A CYTOLOGICAL AND MORPHOLOGICAL STUDY OF TWO POPULATIONS OF TRILLIUM GRANDIFLORUM (MICHX.) SALISB.

by

Richard Alden Giles

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1955

Approved

Richard Alden Giles

Two populations of Trillium grandiflorum (Michx.) Salisb., growing under similar ecological conditions, have been studied intensively for the kind and extent of variation in their morphological and cytological characteristics. Techniques for expression of the shapes of the foliar organs in mathematical terms have been devised, and this has permitted a quantitative comparison of these features. Morphological characters studied included the shape of the leaves, sepals, petals, and ovary, the anther/filament ratio, and the apical and marginal notching of the sepals and petals. Material for cytological analysis was subjected to low temperatures for ninety-six hours and was then prepared according to the standard squash technique, using the Feulgen staining technique.

Leaf shape was found to be due primarily to the interaction of four factors which vary more or less independently of one another. Petal shape appeared to be due mainly to the interaction of two, independently variable factors. Sepal shape seemed to be determined largely by the length/width ratio. Other factors, if they exist, are obscured by it. Ovarial shapes have been classified, primarily on the position of maximum width, into four types. Apical and lateral notching was observed in both sepals and petals. There appeared to be little or no correlation between the two kinds of notching, or between notching in the sepals and in the petals.

Major morphological variations were found to vary independently, indicating their lack of linkage. This, together with the very few significantly large differences in morphological character-

istics, suggests that hybridity has not been a major factor in the development of these populations in recent times.

A standard pattern of differentially reactive segments is present in the chromosomes, and is the same in both populations. Within this standard pattern, several types and degrees of variation in differentiation occur in the two populations, which show little significant difference from one another in this respect. The condition of heteromorphy, in which one homologue shows differential reactivity in certain segments, while the other fails to develop this reactivity in corresponding segments, is considered most useful for comparative purposes. The causal mechanism is unknown, but several possibilities are suggested. Differences in length, staining intensity, and apparent density of the affected segments were noted, and did not appear to differ in the two groups.

No cytological evidence was found which might be considered indicative of hybridity or introgression in these two populations.

A small sample of <u>Trillium flexipes</u> Raf. has been analyzed cytologically and a tentative standard pattern of differentiation established. The distinct difference in number and position of the affected segments, as compared with <u>Trillium grandiflorum</u>, suggests lack of any hybridity between the two, and is significant because the two species grow intermingled in one of the two populations.

This detailed assessment of the characteristics of the plants making up these two populations may now serve as a standard against which other populations may be compared.

BIBLIOGRAPHY

Anderson, Edgar. 1949. Introgressive Hybridization. John Wiley and Sons, Inc., New York, 106pp.

Bailey, Paul C. 1949. Differential chromosome segments in _Trillium erectum_ L. Bull. Torr. Bot. Club. _76_(5): 319-336

______ 1952. Differential reactivity in six species of _Trillium_. Bull. Torr. Bot. Club. _79_(6): 451-458.

Darlington, C. D. and L. LaCour. 1940. Nucleic acid starvation of chromosomes in _Trillium_. J. Genet. _40_: 185-215.

Haga, T. and M. Kurabayashi. 1953. Genom and polyploidy in the genus _Trillium_. IV. Genom analysis by means of differential reaction of chromosome segments to low temperature. Cytologia _18_(1): 13-28.

Kurabayashi, Masataka. 1952. Differential reactivity of chromosomes in _Trillium_. Journ. Fac. Sci., Hokkaido Univ., Ser. V. _6_(2): 233-251.

Wilson, G. B. and E. R. Boothroyd. 1941. Studies in differential reactivity. I. The rate and degree of differentiation in the somatic chromosomes of _Trillium erectum_ L. Can. J. Research, C, _19_: 400-412.

______ and ______. 1944. Temperature-induced differential contraction in the somatic chromosomes of *Trillium erectum* L. Can. J. Research, C, *22*: 105-119.

Woodson, R. E., Jr. 1947. Some dynamics of leaf variation in *Asclepias tuberosa*. Ann. Mo. Bot. Gard. *34*: 353-432.

A CYTOLOGICAL AND MORPHOLOGICAL STUDY OF TWO POPULATIONS OF *TRILLIUM GRANDIFLORUM* (MICHX.) SALISB.

by

Richard Alden Giles

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1955

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

Richard Alden Giles

candidate for the degree of

Doctor of Philosophy

Final examination: May 17, 1955, 2:00 p. m., Botany Seminar Room

Dissertation: A Cytological and Morphological Study of Two Populations of Trillium grandiflorum (Michx.) Salisb.

Outline of Studies:

Botany (Cytogenetics, Morphology, and Taxonomy)

Biographical Items:

Born: December 12, 1917, Cummington, Massachusetts

Undergraduate studies: Massachusetts State College 1935-1939

Graduate Studies: Michigan State College 1939-1941, 1950-1955

Professional Experience:

Undergraduate laboratory assistant in botany (Mass. State College) 1936-1939

Graduate teaching assistant in botany (Michigan State College) 1939-1941

Assistant Professor of Botany and Biology, Michigan State Normal College 1947-1954

Associate Professor of Botany and Biology, Michigan State Normal College 1954-1955

Member of: American Association for the Advancement of Science
Botanical Society of America
Michigan Academy of Science, Arts and Letters
Michigan Education Association
Michigan Natural Areas Council
New England Botanical Club
Sem Bot
Society of the Sigma Xi

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation for the encouragement and help of Dr. G. B. Wilson throughout this investigation. It has been a privilege and a pleasure to work under his guidance.

Thanks are due also to the guidance committee for their helpful criticism and encouragement.

Acknowledgement should also be made to Dr. C. M. Loesell, and others in the administration, who have cooperated in scheduling the author's work at Michigan State Normal College so that time might be available for this study at critical periods.

The photographic work of Mr. Philip Coleman has been most helpful, and grateful acknowledgement for his efforts is hereby made.

Finally, the author offers sincere thanks to his wife, Elizabeth, who not only has given encouragement and help when it was most needed, but also has done the typing of the manuscript, and to the rest of his family, who have willingly made the necessary adjustment in family living which such an investigation demands.

TABLE OF CONTENTS

TABLES

FIGURES

INTRODUCTION

During the past several decades it has become increasingly apparent that botanists who wish to understand the nature and phylogenetic relationship of the kinds of plants with which they work are going to have to modify and change their methods of attack on the problem. Traditionally morphology has been the major avenue of approach, and the individual plant, intensively studied, has been regarded as representative of the species. Gradually there has arisen the realization that morphological characteristics are not the only important criteria to use in delineating species, but that the cytology, the genetic constitution, and the environment of plants also are important in species determination. Further, it is becoming increasingly apparent that we must not content ourselves with study and descriptions of one, or relatively few individuals, but must think and study in terms of groups of individuals, or populations, which more properly represent a species.

The development of these concepts has led to the need for new methods and techniques of study, a need which is far from being fully satisfied at present. The study of populations, for example, requires an expression of the limits of variability in the plants which constitute them, an expression which is largely lacking in the older species descriptions. While it is relatively easy to determine limits for such readily measurable variables as height, length of internode, or diameter of stem, it is a much greater problem to define with adequate exactitude such features as the amount of pubescence or the shape of leaves. Terms such as sparse, dense, moderate, and narrowly oblanceolate to narrowly obovate are often too relative for this sort of approach. These variables should be expressed in such form that they can be determined precisely and compared quantitatively by any number of different investigators. Again, cytogenetics undoubtedly has a great deal to contribute to our knowledge of what constitutes a species, but in many instances has not been used because of the problems involved. Nearly every critical analysis of a plant taxon now includes descriptions of the chromosome complements and, where possible, their behavior. Babcock (1947), in his treatment of the genus Crepis, has given us one of the best examples of this type of approach. In only a few instances, however, has it been possible to do much with the extent of variability in the chromosome complement beyond reporting their number or pairing behavior. If there is a variability in the chromosomes of the plants which make up a species, and there seems to be some evidence to indicate that this is the case in some species, there remains to be done a vast amount of work in this area of investigation.

With these thoughts in mind it seemed that it might be valuable to study comparatively and rather intensively two populations of a species which appears to be quite variable, both from the morphological and cytological standpoint. Trillium grandiflorum (Michx.)Salisb. was the species chosen. Countless notes in the literature attest to its high degree of variability. O. A. Farwell (1919), for example, writing in the Michigan Academy of Science

Papers, describes a woodland in Farmington Township from which he collected and named nineteen different entities, considered by most to be Trillium grandiflorum, but distributed by him among three species, two varieties and fourteen forms. Cytologically, too, T. grandiflorum has much to recommend it for such a study. It has relatively few, rather large chromosomes, different enough morphologically to be easily and certainly recognizable. In addition, as Darlington & LaCour (1938, 1940), Wilson & Boothroyd (1941, 1944), and numerous others have now pointed out, it shows a characteristic differential staining capacity in the various parts of the chromosomes when subjected to low temperatures. This latter capacity might be expected to further assist in determination of the variability present among the chromosomes. Finally, this species is a common one, and thus readily available for study.

Curiously enough, though the literature is filled with notes concerning the more or less isolated, morphological abnormalities in T. grandiflorum, there is little or nothing with respect to the extent of the variation which may be found within a normal population. It seemed valuable, therefore, to attempt to assess somewhat exactly the "normal" variation before proceeding to the more radical variants which have been so frequently reported. This assessment could then serve as a base against which other populations, which might appear to be more variable, could be compared. In attempting these determinations, it seemed better to use two populations, and this procedure has been followed. One is located in the vicinity of Ann Arbor, Michigan, the other near East Lansing, Michigan, approximately fifty miles away. This is somewhat at variance with the more usual procedure in population studies in which a few samples from many groups or populations are employed, as exemplified in Woodson's work (1947) with Asclepias tuberosa and that of Fassett (1941) with Rubus, but it is believed that it is worth while in bringing out certain information which is not otherwise available.

It also seemed desirable to investigate, as far as it was possible to do so in this type of study, the extent to which the variations noted were, or were not, indicators of a possible hybridity in either, or both, of the populations. The populations selected were rather good for this purpose, since one of them was relatively isolated from any possibility of recent contamination by other species, while the other had a number of plants of Trillium flexipes Raf., and its forma Walpolei (Farw.) Fern., growing intermixed with the plants of Trillium grandiflorum. If hybridization between species is frequent in this genus, or if introgression is very active, evidence might be provided by comparative examinations of the two groups of plants.

Since it was necessary to make the comparisons as precise as possible, one of the objectives of the study was to devise techniques for translating such terms as "round-ovate to sub-rhombic oval" used in descriptions of leaves or perianth parts, into a form which could be expressed in quantitative fashion with some degree of preciseness. Very little of this sort of analysis seems to have been attempted previously, though Woodson (1947) did attempt to express the shapes of the bases of the leaves of Asclepias tuberosa in a somewhat similar manner. While it is recognized that different shapes of leaves require different techniques for translation into quantitative expression, it is felt that some of the findings reported here may prove helpful to others engaged in making similar comparisons.

Finally, it seemed useful to examine the morphological evidence for the possible origins of these populations. Several possibilities may be postulated. They may have had a common origin, perhaps being isolated sometime after the retreat of the last glacial ice sheet to cover this part of Michigan. In such an event, they should show many basic similarities. Unless the isolation has been fairly recent, they may also be expected to have some structures which have varied along different lines in the respective populations. On the other hand, they might have arisen independently through hybridization of other species, which might or might not still be present in the area. In this case, there should be some evidence of such origin in the tendency of certain characteristics to "stick together" in the population, rather than being more or less randomly associated with one another and with the other characteristics, as has been pointed out by Anderson (1936, 1939, 1949) and Anderson & Turrill (1938) in their studies on introgressive hybridization. A search for this sort of evidence was made one of the objectives of this phase of the study.

Cytologically, Trillium has received a good deal of attention, particularly since it has been shown to exhibit differential reactivity when treated under proper conditions of cold. Prominent in this respect has been the work of Darlington & LaCour (1938, 1940), Wilson & Boothroyd (1941, 1944), Kurabayashi (1952), Haga & Kurabayashi (1953), and Bailey (1949, 1951, 1952). All have obtained the differential reactivity readily enough, but the interpretations of its cause and significance are not as well agreed upon. Some see in the phenomenon a valuable criterion to assist in species determination. Others believe it has little or no value in this respect (cf. Bailey 1949). It seemed useful then, to investigate as far as was practical, the presence or absence of evidence indicating a possible specific pattern of differential regions in the species T. grandiflorum.

A second phase of the cytological aspect of this study was directed toward assessing the extent and kind of variation in the differential regions within each population and within the two considered together. As has been pointed out in discussion of the objectives of the morphological part of this study, such information should prove useful as a base against which other populations might be compared at a later date.

Investigation was also directed toward the phenomenon which I shall designate "heteromorphism", and which has been variously designated by other investigators as heterogeneity or heterozygosity. This is the condition in which homologous chromosomes in a cell, or in several cells of a plant, show a different pattern of differential reactivity. There has been some controversy over the extent and constancy of heteromorphism, and considerable attention has been directed toward the problem in this study.

Finally, the cytological observations have been studied for the evidence they might present for, or against, hybridity in these populations, and for such evidence as might be found for possible origins of the two. In this connection, brief, and not entirely conclusive, studies were made on the cytology of Trillium flexipes, in order that possible contamination from that species, which grows with Trillium grandiflorum at Ann Arbor, might be more readily recognized. More intensive investigations were not made at this time, since the evidence obtained, though not complete, was sufficient for the purposes noted here. It is hoped that it may form the basis for a future study.

PROCEDURE

A. Location and description of habitat

As has been stated previously, the sites of the two populations studied are located about fifty miles apart by straight line distance, one near Ann Arbor, and one near East Lansing. Both sites are ungrazed woodlots and both have a more or less irregular topography, typical of the morainic country of which each is a part. The flora of the two is somewhat similar, though by no means exactly the same. Examination of weather records for many years past shows the climate to be essentially alike in the two areas. Soil types are quite similar, though there seems to be a higher percentage of clay in the Ann Arbor site. At Ann Arbor another species of Trillium, Trillium flexipes Raf. together with its form, T. flexipes forma Walpolei (Farw.) Fern., grows intermixed with T. grandiflorum. At East Lansing there appear to be no other species present, though there is an area about one mile distant which contains T. flexipes. Basically, then, the two areas are quite similar.

The site at Ann Arbor is located on the farm of Burton Rogers, which lies just south of the University of Michigan experimental forest, known as the Saginaw Forest. The farm extends from West Liberty Road on the north to Scio Church Road on the south, with the woodlot in which this study was made occupying much of the southern half of the farm. The study area appears to be in a transitional stage between a typical oak-hickory forest type and a beech-maple climax. The larger trees are principally oaks (mostly *Quercus rubra* L. and *Q. alba* L., with some *Q. imbricaria* Michx. along the margin) and hickories (*Carya ovata* (Mill.) K. Koch principally but with some *C. cordiformis* (Wang.) K. Koch). There is very little reproduction of the oaks evident. The hickories are reproducing themselves with moderate success in some parts of the woodlot, as judged by the presence of some small trees. Among the younger trees, from three to twenty feet in height, sugar maples (*Acer saccharum* Marsh) are particularly abundant. Beech (*Fagus grandifolia* Ehrh.) and basswood (*Tilia americana* L.) are also represented among the younger trees with a moderately large number of individuals. Ironwood (*Ostrya virginiana* (Mill.) K. Koch) is very abundant throughout. Flowering dogwood (*Cornus florida* L.) forms a very conspicuous understory. Shrubs are not present in any considerable number, being represented chiefly by dogwoods (*Cornus* spp.) and *Viburnum* spp.

In addition to *Trillium grandiflorum* (Michx.) Salisb. and *T. flexipes* Raf., there are several other species which are common in the herbaceous spring flora. Especially abundant are *Claytonia virginica* L., *Erythronium americanum* Ker., (and locally *Erythronium albidum* Nutt.), *Dentaria laciniata* Muhl., *Phlox divaricata* L., *Geranium maculatum* L., *Podophyllum peltatum* L. and several species of violet (*Viola* spp.). Locally abundant are *Cardamine Douglassii* (Torr.) Britt., *Sanguinaria canadensis* L., and *Asarum canadense* L. A number of other species are present but are represented by fewer individuals, and these are somewhat scattered through the whole woodlot.

The soil is basically a Miami loam (cf. Veatch 1930). In general, it is fairly well drained, and is almost universally so in those sections where the Trilliums grow. Normally there is an ample supply of moisture throughout the year, though during one summer it was observed that the soil became very dry, to a considerable depth, through much of the woodlot.

As has been mentioned, the topography is typically moraine, with its many, well drained slopes and several, poorly drained depressions. The latter frequently contain button bush (_Cephalanthus occidentalis_ L.), and apparently are gradually becoming filled with partially decayed organic material. _Trillium_ is found almost wholly on the sides of the slopes.

The site at East Lansing is commonly known as the Sanford Woodlot and is owned by Michigan State College. It is used by the forestry department of that institution in their training program. Very little cutting had been done in the area until the winter and spring of 1954, when one section was cut over quite extensively.

The tree flora appears to be more nearly a stable, beech-maple climax than was the case at Ann Arbor. The large trees are primarily sugar maple (_Acer saccharum_ Marsh) and beech (_Fagus grandifolia_ Ehrh.), and both of these species seem to be reproducing well, as evidenced by large numbers of young specimens. The abundance of large oaks and hickories noted at Ann Arbor is not characteristic of this site. Another difference is seen in the lack of the flowering dogwood (*Cornus florida* L.) which was so abundant there, and in the presence of very few ironwoods (*Ostrya virginiana* (Mill.) K. Koch), as contrasted with the large number of this species found at Ann Arbor. As was also the case there, the shrubs do not form a very important element of the woody flora.

The herbaceous spring flora is quite similar to that described for Ann Arbor with the following exceptions. *Erythronium albidum* Nutt., *Geranium maculatum* L., *Phlox divaricata* L. and *Podophyllum peltatum* L. are distinctly less numerous, while *Hepatica acutiloba* DC., *Dicentra Cucullaria* (L.) Bernh., *Dicentra canadensis* (Goldie) Walp., and *Hydrophyllum canadense* L. are all abundant, or at least locally frequent, at East Lansing and are entirely missing from the flora in the Ann Arbor woodlot. These differences are suggestive of slightly higher moisture levels in the former.

The soils in this woodlot are not strikingly different from those encountered at Ann Arbor, consisting here primarily of one or another of the Fox series of loams. In general, it is probable that there is a higher percentage of sand in these soils and a lower percentage of clays. A good deal of the area is underlain by a heavy, more or less impermeable layer of clay, usually at a depth of about two feet, though this is variable. This, too, is not very different from the conditions met in the other woodlot. Moisture seems to be ample, possibly slightly higher than at Ann Arbor, for the soils did not appear to dry out as much during the one very dry summer referred to in the discussion of that area. Drainage is generally good, excepting in the low spots where

Trillium usually is absent.

The topography of the two areas is very similar. Both are characterized by the presence of several slopes which drain into low, poorly drained depressions, which are often filled with water in the spring, but which frequently dry out more or less completely during the summer months. In both areas, Trillium is usually restricted to the slopes, and is rarely found in the depressions.

Both populations, then, grow under very similar ecological conditions. For all practical purposes, it is probably safe to presume that such morphological differences as may be noted are not very likely to be environmentally caused.

B. Cytological Procedures

Material for cytological analysis in this study has been obtained from two sources, the meristematic region of the roots and the actively dividing cells of young ovules. At the beginning of this investigation, root tips were used to obtain the desired mitotic figures. Two procedures were used in obtaining the tips. In one case, rhizomes were dug in the field, the root tips excised and placed in numbered vials of ice water at once. These vials were then placed in a thermos jug filled with ice. This method presented the problem of finding the rhizomes at the proper time. It soon became apparent that the root tips were most active mitotically after the aerial parts of the plants had withered and died. Unless the position of the plants was marked before the aerial parts disappeared, it was most difficult to find the rhizomes at the time they bore good root tips. To overcome this problem the procedure was then altered to that of digging the rhizomes during the period of late anthesis and transplanting them to flats of soil. These flats could be examined periodically and the root tips gathered when they appeared to be in best condition. New roots appeared on these rhizomes at different times in different rhizomes for a period of about three weeks. They appeared to be most active mitotically when the roots reached a length of between one and two inches, though no quantitative data were collected to support this conclusion. Root tips were excised and treated in the same manner as that described for field collections.

After the vials of root tips were brought in from the field or flats, they were transferred from the jugs of ice to an electric refrigerator (household type) and held at a temperature of about 2 degrees Centigrade. The total period in the vials on ice and in the refrigerator was 96 hours. This was in conformity with the procedure which Wilson & Boothroyd (1941) showed to be most successful in producing good differentiation.

At the end of the refrigeration period, the tips were fixed in a solution of three parts absolute ethyl alcohol and one part glacial acetic acid for at least twelve, but not more than eighteen, hours. After fixation, the material was processed in 1N hydrochloric acid for 8 minutes at a temperature of 60 degrees Centigrade. Staining was by the Feulgen technique. Following staining, the material was thoroughly squashed on a slide, in 45% acetic acid, covered with a cover slip and warmed gently for a few seconds. The slide was then placed in a dish of 95% alcohol, to which a small amount of fast green had been added for counterstaining. After about twelve hours the cover was removed, if it had not already floated off, the excess alcohol was drained off and the material was mounted in diaphane.

Analysis of the material was made using a Spencer microscope with a 10X ocular and a 95X oil immersion objective.

In 1952, Kurabayashi reported considerable success using the ovules from young flowers as a source of his mitotic material. The following spring, 1953, this was attempted in these two populations. The results were good, in general giving mitotic figures in a considerably higher percentage of the slides examined than was the case using root tip material. Differentiation was as good as that in the root tips, and did not appear to differ in kind from that already observed there. It was possible, using this method, to collect material for cytological study and for morphological study at the same time, which was not the case when using root tips. During 1953 and 1954, therefore, all cytological work was done on ovular material.

