A PRELIMINARY BIOSYSTEMATIC STUDY OF *ERYTHRONIUM AMERICANUM* KER. IN THE SOUTHEASTERN UNITED STATES

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An Abstract

Presented to

the Committee on Graduate Studies

Austin Peay State College

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

in Education

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by

Billy Eugene Wofford

August 1967

Variations in the yellow flowering species of the genus Erythronium (Liliaceae) in the southeastern United States, have recently been of considerable taxonomic importance. The existence of these variations between and within single populations of Erythronium americanum has been suggested to the author and served as a basis for the study.

Particular biosystematic emphasis was placed on the purple and yellow anther color forms of Erythronium americanum. Inferences were made from geographical, ecological, cytotaxonomical, anotomical, and morphological data compiled during the course of the study. Statistical analysis were employed where they seemed pertinent.

On the basis of the data assimilated in this study and of the findings of Parks and Hardin (1963), it is this investigator's opinion that Erythronium americanum arose through the hybridization of Erythronium umbilicatum and Erythronium rostratum.

The results of this preliminary investigation also indicate that two distinct biotypes of Erythronium americanum exist and are best distinguished on the basis of anther coloration. In the southeastern United States, it now appears that these two biotypes should be placed in the taxonomic category of forma. However, until variations are sufficiently biosystematically studied throughout the entire range, formal taxonomic recognition will not be presented.

A PRELIMINARY BIOSYSTEMATIC STUDY OF *ERYTHRONIUM AMERICANUM* KER. IN THE SOUTHEASTERN UNITED STATES

A Thesis

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by

Billy Eugene Wofford

August 1967

August 8, 1967

To the Committee on Graduate Studies:

I am submitting herewith a thesis written by Billy Eugene Wofford entitled "A Preliminary Biosystematic Study of *Erythronium americanum* Ker. in the Southeastern United States." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts in Education, with a major in Biology.

William H. Ellis
Major Professor

We have read this thesis and recommend its acceptance:

Fred A. Bunger
Minor Professor

Floyd L. Brown
Third Committee Member

Accepted for the Committee:

William H. Ellis
Director of Graduate Studies

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The author wishes to express sincere appreciation to Dr. William H. Ellis, Professor of Biology, Austin Peay State College, who suggested, aided and counseled him during the course of the study; to Mr. Floyd Brown, Department of Biology, and Dr. Fred A. Bunger, Department of Education, for their suggestions and constructive criticisms of the manuscript. Gratefulness is also extended to Dr. Haskell C. Phillips, Department of Biology, for his aid in photographing the illustrations.

Appreciation is extended to Research Committee of the Tennessee Academy of Science for providing a grant-in-aid which made much of the field work possible.

The author sincerely appreciates the assistance given by Dr. Harry E. Ahles, Department of Botany, University of North Carolina, and Dr. James W. Hardin for making possible the visitation of populations of Erythronium near Raleigh, North Carolina.

The author wishes to thank his wife for helping in every way during the study; his fellow students, especially Lloyd Scott and Billy Evans, for their valuable assistance in much of the field work.

Gratefulness is also extended to the following herbaria for the use of their specimens of Erythronium in

this study. The abbreviations are those suggested by Lanjouw and Stafleu (1959).

DUKE - Duke University

FSU - Florida State University

GA - University of Georgia

GEO - Emory University

MISSA - Mississippi State College

NSC - North Carolina State University

TENN - University of Tennessee

UARK - University of Arkansas

USF - University of South Florida

VPI - Virginia Polytechnic Institute

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

I. INTRODUCTION

Variations in the yellow flowering species of the genus Erythronium (Liliaceae) have recently been of considerable taxonomic importance. Although these plants exhibit certain common characteristics, there usually are distinct morphological variations between or within single populations. The existence of these variations in local populations has been suggested to the author and serves as a basis for the study.

Review of the Literature

The synonymy of Erythronium americanum, taken from Index Kewensis and the Gray Index is listed in Chapter VIII. Pertinent synonyms to this study are cited in this chapter as well as other chapters throughout the discussion.

Eighteenth-century botanists related all the American species of Erythronium to the European species, Erythronium Dens-canis L. However, the American species have either yellow or white tepals while the European plants may be red or purple (Parks & Hardin, 1963).

Harper (1941, 1945, 1949, 1952) comments on the diversity of Erythronium in the eastern United States and recommends that botanists examine populations in their area. Wolf (1941), through his study of Erythronium in Alabama,

divided the genus into three species: Erythronium americanum, Erythronium harperi and Erythronium rostratum. Harper (1945) pointed out what might be another species of Erythronium from plants he observed in Alabama and North Carolina. This undescribed plant showed a distinct morpho-difference in the fruiting capsule from the other species of Erythronium that he had observed.

Parks and Hardin (1963) began their study of the genus with populations around Raleigh, North Carolina. On the basis of morphological and cytological characteristics they described five entities: Erythronium rostratum, Erythronium americanum subsp. americanum, Erythronium americanum subsp. harperi, Erythronium unbilicatum subsp. umbilicatum and Erythronium umbilicatum subsp. monostolum.

Anther coloration within the genus is found to be quite variable. Erythronium rostratum has been reported to have yellow anthers. Yellow or occasionally purple anthers are found in individuals of Erythronium americanum. Erythronium umbilicatum usually has brown or purple anthers, although a few yellow anthered forms may be found. These three species show definite geographic affinities. Erythronium rostratum has been collected in northern and central Alabama and Tennessee and in Arkansas, eastern Texas, eastern Oklahoma and southeastern Kansas. Erythronium

umbilicatum has been collected from eastern West Virginia, western Virginia, eastern Tennessee, North Carolina, South Carolina, central Georgia, eastern Alabama and south to northwestern Florida. On the other hand, Erythronium americanum ranges from northeastern United States and southern Canada from New Brunswick to Ontario and Minnesota, south to North Carolina, Georgia, Alabama, Mississippi and Tennessee.

