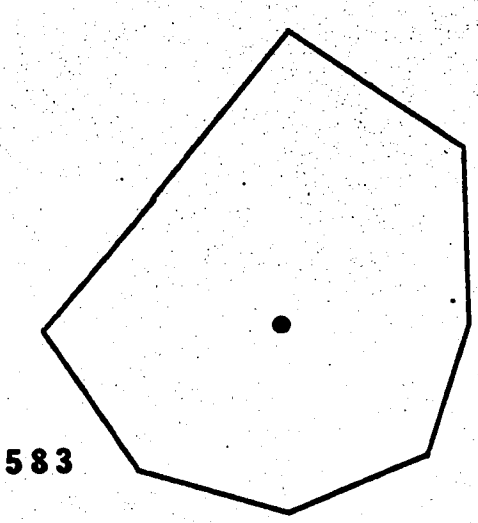
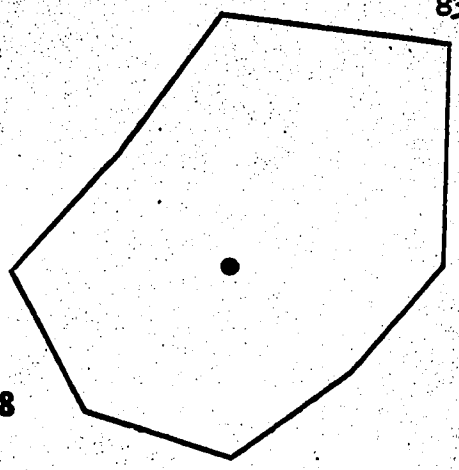
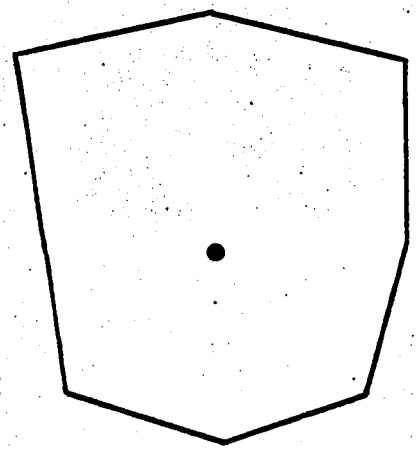
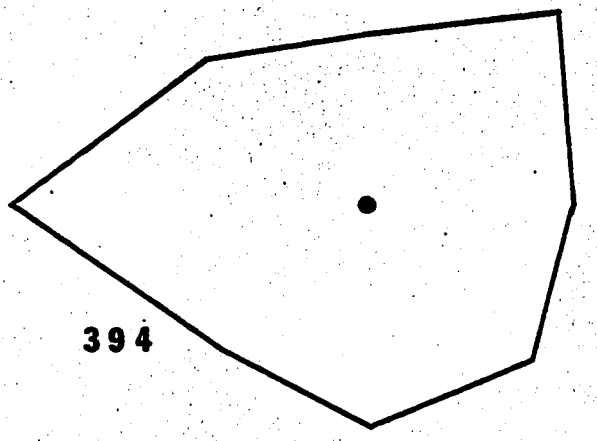


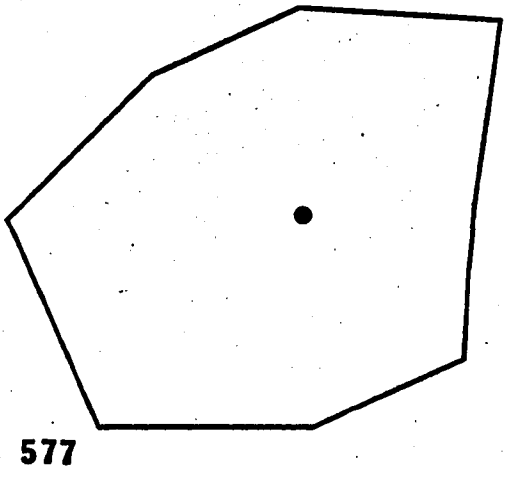
Figure 15F.











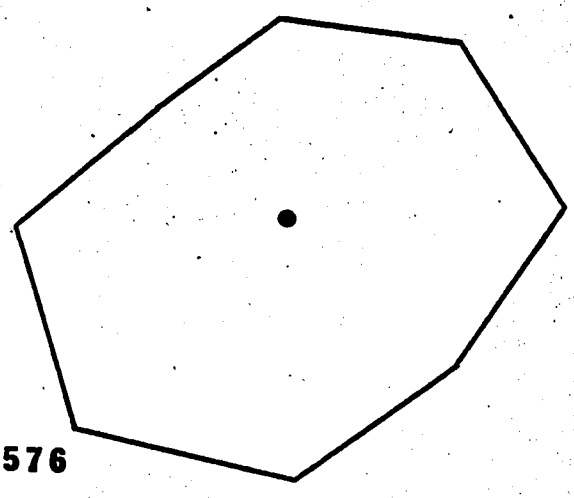


Figure 15G.