An objection to this procedure could be raised on the basis that the figures obtained would not be as certainly representative of the genome of the plant from which the ovules were taken as would the material derived from root tips. This is possible because the older ovules, at least, might be expected to include some figures from the developing embryo or from the endosperm, and these would actually represent the genome of a new plant and not that of the one from which the ovules were taken. The figures from endosperm could be detected readily because of their polyploid nature. They were rarely encountered in the material studied. The figures from the developing embryo would be harder to detect. To minimize this objection, ovules from what appeared to be recently opened flowers were used as much as possible, making it improbable that ovules would contain embryos, or at least that they would contain embryos with sufficient figures to lead to any confusion. Moreover, the use of several ovules on each slide would tend to further minimize this objection, since the genomes of the embryos in two different ovules might be expected to differ from one another. As a matter of fact, however, no differences were detected, and this, in conjunction with the very rare occurrence of any cells which were obviously endosperm, suggests rather strongly that the material studied actually did consist of the tissues of the nucellus and integuments, and hence was representative of the vegetative cells of the plant being studied. Then too, no special attempt was being made to correlate the cytological and morphological conditions in particular plants, so that it did not much matter whether the analysis was made of a potential member of the population, represented by the developing embryo within an ovule, or of a present member of the population represented in these cases by the nucellar and integumentary tissues.

Kurabayashi (1952) apparently refrigerated whole plants in an igloo, placing them in this device the day after the flowers had opened. The technique used here has been somewhat modified from that which he described. Plants were gathered in the field. For the most part selection was made from those whose flowers appeared to have opened recently. It might be mentioned, however, that good results are often obtainable using older flowers, or at the other extreme, unopened buds, so that there is considerable latitude in the choice of material to be collected. The plants were brought into the laboratory, where the ovaries were removed from the flowers and placed in vials of ice water. Observations and measurements were made on the morphological characteristics of the plant, which was then assigned a number. This number was also inserted in the vial with the ovary, so that for each differential pattern the morphological characteristics of the plant which produced it was known.

During the season of 1953, the vials containing these excised ovaries were placed in a household type, electric refrigerator, and maintained there at a temperature of approximately 2 degrees Centigrade for 96 hours. There was undoubtedly some small amount of fluctuation in this temperature during the cooling period. In 1954, the vials were placed in thermos jugs containing ice, and the result appeared to be at least as good as, and probably a little better than, with the material stored in the refrigerator, though no accurate, quantitative comparison was made. The time of cold treatment was 96 hours just as in all of the rest of the material used in this study.

Treatment after refrigeration was the same for the ovular material as that already described for the root tips. It might be noted that one ovary gives ample material for several slides, four to eight ovules per slide proving to be a desirable number. It perhaps should be noted also that better results were obtained when the ovaries were slit open before putting them in fixative. This apparently permits better penetration of the fixative, macerating fluid, and stain.

As has already been noted, collections, using this procedure, were made during both the 1953 and 1954 flowering seasons. In 1954, weekly collections were made for four successive weeks from each of the two populations. These collections were started during the last week in April and covered practically the entire period of anthesis in the two populations.

In collecting material in the field, an attempt was made to get representatives from all parts of the two populations. It is believed that the cytological material is sufficiently randomized in this way, since many plants collected showed little or no mitosis, and others lacked well enough spread metaphase figures to make positive analyses of differential patterns possible. Since there was no way of forecasting which plants might produce good results and which not, the samples reported are presumed to be free from any subjective bias, conscious or unconscious, on the part of the collector.

Since a check on meiosis seemed desirable, an attempt was made to get meiotic material. For this purpose, large rhizomes were collected in the field and transplanted to flats of soil soon after the flowering period had ended. Starting about the first of

September, a few of these rhizomes were examined daily, whenever possible. The bud was opened and a stamen removed. This was fixed, macerated, and stained as described above for the ovular material and root tips. The slides were examined and discarded if meiosis was not present, or made permanent if it was present, by the method noted above for mitosis.

C. Morphological Procedures

1. General considerations

Procedural problems with respect to the morphological portion of this study fall rather naturally under three headings. First, there is the problem of collection and preservation of material for future study. Second, there is the problem of determining which morphological characters are significant to this study. Much of the work of 1951 and 1952 was exploratory in this respect. Third, there is the problem of procedures to be employed in expressing the morphological variables in a form which might lend itself to at least elementary statistical analysis.

The problems concerned with actual collection were primarily those of obtaining collections which would be as nearly truly random as possible. It might be noted that time of collection plays a significant role here, since it was found that some features, considered by many systematists as important, or at least supporting, in determination of species, changed with the age of the plant. It became important to know, therefore, which structures changed and which did not, and collections had to be spread over the whole growing period to care for this.

In each series of collections, an attempt was made to get approximately equal numbers of specimens from each of the major colonies composing the population, plus a number of others from among the plants which were not particularly aggregated in colonies but were scattered here and there in the area. Within this general procedure, actual selection of individual specimens for collection was made by several different methods, in order to try to keep bias as low as possible in the total sample. At some times, a straight line was laid out and the specimen nearest a certain designated distance along that line was collected, followed by a second specimen an equal distance farther along and so on. Where the colonies were fairly dense, specimens were taken by collecting the first plant touched, without looking at it. In some instances several companions, who had no knowledge of the problem, were asked to collect a certain number of specimens from the colony. When the ovaries of the plants were to be used for cytological study, the plants with flowers which appeared to be the youngest open ones were chosen. If several of approximately the same apparent age were present, the first ones seen were taken. It is recognized that bias has not been completely eliminated by these techniques, but it is hoped that it has been reduced to a low enough level so that the samples may be considered reasonably representative of the populations from which they were taken.

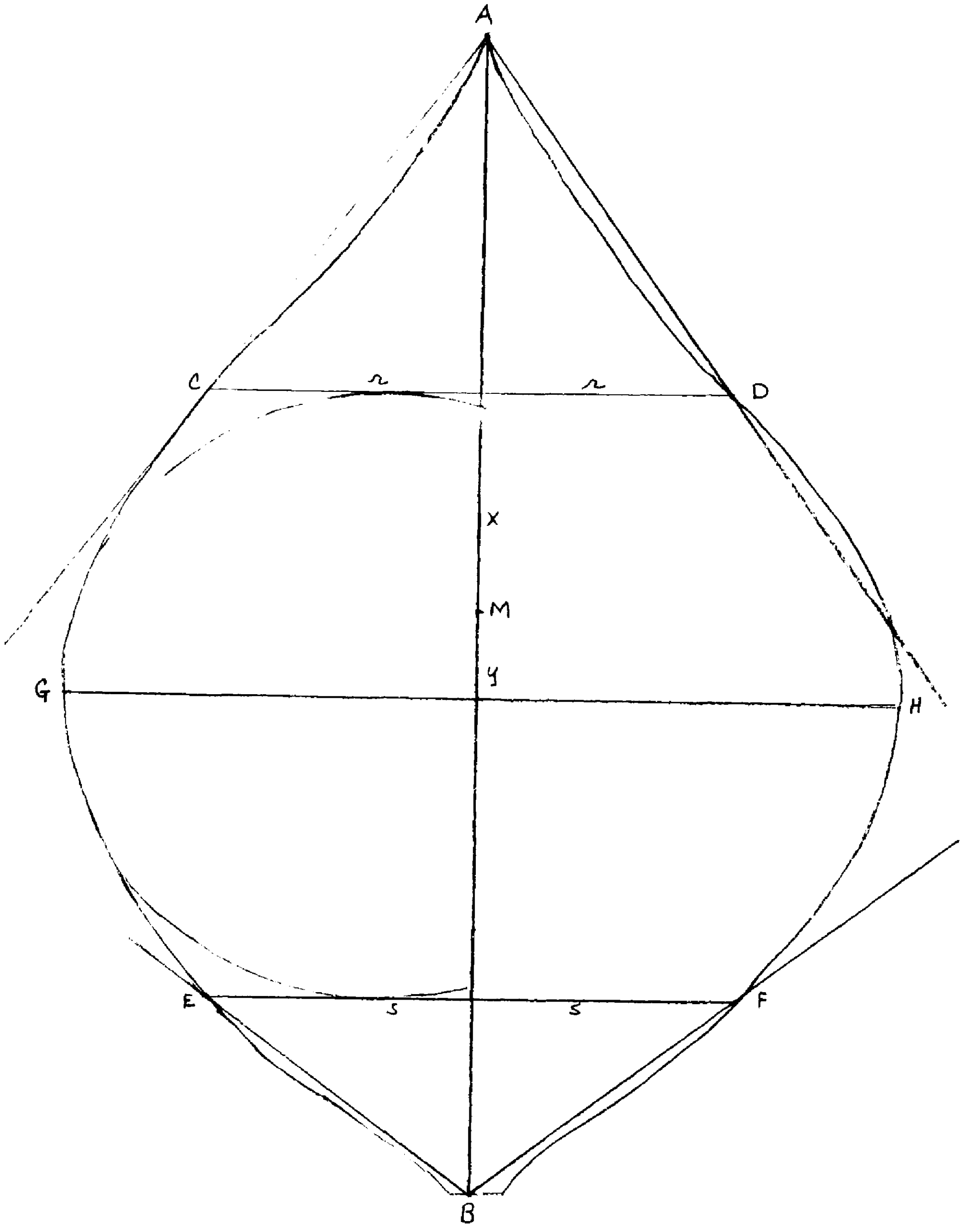
Considering the fact that variations in Trillium have been a source for so many papers, it is interesting to note that the number of variable features is rather smaller in this species than in many others. Most of the variations published have been those dealing with numbers or coloring of the flowering parts. There have been many reports, for example, of plants with green or variegated petals instead of the normally white ones, of plants with no petals, or with four sepals and petals, or with six in each whorl of parts. Indeed, there have been reports made of plants with no perianth parts, with parts in twos, threes, fours, fives, and sixes, and with most of the possible combinations of these, as, for example, three sepals, three petals, four stamens and two carpels. The flowers seem particularly variable in this respect, though as Fernald (1950) puts it in the eighth edition of Gray's Manual, these belong "more to the field of teratology than taxonomy". There were encountered in this study very few such variations, and these consisted mainly of a few plants whose petals bore a bit of green, or whose sepals were marked slightly with white, or whose stamens were either four or five instead of the usual six.

There are, however, variations of another sort, which seem more significant for this problem, even though they are not often, if ever, the source of papers on _Trillium_. They are not sports, nor do they belong to the field of teratology. Instead, they are the normal variations which might be expected to occur in any population of _Trillium grandiflorum_. Moreover, when a population is studied intensively, it becomes apparent that the variations, though not great in number, are considerable in extent. That is, the extremes are quite distinct from one another in most instances.

2. Analysis of leaves

The most obvious variable is probably leaf shape, which may vary from a type which is ovate, sometimes narrowly so, through an almost orbicular, to a rhombic or sub-rhombic type. Indeed, there is so much variation that it has been difficult to find a set of measurements which would satisfactorily express the shape in a form suitable for statistical purposes. After much study, and comparison of numerous specimens, however, it seemed that a solution to the problem might be made, if the shape were regarded as due to three major factors, the body of the leaf, the tip, and the base. It seemed that the same kind of tip, for example, might occur on several different body types, and that a similar situation might exist with respect to the base. Having decided this, the next step was one of determining how to tell what portion should be ascribed to tip, base, or body. After trying several methods, it seemed that best results were obtained when the following procedure was used (cf. Text Fig. 1).

First, using a compass, construct the largest possible arc which will follow the margin of the body of one side of the leaf at its widest part. In some instances the two sides do not appear to be exactly symmetrical, and in such cases I have consistently chosen the side, the center of whose arc is nearer the tip. A vertical line (AB in fig. 1) is then constructed from tip to base. A line (r in fig. 1) tangent to the uppermost part of the arc, and



Text Fig. 1. Procedure for Quantitative Analysis of Leaf Shape.

AB is the line of maximum length. GH is the line of maximum width. M is the midpoint of line AB. Construct an arc with its center on GH such that it follows the natural curve of the leaf as far as possible. Construct line r tangent to the arc at its highest point, intersecting the margins at C and D. Construct a similar line, s, at the lowest point of the arc, intersecting the margins at E and F. The distance from M to line CD is designated as x, and from M to line EF as y. Construct angles CAD and EBF. L/W equals AB/GH, displacement equals x/y, angle T equals CAD/2 and angle B equals EBF/2.

perpendicular to the vertical midline just mentioned, is then drawn. The intersection of this line with the margins of the leaf (C and D in fig. 1) is considered as marking the division between body and tip. While this may seem a trifle arbitrary, in actual practice it works out quite well. A similar line (s in fig. 1) is constructed tangent to the lowermost part of the arc. This, of course, is considered to divide the base from the body of the leaf. Next, the midpoint of line AB (M in fig. 1) is determined by measurement, and then the distances from the intersection of lines r and s with AB to this midpoint. The upper distance, from r to the midpoint, is designated as x, the lower distance as y. Then lines are constructed from the tip of the leaf to the points of intersection of r with the margins, and from the midpoint of the base to the points of intersection of s with the margins. The angle formed by the lines at the tip is designated as angle T, and the angle at the base as angle B. Finally the width of the leaf is measured at its widest part.

Records were made and kept of the ratios between length and width, and between x and y, together with one half of angle T and one half of angle B. The x/y ratio is designated as the displacement factor, since it is indicative of the relative displacement of the bulk of the leaf toward either the base or the tip. One half of the angle is used because that represents the angle at which the tip or base flares, or tapers. By this method, the mean between the two angles which represent the taper is being used.

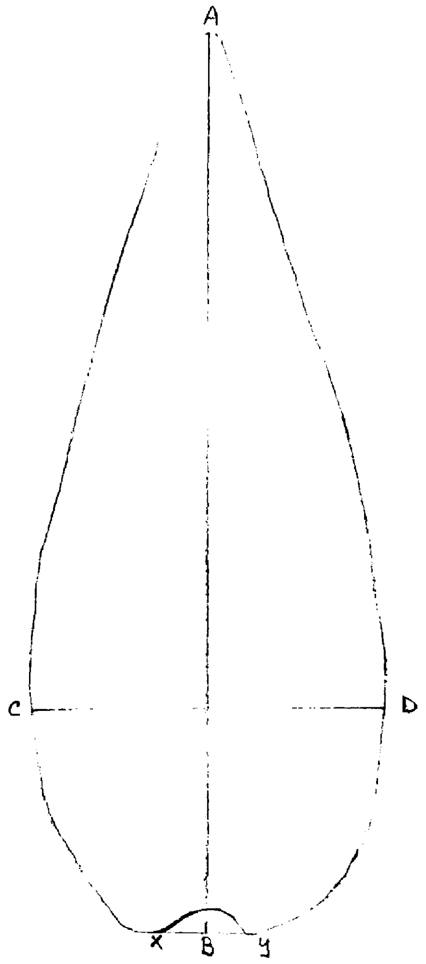
The length/width ratios give a fairly clear indication of whether a leaf is relatively long and narrow or short and broad. The ovate types will generally run toward the higher figures and the orbicular and rhombic toward the lower figures. The x/y ratio indicates something of the relative amount of leaf blade above and below the midline, and has been found to be quite significant in differentiating the various leaf shapes. An ovate leaf might be expected to have a low ratio, an orbicular one a ratio of 1, and an obovate one (if such exists) a high ratio. A low tip angle recording indicates an acuminate tip. Similarly, a rounded base would have a large angle B and a cuneate base a small one.

The leaves used for these determinations were measured from tracings of dried specimens in all but the 1954 collections. The latter were all from tracings of fresh material, done on the day of collection. There seems to be relatively little difference between the fresh and dried material with respect to the various factors considered, which agrees with the results obtained by Woodson (1947) working on Asclepias. In many plants, there does seem to be a certain amount of variation in the leaves of a single plant. In all cases where such variation appeared to be present, the one which was apparently median for the plant was selected. Obviously deformed, wrinkled, or torn leaves were not used.

3. Analysis of sepals

The sepals afford a second set of variations, though the differences noted here are much less than in the leaves. In general, the shape varies from lanceolate, sometimes fairly narrowly so, to some which are probably more properly called ovate. The length/width ratio is apparently the significant variant in determination of shape. Considerable study of the sepal bases was undertaken before it was finally concluded that the differences here are more apparent than real. Trillium flexipes tends to have its widest point somewhat nearer the midpoint than in T. grandiflorum. Because of this, special attention was given to the position of the widest part in the latter. Continued observation has shown that there is very little difference between individuals in this respect. The widest point, in nearly all cases, is located about one quarter of the way from the base to the tip. Records of this feature have been kept, but are not reported here, since they appear to be of little significance. For a time it seemed that there might be some variability in the tips, but further investigation indicated that the type of tip is so closely correlated with the length/width ratio that it is impossible to detect any very significant, independent variation in this character, if such exists. This leaves the length/width ratio as the only major factor in determining sepal shape, and it is the only such factor reported in the tables.

The length of the sepal was determined by measuring the distance from the tip to a line connecting the lowermost point of the two basal lobes (cf. fig. 2). Width was measured at the point of its maximum extent, which usually occurs at or near a point one quarter of the way from the base toward the tip. These measurements were recorded as a ratio, with high figures indicating a long narrow sepal.



Text Fig. 2. Sepal #y516-26 from Ann Arbor Population

L/W ratio 2.73, determined from AB/CD. B is the midpoint of a line connecting the lowermost points of each of the basal lobes, x and y.

Another variable which was noted occasionally was the presence of notches or indentations in the apex or lateral margins of the sepals. A somewhat similar condition was noted in the petals. It appeared earlier in the investigation that the leaves might also be notched, but continued study suggested that the apparent notching in this organ could be more accurately explained as being caused by some growth abnormality due to insect injury, or to tearing as the leaves pushed up through the litter of the forest floor. The notches in the sepals and petals are definitely not of this nature. They may occur in all three sepals, or in only one or two of the set. Their presence has been recorded wherever seen.

Collection and selection procedures were the same as for the leaves. Fresh material was used for all 1954 collections. Otherwise measurements were made on dried material. Because of the small size of the sepals, and the difficulty of determining the measurements accurately on such a scale, enlargements were made, using a projector. Enlargement was to about 4X.

4. Analysis of petals

A third set of variables was noted in the petals. They exhibited a considerable variation in shape, and in addition, differed from one another in some other ways, as, for example, in the presence of a notch at the tip, or of one or two notches in the margin a short distance below the tip. This notching was similar to that found in the sepals, though more prominent here. Coloring was certainly variable, but differed so much with age that it was almost impossible to study it on a population basis. One would have to revisit marked plants daily, in both populations, if adequate information were to be obtained on this point, and since this was not possible at the time of this study, data are not included here. Apparently ruffling of the petals also differs in degree in different plants, but this, too, seemed to differ with the stage of development of anthesis, and so was subject to the same difficulties as posed by variation in color. Moreover, no adequate method for quantitative measurement of the ruffling could be found.

Because the petals are not like either the leaves or sepals in shape, it was necessary to determine anew what factors were present here that might be subject to measurement, and which were influential in determining petal shape. Investigation soon disclosed that the length/width ratio was very significant, much more so than in the case of the leaves. The displacement factor, that is, the relative amount of the petal above and below the midline, was found to be significant here, though perhaps not quite as strongly so as in the leaves. The shape of the petal, however, made the methods used for determination of this factor in leaves impossible of application here, so that new techniques had to be developed. At first it appeared as though there might be other factors involved in determination of the kind of curve or taper between the tip and point of maximum width, and between the base and this same point. It was recognized, of course, that both the length/width ratio and the displacement factor were involved in production of the particular curvature or taper found. Much study, and the use of many different techniques of measurement finally led to the conclusion that these two factors, by their interaction, are almost wholly responsible for these characteristics, and that there either are no independently active causal factors, or if such do exist, they are almost completely obscured by the length/width ratio and displacement of the petal. That this is a reasonable interpretation of the factors influencing shape is suggested by the fact that, given the length/width ratio and the displacement, a petal can be drawn which rather closely duplicates the one from which the data were derived.

The best means of determining the displacement of the petal seemed to lie in the determination of the widest point of petal and then in indicating the relationship of this point to the horizontal midline. If, as frequently happens, the petal appears to be of maximum width for a vertical distance of several millimeters, it is considered to be widest at the midpoint of this vertical distance. The displacement factor is considered to be the ratio between the distance from the midpoint to point of maximum width, and one quarter of the total length of the leaf. The selection of one quarter of the total length rather than one half, the whole length, or any other fraction is purely arbitrary. It was chosen as being a quantity which would show the degree of variation in displacement rather more obviously than would have been the case using the whole length. If the line of maximum width occurs below the midline, negative numbers are used. For example, if a petal

160 mm. long was widest at a point 5 mm. below the midline, the displacement factor would be designated as a minus .125. It is apparent that ovate petals, if such occur, will be designated by relatively high negative numbers, while strongly obovate petals will be indicated by high positive numbers.

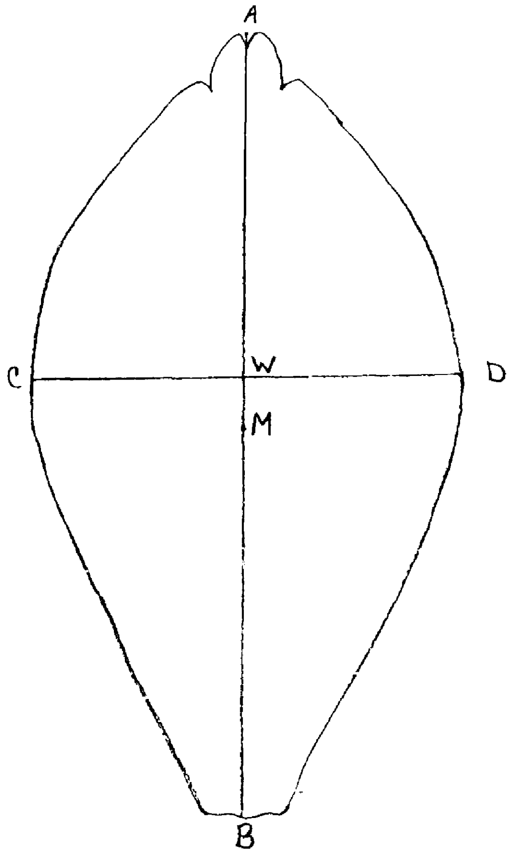
Notches were of two kinds, apical and lateral, and were recorded as being present wherever they were found to occur. No attempt was made to record the depth of notching, though some variability in this feature was noticed. Apical notches were recorded separately from laterals.

Illustration of a petal having both apical and lateral notches, together with an explanation of the determination of its measurements will be found in Text Fig. 5.

Fresh material was used for all 1954 records. Petals were enlarged by means of a projector to about 4X and then traced. Dried material from preceding seasons was wetted by placing it in warm water to which a detergent had been added. The petals were then treated as in the case of the fresh material.