Previous Investigation

Preliminary field work began in March, 1966, as the first leaves appeared. From plants observed in Montgomery and Stewart Counties, Tennessee, both yellow and purple anther color forms of Erythronium americanum were found with the purple anthered form being more common. From field observations, these plants show no apparent ecological specificity. However, a considerable number of morphological variations exist (other than anther coloration between or within populations).

Purpose of the Study

It was the purpose of this study (1) to statistically analyze morphological and anatomical data of both yellow and purple anther color forms of Erythronium americanum; (2) to investigate these two forms cytotaxonomically;

including chromosome counts and cross-breeding experiments (3) and to make an ecological and geographical survey of the yellow flowering Erythronium species of the southeastern United States.

Limitations of the Study

Fresh field material was collected from north and central Alabama, northwest Georgia, North Carolina and Tennessee. Particular emphasis was placed on populations of the two anther color forms of Erythronium americanum in Montgomery and Stewart Counties, Tennessee. Dried specimens of Erythronium were examined from the larger herbaria of the southeastern United States. (See page iv.)

All species of the genus were under investigation with the exception of the white flowering Erythronium albidum.

Definition of Terms

The following is a list of terms used at various points throughout the discussion that are not usually defined in general botany glossaries. These terms are defined for the convenience of the reader and in order that the context in which they are used will be clear.

(1) Allopatric - Taxa of which the geographic ranges do not coincide or overlap.

(2) Anthesis - The period during which a flower is fully expanded and pollination takes place.

(3) Apiculus - An abrupt, short, small tip.

(4) Apomixis - Types of reproduction in which there is no fusion of male and female gametes. This term is also defined by some botanists to include types of vegetative reproduction by runners, stolons, budding, etc.

(5) Biotype - A genetic race of individuals with an extremely high degree of phenotypic similarity.

(6) Cytotaxonomy - The integration of cytology and taxonomy in an attempt to resolve problems of relationships between organisms.

(7) Diploid - An individual having two times the normal haploid number of chromosomes.

(8) Exine - The outer coat of a pollen grain.

(9) Dimorphic - Occurring in two different forms.

(10) Intine - The inner covering membrane of a pollen grain.

(11) Karyology - Study of the chromosomal complex of a group of allied organisms associated with both morphology and chromosome number.

(12) Phenotype - The observable characteristics of

an organism due to the response of genotypic characteristics to the environment.

(13) Polyploid - An organism with more than two times the normal haploid number of chromosomes.

(14) Population - Individuals grouped together that bear a certain temporal or spatial relationship to each other.

(15) Sympatric - Taxa of which the geographic ranges overlap or coincide.

(16) Tepal - A collective term for a sepal or petal in certain members of the Liliaceae.

(17) Tetraploid - An individual having four times the normal haploid number of chromosomes.

II. METHODS AND MATERIALS

Chromosome studies of mitotic tissue of Erythronium americanum and Erythronium rostratum were made from fresh field material collected in Tennessee and Alabama. Sterile bulblets that were not immediately used were grown in the greenhouse at Austin Peay State College in a No. III-complete Hoagland's solution according to Hoagland and Arnon (1938). Stolon tips were used rather than root tips since this tissue is much larger, softer and has more cell divisions. Stolon tip clippings ca. one cm. long were excised in 0.2 per cent colchicine solution and aerated three to five hours. They were then fixed in alcohol-acetic acid (3:1) for one to sixteen hours. Fixation for sixteen hours was preferred over shorter periods (one hour) since this allowed ample time for the middle lamellae to dissolve. The tips were then transferred from the fixative to:

alcohol-acetic acid (1:1)	15 min.
45 per cent acetic acid in water	15 min.
cold 1N HCl	1 min.
hot 1N HCl	3 min.
rinse in distilled water	2 min.
stain in iron-acetocarmine	60 min.
45 per cent acetic acid	5 min.

The darkly stained tip was then placed on a clean slide in a drop of 45 per cent acetic acid. A cover slip

was added and pressure applied by placing a No. 6 solid rubber stopped on the cover slip and pressing firmly to insure adequate spreading of the individual cells. Difficulty in counting was encountered since Erythronium americanum has a relatively large number of chromosomes. This problem was alleviated by covering the oil-immersion lens (100X) with transparent tape and gently touching the cover slip above the individual cell being observed. This method allows the observer to spread the chromosomes as desired. However, care must be taken since too much pressure will rupture the cell membrane and chromosomes will be lost from the field.

The number of stomates per unit area of leaf surface and stomatal size were investigated in each taxon. Stomatal peels of the lower epidermis of fresh and dried specimens were made using Archer's herbarium plastic, (formula: toluene, 720 ml.; methanol, 180 ml.; ethyl cellulose, 250 gms.; Dow resin 275 V-2, 75 gms.) according to the method of Sinclair and Dunn (1961). For fresh material, the leaves were washed in a mild soap solution, to remove debris, and allowed to air dry. They were then flooded with toluene which acts as a solvent for the plastic. Archer's plastic was then applied directly to the leaf surface and spread to the desired thickness with the

edge of an index card. For dried material, the method of Sinclair and Dunn (1961) was slightly modified. A few drops of softening agent for dried plant structures as advocated by Pohl (1954) was added to ca. 100 ml. of cold water and a few leaves were immersed and allowed to soak overnight. This technique was used to return the dried leaves to a turgid state. After the plastic hardened, the imprint was carefully removed with tweezers. Permanent mounts were made by cutting the plastic to the desired size and placing the imprinted surface face down on a clean slide. A cover slip was then added and sealed on all sides with Scotch transparent tape. Measurements of the length and width of the stomatal apparatus and the number of stomates per 0.2 mm^2 of leaf surface were made at 100X magnification. A calibrated ocular micrometer was used for both measurements and counts.