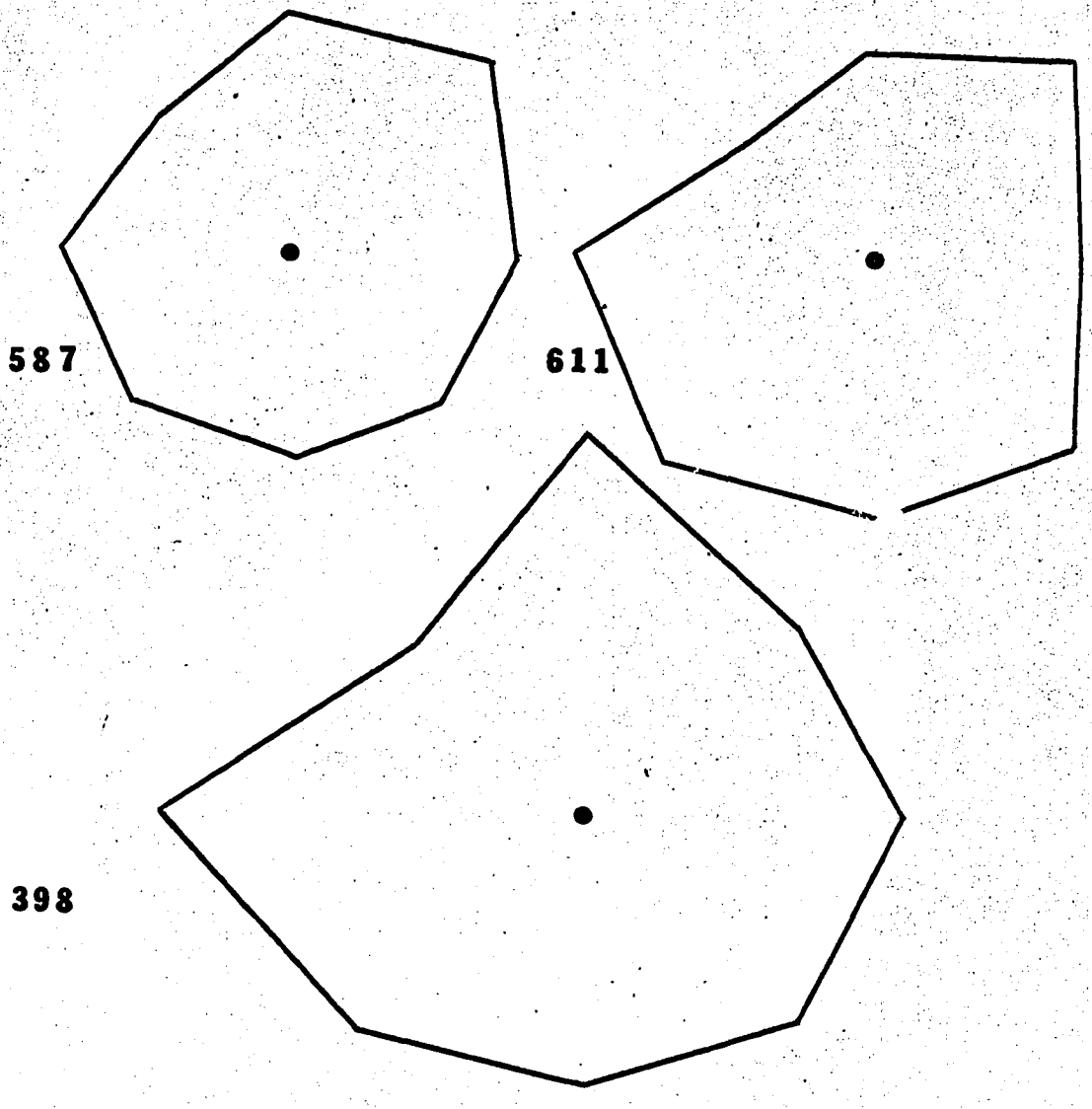
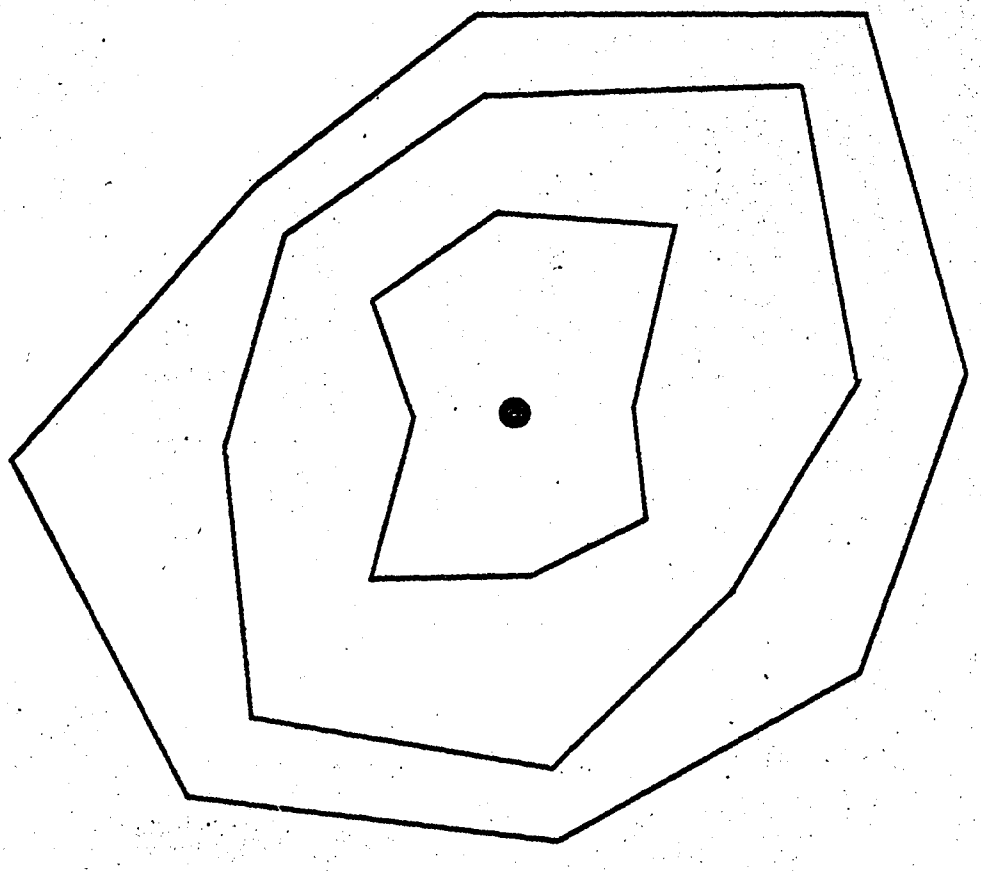