5. Analysis of stamens

Since one of the most obvious differences between _T. flexipes_ and _T. grandiflorum_ appears in the relative lengths of anther and filament, it seemed profitable to inquire into the variation in this regard in the populations concerned. Measurements of each part were made and expressed as a ratio, anther/filament. Fresh material was used in the season of 1954. In previous seasons pressed and



Text Fig. 3. Petal #5921 from Ann Arbor Population

L/W 2.02. Displacement 0.24. AB is the line of maximum length. CD is the line of maximum width. M is the midpoint on AB. W marks the intersection of AB and CD. L/W equals AB/CD. Displacement equals MW/$\frac{1}{4}$AB. Note the presence of an apical notch and two lateral notches. Lateral notches, when present in petals, are nearly always found in this position.

dried material was used. Measurements were made to the nearest millimeter. In the 1954 material the stamens were enlarged by projection and traced, thus giving a more accurate ratio than was the case with the natural size material in which fractions of a millimeter of measurement became relatively more important. Enlargement was about 4X.

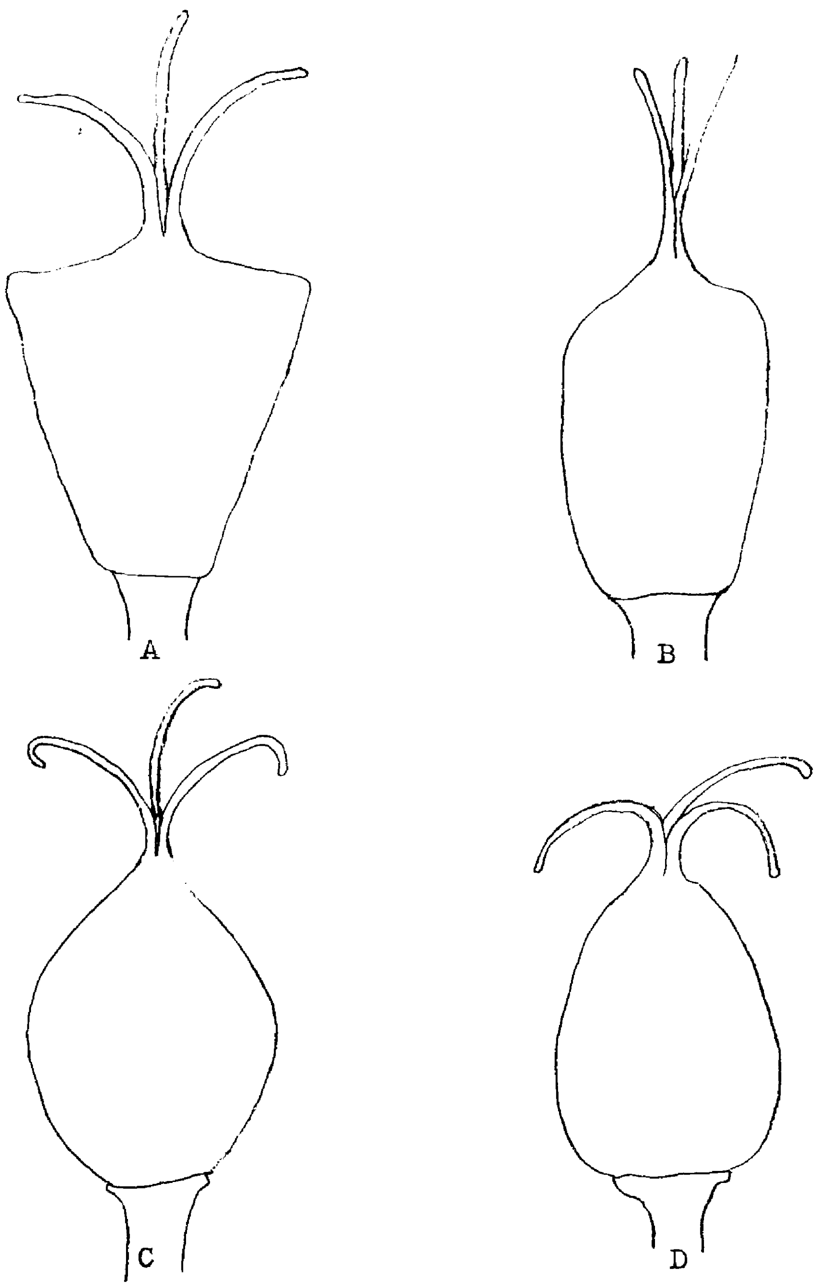
6. Analysis of pistils

The pistil also varies in several features, some of which have been noted in the various keys and manuals of flowering plants. One of these is the recurving of the styles, which in many cases does not exist, the styles being relatively straight. In other cases the styles are definitely recurved. However, it was found that much of this was associated with the stage of anthesis, and since no data were collected from plants which were known to be at exactly the same stage of anthesis, this variable has not been included in this study. The relationship of the length of style to length of ovary has also been used by some taxonomists, but is even more obviously subject to change with age and has not been considered significant enough for inclusion here.

Studies over several seasons have made it quite evident that, while the ovary may increase tremendously in size during the period of a few weeks, it does not change in its basic shape. The lateral, wing-like extensions of the ovary wall tend to make it more difficult to assess accurately the true shape of the ovary, for they are frequently unequal in width and/or spacing. Because of this, the ovary may sometimes appear to have different shapes, depending upon the position from which it is viewed. With a little practice, however, this difficulty can be overcome, and the basic shape determined.

While there is a good deal of intergradation present, the ovaries examined appear to be referable to one of four basic types. These types are based largely on the approximate position of the widest part (cf. Text Fig. 4). One type, designated as type _A_, is characterized by having the widest part at, or near, the top of the ovary. From this point it tapers gradually, but evenly, to the base. The top of the ovary is truncate or nearly so. A second type, designated as _B_, is nearly equal in width throughout its length, usually appearing somewhat columnar. The top is usually somewhat rounded, rather than being truncate as in the preceding type. A third class, designated as _C_, is marked by having the point of maximum width at, or near, the center of the ovary. This type seems to have two extremes, dependent on the relative width of the ovary. If it is very short and broad, the ovary assumes an almost spherical shape, while if it is relatively long and narrow, it may appear more nearly narrowly ellipsoid. No attempt has been made to differentiate between these, or between their intermediates, which occur in considerable numbers, in recording ovary shapes. The fourth type, designated as _D_, has the broadest part of the ovary either at the base, or at least distinctly below the middle. Typically, the appearance is approximately ovoid.

Procedure in recording shapes was to assign each specimen to



Text Fig. 4. Diagrammatic Representation of the Four Major Ovarial Types

A. Truncate or nearly so at the tip, widest near the top, and narrowing gradually all the way to the base.
B. Nearly the same width throughout, appearing almost columnar.
C. Definitely widest at or near the midpoint.
D. Widest at or near the base, narrowest near the tip.

The wing-like ridges which occur on the ovary have been omitted in these diagrams.

one of the four classes described above. In 1954 this was amplified by enlarging the ovaries by projection (4X) and tracing the enlargement for permanent record. Preservation of ovaries by pressing and drying was not particularly successful due to alteration in shape as a result of pressure and shrinkage.

7. Analysis of other features

Both leaves and sepals were examined for stomatal number and size. Since no appreciable differences were noted in the plants studied, about twenty from the two populations, it was presumed unlikely that this was a significant variable.

In the earlier phases of the work, peduncle length was recorded, and compared with length of leaf and petal. Data on this are available, but are not considered significant to this study. This measurement was not continued in later seasons of work. In most instances, measurements, where made, were on fresh material, though a few were made on dried material. It was felt that drying did not appreciably alter the relationships. Relationships between peduncle length and length of stem below the leaves were also determined in a few samples, but did not seem significantly variable apart from differences which seemed primarily ecological.

The presence or absence of anthocyanin in the lower part of the stem was also investigated, but early studies strongly suggested that ecological factors played a major part in its appearance, and further work in this direction was suspended.

OBSERVATIONS AND DISCUSSION

A. Cytological Considerations

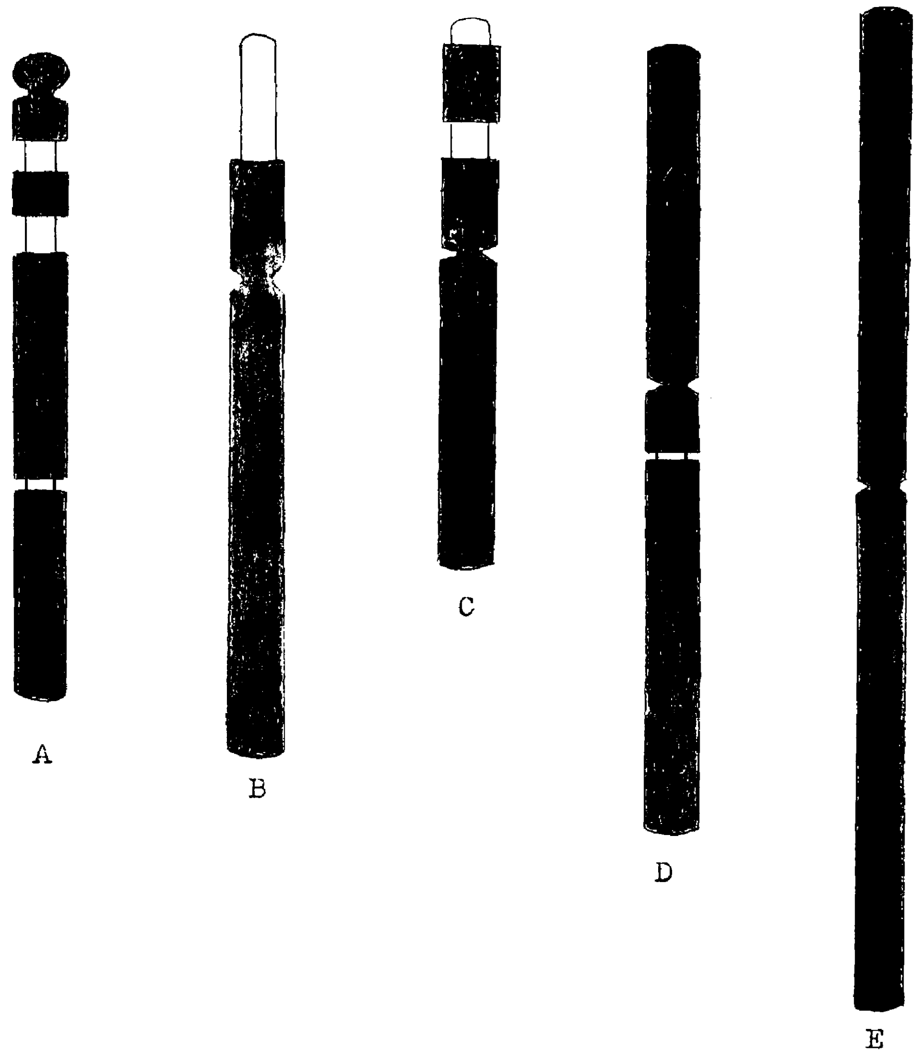
1. Differential reactivity

The genus Trillium has long been recognized as good cytological material, especially with reference to its nuclei, because of the low number and large size of its chromosomes. In addition, it has been known for some time now, that the chromosomes will stain differentially when the cells have been subjected to certain cold treatments. There is still some debate about the constancy, cause, and significance of this phenomenon, though the last fifteen years has seen much light shed on the problem through the efforts of several cytologists (Wilson & Boothroyd (1941, 1944), Darlington & LaCour (1938, 1940), Haga & Kurabayashi (1953), Bailey (1949, 1951, 1952), and others). This study is confined to one species in the genus, Trillium grandiflorum.

In general, the shapes of the chromosomes, and their numbers, are rather remarkably constant within the genus Trillium, though Warmke (1937) found enough minor variations in ratios of arm lengths, presence of satellites, and relative sizes of the complements to enable him to identify the various species which are found on our Pacific Coast. It should be added that Haga and Kurabayashi (1953) have found variable numbers in some of the Japanese species, due to the development of polyploidy. This does not seem to be true of any of our native American species.

In *Trillium grandiflorum* it is possible to recognize the five different types of chromosomes whether or not they are differentiated, and letters have been assigned to each of the types as noted. The most strikingly different chromosome has been designated as the *A* chromosome. It can readily be recognized because of the very great difference in length of the two arms, the shorter one appearing almost like a knob. A second type, designated as *B*, also has the two arms differing considerably in length, though the short arm is very much longer than its counterpart in the *A* chromosome. The long arm, in this case, is between two and three times the length of the shorter one. The kinetochore is much more nearly centrally located in the remaining three kinds. Of these three, the shortest, and the shortest in the whole complement, has been designated as the *C* chromosome. One of its arms is quite visibly shorter than the other, though the difference is not very great. The longest chromosome in the complement, and it is considerably longer than any of the others, has been designated as the *E*. Its kinetochore is the most nearly centrally located of any of the five. The remaining type, designated as *D*, is intermediate in length between the *C* and the *E*, so that it may readily be distinguished from either of them on the basis of length alone, and differs from the *A* and *B* in having a somewhat more nearly median kinetochore. Reference to Text Fig. 5 and Plate I should make these differences clear.

Study of the two populations concerned made it rather clear that a master pattern of differentiation was present in both, and



Text Fig. 5. The Standard Pattern of Differential Segments Established for the Two Populations.

A. The A chromosome has two differential segments relatively close to the kinetochore, and one very narrow segment much farther back in the long arm. The kinetochore is close to the end of the chromosome.

B. The B chromosome has a well marked terminal segment and no others.

C. The C chromosome has both a terminal and an interstitial segment.

D. The D chromosome has one, very narrow, interstitial segment in the long arm near the kinetochore.

E. The E chromosome has no affected regions.

that this pattern was the same for both. The pattern agrees in nearly all respects with the few published accounts that have been made for this species, and very probably represents one which we might consider standard for the species. As will be indicated later, there are some variations within the basic pattern. It is probable that these relatively minor variations have led some authors to reject the idea of a basic pattern for a species. Bailey (1949), for example, has written, "The pattern of differential segments produced by cold treatment cannot be employed as a valid or even useful taxonomic indicator for distinguishing species, for too many differences exist in the regularly occurring pattern in the Canadian Trillium erectum L. and that in plants of the same species growing in Tennessee". Some tendency toward a modification of this position can be noted in a later paper (1952), however, when he says, "The pattern of differential segments produced as a result of cold treatment for 96 hours is different in each of the six species included in this study". Haga and Kurabayashi (1953), working extensively with Japanese species, have taken the opposite stand, saying, "It is established indisputably by the present study that the differentiation is confined to specific segments of the chromosomes" and "Despite these minor variations in the final differentiation, the major patterns are quite constant, so that it can be used for genome analysis". Furthermore, they feel confident enough about its taxonomic value that they have used it as a major criterion in determining ancestry of certain polyploid species with which they are working.

The present work, being concerned with only two populations, not too widely separated, obviously cannot resolve this conflict, though it probably supports the latter viewpoint more strongly than the former. It can help, however, by showing the nature and kind of variation that might be expected to exist within a normal population, something which appears not to have been studied intensively previously.

The basic pattern as it appears in these two populations is illustrated in Text Fig. 5. Typically, there are three differential regions in the A chromosome. Two of them are located rather close together, and near the kinetochore. The third region appears much farther back distally, usually about two thirds of the way. In some cases it is very difficult to distinguish the first region, for there may be only a very small amount of regularly stained material between it and the attachment region. In some cases, too, the first two regions are only indistinctly separated from one another, appearing almost like one continuously differentiated region. The third region is regularly a much narrower one than either of the other two.

The B chromosome has a well marked, terminal, differential segment in the short arm. No other differentially reactive segments have been noted in either arm.

The C chromosome is marked with differential segments occurring both terminally and interstitially in the shorter arm. The interstitial may appear very long at times, looking almost as though it were a terminal, but is usually shorter and quite obviously interstitial. No regions have been detected either terminally or interstitially in the longer arm.

The D chromosome is marked by a very narrow differential segment in the long arm, located fairly near the kinetochore, though not close enough to be confused with it. This segment is usually the narrowest of the complement. No other differentiated segments have been observed in the D chromosome in these two populations, though some other investigators have reported a terminal segment in the long arm.

The E chromosome is completely lacking in any differential segments. It might be interesting to note at this point that *Trillium flexipes*, which grows intermingled with *Trillium grandiflorum* at Ann Arbor, shows well marked regions in both arms of the E, and this serves as an easy way of distinguishing the two. It might be presumed that, if the latter were being contaminated by the former, differential regions might be discovered in this chromosome.

This standard pattern, which has just been described, became evident rather early in the study. It also became evident, however, that by no means all of the chromosomes showed the full complement of differential segments which constitute the master pattern. Variations were noted from plant to plant, some showing all of the regions affected, and some showing only a few. It should be emphasized that each plant had its characteristic pattern, which was constant for all the cells. If the distal region was missing in one, but not both, of the A chromosomes in one cell, the same condition was found in the other cells. Thus it was possible to determine a definite pattern for each plant. Observations on a number of plants from each population which showed particularly well spread and stained figures are reported in Tables I, II, and III. These plants are from several different years, and from each week of the flowering season. It is believed that they constitute a thoroughly random sample.

Table I shows the percentage of possible regions actually observed in plants from both populations, using the basic pattern as a standard for comparison.

It can be seen that only about 60% of the potential regions were actually differentiated, which gives some suggestion of the variability encountered. It is also apparent that some chromosomes realize a much higher percentage of the potential than others. In this respect, the B leads all the others, its differential segment having been noted as present in all plants examined. The C shows the least development of potential regions, occurring with something less than half the maximum frequency. It should be pointed out, perhaps, that in setting up the standard against which to compare, each plant was considered to have fourteen differential regions, three in each A (6), one in each B (2), two in each C (4), and one in each D (2). The total possible number of standard regions in a population would then be the number of plants multiplied by fourteen. A similar procedure was used for each chromosome.

Comparison of the two populations as to degree of differentiation, shows no significant difference, either with respect to total

TABLE I

PERCENT OF STANDARD DIFFERENTIAL REGIONS OBSERVED

CHROMO-SOME	ANN ARBOR			EAST LANSING		
	Number Regions Found	Number Standard Regions	Percent of Standard	Number Regions Found	Number Standard Regions	Percent of Standard
A	151	246	61.4	154	270	57.1
B	82	82	100.0	90	90	100.0
C	83	164	50.6	75	180	41.7
D	56	82	68.3	58	90	64.5
E	none	none		none	none	
TOTAL	372	574	64.8	377	630	59.9

This table is based on 41 plants from the Ann Arbor population, and 45 plants from the East Lansing population. Standard regions are those differential regions considered to be characteristic for each kind of chromosome (cf. Text Fig. 5).

TABLE II

FREQUENCY OF DIFFERENTIAL SEGMENTS IN A, C, AND D, CHROMOSOMES

Possible positions of differential segments		Number of plants showing these positions		
Chromosomes A	A'	Ann Arbor	East Lans.	Total
0	0	-	-	-
0	1	-	-	-
0	2	1	-	1
0	3	-	-	-
0	1,2	4	11	15
0	1,3	-	-	-
0	2,3	-	-	-
0	1,2,3	11	3	14
1	1	-	1	1
1	2	-	-	-
1	3	-	-	-
1	1,2	1	4	5
1	1,3	-	-	-
1	2,3	-	-	-
1	1,2,3	3	2	5
2	2	1	7	8
2	3	-	-	-
2	1,2	-	-	-
2	1,3	1	-	1
2	2,3	-	-	-
2	1,2,3	-	-	-
3	3	-	-	-
3	1,2	3	-	3
3	1,3	-	-	-
3	2,3	-	-	-
3	1,2,3	-	-	-

Possible positions of differential segments		Number of plants showing these positions		
Chromosomes A	A'	Ann Arbor	East Lans.	Total
1,2	1,2	4	3	7
1,2	1,3	-	-	-
1,2	2,3	-	-	-
1,2	1,2,3	8	9	17
1,3	1,3	-	-	-
1,3	2,3	-	-	-
1,3	1,2,3	-	-	-
2,3	2,3	-	-	-
2,3	1,2,3	-	-	-
1,2,3	1,2,3	4	5	9
Chromosomes C	C'			
0	0	-	3	3
I	I	14	15	29
T	T	7	1	8
IT	IT	-	-	-
0	T	-	-	-
0	I	8	15	23
0	IT	-	2	2
I	T	3	3	6
I	IT	9	6	15
T	IT	-	-	-
Chromosomes D	D'			
0	0	8	5	13
0	I	10	22	32
I	I	23	18	41

Differential Regions		Total Occurrences		
Chromosome	Position	Ann Arbor	East Lans.	Total
A	1	57	62	119
A	2	58	68	126
A	3	36	24	60
C	I	57	62	119
C	T	26	13	39
D	I	56	58	114

In chromosome A:
Position 1 is close to kinetochore
Position 2 is slightly farther back distally
Position 3 is in distal 1/3 of long arm

In chromosome C:
T indicates terminal region
I indicates interstitial

Observations based on 41 plants from Ann Arbor, 45 from East Lans.

TABLE III

DEGREE OF HETEROMORPHY IN CHROMOSOME PAIRS

CHROMOSOME	COMPLETELY HOMOMORPHIC				HETEROMORPHIC FOR ONE POSITION				HETEROMORPHIC FOR TWO POSITIONS				HETEROMORPHIC FOR THREE POSITIONS			
	Number		Percent		Number		Percent		Number		Percent		Number		Percent	
	AA	EL	AA	EL	AA	EL	AA	EL	AA	EL	AA	EL	AA	EL	AA	EL
A	9	16	21.9	35.6	9	13	21.9	28.9	8	13	19.5	28.9	15	3	36.6	6.7
B	41	45	100.0	100.0												
C	21	19	51.2	42.2	17	21	41.5	46.7	3	5	7.3	11.1				
D	31	23	75.6	51.2	10	22	24.4	48.8								
E	41	45	100.0	100.0												
Total	143	148			36	56			11	18			15	3		

differentiation of the complement, or to differentiation of each type of chromosome. The two populations show the greatest divergence from one another in the C chromosome, but application of the Chi-square test indicates that this difference is not significant.

At first glance, it might appear that the establishment of a basic pattern must require examination of a rather large number of plants in a population. Actually, this does not seem to be the case. Reference to Table II makes this clear. It can be seen from that table that the least commonly differentiated segment is the terminal C. Yet it occurs some 39 times in the 86 plants recorded, and in 31 of the 86 plants. This is somewhat more than one third of the plants examined. It might be expected, therefore, that a moderate sized sample, if random, should show evidence of a terminal differential segment. The more frequently differentiated segments would, of course, require even smaller samples in order to be detected.