Pollen viability tests were conducted with both yellow and purple anther forms of Erythronium americanum. The aniline-blue lactophenol stain was used according to the formula of Maneval (1936) and consists of the following:

phenol crystals (melted)	20 ml.
lactic acid	20 ml.
glycerine	40 ml.
distilled water	20 ml.
1 per cent aqueous aniline blue	5 ml.

Pollen was placed on a slide and washed with 95 per cent ethyl alcohol. Then a drop of aniline-blue lactophenol

stain was added and covered with a coverslip. The stain takes from 12 to 24 hours to penetrate the pollen grains. A total of 774 grains of the purple anthered and 936 grains of the yellow anthered form were randomly counted and evaluated. Those pollen grains that are fertile stained blue, the sterile grains remained unstained.

Measurements of pollen grain lengths were made from both yellow and purple anthered forms of Erythronium americanum. Prior to examination, the pollen grains were stained according to Wodehouse (1935). A small amount of pollen was placed on a clean slide and a drop of alcohol added to remove oily and resinous substances. Glycerine jelly (ca. one drop) was then added and passed over a bunsen burner until liquefied, after which a few drops of a saturated solution of methyl green in 50 per cent alcohol was added. A cover slip was placed on the slide and the slide was allowed to cool overnight before being evaluated. This stain as indicated by Wodehouse (1935) is superior in that pollen grains rarely become overexpanded. Measurements were made with a calibrated ocular micrometer at 100X magnification.

III. ECOLOGICAL AND GEOGRAPHICAL CONSIDERATIONS

The yellow flowering species of Erythronium of the southeastern United States occupy a wide variety of habitats. They are found in rich woods, ravines, shaded and open slopes of floodplains of creeks, moist and occasionally on dry, exposed sites. They have been observed in sandy loam, loam, and calcerous soils.

Erythronium rostratum is found in Tennessee, northern and central Alabama, Louisiana, Oklahoma, Arkansas and Missouri. Kral (1966) later reported this species from extreme eastern Texas. (Sabine Co., Kral 23369, San Augustine Co., Kral 23355) More collections and study of this species are needed since it shows an interesting disjunct distribution.

Erythronium umbilicatum subsp. umbilicatum occurs from eastern West Virginia, western Virginia, eastern Tennessee, North Carolina, South Carolina, central Georgia, eastern Alabama and south to northwest Florida. This subspecies exhibits a wide range of moisture tolerance since it occurs on both slopes and floodplains of creeks and/or on dry exposed rocky slopes (Parks and Hardin, 1963).

Erythronium umbilicatum subsp. monostolum has been reported only from eastern Tennessee and western North

Carolina. This subspecies has a more limited ecological tolerance than subsp. umbilicatum. It is found in rich woods and slopes at high elevations only if moisture is sufficient. These two subspecies may be considered sympatric if a large area is considered, but since mixed populations have never been reported they are then considered allopatric, in a strict sense (Parks and Hardin, 1963).

Erythronium americanum ranges from Minnesota to Maine, south to North Carolina, Georgia, Mississippi and Missouri. Parks and Hardin (1963) report Erythronium americanum subsp. harperi from northern Alabama and Tennessee.

Erythronium americanum occurs in moist ravines and on floodplains and north facing slopes of creeks. Infrequent individuals are occasionally found on drier, more exposed sites.

Common associates of Erythronium americanum are Sanguinaria canadensis, Anemonella thalictroides, Pachysandra procumbens, Viola papilionacea, Trillium cuneatum, Trillium recurvatum, Claytonia virginica, Dentaria laciniata, Erigenia bulbosa, Asarum canadensis, Delphinium tricorne and Hepatica acutiloba. The occurrence of various species of Aesculus with Erythronium is also worthy of note. Aesculus sylvatica, Aesculus pavia and Aesculus glabra were

commonly found in association with Erythronium umbilicatum, Erythronium rostratum and Erythronium americanum, respectively. With reference to the geographical distributions of Aesculus as proposed by Hardin (1957), correlation between the geographical distribution of these respective species are only slight. Although Aesculus appears to have a wider ecological tolerance than Erythronium, the niches filled by the respective species are somewhat similar.

In this phase of the study, emphasis was placed on the approximate relative frequency of purple versus yellow anthered forms of Erythronium americanum within a single population. Parks and Hardin (1963) state that yellow is the more common anther color with occasional individuals having purple anthers. A total of 103 plants from a population in Stewart County, Tennessee, were marked prior to flowering to obtain a rough indicator of relative frequency of the two anther color forms within a population. Of these, 76.7 per cent had purple anthers and 23.3 per cent had yellow anthers. Little or no emphasis can be placed on frequency data in view of the fact that one population in Montgomery County, Tennessee, was observed in which the yellow anthered form was completely absent.

Herbarium specimens were also examined with the anticipation that inferences could be made from the distribution of both forms. It was found, however, that sufficient

data could not be collected since pressed material of the yellow anthered form turns dark with age. More research dealing with distribution in relation to anther color is planned in the future.

IV. CYTOTAXONOMY

In this phase of the study, chromosome counts of *Erythronium rostratum* and both yellow and purple anther color forms of *Erythronium americanum* were made. Preliminary breeding experiments were also carried out between the two anther color forms of *Erythronium americanum*.