Figure 15H.

Figure 16. Polygonal graphs of the mean values of (l. to r.) B. cordifolia, B. caerulea-grandis and B. populifolia.



coordinates in Figure 17 were bract length and style length. The key to the characters is given in Figure 17. Each individual is represented by a spot on the graph. As illustrated in Figure 17, B. populifolia differs markedly from B. cordifolia not only in bract length and style length but also in the other characters represented by the appendages to the spots on the graph. Betula caerulea-grandis bridges the gap between B. cordifolia and B. populifolia. In the second scatter diagram (Figure 18), style length and catkin length are used as the coordinates. Betula populifolia and B. cordifolia are again easily differentiated using style length and catkin length as the coordinates. Betula caerulea-grandis is intermediate between B. populifolia and B. cordifolia.

Pollen grain stainability.

The percentage of stainable pollen for the accession numbers tested is listed in Table 5. It is shown that in all cases over 85% of the pollen grains examined were stainable.

Geographical distribution of the species.

Three collections of Betula species from eastern Canada and one from Vermont were mapped (Figure 19). The species collected were B. populifolia, B. caerulea, B. caerulea-grandis and B. cordifolia.

Figure 17. Pictorialized scatter diagram of B. populifolia, B. caerulea-grandis and B. cordifolia, using bract length and style length as coordinates.

Key to pictorial scatter diagram.

Species	Symbol	Catkin length (cm)	Symbol
B. cordifolia	●	less than 2.5	○
B. caerulea-grandis	⊛	2.5 - 3.49	⊙–
B. populifolia	○	greater than 3.49	⋈
a/m ratio		**Achene length (mm)**	
greater than 1.33	○	less than 1.71	○
1.00 - 1.33	–○	1.71 - 2.3	♀
less than 1.00	⋈	greater than 2.3	♀
Achene width (mm)		**Mean values**	
less than .97	○	B. cordifolia	●
.97 - 1.40	○	B. caerulea-grandis	⊛
greater than 1.40	⧖	B. populifolia	○