Once having established a standard, or basic, pattern for the populations, it becomes apparent that there are many variations within that pattern. Obviously, it is important to know their extent. It should be emphasized that the variations dealt with here are variations from plant to plant, rather than between the cells of a plant. The latter was found to be quite rare in this material. Most of the cells of a plant could be counted on to show the same differential pattern. Therefore, in the tables concerning these variations, the numbers refer to the number of plants showing a particular variation rather than to the number of cells.

The most prominent variation is the failure of one or more of the standard regions to differentiate. This may take place in both homologues, or in only one of the pair. Table II shows all the possible variations of this nature, and the number of each found in a representative sample taken over a period of several years and several different weeks of the growing season. It is apparent at once that a number of the possibilities are not realized at all, and that a number of others are of rare occurrence. For instance, in the A chromosomes there are 36 possible variations within the basic pattern. In the plants observed only one third of these possibilities were recorded. Furthermore, almost two thirds of the plants fall into one or another of four different patterns. Rather large percentages were found to have all three regions present in one homologue and either two or three regions in the other. Another major concentration was noted around the patterns in which one homologue had no regions and the other had either two or three regions clearly marked. The C chromosomes show a considerably higher percentage of the possible variations actually occurring, but again there is a marked tendency to concentrate around two or three of those possibilities. The D has only limited possibilities for variation, since it has only one potentially heterochromatic region.

Some investigators (eg. Darlington & LaCour (1940)) have suggested that in the species they investigated, the terminal segments were more variable in their occurrence than were the interstitials. This did not seem to be the case with the populations studied here. The B chromosome, which has the longer terminal segment, was found to show differentiation in all of the specimens examined. Thus, as far as occurrence goes, it is one of the least variable of the segments. The C chromosome is almost exactly the opposite, its terminal segment occurring less frequently than any other differentiated region in the complement. This species, then, does not seem to bear out the suggestion made by these authors. It should be noted, however, that the B terminals did exhibit some differences other than mere presence or absence. They sometimes appeared to be of different lengths, showed variation in intensity of staining, or exhibited qualitative differences in apparent consistency of the part involved. A further discussion of such differences will be presented in a later paragraph.

The explanation for these differences between chromosomes, and between patterns in the various plants studied is not clear. It has been suggested by several, and substantiated by critical measurements in the species Trillium erectum, (Wilson & Boothroyd (1944)), that the differences between homologues may be explained on the basis of loss of the whole or a part of the normally differentiated segment. It is postulated by some that terminal segments are lost more readily than interstitials, and that distal segments are more readily lost than proximal ones. As noted, we find no conclusive evidence for the former position. It is true that the distal segment in the A chromosome is found less often than either of the two much more proximally located ones. It is also noteworthy that in the D chromosome, the differential segment which is located quite near the kinetochore, occurs with approximately the same frequency as do the proximal two segments of the A, which are somewhat similar in their distance from the kinetochore. Thus, the position that distal segments are lost, or at least fail to appear, more regularly than the more proximally located ones does receive some support from this work. The fact that the more proximal number one position in the A is slightly less frequent than the somewhat more distally located number two can hardly be regarded as significant since they both occur close together, and they differ by only a very few occurrences. The fact that the distal, number three position in the A is much shorter than the other two, and thus is more readily obscured by twisted or overlapping chromosomes is of minimum significance due to the procedure used of examining many cells from each plant, and determining a pattern for the plant on that basis. Thus, if it were to be obscured for one of these reasons in some cells, it would become apparent in others if it were present. This is borne out by the observation that the segment in the D, which is the narrowest of all, was detected an appreciably greater number of times than was the number three position in the A.

The evidence noted here with respect to the terminal segments suggests that loss may be more closely connected with distance from the kinetochore, rather than by the mere fact of its being a terminal segment instead of an interstitial. The B terminal is relatively much closer to the kinetochore than is the C, and on the basis of the hypothesis might be expected to be of much more frequent occurrence, as it is. That this explanation is not completely satisfactory is indicated by the fact that the B segment is not so close as to necessitate supposing it to have a universal occurrence, which is the condition reported. Further, many more species must be intensively studied for this kind of information before such explanation can be considered more than suggestive. Finally, while there is some indication of an order of loss, there is no suggestion of what the initiating agent of such loss may be.

An alternative hypothesis is the one which suggests that presence or absence of differential regions is controlled by one or more alleles or sets of alleles. We might postulate, for example, a gene (more probably several genes) whose presence causes the retarded contraction, or possibly even elongation, and lessening of staining capacity in the distal differential segment of the A chromosome. We might then postulate that its allele does not result in these phenomena. If the former gene is present, the A will have a distal differential region, if its allele is present, that region will be lacking. Differences in length of segment, and intensity of stain, which are often observed, could then be accounted for in either or both of two ways. The two alleles might act quite differently under differing environmental conditions. For example, neither might be able to induce the phenomena we associate with differential regions at a temperature of twenty degrees, while at three degrees one might be able to induce a strongly marked region while the other still could not bring about the characteristic failure to contract and take up stain.

A few degrees difference in temperature, or a few hours difference in time of treatment might then alter considerably the capacity of a gene to bring about these changes. An attempt has been made to minimize this effect by keeping conditions as nearly uniform as possible during treatment, but many other possible factors cannot be controlled, as, for example, the soil conditions in which the plant is growing, its age, vigor, and rate of metabolism. Any one of these could conceivably alter gene action, though at this point this is purely speculative. A second way in which differences in length or intensity of stain might be accounted for would be to suppose that these phenomena are products of the actions of several genes acting in a cumulative fashion, assuming again that certain genes are effective, while their alleles are not. Various combinations of effective genes and their "ineffective" alleles would then result in varying degrees of contraction and staining in the affected regions. The weakness in this hypothesis seems to lie chiefly in the fact that there is strong evidence that real differences in chromosome length occur between those which exhibit differential regions and those which do not, indicating an actual loss of chromosome material. It might very well be true, however, that if a region is present, the degree to which it is observable is due to this sort of action.

If the "loss hypothesis" is correct, that is, absence of differential regions in a chromosome is due to actual loss of a region rather than to its failure to differentiate, then heteromorphic pairs (homologues with one or more regions lacking in one but not both) cannot be used as indicators of hybridity. In comparing populations, we could detect hybridity or difference in origin only when a differential region was present in one population and not in the other. If the alternative hypothesis is correct, the use of heteromorphic pairs as indicators is not so clear. It is quite possible, though by no means certain, that they might be usable in this fashion. There are several indications that they are doubtful indicators in our material, in any event.

Fortunately for the purposes of this work, it is not necessary to determine which of these hypotheses is correct, nor whether, if the latter one is more acceptable, heteromorphic pairs are indicators of hybridity. It is desirable to know whether these populations are hybrid, or whether their origin is the same, but that can be determined without knowing which hypothesis is correct. If failure of segments to appear is due to loss, as has been explained, differences in origin or hybridity of the two populations would be shown only by the occurrence of a differential region in one population and not the other. This has not been discovered in any of the material examined, and we may then assume, on this basis, that the two populations are essentially similar and of common origin, and that if any hybridity was involved it must have been before they separated from a common stock. If, on the other hand, the heteromorphic pairs may be used as indicators, then the fact that the two populations show about the same amount of heteromorphy is suggestive again that they are not significantly different, but are likely of common origin, differing only as any two isolated groups of similar origin might differ.

Table III shows the degree of heteromorphy in the two populations. It will be noted that in the two populations taken together the number of completely homomorphic pairs in the A, C, and D chromosomes, represents between 45 and 50% of the total. That is, about half of these pairs are homomorphic. The B and E chromosomes are, of course, wholly homomorphic. The remaining pairs, as can be seen, are heteromorphic in differing degrees. Maximum possible heteromorphy was shown in less than one quarter of the pairs.

Of the three chromosome pairs which exhibit well marked heteromorphy, the A chromosome shows the greatest amount, both from the standpoint of numbers with the maximum possible amount, and from the standpoint of total heteromorphy observed. It will be noted that the A chromosome may be heteromorphic for one, two, or three regions. Here the latter condition is termed maximum possible, while total heteromorphy would include those pairs which showed the two lesser degrees, as well as those exhibiting the maximum condition. The D chromosome shows the least total heteromorphy though it is not significantly different from the C in this respect when tested by the Chi-square method. The A, of course, is significantly different from either the C or the D. A great deal of this difference is due to the greater number of regions in the A and hence the greater number of possibilities for heteromorphy. Actually it is somewhat more surprising to find the close correspondence of the C and D than it is to find the wide divergence between the A and the other two.

Comparisons have been made between the two populations with respect to the frequency of occurrence of the various regions, the various combinations in which they occur, and the degree of heteromorphy. For the most part (see Tables I, II, III), the differences are not of large degree. In general, the two correspond quite closely, varying no more than might be expected in two populations which probably have been separated from a common ancestral stock for a considerable number of generations. It might be anticipated that in the time probably involved, each population would tend to vary somewhat from its ancestral condition, and it would be logical to expect that the variations might be somewhat different in the two. Hybridization, if it occurred after separation, might be expected to increase the differences between the two. The observation of very few significant differences, therefore, is taken to further indicate a lack of hybridity in these populations in any very recent time.

Reference to Table I shows that correspondence between the two populations is close in nearly all instances. That is, the regions occur with about equal frequency in the two. This is true when we consider the total number of occurrences, or the occurrences in each type of chromosome. The C chromosome shows the greatest difference, the Ann Arbor population showing about 51% of the standard regions as compared with about 42% at East Lansing. Application of the Chi-square test shows that there is no significant difference between the two populations with respect to frequency of differential regions, either in the complement considered as a whole, or in any of the five different types of chromosomes considered singly. It seems rather doubtful that such conditions would prevail were the two populations resultants of hybridity occurring since their separation from a common ancestral stock.

More differences between the populations are noted when the minor patterns within the species pattern are considered (cf. Table II). Some of these may be of high enough order to be of statistical significance. The pattern in which one A homologue shows no regions and the other shows all three is a good example. This condition was noted in eleven of the forty-one plants recorded in the Ann Arbor population, and in only three of forty-five in the one at East Lansing. Application of the Chi-square test gives a value of 6.39, which is considerably above the critical value of 3.84 for the 5% level of significance. Other patterns which exceed the critical value, though to a lesser degree, are the one in which the number two region and no others is present in both A homologues, the occurrence of both terminals and no interstitials in the C chromosome, and the presence of an interstitial in one D homologue but not the other. No other patterns occur in significantly different numbers in the two populations.

If each region is considered separately, and its total number of appearances in each population is compared, the Chi-square test suggests that significant differences may be present in the number three region of the A, and in the terminal segment of the C. Values obtained are 5.61 and 7.29 respectively.

Comparison of the populations with respect to degree of heteromorphy and homomorphy suggests two possibly significant differences between the two. The D chromosome appears to be homomorphic in an appreciably greater number of plants in the Ann Arbor population, and the A chromosome, while not differing significantly in overall heteromorphy in the two, does appear heteromorphic for all three positions in a very much greater percentage of the Ann Arbor plants than in those from East Lansing. In all other comparisons of this kind there appears to be no significant difference between the two populations.

The cytological evidence presented in these first three tables, then, does show some significant differences between the Ann Arbor and the East Lansing plants. Such differences should be expected in two isolated populations, and it seems to me that the noteworthy point is not that such differences do exist, but that they are, if anything, of somewhat smaller order than might be expected. Certainly there is little to suggest that they are the result of any recent hybridity or that there is now at work any introgression from other species. This is particularly significant with respect to the Ann Arbor population where Trillium flexipes is growing intermixed with the Trillium grandiflorum.

It might be noted here that, on the basis of relatively small samples, the overall pattern for Trillium flexipes appears to be quite different from that of Trillium grandiflorum. In many respects it appears to be closer to the pattern established for Trillium erectum by Wilson and Boothroyd (1941). The situation is somewhat complicated by the fact that the forma *rubra* occurs here, as well as the more typical, white flowered form. It is not yet wholly clear whether the differential pattern is significantly different in these two forms. Some indications of the possible pattern may be obtained from the following description of the genome as it appears to me from limited examination. The *A* chromosome is much like that of *T. grandiflorum* except that it rather consistently has one long region near the kinetochore, rather than the two separated regions noted in that species. The third region is also present here. The *B* chromosome has two interstitials very close to the kinetochore, one on either side of it. This, of course, is quite different from the condition in *T. grandiflorum*. In addition, it is doubtful whether a terminal differential segment is present, though the short arm does appear somewhat knobby in several preparations. The *C* chromosome appears to have only a terminal segment in the short arm, with no interstitials being detected. This too, differs from the condition in *T. grandiflorum* where there are both interstitial and terminal segments, but the former is much more frequent than the latter. The *D* chromosome differs in having a definitely affected terminal segment in the short arm, and in the apparent lack of any interstitial. The *E* chromosome, which in *T. grandiflorum* is consistently unaffected, shows one interstitial in the shorter arm, and two in the longer. In addition, in the forma *rubra* at least, there appears to be a rather consistent occurrence of one or more, apparently centric fragments. A number of additional samples will have to be taken before they can be considered to be characteristic of the species or form however. In any case, it is apparent that there are present enough differences, including a number of differential regions which are not found in T. grandiflorum, so that any hybridity between the two ought to be fairly readily detected cytologically, especially in as extensive a sampling as was undertaken here. No such evidence was found in any of the material. This is particularly interesting in view of the fact that there does seem to be a definite overlapping of the two species in some of their morphological characteristics, especially with respect to leaf shape, and to a lesser extent with anther/filament ratios. This is another example of the manner in which cytological evidence can be of value taxonomically. Morphologically there is overlap between the two species. Cytological evidence suggests fairly strongly that such overlap is not due to hybridization.

While the variations noted above are the only ones that have been quantitatively recorded, there are some others which deserve mention. One of the most obvious is difference in length of the heterochromatic regions. A second is the difference in staining capacity. A third is an apparent qualitative difference in composition of the affected region apart from the staining capacity. These may, or may not, be significant as indicators of differences between homologues, but being present should be considered.

The difference in length of the heterochromatic regions is a fairly common, but very difficult one to assess accurately. There seem to be several factors at work affecting length, some of which may be genetic, and some possibly environmental. Furthermore, there are several kinds of differences, such as differences from cell to cell in a plant, differences between plants, and differences between homologous chromosomes in a plant. It is probable that these several types of differences have different causal agents. Lastly, types of chromosomes are not alike in the frequency or intensity of expression of length differences. The B chromosome, for example, varies tremendously in the apparent length of its terminal differential segment. It sometimes appears quite short, but occasionally appears extremely long. The C chromosome is the one most likely to show distinct, and consistent differences in length of interstitial zone between homologues. The D chromosome shows the least difference in length of interstitial segments. There is rarely any observable variation in it.

Some variation between cells in length of chromosomes is undoubtedly due to the effects of the cold treatment. Boothroyd (1953) has shown, for instance, that the earlier in prophase the cold treatment is initiated, the more differentiation is likely to occur. Undoubtedly cells are at different stages of mitosis when cold treatment is initiated, and it is natural to expect some variation in degree of differentiation, and hence length of affected segments, between cells. Much of the difference in length of B terminal segments may probably be properly attributed to this, the B seeming to be particularly sensitive in this respect. It might not be unreasonable to suggest at this point that metabolic activity of the cells also could very possibly affect the differentiation, including length of segment. Suggestive in this connection is the report by Ris (1951) that the metabolic rate in cells definitely affects the length of the chromonemata and the intensity of the Feulgen reaction in many organisms. Environmental differences, then, through their influence on metabolism, might be a contributing factor, and such things as temperature, moisture, minor soil differences, etc. could result in some minor differences in apparent length of segment. Under this hypothesis other conditions such as health and vigor of the plant, age, etc. would also play a part. Much physiological research needs to be done on this problem, as well as considerably more critical work on the actual length, and variation of same, of the differential segments. This would, however, very readily explain differences in length between different plants.

The difference in length of differential segment in two homologues in the same cell can scarcely be accounted for by either of the hypotheses mentioned above. This seems to be more directly controlled by gene action. Such differences occur with great enough frequency to suggest that they may be of some value in determining relative heteromorphy, but no quantitative record was kept. This was in part due to difficulty of determining exactly when there was, and when there was not, such a difference. In general, slight differences can be more readily detected in material which is not highly contracted. Since there is difference in contraction between the cells of a plant, as well as differences in average contraction of the chromosomes between plants, recording of differences in length of heterochromatic regions between homologues would be only approximate at best. A very refined method of measurement, including provision for measurement of twisted and vertically deviating portions of segments, would have to be worked out and applied, and there would have to be a large number of excellently spread metaphases in order that a statistically significant pattern might be established for each plant. The value of the additional data that might be obtained in this manner was not considered to be of sufficient importance to the problems considered in this work to warrant the expenditure of time which it would necessitate.

Study of the C interstitial and the B terminal, in which the differences in length of affected regions between homologues occurs most frequently, suggests that all degrees of difference may exist, from a condition in which the difference is barely detectable to one in which it is very obvious. It is also probable, though not quite as certainly so, that the degree of difference is fairly constant for a given plant, though differences in chromosome contraction between the various cells may obscure this constancy. The mechanism for such a phenomenon is not entirely clear, but it may be suggested that the heterochromatic regions are under the influence of a number of genes, acting in a cumulative fashion. Loss of one or a few might then be expected to result in a shorter region. Alternatively, of course, it may be that small parts of these regions are lost from time to time, without necessarily losing any of the genes which control their differential reactivity. This too would result in a consistent difference in length between homologues. Wilson and Boothroyd (1944) have shown, by using critical measurements, that when the majority of cells in a plant show a lack of a particular differential segment, that lack does represent an actual decrease in overall length of the chromosome. This does not appear to be the case in situations where some cells show the differential segment, and others in the same plant do not. If whole regions can be lost, as they suggest, it seems reasonable to assume that parts of regions may be lost as well as whole regions. This might explain the variations in length which have been noted between homologues, and between plants.

It is, of course, well known that variations in cold treatment, with respect to both temperature and time, can result in variation in the amount of differential reactivity, and hence in the length of the affected segments. Since materials reported on here were all subjected to the same time of treatment, and degree of cold, it is presumed that these factors are not of great consequence in bringing about the observed variations in length.

Summarizing the observations and conclusions about the variations in length of differential segments, it may be stated that several types of variations seem to be present. It is felt that these may have different causes. Variations between homologues may have a genetically controlled origin, but because there seems to be evidence of a real difference in total length of the two members of such a pair, it is felt that it is more likely that there is an actual loss of much, or little, of a heterochromatic segment. Such losses probably do not occur very frequently, but when they do occur, presumably continue in the shortened condition through succeeding generations. It appears that losses of heterochromatic material may vary from very minor portions of a segment to loss of almost the complete segment. Whether major losses have resulted from a series of small losses gradually accumulating to major proportions, or whether they result only from a single loss, or from a combination of both is not known. The reason for more frequent observation of this phenomenon in the *C* interstitial and in the *B* terminal probably lies in the fact that these two are typically the longest segments. The other segments, especially the distal *A* and *D* interstitial are so narrow that loss of any portion of the segment would result in almost complete elimination of it. The causal mechanism of such loss is unknown at present. Variations from cell to cell, and in the general amount of contraction from plant to plant are presumed to be due to several other causes, and are not regarded as being of the same degree of constancy as those just noted between homologues. Major causal agents are most likely the stage of mitosis at the time of beginning of the cold treatment, and possibly the metabolic rate of the cell or plant as it, in turn, may be affected by age, temperature, soil conditions, general vigor of the plant, or other environmental conditions. Though no accurate record was kept, it is felt that the two populations show about equal quantity and kind of variation in length of differential segments, almost certainly not varying in this respect to any greater degree than in the characters reported in Tables I, II, and III, and discussed previously.

There seem to be occasional differences in the staining capacity of differential segments in different plants and in different cells of the same plant. There is some indication that this may be correlated to a limited degree with the differences in length noted in the preceding paragraphs, though this has not been verified statistically. There is often a certain constancy about this type of difference too, when it occurs between homologues in the cells of a particular plant. In several cases there was noted a very distinct region in one homologue, showing practically no stain whatever, while the corresponding region in the other homologue showed a definite stain, though not as great in intensity as in the non-differential segments. In other cases, both homologues showed some staining capacity in the differential segments, but the stain was somewhat more intense in one than in the other. In general, the degree of difference appeared to be fairly nearly the same throughout the cells of a plant, though it must be admitted that there is some element of subjectivity in determination of the degree of staining capacity present. Because there are a fair number of these cases in which homologues show a difference in staining capacity in the cells of a plant, it seems rather likely that this, too, is controlled by one or more pairs of allelic genes. Whether they are the same genes that control length of the segments has not been determined at this point.

The last, and least objectively determinable variation, is the observed qualitative difference in composition of the heterochromatic regions. The regions may show little difference in stain intensity or in contraction from the condition present in the non-differentiated parts of the chromosomes, yet be readily recognizable as being unlike the latter. This appears to be due to a difference in apparent density. It was noted most frequently in the _B_ terminal segments, though it was not common even there. Other segments exhibited the phenomenon only rarely. It is quite probable that it has but little significance for this work. It did occur in both the East Lansing population and the one at Ann Arbor, but occurrences were too infrequent to make any judgment as to comparative frequencies in the two. There were no cases where it could be ascertained definitely that the segment of one homologue differed significantly in density from that of the corresponding segment in the other homologue, but this would be particularly difficult to determine, and such differences might be present without being detected. It is possible, of course, that such a condition marks the very lowest degree of differentiation which can be detected, a sort of first step, which is followed by decrease in intensity of stain and differential contractility. Some support for this view is lent by the observation that, as a general thing, the phenomenon is encountered only when other differential regions in the complement also show some lessening of differential reactivity.

2. Meiosis

Although there was little indication cytologically that hybridization might have been present in either of the populations, it was felt that it might be advisable to check meiosis in several of the plants from each population. It was discovered that microsporogenesis occurs during the second or third weeks in September here, and is over in nearly all plants in a rather brief period. In the material examined, pairing seemed to be quite normal, there being no evidence of hybridity in any of the samples from either population. This, of course, verifies what was indicated in the study of the other aspects of their cytological condition, so that it seems we should be reasonably confident that hybridity has not played any major role in the present composition of either of the populations studied.