Chromosome Counts

A review of the cytotaxonomic literature revealed several karyological studies of the *Erythronium* species of the eastern United States. Darlington and Wylie (1955) report a basic number of n = 12 for the yellow flowering species. Cooper (1939) reports a basic number of n = 11 for *Erythronium albidum*. Parks and Hardin (1963) found *Erythronium rostratum* and *Erythronium umbilicatum* to be diploids with 2n = 24 chromosomes. Smith (1955), Parks and Hardin (1963) and Haque (1951) found *Erythronium americanum* to be a tetraploid with 2n = 48 chromosomes.

For the present study, sterile bulblets were collected in Tennessee and Alabama (Table I). Stolon tips, one cm. long, were pre-treated in colchicine for three to five hours prior to staining. Iron-acetocarmine prepared according to Belling (1926) was found to be satisfactory

TABLE I

CHROMOSOME COUNTS DERIVED FROM STOLON TIPS OF *ERYTHRONIUM ROSTRATUM* (R) AND *ERYTHRONIUM AMERICANUM*, YELLOW ANTHERS (Y), PURPLE ANTHERS (P)

Taxon	State	County	Date	Chromosome count
R	Alabama	Colbert	30 Mar 1967	24
Y	Alabama	Marion	31 Mar 1967	48
Y	Tennessee	Stewart	1 Apr 1966	48
Y	Tennessee	Montgomery	31 Mar 1966	48
Y	Tennessee	Stewart	4 Apr 1967	48
Y	Tennessee	Montgomery	5 Apr 1967	48
P	Tennessee	Stewart	31 Mar 1966	48
P	Tennessee	Montgomery	30 Mar 1967	48
P	Tennessee	Stewart	31 Mar 1966	48
P	Tennessee	Montgomery	29 Mar 1967	48

for staining the chromosomes. Subsequent chromosome counts of Erythronium rostratum were found to be 2n = 24 chromosomes (Figure 1). Counts for both anther color forms of Erythronium americanum were 2n = 48 chromosomes. Figure 2 shows the mitotic chromosomes of the purple anthered form and Figure 3 shows the mitotic chromosomes of the yellow anthered form. Photographs were made at ca. 2000X magnification with the aid of a Unitron BU-13 photomicroscope.

Breeding Studies

Preliminary cross-breeding experiments between the two anther color forms of Erythronium americanum were carried out during the course of the study. Although it was assumed that these two entities freely interbreed, the possibility of cross-sterility existed. Crosses were made under field conditions since flowering plants cannot be produced from seeds in less than four years (Blodgett, 1900). Immature anthers were removed from flower buds of four plants of both forms from a large population in Stewart County, Tennessee. The plants were then immediately covered with gallon jugs raised slightly above ground level to prevent cross-pollination by insects, wind, etc. During anthesis, pollen transfers were made and plants again covered. Two plants of both forms set seed while the other two were abortive.