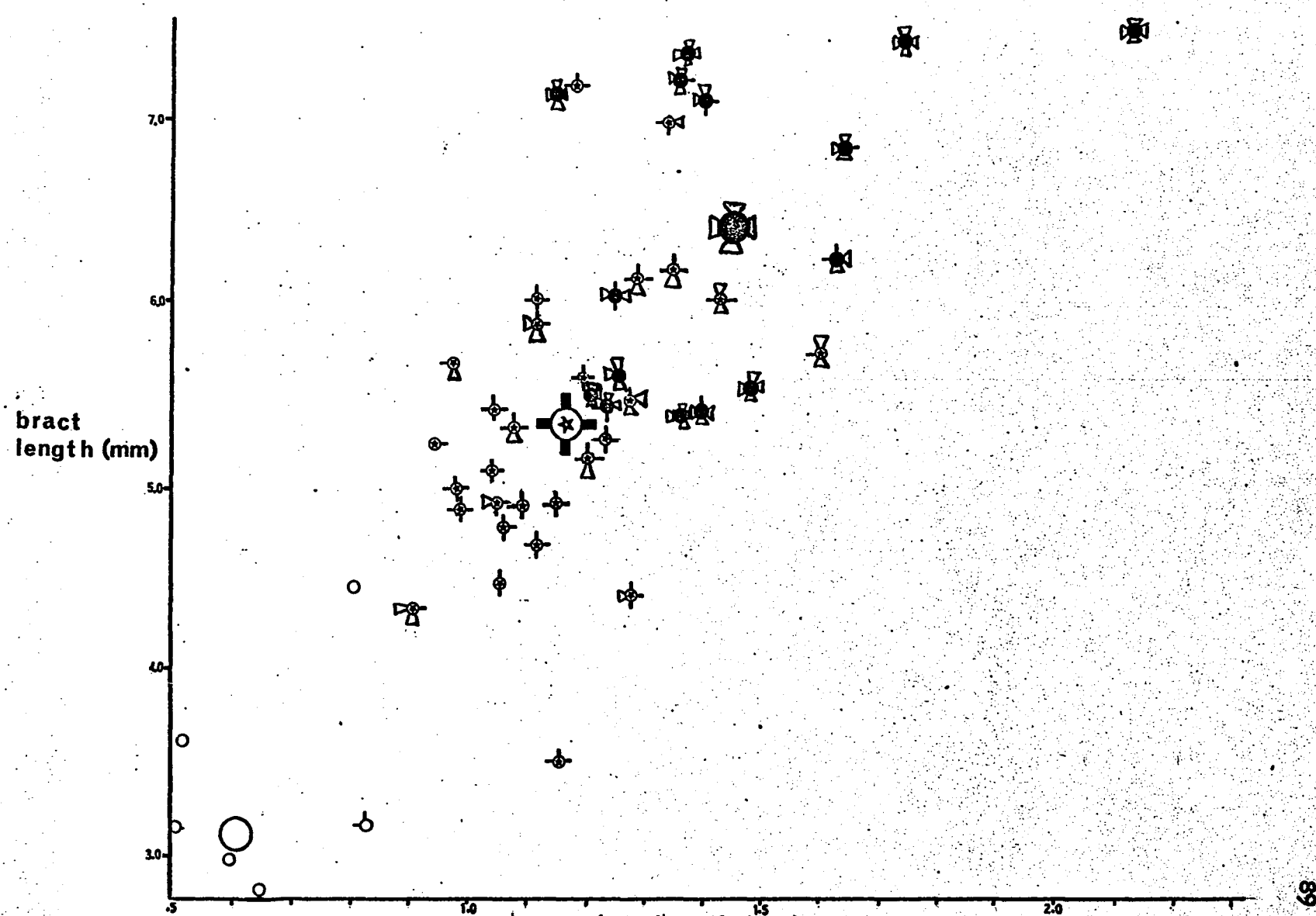

bract
length (mm)
7.0
6.0
5.0
4.0
3.0
.5
1.0
1.5
2.0
style length (mm)

Figure 18. Pictorialized scatter diagram of B. populifolia, B. caerulea-grandis and B. cordifolia, using style length and catkin length as coordinates.

Key to pictorial scatter diagram.