B. Morphological Considerations

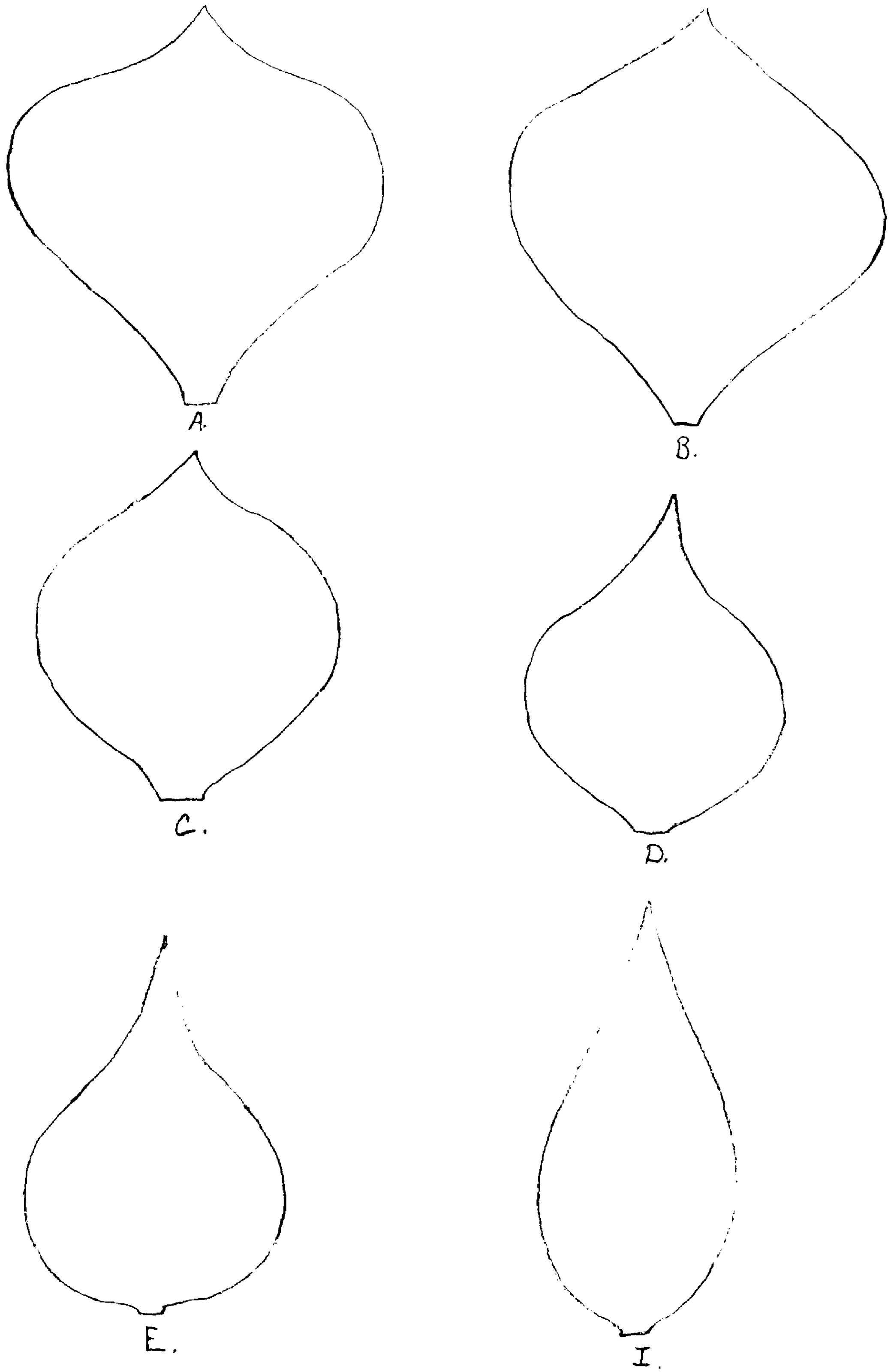
1. Introductory Comment

It has been pointed out previously that Trillium grandiflorum does not vary in many morphological characters, though the few in which variation is noted have a wide range of variability. Furthermore, it has become evident that a factor which might appear to be a single variable may often be the resultant of the interaction of several other factors varying more or less independently from one another. Variations in leaf shape, for example, appear to be determined not by one, but by four separate factors. In general, the factors selected for inclusion here are those which seem to be least sensitive to seasonal changes. On this basis such variables as recurved styles, anthocyanin in the stem, peduncle length, and some others have been omitted from this discussion. The characteristics retained include the following: leaf shape, sepal shape, petal shape, anther/filament ratio, notching of sepals and petals, and shape of the ovary. Data on these are summarized and presented in tabular form.

Tables IV through VII include data from one season's collections only, since it was only in 1954 that conscious attempts were made to collect from the two areas at as nearly the same time as was possible. Examination of the first two year's collections led to the belief that the time of collection might influence some of the factors being studied. Therefore, if accurate comparisons between the two populations are to be made, they should be based on plants in comparable stages of seasonal growth. If one population has an excessively high number of collections from early in the season, and the other has a majority of plants collected late in the season, a greater difference than is actually present may be indicated in the means of some of the factors involved. In general, collections made at presumably comparable periods of growth, though in different years, show similar means in the factors under consideration here. The closest correspondence of this kind is in that of collections made on May 9, 1952 and the same date in 1954, which agree exactly in L/W ratios of their leaves. In no case is there a significant difference between the means for the collections made in the previous seasons and the comparable ones for the season of 1954. Also there are only a very few plants recorded for those years which fall outside the range of variability established for the season of 1954.

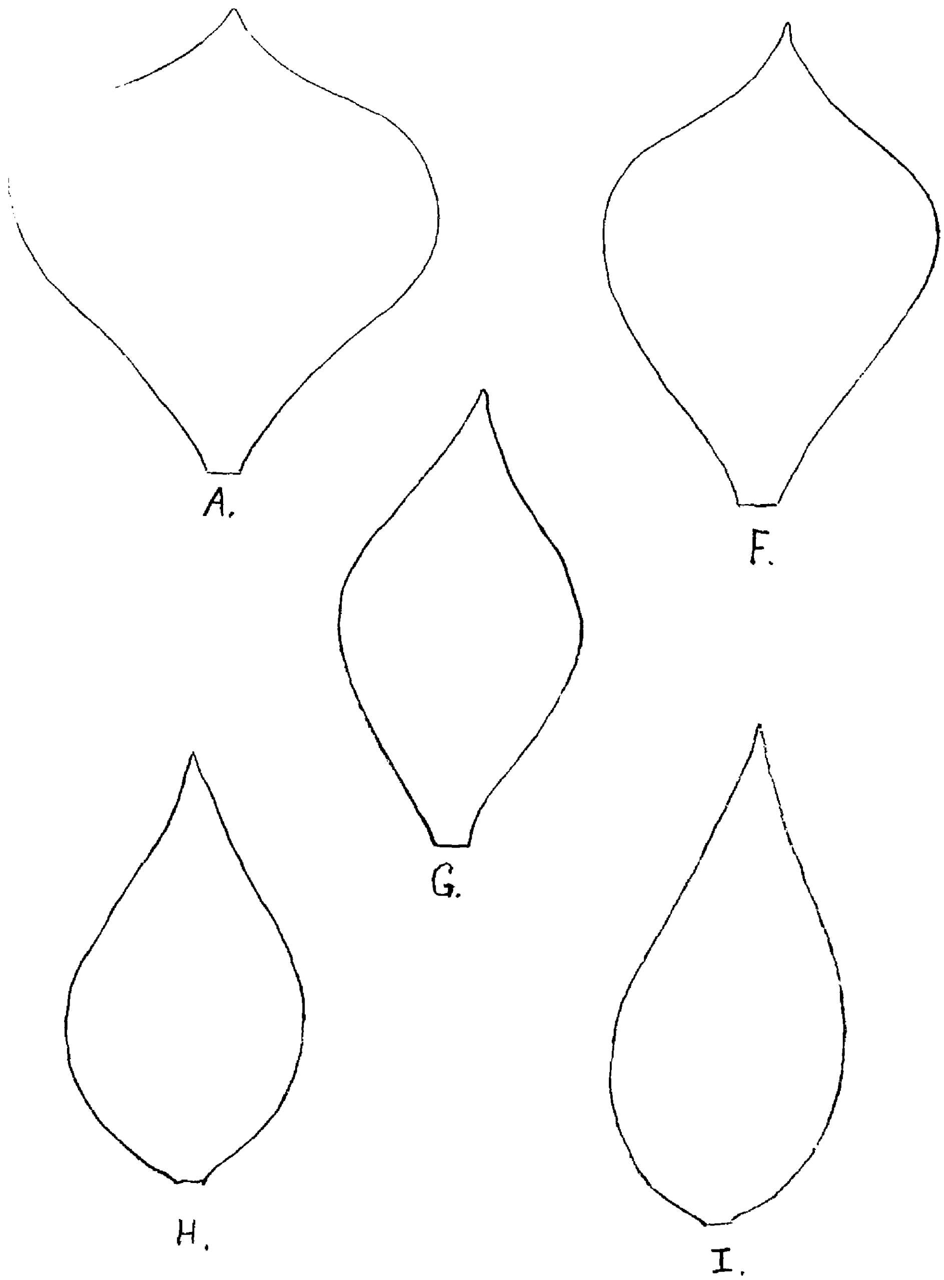
2. Analysis of Data on Leaves

The procedure for translation of shape of leaves into a set of measurements has already been described. It may be recalled at this point that the shape of the leaf is considered as being determined by the type of body, tip, and base. The body shape is determined by two factors, L/W and displacement, which have been found to vary quite independently of one another. The tips and bases also vary independently of one another, but are apparently somewhat limited in the extent of their variability by the L/W and displacement factors. This sort of mechanism provides for a rather wide range of leaf shapes, as is illustrated in Text Figures 6 and 6a which show several of the many types encountered in these two populations. Two of the several extremes of the range of variability are indicated at A and I with the other letters representing intermediates. The latter could be arranged in two or more series running from A to I, though there is no evidence that such series actually exist. It will be noted that type A has a low length/width ratio, a relatively wide tip angle (acute but not acuminate), a narrow (cuneate) base, and a high displacement factor (the x/y ratio referred to in the section on procedure). The latter factor suggests the amount of leaf blade above the midline, and is so-called because it indicates the amount of displacement



Text Fig. 6. Series of Variations in Leaf Shapes.

Explanation in text.



Text Fig. 6a. Series of Variations in Leaf Shapes.

Explanation in text.

of the bulk of the leaf toward the tip or the base. It might be pointed out here that this type of leaf is similar to that found in many of the T. *flexipes* plants.

Type B is similar to A except for a reduction in the displacement factor, that is, a shifting of the bulk of the blade toward the base, and some broadening of the base. Raising of the L/W ratio from a type like that represented at B would then result in a leaf similar to that represented at C. Further broadening of the base and narrowing of the tip would result in types like those shown at D and E. Further increase in the L/W ratio would then result in the extreme type noted at I. This comparison should not be construed to indicate a belief that these types were derived from one another in an evolutionary sense. It is simply a convenient way of showing how modifications of one or two factors can produce quite different shapes.

If, instead of lowering the displacement factor from a type like that in A, it remained unchanged, while the L/W ratio increased, a type similar to that at F would be produced. It might be noted that such increase in L/W would probably interact with the tip to cause some narrowing of the angle there. Further displacement downward would result in a shape something like that at G. Again such a downward displacement might be expected to result in a further narrowing of the tip angle, as has been indicated. Observation has shown that this need not be so, for there are types intermediate between these two. Further downward displacement would almost certainly result in broadening of the base, and a type like that at H might result. Further narrowing of the tip angle, plus increase in L/W ratio, would produce a type like that at I. It is apparent, of course, that A and I contrast markedly with one another with respect to each of the four factors, A having high displacement factor, low L/W ratio, wide tip angle and narrow base, while I has low displacement factor, high L/W ratio, narrow tip angle and wide (rounded) base.

It would be convenient if there were some way to express the total shape, resulting from the interaction of the four factors mentioned, by some index number. Index numbers have been used in a number of population studies involving hybrid entities (cf. Anderson & Turrill (1938), Anderson & Whittaker (1934)). The usual procedure is to divide each factor into several classes on the basis of the intensity of its expression, and then to assign an index number to each class. Totalling the numbers for all the factors in a plant gives an index number for the whole plant. This usually results in some plants with very low numbers, some with very high numbers, and a considerable number of intermediates. Looking at these numbers one can tell a good deal about the characteristics of the plant in question. It might be, for example, that a low number would indicate the extreme represented by long narrow leaves, a high degree of pubescence, white flowers, and short internodes, while high numbers would indicate the opposite conditions. Such methods were found to be of limited use here because the factors appear to be varying independently, rather than tending to stick together as they might be expected to do in hybrid populations. This, in itself, may be taken as indication of the fact that we are dealing with stabilized populations rather than with recently hybridized groups, or groups in which introgression is now actively taking place.

Samples of 100 plants from each population, taken in equal numbers at each of the four weekly intervals, were analyzed statistically for the extent of correlation between the factors considered responsible for determination of leaf shape. Several other check samples were taken from previous years' collections and similarly analyzed. From these studies it was quite apparent that almost no correlation exists between the length/width ratios and the displacement factors. There is evidence of some correlation between the former and both the tip angles and the basal angles, though it apparently is not of very high order. A somewhat similar relationship exists between the displacement factor and the tip and basal angles. This might be predicted when it is realized that, although there can be some variability in tips and bases without any alteration of either the length/width ratio or the displacement factor, the two latter do set limits to the extent of this variability. The fact that a number of instances of fairly extensive differences in tips and bases do occur in leaves with essentially the same length/width ratios and displacement factor, is taken to indicate that the taper of these parts is under different genic influence. Length/width ratios do not set any limits on the displacement factor, nor does the latter restrict the former in any way.

Statistical comparisons of the two populations have been made with respect to the four factors which have been found important in determining leaf shape. These are tabulated in Tables IV, IV-A, and IV-B. In addition the population means for each of the four factors have been determined for both populations, and these figures have been used to reconstruct the leaf which may be regarded as "typical" or average for each group. These reconstructions are pictured in Text Fig. 7. Some of the extreme variations from the average are depicted in Text Figs. 8, 9, 10, and 11, though, as will be pointed out, great care should be used in employing the latter in any comparative way. Because another species, Trillium flexipes, grows intermingled with the plants studied at Ann Arbor, small samples of that species have been analyzed, means established, and a reconstructed "typical" leaf shown in Text Fig. 12. Text Figs. 13 and 14 are used to show some of the variations in this species.

Examination of the "typical" leaves from each of the populations, as shown in Text Fig. 7, emphasizes the rather close similarity of the two, especially from the standpoint of their mean shape. A quick glance might suggest that there are scarcely any differences between them. A closer examination would reveal that the Ann Arbor plant does have a lower displacement, broader base, narrower tip, and slightly higher length/width ratio. But the differences in each case are so slight that it is extremely doubtful that they could be considered at all significant. They certainly appear as though they belonged to the same population.

TABLE IV

LEAF MORPHOLOGY DATA -- SUMMARY OF SAMPLES FROM TWO POPULATIONS

SERIES	N	AREA	LENGTH/WIDTH		DISPLACEMENT		TIP ANGLE		BASAL ANGLE	
			X	S.E.	X	S.E.	X	S.E.	X	S.E.
E424	24	E.L.	1.48	.043	.54	.035	32.3	.88	51.5	1.18
E51	25	E.L.	1.45	.041	.61	.035	33.4	1.15	50.7	1.49
E58	25	E.L.	1.39	.037	.69	.035	36.4	.90	51.0	1.41
E515	25	E.L.	1.47	.031	.62	.037	34.2	.88	49.3	1.27
Y425	25	A.A.	1.56	.035	.50	.027	29.8	.80	56.2	1.39
Y53	25	A.A.	1.43	.031	.57	.039	33.4	.78	50.7	1.16
Y59	25	A.A.	1.51	.031	.57	.029	31.9	.82	52.6	1.20
Y516	25	A.A.	1.47	.037	.64	.022	34.8	1.02	48.9	1.00

TABLE IV-A

STATISTICAL COMPARISON OF TWO POPULATIONS WITH RESPECT TO LEAF CHARACTERS

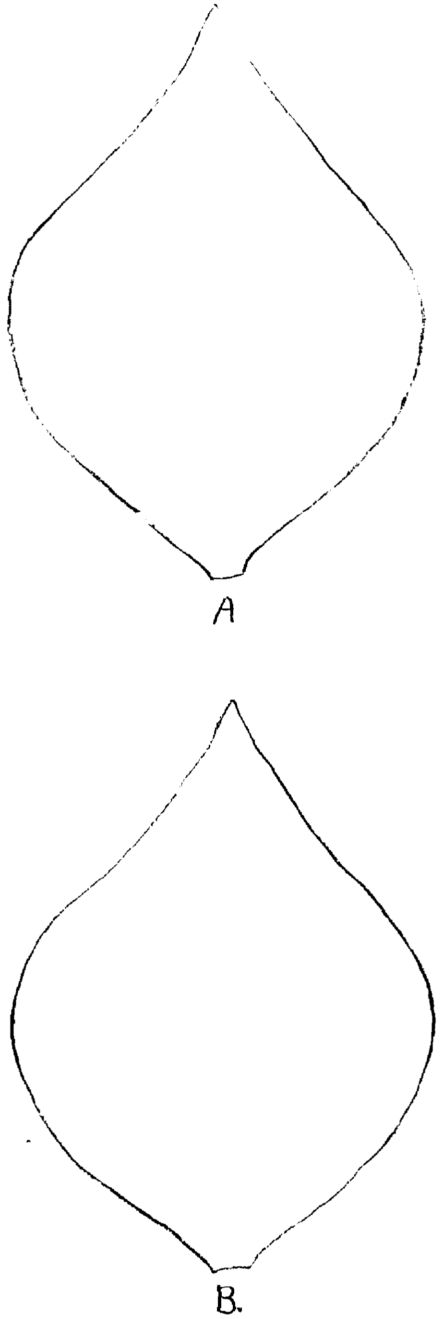
SERIES	N	$\overline{X}$	S.D.	S.E.	D_x/SE_d	t_{05}
			Length/Width Ratio			
E424	24	1.48	.21	.043	.28	2.06
Y425	25	1.56	.17	.055		
E51	25	1.45	.20	.041	.07	2.06
Y53	25	1.43	.15	.031		
E58	25	1.39	.18	.037	.46	2.06
Y59	25	1.51	.15	.031		
E515	25	1.47	.15	.031	.00	2.06
Y516	25	1.47	.18	.037		
			Displacement Factor			
E424	24	.54	.17	.035	.16	2.06
Y425	25	.50	.13	.027		
E51	25	.61	.16	.033	.15	2.06
Y53	25	.57	.19	.039		
E58	25	.69	.17	.035	.47	2.06
Y59	25	.57	.14	.029		
E515	25	.62	.18	.037	.08	2.06
Y516	25	.64	.11	.022		

N indicates the number of leaves in the series. X indicates the mean of the series. S.D. indicates the standard deviation. S.E. indicates the standard error. D_xSE_d indicates the difference between the two means divided by the standard error of that difference. t is the Fisher's t value at the 5% level of significance. If D_xSE_d exceeds t, the difference between the means is significant.

TABLE IV-B

STATISTICAL COMPARISON OF TWO POPULATIONS
WITH RESPECT TO LEAF TIPS AND BASES

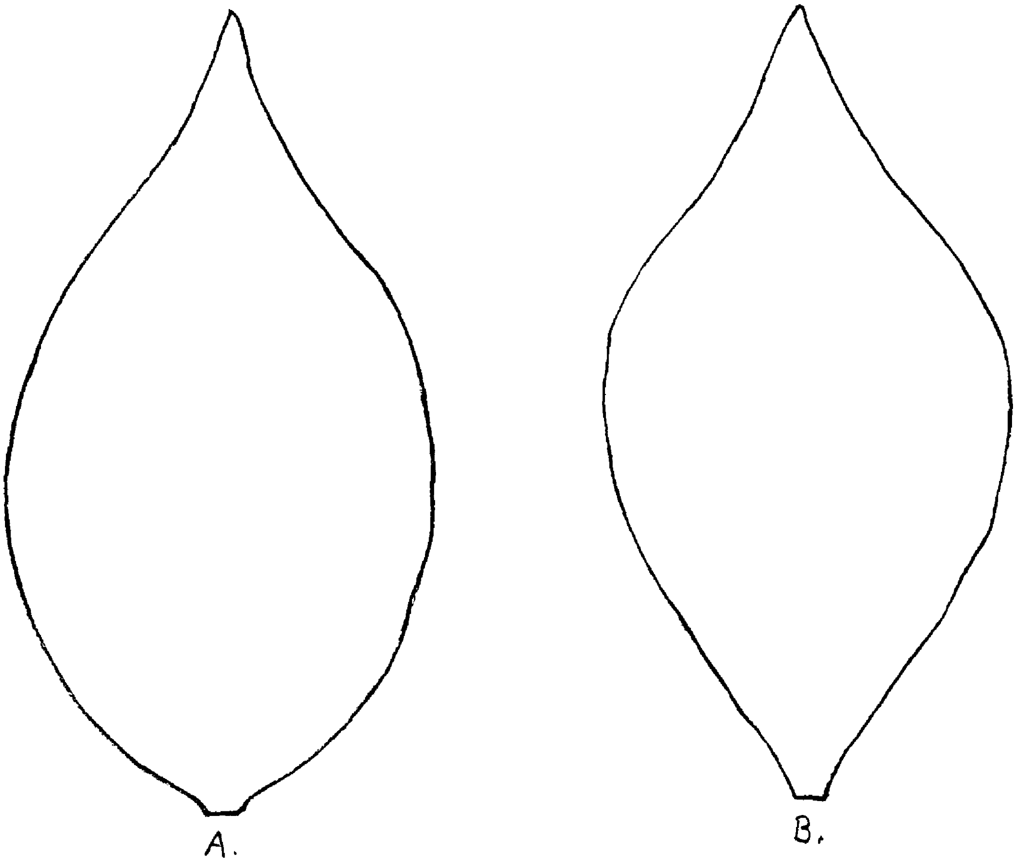
SERIES	N	$\overline{X}$	S.D.	S.E.	D_x/SE_d	t_{05}
Tip Angle						
E424 Y425	24 25	32.3 29.8	4.3 3.9	.88 .80	1.93	2.06
E51 Y53	25 25	33.4 33.8	5.6 3.8	1.15 .78	0.00	2.06
E58 Y59	25 25	36.4 31.9	4.4 4.0	.90 .82	3.44	2.06
E515 Y516	25 25	34.2 34.8	4.3 5.0	.88 1.02	.44	2.06
Basal Angle						
E424 Y425	24 25	51.5 56.2	5.8 6.8	1.18 1.39	2.92	2.06
E51 Y53	25 25	50.7 50.7	7.3 5.7	1.49 1.16	0.00	2.06
E58 Y59	25 25	51.0 52.6	6.9 5.9	1.41 1.20	.99	2.06
E515 Y516	25 25	49.3 48.9	6.2 4.9	1.27 1.00	.26	2.06



Text Fig. 7. "Typical" Leaves Reconstructed Using Means of the Samples Recorded in Table IV.