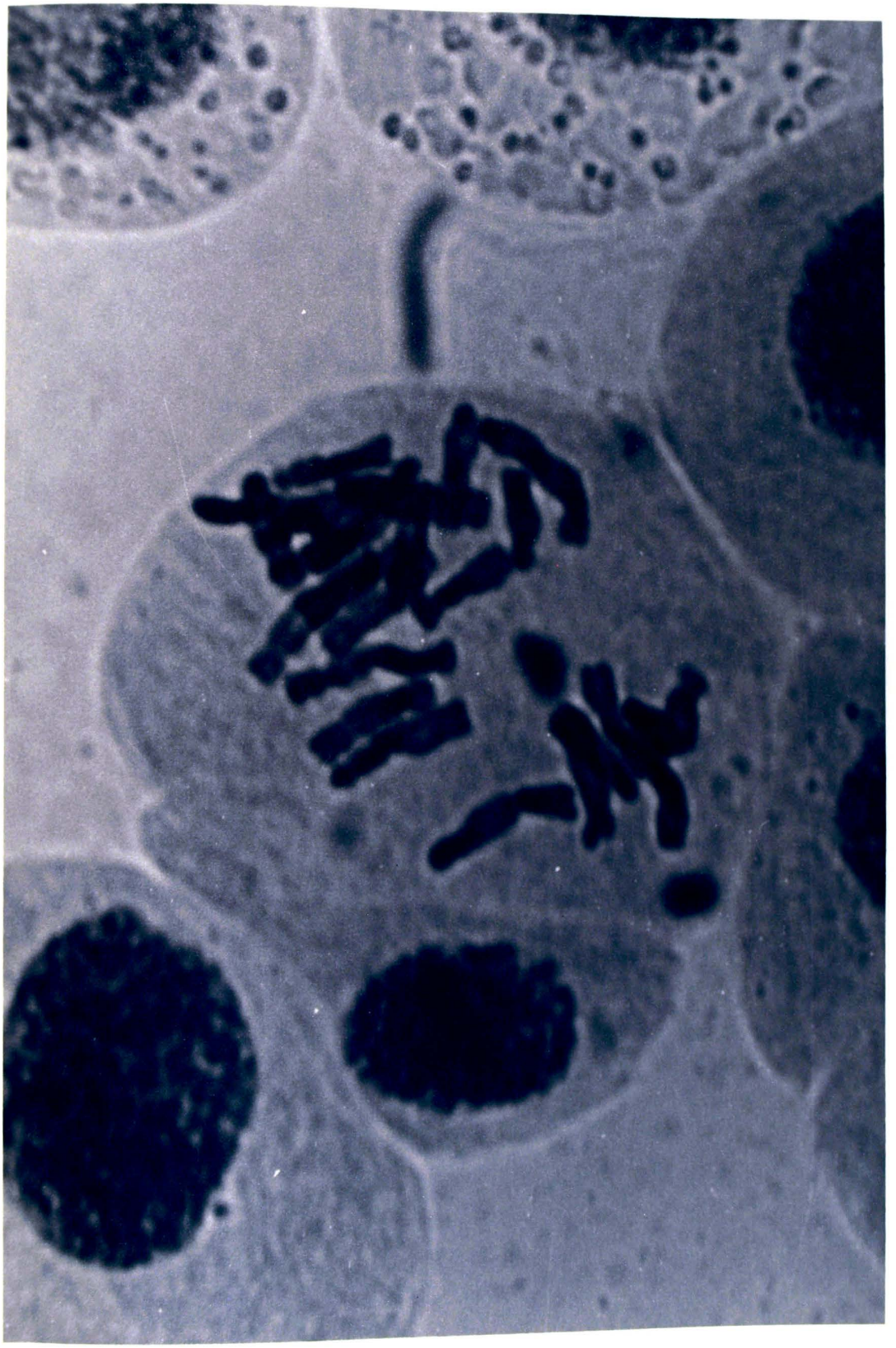

Figure 1. Mitotic Chromosomes of Erythronium rostratum.

Figure 2. Mitotic Chromosomes of the Purple Anthered Form of Erythronium americanum.

Figure 3. Mitotic Chromosomes of the Yellow Anthered Form of Erythronium americanum.

Little emphasis can be placed on this phase of the study since limited data were collected and it is not yet known if these seeds are viable. More investigations are planned in the future.

V. STOMATAL STUDY

Stomatal peels for microscopic examination were made using Archer's herbarium plastic according to the method of Sinclair and Dunn (1961). The number of stomata per unit area of leaf surface and stoma length and width measurements were made at 100X magnification from lower leaf surface peels with the aid of an ocular micrometer. Stomata were also found on the upper leaf surface of the three species investigated, but no measurements or counts were made. It is quite evident from observations, however, that there are fewer stomata per unit area of leaf surface on the upper epidermis than on the lower epidermis. These stomata also appear to be raised above the other epidermal cells as illustrated by Sinclair and Dunn (1961) while those of the lower leaf surface appear to be even with the other epidermal cells.

Correlations between stomatal size and distribution with ploidy level has been used in recent years as an important taxonomic tool by Stone (1961) and Sax and Sax (1937). In an effort to give more supporting evidence to the apparent intermediacy of Erythronium americanum to Erythronium rostratum and Erythronium umbilicatum as advocated by Parks and Hardin (1963), the number of stomata per

0.2 mm^2 of leaf surface was counted. A total of 140 counts from ten individuals (seven counts each) from both yellow and purple anthered forms of Erythronium americanum and 28 counts from four individuals (seven counts each) of Erythronium rostratum and Erythronium umbilicatum were made.

A total of 100 measurements of the length and width of the stomatal apparatus from ten individuals of both yellow and purple anther color forms of Erythronium americanum were made.

Mean values (Table II) of stomata measurements and counts of the anther color forms were subjected to the t-test of significant differences between means according to the method of Underwood, et al. (1954). Statistical analysis indicates that there is no significant difference between stomatal length and width (less than 95 per cent confidence level) of the two entities investigated. The analysis of the number of stomata per 0.2 mm^2 indicates a highly significant difference (greater than 95 per cent confidence level) between the two forms. Insufficient data, at the present time, does not warrant statistical analysis of the number of stomata per unit area of leaf surface of Erythronium rostratum and Erythronium umbilicatum. Mean values, (Table II) however, apparently indicate significant differences between the three taxa.

TABLE II

MORPHOLOGICAL DATA OF ERYTHRONIUM UMBILICATUM (U); E. ROSTRATUM (R) AND E. AMERICANUM: PURPLE ANTHER (P), YELLOW ANTHER (Y)

Measurement or count	Taxon	Range	Mean	SD	SE
Stomata per 0.2 mm^2	(P)	15 to 28	21.0	3.7	0.44
	(Y)	13 to 23	17.3	2.4	0.29
	(U)	26 to 43	33.8	-	--
	(R)	24 to 22	27.6	-	--
Pollen Grain Length	(P)	85 to 105u	94.2	4.8	0.39
	(Y)	85 to 104u	93.6	4.7	0.38
Stomatal Length	(P)	75 to 95u	83.7	5.3	0.53
	(Y)	75 to 91u	83.3	4.5	0.46
Stomatal Width	(P)	40 to 52u	45.3	3.2	0.33
	(Y)	39 to 50u	43.8	2.7	0.27

The variation in both the stomatal counts and measurements of the taxa investigated is shown graphically in Figure 4, according to the method of Dice and Leraas (1936), as modified by Hubbs and Perlmutter (1942). The horizontal line indicates the range, the central vertical line the sample mean, the clear rectangles one standard error on either side of the mean, and the shaded rectangles one standard deviation on either side of the mean.