Species	Symbol
B. cordifolia	
B. caerulea-grandis	
B. populifolia	

a/m ratio	
greater than 1.33	
1.00 - 1.33	
less than 1.00	

Achene width (mm)	
less than 0.97	
0.97 - 1.40	
greater than 1.40	

Bract length (mm)	Symbol
less than 3.6	
3.6 - 5.49	
greater than 5.49	

Achene length (mm)	
less than 1.71	
1.71 - 2.3	
greater than 2.3	

Mean values	
B. cordifolia	
B. caerulea-grandis	
B. populifolia	

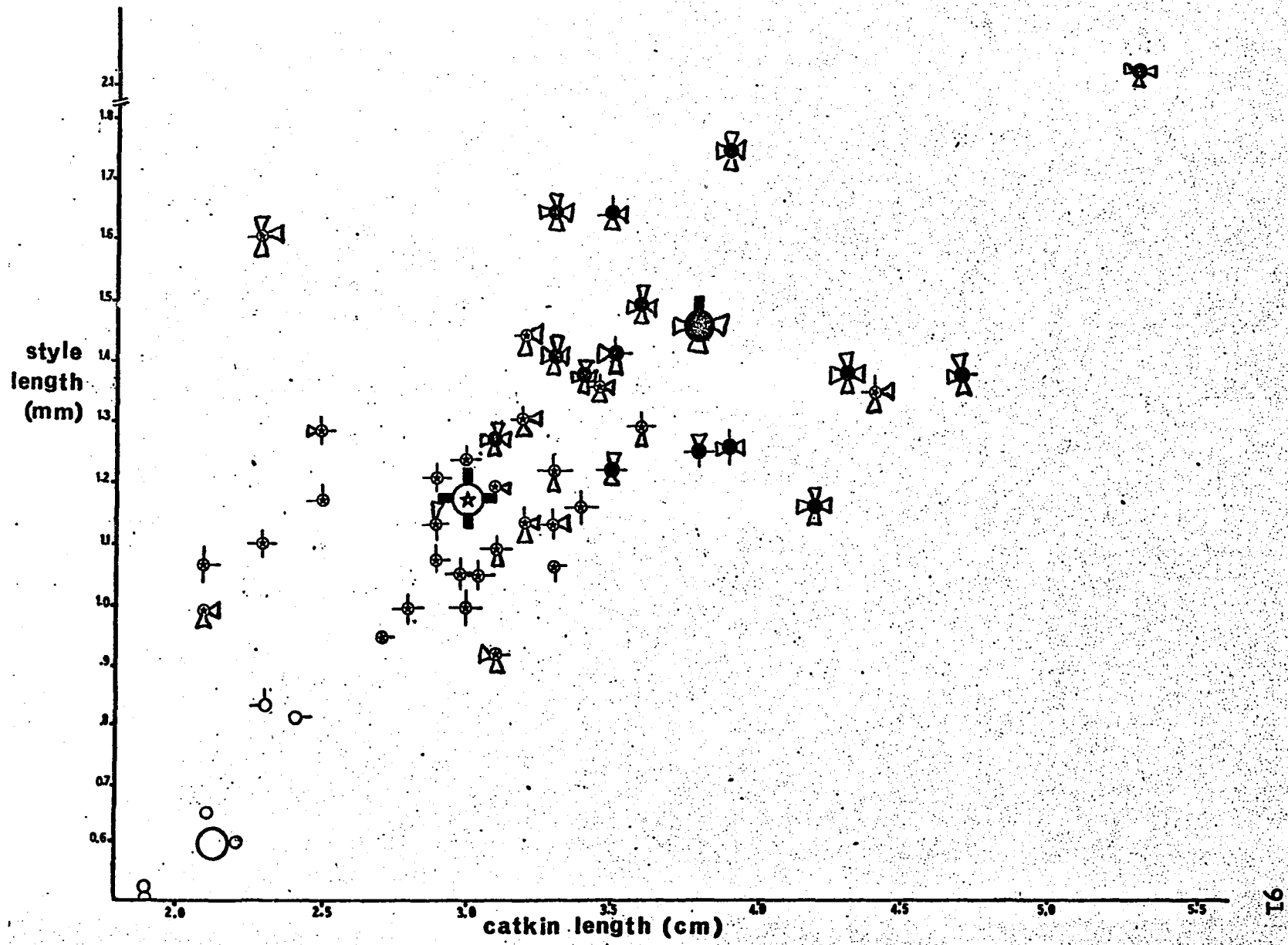

style length (mm)
catkin length (cm)
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
0.6
0.7
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
2.1

Table 5. Percentage stainable pollen with fast green-lactophenol.

	B. populifolia	*B. caerulea-grandis*	*B. cordifolia*	*B. papyrifera*
Percent stainable pollen	87.95	93.27	98.10	99.10

Table 6. DNA density ratios for *B. populifolia*, *B. caerulea-grandis*, and *B. cordifolia*. Two ratios were obtained for each comparison from different accession numbers for the given species.

Species compared	Ratios
B. populifolia and *B. caerulea-grandis*	1.01:1 1.02:1
B. cordifolia and *B. caerulea-grandis*	0.94:1 0.87:1
B. populifolia and *B. cordifolia*	1.07:1 1.10:1

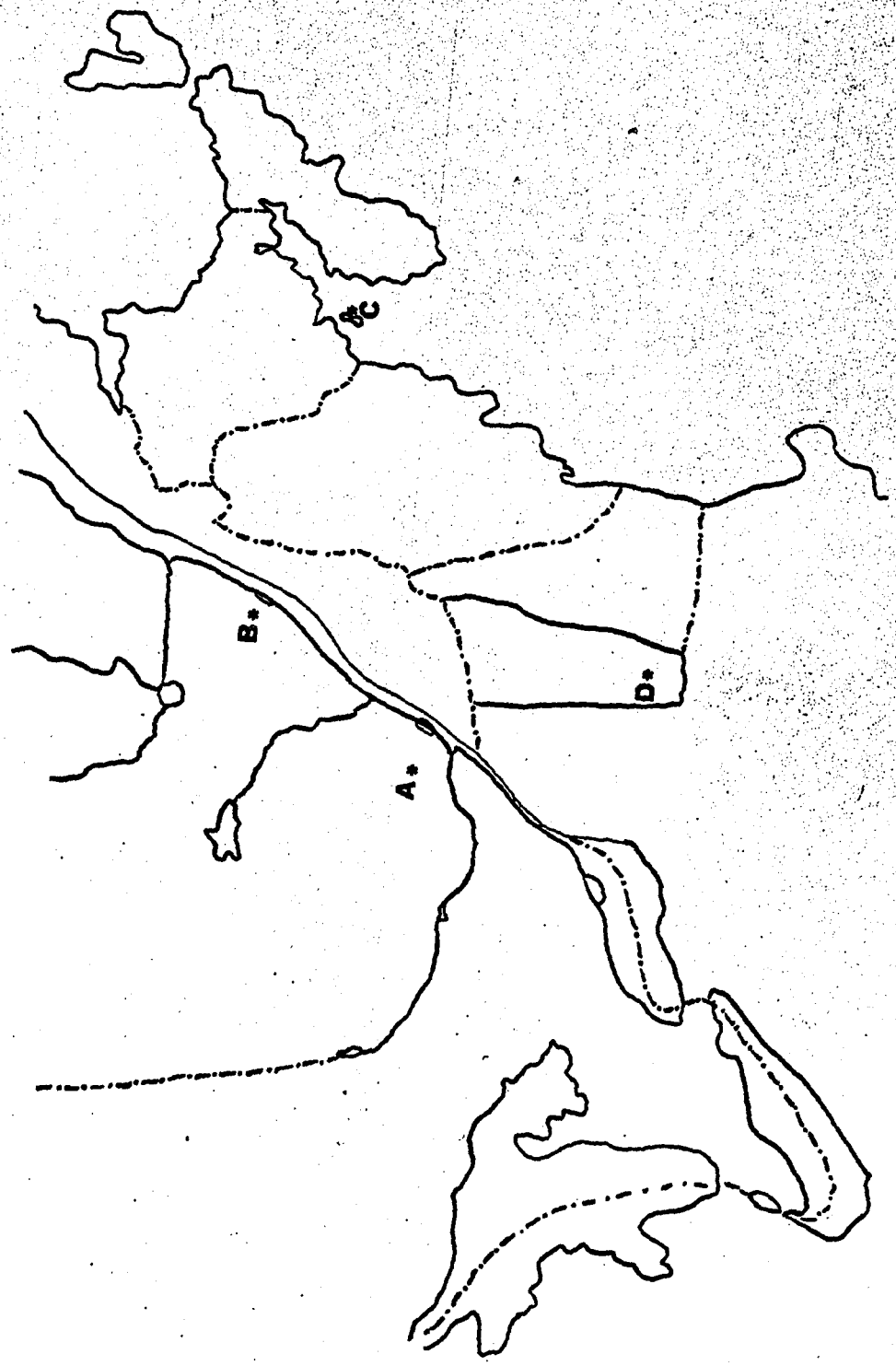
Figure 19. Geographical distribution of birch populations.