A. Leaf from East Lansing Population.
L/W 1.45; displacement .615; tip 34; base 50.6.

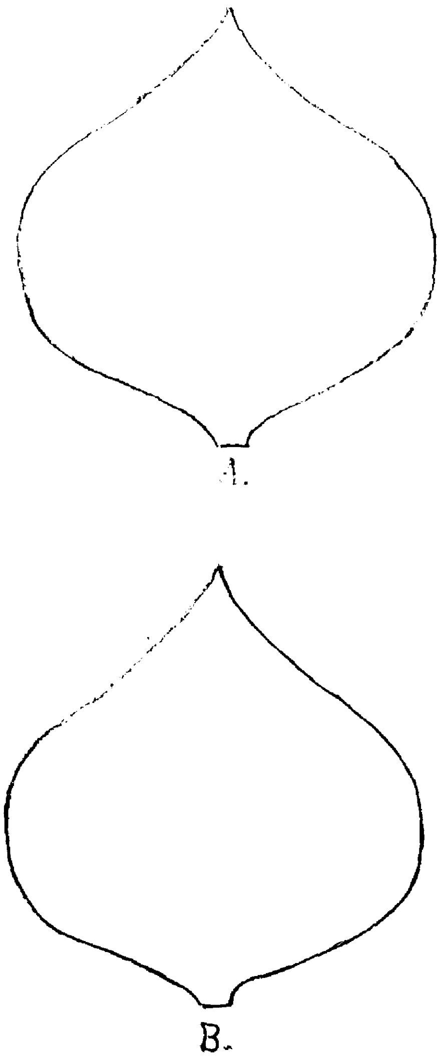
B. Leaf from Ann Arbor Population.
L/W 1.49; displacement .57; tip 32.5; base 52.



Text Fig. 8. Leaves with Maximum L/W Ratio.

A. Leaf from Ann Arbor Population.
L/W 2.05; displacement .52; tip 28; base 55.

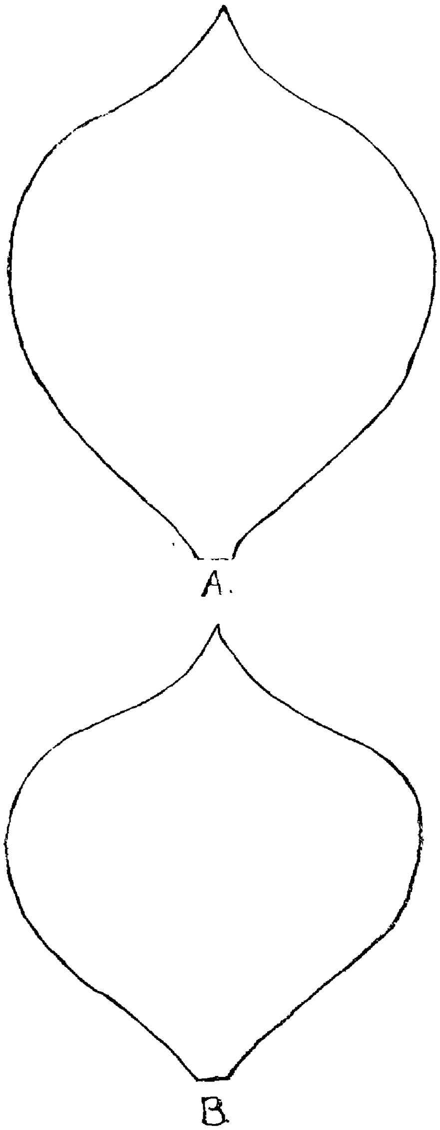
B. Leaf from East Lansing Population.
L/W 2.06; displacement .98; tip 24; base 35.



Text Fig. 9. Leaves with Minimum L/W Ratio.

A. Leaf from Ann Arbor Population.
L/W 1.12; displacement .70; tip 44; base 57.

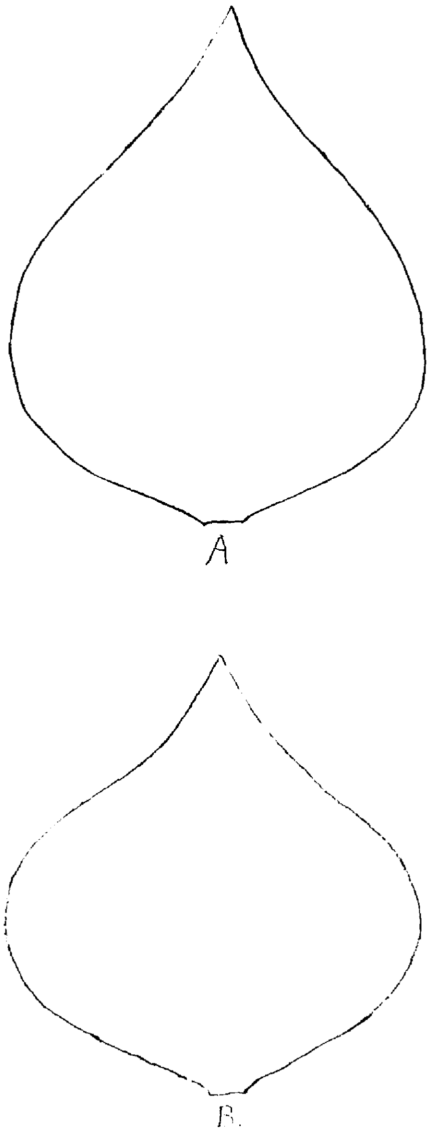
B. Leaf from East Lansing Population.
L/W 1.12; displacement .49; tip 42; base 62.



Text Fig. 10. Leaves with Maximum Displacement.

A. Leaf from Ann Arbor Population.
L/W 1.40; displacement 1.10; tip 55; base 51.

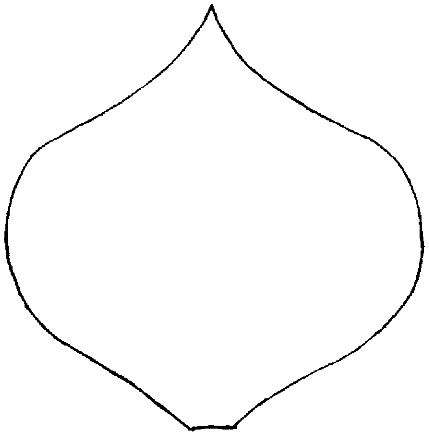
B. Leaf from East Lansing Population.
L/W 1.19; displacement 1.21; tip 45; base 47.



Text Fig. 11. Leaves with Minimum Displacement.

A. Leaf from Ann Arbor Population.
L/W 1.33; displacement .21; tip 35; base 66.

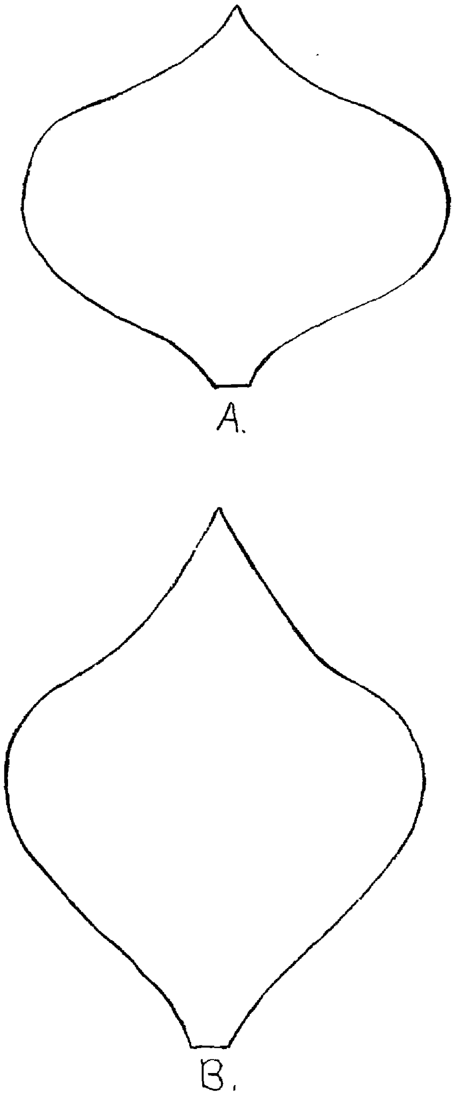
B. Leaf from East Lansing Population.
L/W 1.15; displacement .19; tip 41; base 61.



Text Fig. 12. _Trillium flexipes_ Leaf

Reconstructed from the means of the four factors determining leaf shape in a small sample from the population at Ann Arbor.

L/W 1.15; displacement .85; tip 43; base 49½.

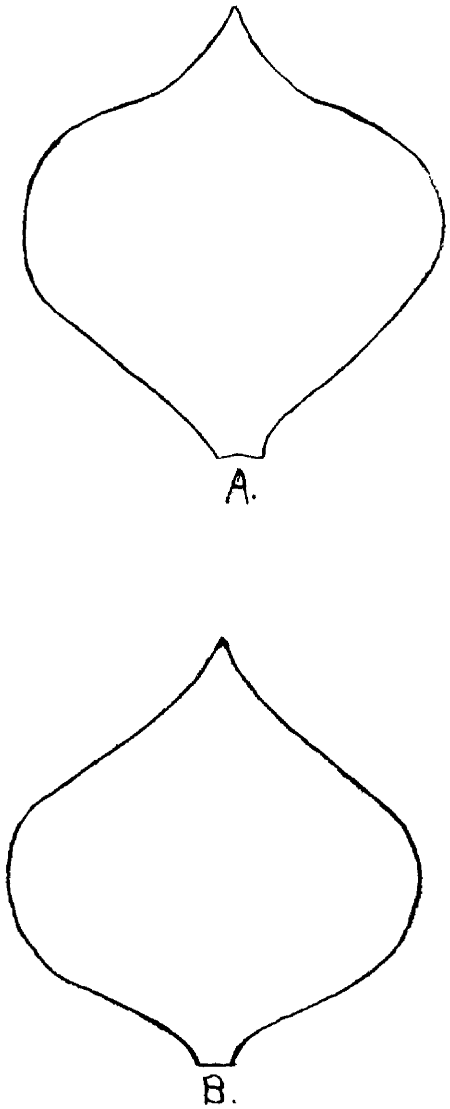


Text Fig. 13. Variations in Leaf Shape in *Trillium flexipes*.

Extremes in L/W Ratios.

A. Leaf with Low L/W Ratio.
L/W 1.01; displacement .93; tip 51; base 53.

B. Leaf with High L/W Ratio.
L/W 1.44; displacement 95; tip 35; base 40.



Text Fig. 14. Variations in Leaf Shape of _Trillium flexipes_.
Extremes in Displacement.

A. Leaf with High Displacement.
L/W 1.17; displacement 1.19; tip 42; base 45.

B. Leaf with Low Displacement.
L/W 1.07; displacement .60; tip 44; base 55.

When these two leaves are compared with the "typical" leaf of *Trillium flexipes* (cf. Fig 12), basic differences are apparent at once. The latter has a considerably lower length/width ratio, a much higher displacement, and a broader tip. These differences are of an order which is considered significant, so that one should be able to determine which species he has by determining the mean shape from an adequate sample. It must be pointed out, however, that the shape of leaf of one or a few specimens cannot be relied upon for satisfactory separation. There is a considerable overlapping in the ranges of the deviations from the mean in the two species. The leaf from the Ann Arbor population shown in Fig. 9, for example, is very close to typical *Trillium flexipes* in both length/width ratio and tip angle, while the leaf from the East Lansing population shown in Fig. 10 is very like *Trillium flexipes* in all respects excepting displacement, where it has a higher value, and the higher values for displacement are usually associated with *Trillium flexipes* rather than *Trillium grandiflorum*. It would be impossible in these cases to suggest to which species the plants belonged on the basis of leaf shape, though such characters are often used as points of some importance in keying out these plants in some of the "manuals". It is worth noting, perhaps, that this overlapping of shapes between the two species is probably not significantly different in the two populations. That is, there are at least as many plants in the East Lansing samples which have leaves resembling those of some of the *Trillium flexipes* plants as there are in the Ann Arbor samples. If there is any tendency present at all, it is for the plants at East Lansing, rather than the plants at Ann Arbor, to resemble Trillium flexipes more closely, and this in spite of the fact that it is at the latter location that the two species grow intermixed. This simply strengthens the impression that, while overlap occurs in the leaf shapes of the two species, there does not appear to be any evidence that this may be due to hybridity between the two.

While these figures by no means exhaust the extremes of variability found in the population, they will give some idea of how one of the factors may remain constant but variation in the others give rise to quite different shapes. The two leaves having the largest L/W ratios are depicted in Fig. 8, one from each population. It is quite obvious that they differ somewhat in general shape because, while essentially the same length and width, they do vary considerably in displacement and in type of base. The differences shown here seem to be rather consistently present in other leaves with high L/W ratios. That is, the long narrow leaves in the Ann Arbor population have a lower displacement and broader base than do similar leaves in the East Lansing population. Such consistency is not present, however, in the leaves shown in Fig. 9, representing the leaf in each population with the lowest L/W ratio. These figures would suggest that relatively short and wide leaves in the East Lansing population tend to have lower displacement and wider bases than their Ann Arbor counterparts. Study of the samples discloses that this is not by any means always true, for a number of leaves are present in the East Lansing population with

L/W ratios similar to that of the leaf figured, but with displacement more nearly like that figured for the Ann Arbor plant. Several others agree essentially with the appearance of the leaf shown in this plate. This points up the fact that, while these are presented in pairs, they should not be considered as necessarily representative of all the types which may occur at the extreme of variability of one of the shape factors, and hence should not be used as a basis for comparison of extremes of population shapes.

Fig. 10 shows types with maximum displacement. Other leaves with high displacements vary quite widely with respect to the other characters, so that these figures are representative of only two of several types that can be found. A similar situation seems to exist with respect to the leaves shown in Fig. 11. In both populations there are a number of other leaves with low displacement which are quite variable with respect to the other characters, so that the leaves shown here are again representative of only two of several types which have a very low displacement. There appears to be no significant difference in this type of leaf in the two populations.

It has been pointed out previously that the shapes of _Trillium flexipes_ leaves are, in some cases, similar to those of _Trillium grandiflorum_. Comparison of Figs. 13 and 14 with those just discussed should make this somewhat more evident.

Examination of the three tables concerned with leaf shapes again points up the similarity of the two populations. The means for the four factors do not appear to be very different, and the variation as indicated by the standard error is also very close in the two. The latter does seem to indicate a rather consistent, though statistically insignificant, tendency toward more variation in the East Lansing plants. This would be just the reverse of the expected condition, if there were any present species hybridity or introgression occurring, since these plants are much more isolated from other species of Trillium than are the ones at Ann Arbor.

Table IV-A is a comparison of samples collected from the two populations at weekly intervals through the period of anthesis in 1954. The paired recordings represent collections made on one day at East Lansing, and the following day at Ann Arbor. As nearly as is possible with field collections, therefore, they represent comparable stages of growth and development. There seems to have been very little difference in the time of beginning and ending of anthesis in the two areas, even though they are separated by more than fifty miles, and one station is considerably farther north than the other. This type of pairing was used to avoid, as much as possible, any influence on shape that might result from differential growth of one part or another during different stages of development. Initial experience had suggested that the Ann Arbor population, during early anthesis, might tend to have a larger proportion of long, narrow leaves with comparatively lower displacement than was the case later in the season. The tendency was not so noticeable in the East Lansing preliminary surveys. Examination of the data collected in 1954 and recorded in Table

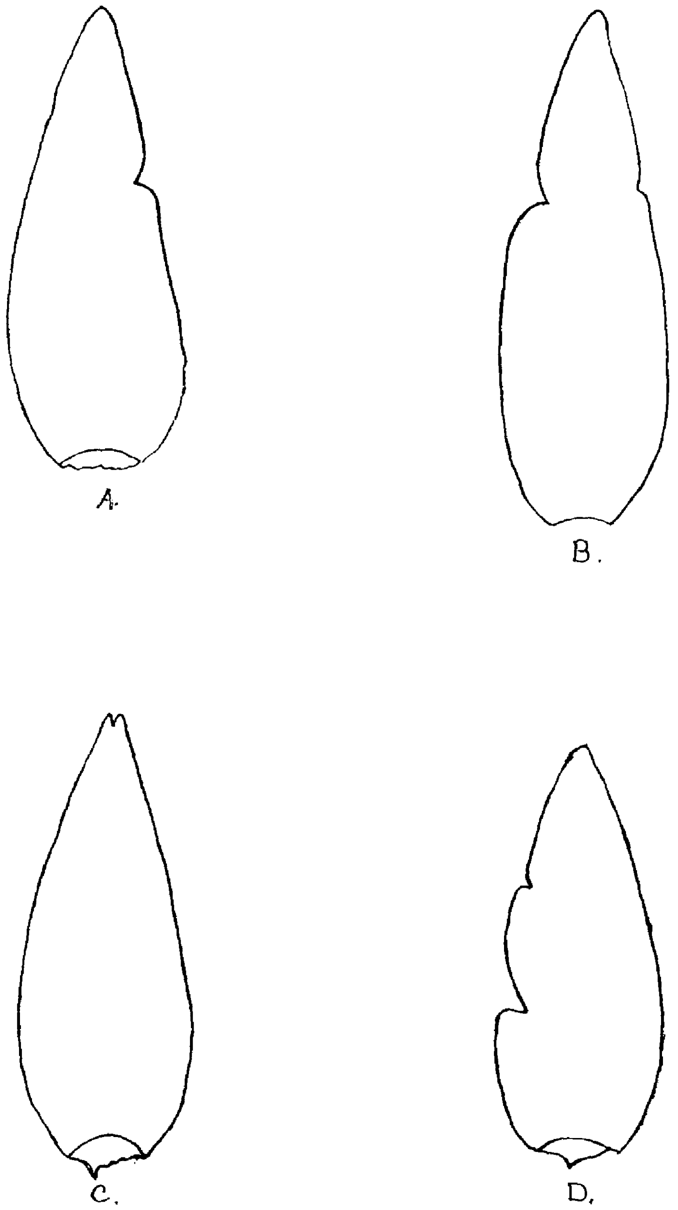
IV-A gives limited support to this idea, though the differences are not large enough to be considered statistically significant at the 5% level.

In L/W ratio and in displacement the two populations show no statistically significant difference during any part of the flowering period. In tip angle and basal angle the situation is not as clear statistically. In each case, one of the four weekly collections shows what might be considered a statistically significant difference, at the 5% level of significance. However, in each case too, one of the weekly collections showed no differences in the mean. Figures compiled for the four sets of samples considered together, and not included in the table, indicate no significant difference exists for the season as a whole. In all four factors, there appears to be a greater difference between the populations during the first and third weeks than during the second and fourth weeks. Whether this is a real difference, or only a matter of chance cannot be determined without similar collections from several different seasons, data which are not available at this time. It might be pointed out here that, when we compare the data for the tips in the Ann Arbor population for the second, third, and fourth weeks with that for the first, statistically significant differences are found in the second and fourth weeks, though not in the third. In the case of the base, statistically significant differences are found between those plants of the first and each of the other three weeks. The situaticn at East Lansing is not similar, for there, significant differences are found only between the tips from the first and third weeks, and none in the bases. The explanation for this is not clear, though it is suggestive that the leaves of early flowering plants at Ann Arbor tend to be of somewhat different shape than those which come from the plants flowering later. This may possibly be associated with the age of the plant in years, though few data are at hand to verify that supposition. It did seem, however, that there might be a tendency for the earlier flowering plants to be smaller and presumably from younger rhizomes. That the leaves are smaller in the first week's collections and that the difference in size is more marked at Ann Arbor than at East Lansing is indicated by the following measurements of mean lengths and widths of the leaves collected. At Ann Arbor the mean length at time of collection of the first plants was about 77 cm. One week later the mean was 105 cm., which was fairly close to the maximum mean of 112 cm. recorded in the final week. Comparable figures in the East Lansing population show 84 cm. the first week, 98 cm. the second week, and a maximum 110 cm. in the third week. The widths at Ann Arbor are 52 cm. for the first week, 75 cm. for the second, and a maximum of 78 cm. in the fourth week. At East Lansing the width was 57 cm in the first week, 69 cm. in the second, and 81 cm., the maximum, in the third week. It is apparent that in this season, at least, the Ann Arbor population starts out with smaller leaves than at East Lansing, and builds up to nearly maximum size during the first week, while at East Lansing the leaves of the earlier flowering plants are correspondingly larger, and build up to a maximum size over a longer portion of the growing season. These differences might well be expected to reflect themselves in the comparisons made between the populations during different periods of the growing season and between samples from the same population at different periods. It is probable that this differential growth is responsible for the apparently significant differences obtained between collections made from the same population at different times, and quite possibly for the differences noted between the two populations in the two instances where they appear significant. I do not believe that there is any evidence here to suggest that the two are essentially different. It does point up the necessity, however, of taking samples from plants in similar stages of anthesis, if populations are to be compared accurately, or better still, that samples be taken during several different periods of anthesis in each population. Continued study may indicate that these differences in growth are genetically controlled, and are as significant in a comparison of populations as are factors such as leaf shape, petal shape, or anther/filament ratios. On the other hand, they may be reflections of an environmental difference which was not closely studied in this work.