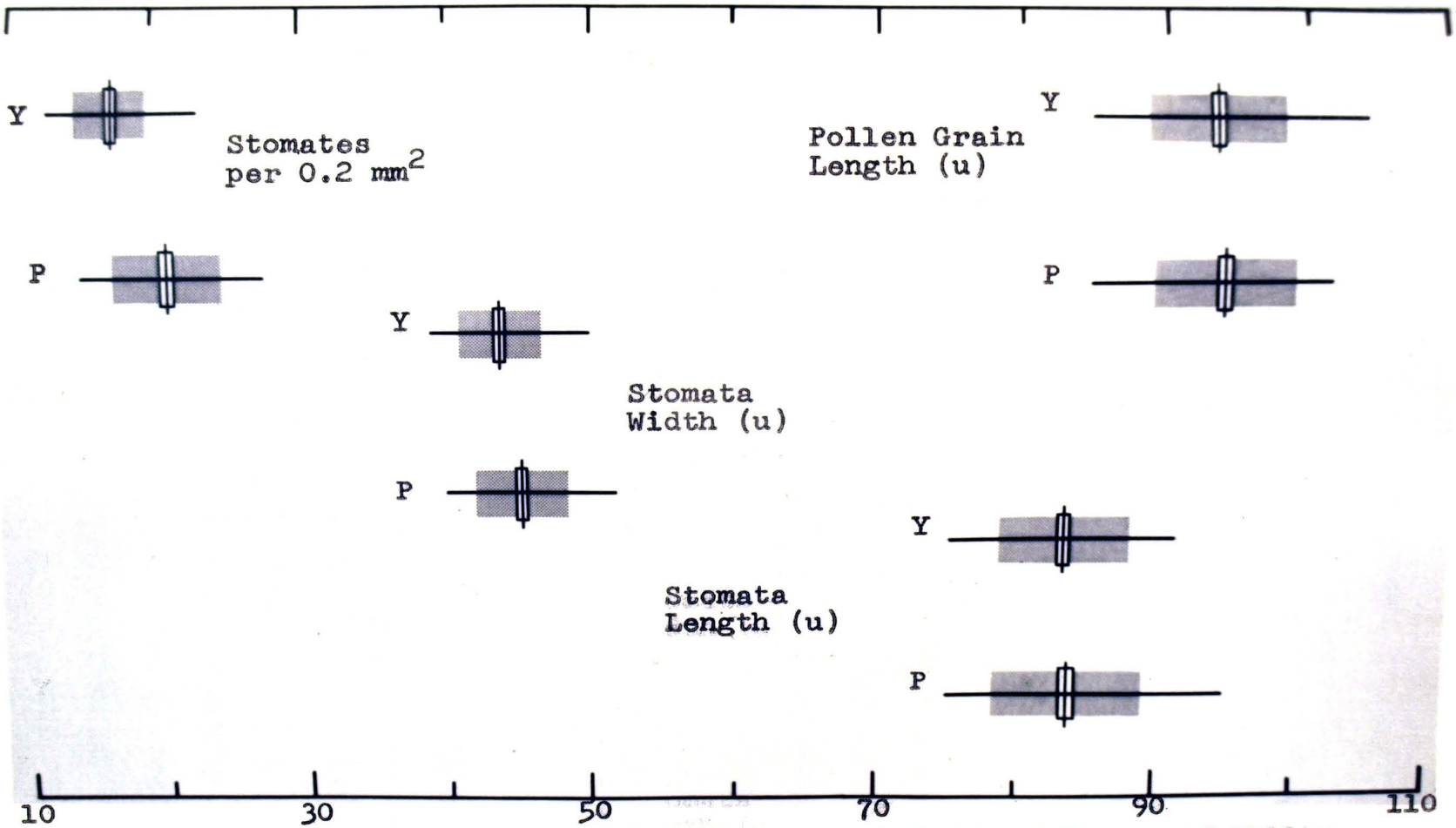

Figure 4. Ranges, Means, Standard Deviations, and Standard Errors of Pollen Grain Length, Stomata Length and Width, and the Number of Stomates per 0.2 mm^2 of Erythronium americanum, Yellow Anthers (Y), and Purple Anthers (P).

VI. POLLEN STUDY

In this phase of the study, two approaches were used in an attempt to gain a better understanding of the variation in Erythronium americanum. Pollen fertility tests were made and pollen grain lengths were measured in both purple and yellow anthered forms.

Pollen Fertility Tests

Pollen fertility tests have been used in recent years as an important taxonomic tool by Owczarzak (1952), Maneval (1936), King (1960) and Hauser and Morrison (1964). Stained pollen grains cannot be equated with viable pollen grains but this technique can be used as a rough indicator of relative fertility. Fresh pollen grains of Erythronium collected in Montgomery County, Tennessee, were stained according to the method of Maneval (1936). A total of 774 randomly selected grains of the purple anthered Erythronium americanum were evaluated. Of these, 693 were stained while 81 remained unstained, representing 89.53 per cent relative fertility. A total of 936 randomly selected grains of the yellow anthered Erythronium americanum were also evaluated. Of these, 871 were stained and 55 remained unstained, representing 93.05 per cent relative fertility.

Pollen Size

Pollen size has frequently been employed as a tool for delimiting taxonomic entities by Whitehead (1963, 1965), Buell (1946a) and Cain (1940). In the present investigation, measurements were made of the pollen grain length of both anther color forms of Erythronium americanum. Prior to examination, the grains were stained according to the method suggested by Wodehouse (1935). This is a methyl green stain that stains only the exine while leaving the intine and cell contents uncolored. This treatment was found to be satisfactory for gross morphology study and measurements. A total of 150 grains were examined and measurements were made of both anther color forms. From each specimen selected, 15 grains were randomly selected and evaluated. Cain and Cain (1944) state that 150 grains constitute an adequate sample for size frequency studies. Measurements were made at 100X magnification with the aid of a calibrated ocular micrometer.

Pollen grains of both forms were found to be one-sulcate as previously stated by Erdtman (1952).

Mean values (Table II, Chapter V) of pollen grain length of both anther color forms were subjected to the t-test of significant differences between means, according to the method of Underwood, et al. (1954). Statistical

analysis indicates that there is no significant difference between the pollen grain length (less than 95 per cent confidence level) of the two entities investigated.