A. Lac Carré, Quebec.

B. Valcartier, Quebec.

C. Grand Manan Island, New Brunswick.

D. Vermont.


B&C
B*
D*
A*

Cytophotometric observations.

The results of the studies on the density of DNA among closely related birch species indicate that B. populifolia, B. caerulea-grandis and B. cordifolia have very similar genetic makeups. Table 6 presents the results of the comparison of the relative densities of DNA among B. populifolia, B. caerulea-grandis and B. cordifolia. It may be seen that the relative densities of the three species are almost identical.

Thin-layer chromatography.

The results of the thin-layer chromatography are shown in Figures 20, 21 and 22. Figure 20, a photograph of the fluorescent compounds in leaf extracts, shows the differences in spot intensity among B. cordifolia, B. caerulea-grandis and B. populifolia. After spraying the plates with concentrated sulphuric acid many more spots were visible under long-wave ultraviolet light than on the nonsprayed plates especially between R_f 0.5 and 0.8. At the same time there was some deterioration in the spots between R_f 0.0 and 0.3 with the spraying. However, the increased number of easily visible spots between R_f 0.5 and 0.8 under ultraviolet after spraying more than compensated for the deterioration in those close to the origin when trying to differentiate between the species (Figure 21). In Figure 21 the differences among the species under long-wave ultraviolet

Figure 20.
Chromatogram showing pattern of fluorescent compounds for (l. to r.) B. cordifolia, B. caerulea-grandis and B. populifolia

Figure 21. Patterns and spot colors of chromatograms of leaf extracts under long-wave ultraviolet light after spraying with concentrated sulphuric acid.

A. B. caerulea-grandis, Acc. No. 601

B. B. populifolia, Acc. No. 596

C. B. cordifolia, Acc. No. 581

Spot colors:

P, pink

B, blue

W, white

Y, yellow

Bl, black

b, bright

f, faint

v, very

W B fP
vfP fP B bP bP bP bP fP W bY fP Y Bl Bl Y
A
B vfP
fP fP B vfP bP bP bP bP fP W fP W fP Y Bl Bl Y
B
W B fP
vfP fP W fP fP bP W fP W fP fP W vfW Y Bl
C
1.0 .9 .8 .7 .6 .5 .4 .3 .2 .1 .0

Figure 22. Patterns and spot colors of chromatograms of leaf extracts under visible light after spraying with concentrated sulphuric acid.

A. B. caerulea-grandis

B. B. populifolia

C. B. cordifolia

Spot colors:

P, pink

Br, brown

W, white

Y, yellow

V, violet

R, red

G, green

b, bright

f, faint

l, light

v, very

fIBr
fG
Y-Br
fP
dV
P
R-Br
P-G
lG
P
lG
lP
bR
Y
bY
Y-Br
Y
Y
lBr
Br
dBr
A
Y-Br
fP
dV
P
P
lR-Br
vfG
P-G
lG
P
lG
P
bR
Y
lY-Br
Y
Y
Y
lBr
Br
dBr
B
fIBr
Y-Br
P
dV
bP
dR-Br
G
P
lG
bP
fY
Y
Y
Y
vfW
Y
lBr
dBr
C
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0

light after spraying with concentrate sulphuric acid may be seen. One of the two consistent differences between B. cordifolia and B. populifolia is the very bright pink spot at R_f 4.0 B. populifolia which is not seen in B. cordifolia. The other difference is in a tiny white spot at R_f 9.5 which is seen in B. cordifolia and not in B. populifolia. Therefore, it is possible to identify B. populifolia by the presence of a bright pink spot at R_f 4.0 and the absence of a white spot at R_f 9.5. The reverse arrangement of spots characterizes B. cordifolia. It is seen in Figure 21 that B. caerulea-grandis has a bright pink spot at R_f 4.0 and also a white spot at R_f 9.5.