It seems likely then, that there is no significant difference in the populations at East Lansing and Ann Arbor with respect to leaf shape in general. The two basic factors in determining leaf shape, L/W ratio and displacement, show a very close correspondence, while the secondary features, tip and base, are considerably less similar, though not significantly different when considered over the season as a whole. All four factors tend to vary somewhat during the period of anthesis, but the tips and bases seem more likely to vary with the period of anthesis than does the L/W ratio or the displacement. Collections from samples taken very early in anthesis should not be compared with samples from other populations taken at later periods, especially if comparisons between tips and bases are being made, since significant differences may appear in these characters in the same population at different periods. Significant differences are present between population means in Trillium grandiflorum and Trillium flexipes, which grow intermingled at Ann Arbor, especially with respect to L/W ratio, displacement, and tip angle. There is some overlap, however, between individual members of these populations with respect to each of the four factors. Finally, there is almost no correlation between the two major factors in determination of leaf shape, and only moderate correlation between them and the tips and bases. Seemingly, L/W ratios and displacement set limits within which the tips and bases may vary, and thus the latter are, in part, a function of the former. Within these limits, however, the tips and bases seem to vary more or less independently. Thus, there seems to be none of the tendency for some or all of these factors to stick together as might be expected if either or both populations were hybrid in nature. It would seem, rather, that they represent two presently isolated groups of a stabilized species, varying no more than one might expect a group to do in the time during which this isolation may have existed.

3. Analysis of Data on Sepals

The sepals show much less variability than the leaves, and are apparently under the influence of fewer shape-determining factors. The principal differences seem to be associated with the length/width ratio, with any secondary factors which might be present obscured by it. The widest part is almost always located about one quarter of the distance from the base to the tip. A second factor, not associated with shape, is the definite notching of the margin, either laterally or apically (cf. Fig 15). The lateral notches appear to be fairly constant in position, being found most often between one half and two thirds of the way up the margin. Rarely they may occur about one third of the way up the margin. Usually they occur on one side of the sepal only, but several cases were noted in which they occurred on both margins. The depth varies considerably, in some cases being quite deep, while at the other extreme, some are so shallow as to be hardly noticeable. There is also some variation in the number of sepals which are affected in a plant. In some plants only one of the three will be notched, but more often two or all three will be affected. Possibly significant is the fact that lateral notches are found more often in the latter half of the flowering period. This might be considered as suggesting that environment may play a considerable part in the expression of this genically controlled trait. The relative constancy of its position is strong indication that it is under genic control. Apical notches also vary considerably in depth. They, too, are more apt to be found later in the



Text Fig. 15. Sepals Showing Apical and Lateral Notching.

A. One lateral notch in the position most frequently noted.
B. Lateral notches on opposite sides of sepal.
C. Typical apical notch.
D. Lateral notches on the same side; one deeply notched, one very shallowly notched.

These types represent the most frequently noted conditions, with the exception of D which is quite rare.

season, but this charactersitic is not so marked as it was in the case of lateral notches. In only one case were sepals found with both lateral and apical notches.

Examination of the data presented in Tables V and V-A makes it quite clear that the two populations studied here are very much alike as regards sepal shape. There is some range of mean from sample to sample, but the range is not particularly greater in either population, and the average of the means is very close in the two. Extremes of variation in shape are illustrated in Text Fig. 16. It happened that both of the extremes were found in the Ann Arbor population. However, the extremes in the East Lansing population are almost as great, 2.29 as compared with 2.22, and 4.67 as compared with 4.74.

Comparison of the sepals from the two populations at weekly intervals through the period of anthesis are recorded in Table V-A. Application of the t test indicates that in none of the pairs is there any reason to suppose that they come from significantly different populations.

There is indication of some correlation between the L/W ratio of the sepals and the same ratio in the leaves. This is not an unlikely situation, since it might be expected that the genes controlling length are very likely active in producing greater length in all competent tissues, in whatever organ they may be found. On such a basis one might predict that there would also be a correlation between the L/W ratio of sepals and petals, and such a correlation is found to be present.

TABLE V

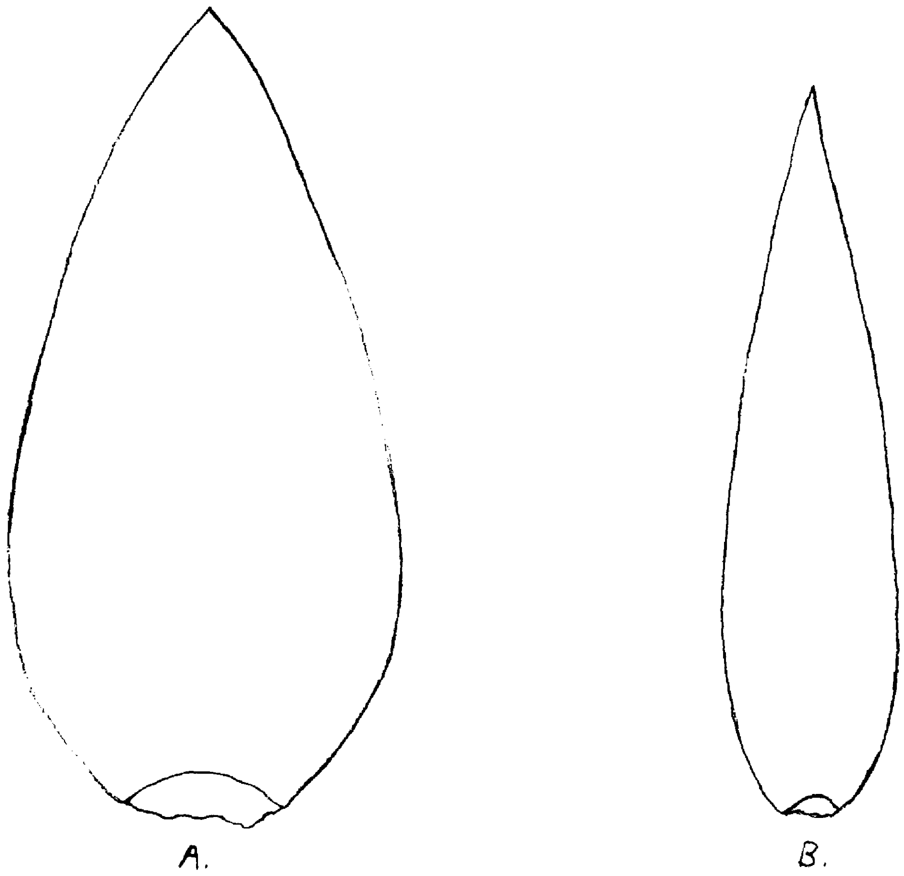
SEPAL MORPHOLOGY DATA -- SUMMARY OF SAMPLES FROM TWO POPULATIONS

SERIES	N	AREA	LENGTH/WIDTH		NOTCHES	
			$\bar{X}$	S.E.	LATERAL	APICAL
Y425	25	A.A.	3.56	.108	1	1
Y53	25	A.A.	3.11	.098	3	4
Y59	25	A.A.	3.19	.094	3	8
Y516	25	A.A.	3.18	.092	10	3
E424	25	E.L.	3.34	.102	0	1
E51	25	E.L.	3.32	.102	3	1
E58	25	E.L.	2.94	.086	5	2
E515	25	E.L.	3.07	.082	9	2

TABLE V-A

STATISTICAL COMPARISON OF TWO POPULATIONS WITH RESPECT TO SEPAL L/W RATIOS

SERIES	N	$\bar{X}$	S.D.	S.E.	D_x/SE_d	t_{05}
E424	25	3.34	.50	.102	.48	2.06
Y425	25	3.56	.53	.108		
E51	25	3.32	.50	.102	.47	2.06
Y53	25	3.11	.48	.098		
E58	25	2.94	.42	.086	.59	2.06
Y59	25	3.19	.46	.094		
E515	25	3.07	.40	.082	.26	2.06
Y516	25	3.18	.45	.092		



Text Fig. 16. Extreme Variation in Sepal Shape.

A. Sepal showing minimum L/W ratio (2.22), found in the Ann Arbor population.

B. Sepal showing maximum L/W ratio (4.74), also found in the Ann Arbor population.

Correlation between L/W ratios and notching in the sepals appears to be almost completely lacking. Notches are found in sepals with a L/W ratio as high as 4.27 and as low as 2.30, and are well scattered throughout the range between these limits.

There appears to be no significant difference between the two populations with respect to the lateral notches, for these occur with remarkably similar frequency in the two. There does appear to be a difference in the frequency of occurrence of apical notches, however. In nearly 150 plants from each population notches are found more than three and one half times as frequently in the Ann Arbor population. The Chi-square test indicates that this difference is of high enough order to be considered significant, and that the two populations, therefore, are different with respect to this one feature. It must be pointed out, however, that such conditions may be expected in isolated populations, and unless supported by several other significant differences, can hardly be used to suggest that the two have had anything other than a common origin, with little or no hybridization or introgression since their isolation.

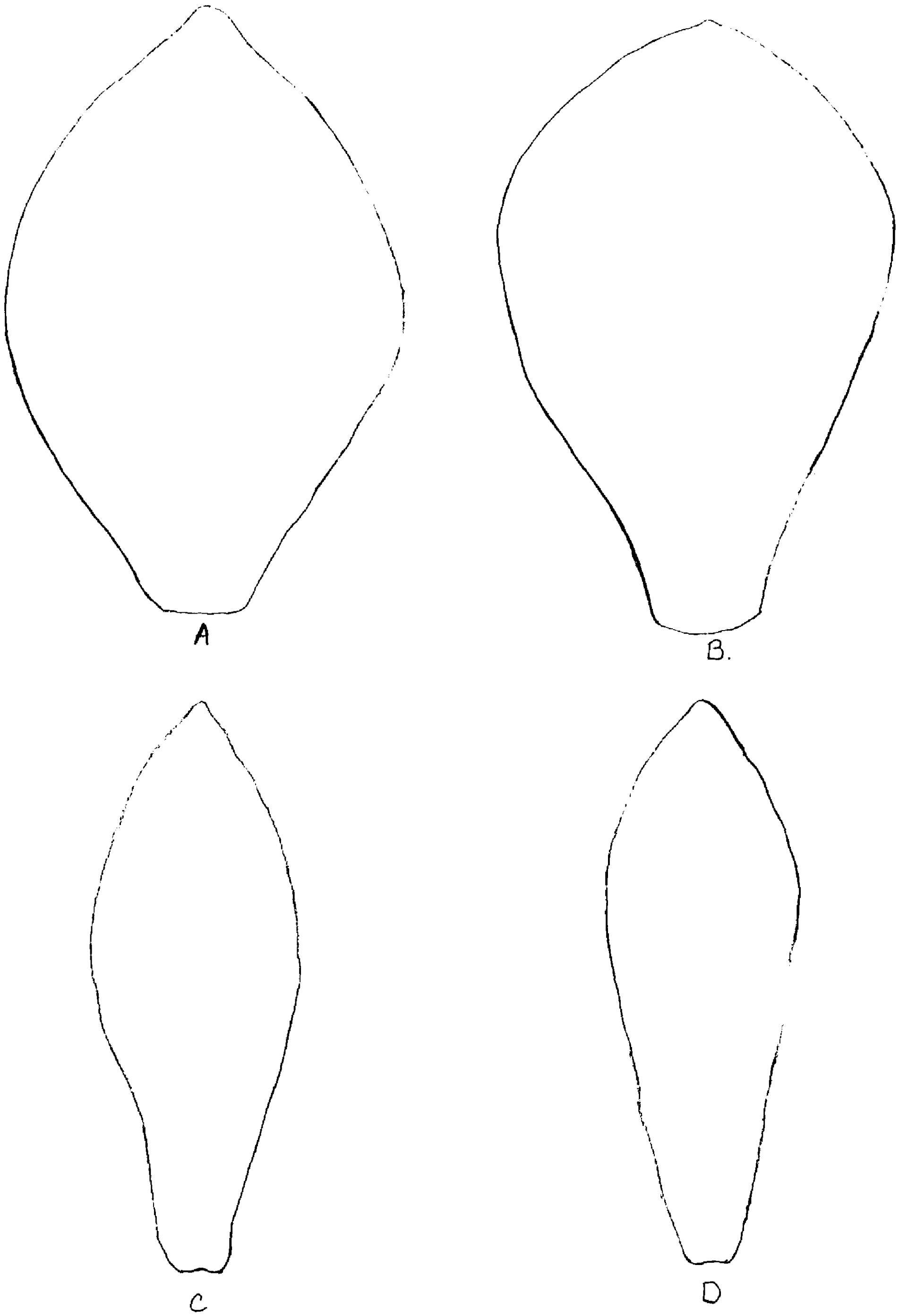
Conditions in the sepals, then, tend to confirm the evidence from the leaves, and one must remain, for the most part, rather more impressed by the similarity of these two populations than by their differences.

4. Analysis of Data on Petals

The shape of the petal, as has been pointed out in discussion of procedure, appears to be due primarily to the interaction of the L/W and the displacement factors. There is considerable variability, as can be seen in Text Fig. 17. In general, there seems to be more variation in shape than in the sepals, but less than in the leaves. This might be ascribed to the fact that petals probably have more factors involved as determiners of shape than do the sepals, but less than the leaves. In the only measurement which is common to all three plant structures, leaves, sepals, and petals, the L/W ratio is found to be most variable in the sepals, and least variable in the leaves. This would seem to support the contention that the number of factors involved in production of the shape of these structures brings about more apparent variability in shape than does greater variability in any one factor.

There does not seem to be any significant correlation between the L/W factor and the displacement, which parallels the situation found in the leaves. In the population, then, we might expect to find plants with petals which approached each of four extremes: (a) those which are long and narrow and have a high displacement; (b) those which are long and narrow but have a low displacement; (c) those which are relatively short and wide and have a high displacement; (d) those which are short and wide and have a low displacement. These might be designated as approaching an oblanceolate, a narrowly elliptical, an obovate, and a broadly elliptical shape (cf. Fig. 17).

Tables VI and VI-A summarize the data on the collections of



Text Fig. 17. Extremes of Variation in Petals.

A and B drawn to same scale. C and D drawn to half that scale. A shows low L/W ratio, low displacement; B shows low L/W ratio, high displacement; C shows high L/W ratio, relatively low displacement; D shows high L/W ratio, relatively high displacement. A and C from East Lansing, B and D from Ann Arbor.

TABLE VI

PETAL MORPHOLOGY DATA -- SUMMARY OF SAMPLES FROM TWO POPULATIONS

SERIES	N	AREA	LENGTH/WIDTH		DISPLACEMENT		NOTCHES	
			$\bar{X}$	S.E.	$\bar{X}$	S.E.	LATERAL	APICAL
E424	25	E.L.	2.28	.063	.35	.020	1	12
E51	25	E.L.	2.17	.063	.37	.022	6	18
E58	25	E.L.	2.04	.065	.36	.033	4	10
E515	25	E.L.	2.13	.084	.30	.037	0	9
Y425	25	A.A.	2.14	.051	.33	.029	5	21
Y53	25	A.A.	1.89	.043	.30	.027	3	19
Y59	25	A.A.	2.12	.047	.34	.031	4	14
Y516	25	A.A.	2.22	.078	.43	.029	12	11

TABLE VI-A

STATISTICAL COMPARISON OF TWO POPULATIONS WITH RESPECT TO PETAL CHARACTERS

SERIES	N	$\overline{X}$	S.D.	S.E.	D_x/SE_d	t_{05}
LENGTH/WIDTH						
E424 Y425	25 25	2.28 2.14	.31 .25	.063 .051	.42	2.06
E51 Y53	25 25	2.17 1.89	.31 .21	.063 .043	.86	2.06
E58 Y59	25 25	2.04 2.12	.32 .23	.065 .047	.24	2.06
E515 Y516	25 25	2.13 2.22	.41 .38	.084 .078	.22	2.06
DISPLACEMENT						
E424 Y425	25 25	.35 .33	.10 .14	.020 .029	.09	2.06
E51 Y53	25 25	.37 .30	.11 .13	.022 .027	.32	2.06
E58 Y59	25 25	.36 .34	.16 .15	.033 .031	.08	2.06
E515 Y516	25 25	.30 .43	.18 .14	.037 .029	.51	2.06

1954 and record statistical comparisons between the two populations with respect to petal shape and notching. Once again, there appears to be no significant difference between the two, either with respect to the L/W or the displacement factor. The seasonal variation which seemed to be present in these factors in studies made of the leaves does not appear to occur in the petals, but this should be taken as suggestive only, for the sampling needs to be more extensive and over more seasons before more positive statements can be made. When the Chi-square test is applied to the data on notching, however, there does appear to be a significant difference between the two populations at the 5% level of significance, though not at the 1% level. The critical value for the 5% level is 3.84. The calculated values are 5.29 for the lateral notches and 5.22 for the apical notches. Critical value at the 1% level of significance is 6.63.

As with the sepals, the notches may appear either apically or laterally or both. The latter condition is somewhat more frequent in the petals than it is in the sepals. The positions are relatively constant for the laterals, the notches usually appearing in about the place indicated in Text Fig. 5. Rarely they may occur farther down on the margin. As with the sepals, the notches do not appear to be correlated with either the L/W ratio or the displacement. It might be reasonable to suspect that the same factor which produced notches in the sepals would also produce notches in the petals, but study suggests that this is not the case. In samples of 100 plants from each of the two populations, 48 were found to have lateral notches in the sepals, the petals, or both. Of these 48 plants only 4 showed notches in both petals and sepals. Similarly, 120 plants possessed apical notches in the sepals, the petals, or both. Of these 120 plants, only 14 were notched in both sepals and petals. On this basis it seems quite likely that the factors producing notches in the sepals are not the same as those in the petals, and they are apparently acting independently.

While no records have been kept on their variability, there are two other characteristics of the petals which are deserving of consideration at this point. These are the color of the petals and the ruffling which is so often present. Both seem quite definitely correlated with the stage of development of the plant, often varying in the intensity of their expression with the stage of anthesis.

It is a readily observable fact that in any population of Trillium grandiflorum many of the flowers appear to turn pink as anthesis progresses. Investigation of these two populations suggests, however, that some plants may retain the white color in their petals throughout the period, finally withering and turning brown without a trace of pink ever appearing. The explanation for this difference is not clear, nor is it clear why the petals of some plants will be pure white at first and yet become quite a deep pink before they wither and drop off. Still other plants have been observed whose petals are pink even in the bud. Though a great deal of further study is needed before an adequate explanation can be presented, it may be noted that there seems to be some indication that the phenomenon must be, at least in part, genetically controlled, and that some of the variability is due to direct gene action. Some other factor seems to be present, whether genetic or environmental is not clear, which affects the timing of the expression of such color genes as may be present. In any case, because of this differential in time of expression, comparisons between populations with respect to this character must always be subject to the criticism that samples being compared were not taken from plants in the same stage of anthesis, and hence an apparent difference may not be real at all.

The phenomenon of ruffling in the petals is also a difficult one to analyze. It is quite apparent that the number of plants with petals which show this feature rises sharply as the season progresses. It was a particularly troublesome character in this study since it made determination of shape much more difficult, especially if dried material was being used. Since it characteristically increases in intensity with the increase in size of the petals, it might be suggested that it could result from unequal growth rates in the cells of certain regions of the petals. This suggestion must be considered very tentative, however, and in need of a great deal more experimental work, especially from the anatomical standpoint.

While strict comparisons between the two populations are impossible with respect to these two characters, general observation suggests that the two populations probably do not differ significantly in either ruffling or color. Certainly there is no marked difference between them.

It may be concluded, then, that the petals seem to follow the pattern established in the study of the sepals and the leaves. The factors that seem to be genetically controlled, and subject to measurement, appear to be acting independently of one another and do not tend to "stick together" as might be the case if hybridity were involved. Furthermore, there is no evidence to suggest that the two populations are significantly different with respect to the determiners of shape, though the evidence is less conclusive in the notching. However, one would expect to find some differences in two populations which have been isolated from one another as long as these probably have, and it is probable that the differences in notching are of this sort.

5. Analysis of Data on Stamens

The fact that anther/filament ratio was so distinctly different in Trillium flexipes suggested that it might be worthwhile checking this factor in any comparison of the two populations of Trillium grandiflorum. Studies of the collections of the first several seasons gave the impression that there might be seasonal differences in this ratio. However, when weekly collections were made in 1954, the resultant data did not give any conclusive evidence of such condition. If there is such a difference, and I am not convinced yet that there is not, it must result from a brief, but very rapid growth of the anther, which is not matched by the filament until a bit later. It is suggested that this usually takes place fairly early in the flowering period, but may vary from just after opening of the first flowers in the population to a period of a week or ten days after that. It would be interesting to trace this development daily in a designated sample group through a period of several flowering seasons. Certainly there is no statistical evidence to suggest such a difference at this time. In this study, it is not especially important that the answer to this problem be known, for the populations appear to behave alike, and it does not appear to interfere appreciably with making comparisons between the two.

As may be noted from a consideration of Tables VII and VII-A, the two populations are not significantly different in their means, either for any particular period, or for the whole season. Once again, it is a condition of marked similarity rather than one of considerable dissimilarity. Certainly there is nothing here to suggest anything other than that they are two separate segments of a stable population. They both are quite distinct from Trillium flexipes, which is normally characterized by a ratio which is two or more times as great as that established for Trillium grandiflorum, as studied here. As might be expected, there is some overlap, for occasionally the ratio in the former species will get as low as 1.75, and the ratio in the latter may go as high as 2.10. This is almost certainly the overlapping of the ranges in variation between two distinct species, rather than a phenomenon associated with hybridity, however, as is at least partially evidenced by the very small numbers of specimens found

TABLE VII

ANTHER/FILAMENT RATIOS

SUMMARY OF SAMPLES FROM TWO POPULATIONS

SERIES	N	AREA	LENGTH/WIDTH	
			$\bar{X}$	S.E.
E424	25	E.L.	1.51	.061
E51	25	E.L.	1.36	.047
E58	25	E.L.	1.34	.043
E515	25	E.L.	1.36	.057
Y425	25	A.A.	1.32	.049
Y53	25	A.A.	1.51	.063
Y59	25	A.A.	1.40	.053
Y516	25	A.A.	1.28	.020

TABLE VII-A

STATISTICAL COMPARISON OF TWO POPULATIONS WITH RESPECT TO STAMENS

ANTHER LENGTH/FILAMENT LENGTH

SERIES	N	$\bar{X}$	S.D.	S.E.	D_x/SE_d	t_{05}
E424 Y425	25 25	1.51 1.32	.30 .24	.061 .049	.57	2.06
E51 Y53	25 25	1.36 1.51	.23 .31	.047 .063	.45	2.06
E58 Y59	25 25	1.34 1.40	.21 .26	.043 .053	.19	2.06
E515 Y516	25 25	1.36 1.28	.28 .10	.057 .020	.29	2.06

with ratios falling into this intermediate category between the two species.