Variation in the measurements between the two entities is shown graphically in Figure 4 (Chapter V) according to the method of Dice and Leraas (1936), as modified by Hubbs and Perlmutter (1942).

VII. MORPHOLOGICAL AND TAXONOMIC CONSIDERATIONS

In this phase of the study, a number of morphological characteristics of both yellow and purple anther forms of Erythronium americanum were subjected to statistical analysis. Field observations were also made for the purpose of morphologically comparing Erythronium americanum subsp. americanum and subsp. harperi. Variations encountered from field observations, and their apparent taxonomic importances, will also be discussed in this chapter. All measurements were made from fresh specimens collected in Montgomery and Stewart Counties, Tennessee.

A total of 54 measurements of sepal length, sepal width, petal length and petal width were made of both yellow and purple anther color forms of Erythronium americanum. Petal and sepal length measurements were made from the point of attachment to the peduncle to the apex. Petal and sepal width measurements were made at the widest portion, approximately one cm. from the point of attachment to the peduncle.

Variation in the measurements between the two entities is shown graphically in Figure 5, according to the method of Dice and Leraas (1936), as modified by Hubbs and Perlmutter (1942). The horizontal line indicates the range, the central line the sample mean, the clear rectangles one

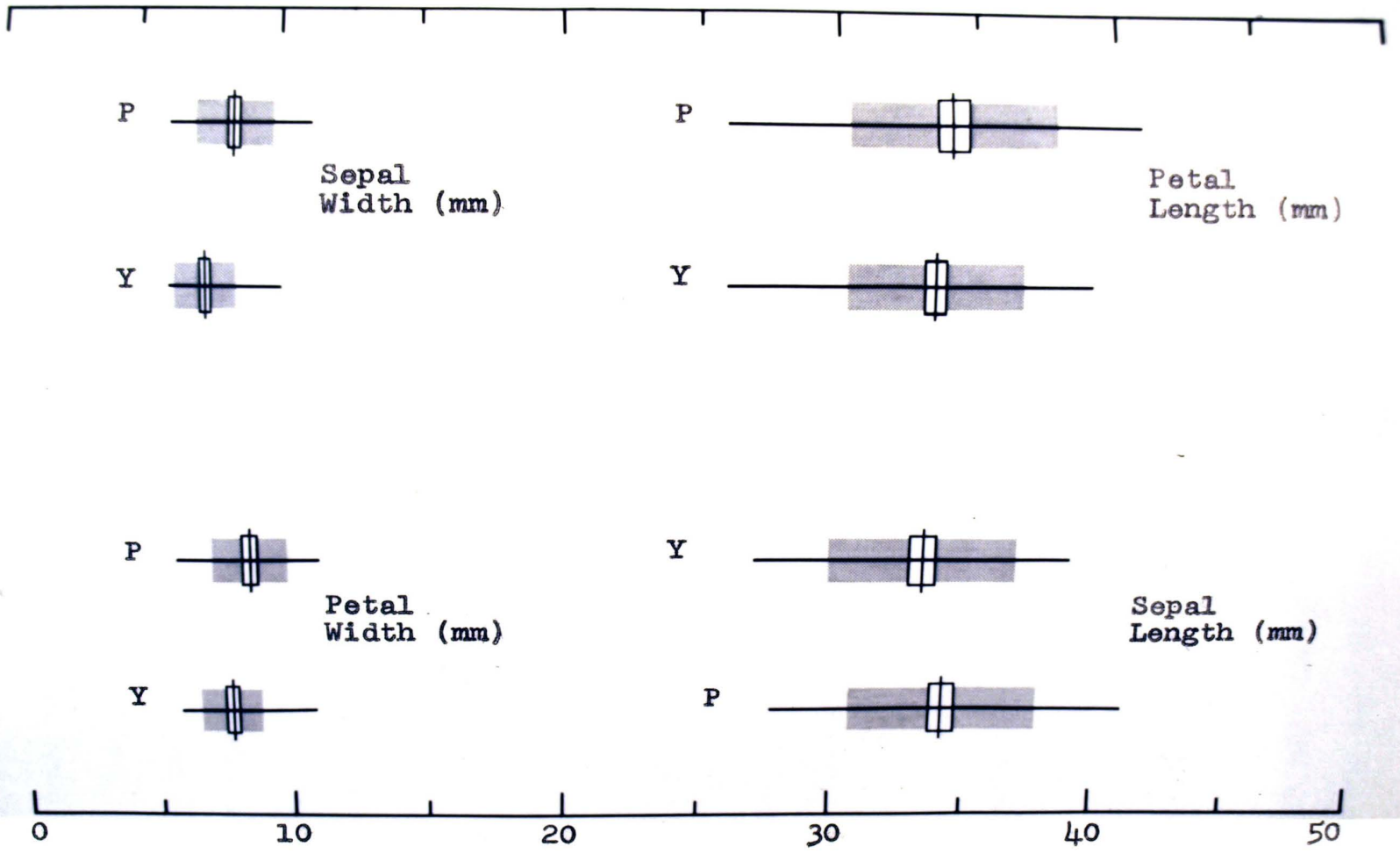

Figure 5. Ranges, Means, Standard Deviations, and Standard Errors of Petal and Sepal Length and Width Measurements of Erythronium americanum, Yellow Anthers (Y), and Purple Anthers (P).

standard error on either side of the mean, and the shaded rectangles one standard deviation on either side of the mean.

A total of 66 measurements were made of the peduncle and leaf length of the purple anthered form, and 51 peduncle, leaf measurements were made of the yellow anthered Erythronium americanum. Peduncle length measurements were made from the point of leaf sheathing to the base of the ovary. Leaf length measurements were made from the point of leaf sheathing to the apex of the largest leaf.