When the plates were sprayed with concentrated sulphuric acid a number of spots became visible even under normal light conditions. Figure 22 is a tracing of the pattern which appeared under visible light after the plate was sprayed with concentrated sulphuric acid. This is in contrast to the limited number of spots seen under visible light on a nonsprayed plate. The differences noted above between B. populifolia and B. cordifolia under ultraviolet light were also noted under visible light. In this case, there is a bright red spot in B. populifolia and B. caerulea-grandis at R_f 4.0 which has no counterpart in B. cordifolia. Betula cordifolia has a faint brown spot at R_f 9.5 which is missing in B. populifolia but which is present in B. caerulea-grandis.

DISCUSSION

The blue birches, Betula caerulea and B. caerulea-grandis, first discovered and named by William H. Blanchard in 1904, have been the object of controversy concerning their origin. Blanchard (1904a, 1904b) considered B. caerulea and B. caerulea-grandis to be two distinct species. Sargent (1922) considered them both to be hybrids and he considered B. caerulea-grandis to be a larger variety of B. caerulea. Sargent concluded that the parental species involved in the production of these hybrid taxa were B. papyrifera, the paper white birch, and B. populifolia, the grey birch. When Woodworth (1929) published the diploid chromosome numbers of different birch species he listed B. papyrifera with $2n = 70$, B. cordifolia with $2n = 56$, B. caerulea with $2n = 28$, B. caerulea-grandis with $2n = 28$ and B. populifolia with $2n = 28$. Brittain and Grant (1965b) in their study of B. papyrifera and B. cordifolia found that the somatic chromosome number of B. papyrifera was generally 70 or 84, though some individuals were found with $2n = 56$. With few exceptions, B. cordifolia was found to have a diploid chromosome complement of 28.

If the proposal of Sargent (1922) is correct, namely, that B. caerulea and B. caerulea-grandis are both hybrids between B. papyrifera and B. populifolia, then the chromosome numbers of the postulated

parents are important factors to be considered. In the case of Betula, if one parent of a hybrid with 28 chromosomes, itself has 28 chromosomes, then the other parent should also have 28 chromosomes. Betula papyrifera has never been reported to have a somatic chromosome number of 28. Nevertheless, Brayshaw (1966) apparently not fully considering the cytological implications of his conclusions regarding the origin of the blue birches, considered B. caerulea and B. caerulea-grandis to be a hybrid swarm between the white and grey birches without distinguishing between B. papyrifera and B. cordifolia.

For many years the white birches were not fully distinguished one from another. Betula cordifolia was considered to be a variety of B. papyrifera as it closely resembled it in many characters. Certain features were different enough for Rosendahl (1928) to propose that B. cordifolia be given full specific status. From their studies on B. papyrifera and B. cordifolia Brittain and Grant (1965b) also believed that B. cordifolia was a species distinct from B. papyrifera.

If B. cordifolia is substituted for B. papyrifera in the proposal that B. caerulea and B. caerulea-grandis are hybrids between B. B. papyrifera and B. populifolia, then the cytological problem of chromosome number is solved, as all taxa have $2n = 28$. Betula cordifolia has the features of white birch which are found in modified form in the hybrid B. caerulea and B. caerulea-grandis. From Table 4 it is seen that when the hypothetical hybrid mean values for a cross

of B. papyrifera and B. populifolia are compared to the hypothetical mean values for a cross of B. cordifolia and B. populifolia the means determined for the cross of B. cordifolia and B. populifolia are more similar to the actual values of B. caerulea-grandis. It is for these reasons that it is proposed that B. caerulea-grandis is a hybrid from a cross between B. populifolia and B. cordifolia. Preliminary experiments with B. caerulea indicate that it is also of hybrid origin but no definite conclusions have been reached.

Further evidence to support the proposal that B. cordifolia is a parental species in the production of B. caerulea-grandis come from comparisons of the morphological characters by the polygonal graph method. In ten quantitative traits measured in B. populifolia, B. caerulea-grandis and B. cordifolia it may be seen (Figure 16) that B. caerulea-grandis is intermediate in all characters between B. populifolia and B. cordifolia.