6. Analysis of Data on Pistils

Finally, there does appear to be a definite difference in shape of the ovary in the various plants of the populations being studied. This is not a characteristic for which I have been able to devise any adequate technique for exact, quantitative measurement. Yet there are so few morphological variables which may be used for comparative purposes that it seemed wise to include this one here.

When the ovaries of the samples collected weekly during 1954 are classified according to the system described in an earlier part of this work, it can be seen that the two populations are quite similar with respect to ovarial shape. Included in type A (cf. Fig. 4) were 20 plants from the East Lansing population, and 26 from Ann Arbor; in type B there were 17 from East Lansing and 27 from Ann Arbor; in type C there were 57 from East Lansing and 44 from Ann Arbor; type D included 6 from East Lansing and 3 from Ann Arbor. When tested according to the Chi-square test, it is observed that none of the four types appears to come from significantly different populations. The calculated figures are: for type A 0.71, for type B 2.36, for type C 2.88, and for type D 0.48. Since the critical value at the 5% level of significance is 3.84, it is clear that these populations are not significantly different statistically. While these figures are less reliable than those recorded for the other factors studied, because of the greater chance of subjectivity in classifying intermediate types, they tend to conform to the findings for other characteristics generally, and probably may be safely said to strengthen the concept that these two populations are actually two segments of one large, relatively stable population which is the species *Trillium grandiflorum*.

7. Correlations between Variables

Since the tendency of several different factors to "stick together" has been shown to be a characteristic of hybrid populations, it seemed advisable to determine whether the variables studied here tend to behave in that fashion. Some indication of correlation, or lack of it, has already been presented in the analysis of the morphology of the plants in these populations. More will be presented below.

As has been pointed out, there is evidence of some correlation between the L/W factor and the tip and basal angles in the leaves. In both cases it is a negative correlation. A sample of 100 plants from both populations, and including specimens from early, middle, and late stages of anthesis, shows a correlation factor of minus .457 between L/W and the basal angle. A similar sample analyzed for correlation of L/W and the tip angle shows a factor of minus .756. This suggests that these three are at least moderately interdependent. As previously indicated, this is about what we might expect, since the L/W factor sets limits within which the tips and bases may vary.

The correlation between displacement and tips and bases presents a somewhat different situation. Here, there is a fairly well marked indication of a positive correlation between the displacement and the tip (r equals .632), but correlation between the displacement and the base is questionable (r equals minus .175). There seems to be a tendency for the displacement to be more limiting in its influence on the tip than on the base. This condition may be parallel to that of the L/W ratio, though the difference between the limiting effect of L/W on bases and on tips does not appear to be as great as in the case of the displacement factor. In short, it seems probable that both the displacement and L/W factors set some sort of limit on the extent of independent variation in the tip. They do not seem to limit independent variation in the bases as much, due especially to the low degree of interdependence between the base and the displacement.

The L/W factor and the displacement show almost no evidence of correlation with one another, either in the leaf (r equals .002) or in the petal (r equals .035). The evidence that these are varying independently seems quite strong.

It was thought quite probable that the same factors that produced long narrow leaves might also tend to produce similar sepals and petals. Determination of the correlation factors in a number of samples indicates that this figure will average near .650 for petals and about .550 for sepals. Correlation factors determined for the L/W between sepals and petals also lies in this same general range. Apparently, at least a moderate degree of correlation does exist, presumably because a single set of factors is expressed in each of the three.

The same sort of conjecture might be made with respect to the displacement factor. However, the evidence obtained here points strongly to the fact that the factors that control the displacement in the leaf are not the same as those which exercise control in the petal. The correlation coefficient obtained is a negative .050.

Since the displacement factor is apparently not under the same control in petals and leaves, it is possible that a linkage might exist between the L/W factor in leaves and the displacement factor in petals, though the indication of some dependence in L/W between leaves and petals, coupled with the apparent independence of L/W and displacement in petals, would suggest that this might be a limited possibility. It was found that the coefficient in the sample tested was .136, so that it seems likely there is independence here. The reverse situation in which petal L/W is checked against leaf displacement also shows a very low correlation coefficient (r equals -.049). When sepal L/W is used, the figure is .134. It seems fairly safe to conclude, therefore, that the L/W and displacement are not linked in any of the several possible ways.

Notching appears to be quite unrelated to the L/W ratio of leaves, sepals, or petals, or to the displacement factor in leaves or petals. A number of possible combinations exist and all have been checked using the 200 plants collected in 1954, and in some cases other collections as well. This is true of both apical and lateral notches, each of which had to be considered, since the two apparently are not under the same control, and vary independently.

Similar studies were made with the ovarial shapes in comparison with the L/W, displacement, apical notching and lateral notching. No evidence could be found to suggest that any of these factors were linked. For example, in a sample of 200 plants, the mean leaf L/W of those plants with type A ovary was 1.55 as compared with a mean of 1.47 for the whole sample, and in a sample of 100 plants, the mean L/W for those plants with type B ovary was 1.47 as compared with a mean of 1.45 for the whole group. Considering another example, it was noted that in a sample of 200 plants, about 23% of them had type A ovaries. If apical notches were linked with this type ovary, and all plants with apical notches were examined, the percentage showing type A ovaries should be considerably in excess of 23%. The percentage actually observed was about 25%, suggesting that there was little correlation between these factors. Similar examples could be given with respect to other possible linkages. The only possible conclusion of these observations seems to be that there was little or no tendency for the shape of the ovary to be linked with any of the other characteristics examined.

From all of these examinations it seemed clear that, with the exception of the possible linkages between L/W and leaf tips and bases, between displacement and tips, and between L/W in leaf, sepal, and petal, there is fairly good evidence that all the characters studied are varying independently, and are not linked, as one would expect some of them to be, if these were hybrid populations. Moreover, it seems quite probable that the correlation between the L/W of the three foliar organs is due more to the fact of each being an expression of the same set of controlling genes than to linkages between different sets. Therefore, it must be concluded that the morphological variability which is most apparent in Trillium grandiflorum is due primarily to a set of factors which are transmitted independently of one another.

SUMMARY

1. A comparative study of the external morphology and the cytology of the chromosomes of two populations of Trillium grandiflorum has been undertaken, the two populations being located in similar ecological situations, but isolated from one another by a distance of about 50 miles.

2. Techniques for expressing the factors which are responsible for the shape of the leaves, sepals, and petals in mathematical terms have been devised and used for comparative studies and statistical analysis.

3. Ovular and root tip material for cytological analysis has been prepared by squash technique after having been subjected to temperatures of zero to two degrees Centigrade for 96 hours. The Feulgen staining technique has been employed.

4. A standard pattern of differentially reactive regions is present in the chromosomes, and is the same in both populations.

5. Frequent variations occur within the standard pattern, and are usually constant within the cells of a given plant, but may vary from plant to plant.

6. Several types and degrees of variation in differentiation are present in the two populations, which show little significant difference from one another in this respect.

7. The most useful type of chromosome variation for comparative purposes is the condition designated as heteromorphy, in which one homologue shows differential reactivity in certain

segments, while the other fails to develop this reactivity in one or more of the corresponding segments. The causal mechanism is unknown, but it is suggested that it may be due to loss of the differential segments, in which case it is not useful as an index of hybridity, or to the presence of different sets of allelic genes, which might make it useful as an index of hybridity. Possibly, both of the proposed causal factors may be operative in these populations. Since the heteromorphy does not differ significantly in the two populations, it appears that hybridity is not indicated in these populations.

8. Several other types of variation are present, but are regarded as being less reliable than the type of heteromorphy described above as criteria for comparisons between populations. These include variations in length of differential segments, variations in staining intensity, and variations in apparent density of the segments. All of these may occur between homologues. This type of variation shows more tendency to vary from cell to cell in a plant than was the case with the kind of heteromorphy in which segments are completely lacking.

9. A small sample of Trillium flexipes has been analyzed, and, while not enough specimens have been examined to permit positive determination of a standard pattern of differential reactivity in the chromosomes, it is certain that this species is distinctly different from Trillium grandiflorum in the number and position of its differential segments.

10. No cytological evidence is present which might be con-

sidered indicative of hybridity or introgression in either of these populations,

11. The morphological characteristics analyzed include leaf shape, sepal shape and notching, petal shape and notching, ovarial shape, and anther/filament ratio. Several other characteristics were studied but rejected as being unreliable criteria for use in comparing populations.

12. Considerable variation occurs in the shapes of the structures studied but generally they form a nearly normal distribution.

13. Leaf shape was found to be due primarily to the interaction of four factors. These are the length/width ratio, the displacement, the tip angle, and the basal angle. The first two vary independently of one another but probably set limits within which the latter may vary more or less independently. Because of the several factors involved, leaf shapes do not vary between two well defined extremes, but range between a number of extremes. No significant differences in leaf shapes, or in the extent of their variation, are found when the two populations are compared.

14. Sepal shape was found to be due primarily to the L/W ratio. Other factors, if they exist, are obscured by it. Previously unreported notching, both apical and lateral, was observed to be present in well defined positions in a number of specimens. Apical and lateral notching vary independently of one another and of the L/W ratio. No significant differences in kind or extent of variation were noted in the two populations.

15. Petal shape was found to be due to two major factors, the L/W ratio and the displacement, which vary independently. Apical and lateral notches occur, apparently varying independently of one another and of the displacement and L/W. No correlation between notching in sepals and in petals was observed. Color and ruffling of the petals appear to vary considerably with the period of anthesis, and possibly with other factors.

16. The anther/ filament ratio is essentially the same in both populations.

17. Ovarial shapes have been classified, primarily on the basis of position of maximum width, into four types. These do not seem to be correlated with the other morphological factors studied.

18. Major morphological variations were found to vary independently, indicating their lack of linkage. This, together with the very few significantly large differences in morphological characteristics, suggests that hybridity has not been a major factor in the development of these populations in recent times.

19. This detailed assessment of the cytological and morphological characteristics of the plants making up these two isolated populations may now serve as a standard against which other populations of this species may be compared.

PLATE I

Differential Reactivity of Chromosomes of *Trillium grandiflorum*

These figures show chromosomes from a single cell, and are arranged with kinetochores on approximately the same level in each row.

Fig. A. A chromosomes, heteromorphic, with differential segments in one member of the pair and not in the other.

Fig. B. B chromosomes with both terminals differentiated.

Fig. C. C chromosomes showing interstitial, differential segments which differ in length in the two chromosomes.

Fig. D. D chromosomes with interstitial, differential segment in each long arm, but with one more markedly differentiated than the other.

Fig. E. E chromosomes showing greater length and lack of differentiation.

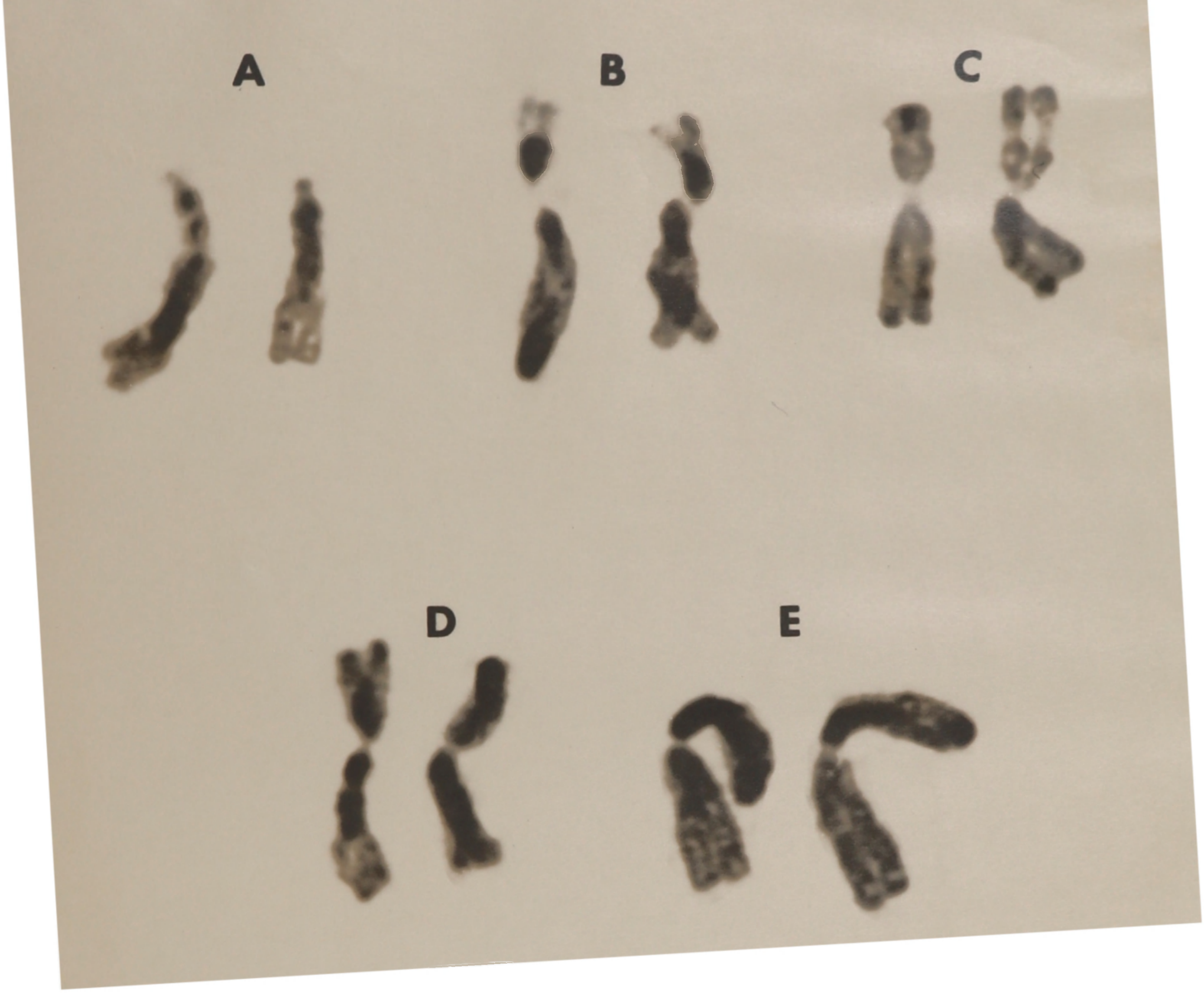

A
B
C
D
E

PLATE II

Cells Showing Variations in Differential Reactivity of Chromosomes

Fig. 1. Metaphase. Note especially the well defined, interstitial segment in the C chromosome at the top of the figure, the clearly marked D chromosome at the upper left and the two well differentiated A chromosomes at the bottom and right center of the figure. An acentric fragment is present in this cell.

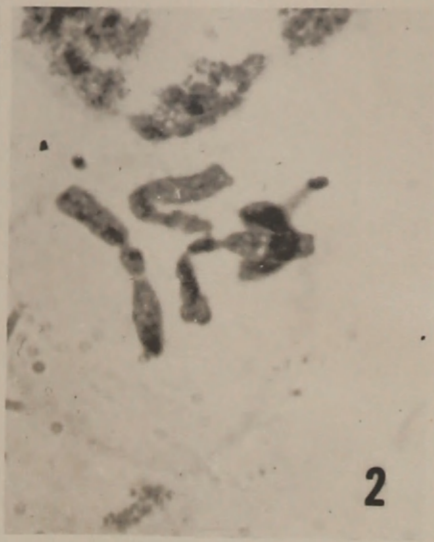
Fig. 2. Portion of a metaphase figure showing homomorphic D chromosomes with clearly marked interstitials.

Fig. 3. Metaphase, showing well contracted chromosomes with several clearly marked differential segments. Note especially the two B chromosomes at the left center of the figure.

Fig.4. Anaphase, somewhat scattered, showing A chromosomes well differentiated in proximal but not distal segment; B chromosomes with long terminal segmets; C chromosomes heteromorphic, with terminal and interstitial segments in one, interstitial only in the other; D chromosomes homomorphic with the interstitial segment evident in both; E chromosomes wholly unaffected.

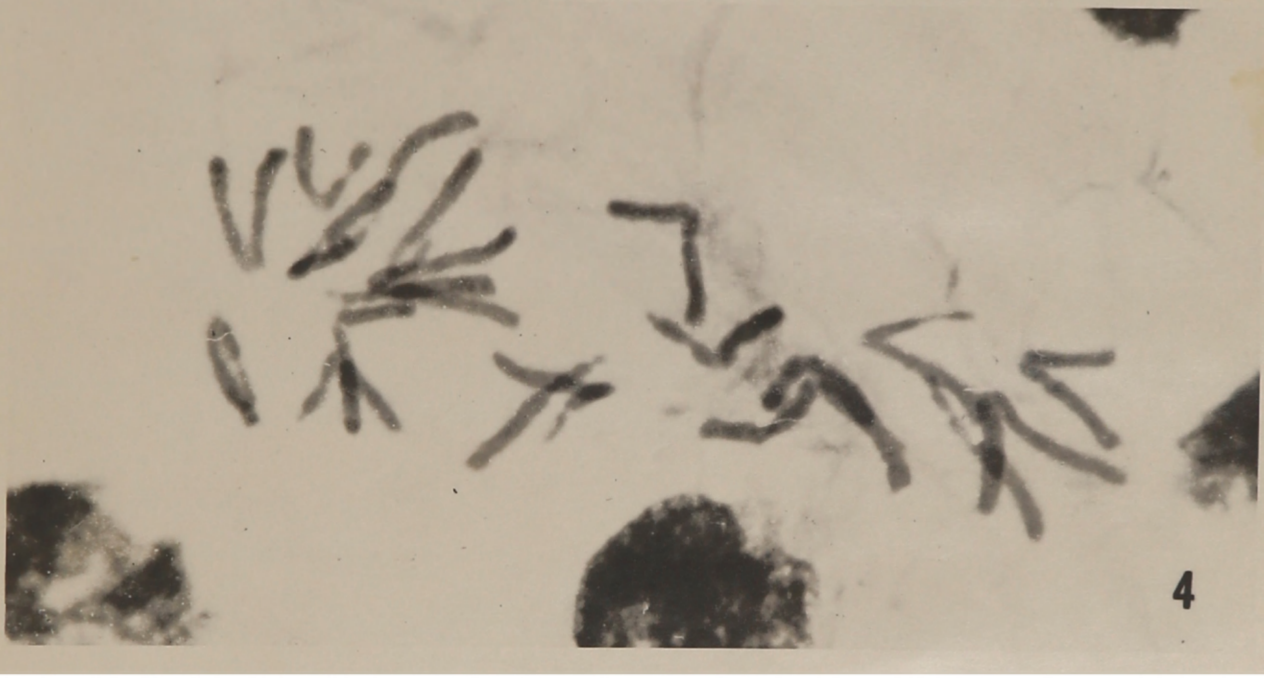
Note: The cells shown in these figures were obtained from different plants.

1



2

3



4

BIBLIOGRAPHY

Anderson, Edgar. 1936. Hybridization in *Tradescantia*. Ann. Mo. Bot. Gard. **23**: 511-525.

—————— 1939. Recombination in species crosses. Genetics **24**: 668-698.

—————— 1949. Introgressive Hybridization. John Wiley & Sons, Inc., New York, 106 pp.

—————— and L. Hubricht. 1938. The evidence for introgressive hybridization. Am. J. Bot. **25**: 396-402.

—————— and W. B. Turrill. 1938. Statistical studies on two populations of *Fraxinus*. New Phytologist **37**: 160-172.

—————— and T. W. Whittaker. 1934. Speciation in *Uvularia*. Journ. of Arn. Arb. Harvard Univ. **15**: 28-42.

Babcock, Ernest B. 1947. The Genus *Crepis*. Univ. of Cal. Press, Los Angeles.

Bailey, Paul C. 1949. Differential chromosome segments in *Trillium erectum* L. Bull. Torr. Bot. Club. **76**(5): 319-336.

—————— 1951. A study of the chromosome morphology of some species of *Trillium*. Bull. Torr. Bot. Club. **78**(4): 324-330.

—————— 1952. Differential reactivity in six species of *Trillium*. Bull. Torr. Bot. Club. **79**(6): 451-458.

Boothroyd, E. R. 1953. The reaction of *Trillium* pollen-tube chromosomes to cold treatment during mitosis. Journ. of Heredity **44**: 2-9.

Darlington, C. D. 1937. Recent advances in cytology. Blakiston Co., Philadelphia. 671 pp.

______ and L. LaCour. 1938. Differential reactivity of the chromosomes. Ann. Bot. (n.s.) 2: 615-635.

______ and ______ 1940. Nucleic acid starvation of chromosomes in Trillium. J. Genet. 40: 185-213.

Farwell, O. A. 1919. The Trillium grandiflorum group. Ann. Rep. Mich. Acad. Sci. 20: 155-159.

Fassett, Norman C. 1941. Mass collections: Rubus odoratus and Rubus parviflorus. Ann. Mo. Bot. Gard. 28: 299-374.

Fernald, M. L. 1950. Gray's Manual of Botany. Eighth Edition American Book Co. 1632 pp.

Haga, T and M. Kurabayashi. 1953. Genom and polyploidy in the genus Trillium. IV. Genom analysis by means of differential reaction of chromosome segments to low temperature. Cytologia 18(1): 13-28.

Kurabayashi, Masataka. 1952. Differential reactivity of chromosomes in Trillium. Journ. Fac. Sci., Hokkaido Univ., Ser. V. 6(2): 233-251.

Ris, Hans. 1951. Recent work on the chemistry of chromosomes. Symposium on Cytology, Mich. State Coll. Press: 21-36.

Stebbins, G. L., Jr. 1950. Variation and evolution in plants. Columbia Univ. Press, New York, 643 pp.

Veatch, J. O. 1930. Soil survey of Washtenaw County, Michigan. Mich. Ag. Exp. Sta., Bureau of Chem. and Soils, East Lansing, Michigan. 47 pp.

Wilson, G. B. and E. R. Boothroyd. 1941. Studies in differential reactivity. I. The rate and degree of differentiation in the somatic chromosomes of Trillium erectum L. Can. J.

Research, C, 19: 400-412.

______ and ______ 1944. Temperature-induced differential contraction in the somatic chromosomes of *Trillium erectum* L. Can. J. Research, C, 22: 105-119.

Warmke, H. E. 1937. Cytology of the Pacific Coast Trilliums. Am. J. Bot. 24: 376-383.

Woodson, R. E., Jr. 1947. Some dynamics of leaf variation in *Asclepias tuberosa*. Ann. Mo. Bot. Gard. 34: 353-432.