In the four locations studied, Lac Carré, and Valcartier, Quebec, Grand Manan Island, New Brunswick, and Vermont, the four species B. papyrifera, B. populifolia, B. caerulea-grandis, and B. cordifolia, were found together. Although the production of hybrid seed is not uncommon, the production of a hybrid swarm of trees is more rare. When B. cordifolia and B. populifolia hybridize to produce hybrid seed, the survival rate of seedlings would probably be quite low. However, when the habitat has been disturbed, generally by farming,

ecological situations intermediate to those which are suitable for the parental species become available (Heiser, 1949). Hybrid seeds, germinating in these areas, generally have a much better chance of survival. In all four locations studied, the habitats have been altered by man. In southern Vermont, in the Laurentians and on Grand Manan Island, farming has cleared away the forest cover and changed the ecology of these areas. In Valcartier, the land has been farmed and, in addition, military manoeuvers have been carried out for at least thirty years creating differences in the habitat.

When the species were examined to determine if the location affected the variability of the characters studied, the mean guard cell lengths were significantly different in a comparison of B. populifolia populations from the Laurentians and Grand Manan Island. This would indicate that the mean guard cell lengths were at opposite ends of the range obtained for this character. The guard cell lengths did not differ significantly from one location to another in other species.

It is not known if this difference in guard cell length between the Laurentian and Grand Manan Island populations is caused by the influence of the climate, or if there is an inherent plasticity within the species for that character. For B. cordifolia and also for B. caerulea-grandis the bract length and the number of veins varied significantly, although the two populations at variance were not

necessarily the same. It appears then that bract length and the number of veins on a side of a leaf are more variable in B. cordifolia and B. caerulea-grandis than are the other characters.

The morphological variation exhibited by some herbarium specimens presented problems of classification by morphological studies alone. When the hybrid index values were calculated for individuals which were difficult to morphologically classify, it was found that they were within the range indicative of hybridization. But they tended to cluster near the extreme ends of the range either nearer to the B. cordifolia parental range or to the B. populifolia parental range. It was thought that these individuals were possible backcross individuals accounting for the clustering of their hybrid index values at the extremes nearest the range of those of their parental populations.

From the pictorialized scatter diagrams it may be seen that B. populifolia and B. cordifolia are well differentiated and do not overlap. Betula caerulea-grandis bridges the gap between the two parental species and also displays quite a range of variation in character association. There are some individuals classified as B. caerulea-grandis which may be backcrosses to the parental species.

The relative densities of the DNA of the different birch species examined have been shown to be essentially identical (Table 6). This

indicates that the genomes which comprise B. caerulea-grandis did not come from a parent of higher chromosome number as this would have shown up as a higher density. The cytophotometric method has made it possible to reliably estimate the number of chromosomes in an individual and to save considerable time in making a greater number of preparations that would have been necessary in order to actually find and determine the number of chromosomes in the conventional manner.

The results of the chromatography as seen in Figures 20, 21 and 22 give very direct evidence to support the view that B. caerulea-grandis is a hybrid of a cross between B. cordifolia and B. populifolia. The pink spot which occurs in B. populifolia and not in B. cordifolia and the white spot which occurs in B. cordifolia and not in B. populifolia are both present in the chromatogram of B. caerulea-grandis.

The chromatographic pattern shown for each species is a start at presenting a biochemical picture of the species in order to identify and differentiate them. More chromatographic patterns must be studied before the spots characteristic of each condition can be conclusively determined but the results with B. caerulea-grandis, B. cordifolia and B. populifolia indicate that with further studies chromatography will be of great value in differentiating birch species and hybrids.

SUMMARY AND CONCLUSIONS

1. Collections of *Betula cordifolia*, *B. caerulea*, *B. caerulea-grandis*, *B. populifolia*, and *B. papyrifera* were made in 1966 and 1967 from four localities, namely, Valcartier, Quebec; Lac Carré, Quebec; Grand Manan Island, New Brunswick; and Vermont.

2. Studies were made on leaf characters including leaf tip attenuation, number of veins and serrations on one side of a leaf, guard cell lengths, and on fruiting characters including the length of the mature female catkin, the length and shape of the bracts, the length and width of the achene, the length/width ratio of the achene and the style length. The measurements were subjected to a statistical analysis.

3. The somatic chromosome numbers for the following species were reported: *B. cordifolia*, $2n = 28$; *B. caerulea-grandis*, $2n = 28$ and *B. populifolia*, $2n = 28$. Karyotypes of chromosome complements were presented.

4. From a statistical analysis of the populations, and using hybrid index values, polygonal graphs and pictorialized scatter diagrams, it was considered that *B. caerulea-grandis* is a hybrid of a cross between *B. populifolia* and *B. cordifolia*.

5. Thin-layer chromatographic patterns of visible and fluorescent compounds in leaf extracts were presented. Specific differences were found which distinguish B. cordifolia from B. populifolia and which show that B. caerulea-grandis is a hybrid of B. cordifolia and B. populifolia.

6. More spots became visible on the plates under ultraviolet and visible light when plates were sprayed with concentrated sulphuric acid.

7. The relative density of the DNA in root tip cells of B. caerulea-grandis, B. cordifolia and B. populifolia was reported. Comparisons among the three species indicated that there was no significant difference in the density of the DNA.

8. The density of the DNA as determined for individuals of known chromosome number, was used as a standard in a comparison with the density of the DNA from individuals of undetermined chromosome number. In this way the cytophotometric method provided a reliable estimate of the chromosome number.

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