Fruit length and width measurements were made from 57 purple anthered and 70 yellow anthered forms of Erythronium americanum. A length-width ratio was calculated by dividing the width into the length.

Variation in the measurements between the two entities is shown graphically in Figure 6.

Mean values (Table III) of petal length and width, sepal length and width, leaf length, peduncle length and fruit length-width ratio were subjected to the t-test of significant differences between means, according to the method of Underwood, et al. (1953). Statistical analysis indicates that there is no significant differences (less than 95 per cent level of confidence) between petal length, sepal length, leaf length, and fruit length-width ratio of

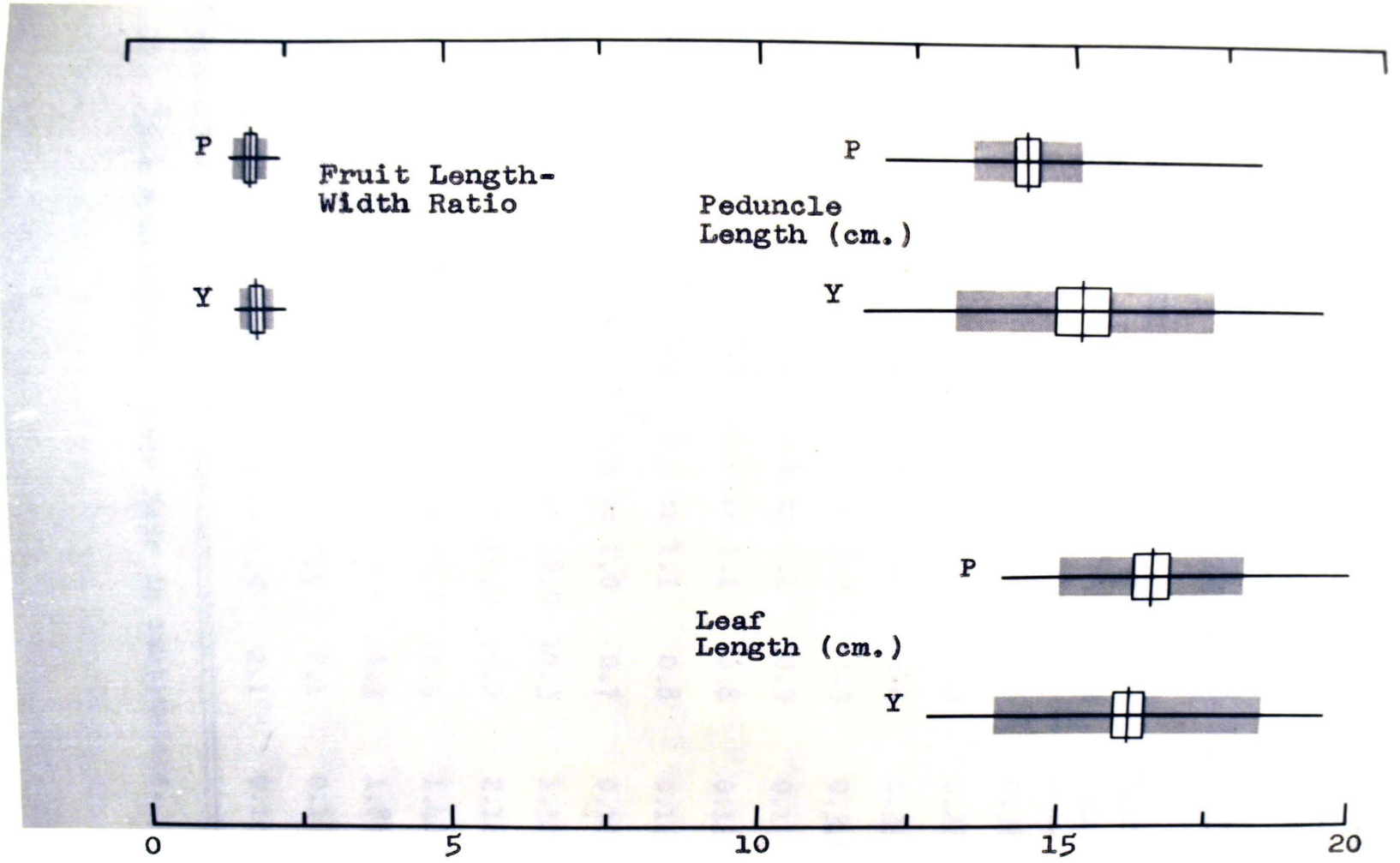

Figure 6. Ranges, Means, Standard Deviations, and Standard Errors of Fruit Length-Width Ratio, Peduncle Length, and Leaf Length of Erythronium americanum, Yellow Anthers (Y), and Purple Anthers (P).

TABLE III

MORPHOLOGICAL DATA* OF ERYTHRONIUM AMERICANUM: PURPLE ANTHER (P), YELLOW ANTHER (Y)

Measurement	Taxon	Range	Mean	SD	SE
Petal Length	(P)	2.6 to 4.1	3.3	0.38	0.05
	(Y)	2.6 to 3.9	3.3	0.28	0.04
Sepal Length	(P)	2.8 to 4.1	3.4	0.35	0.05
	(Y)	2.7 to 3.9	3.3	0.34	0.05
Petal Width	(P)	0.6 to 1.1	0.9	0.13	0.02
	(Y)	0.6 to 1.1	0.8	0.12	0.02
Sepal Width	(P)	0.6 to 1.1	0.8	0.12	0.02
	(Y)	0.6 to 1.0	0.7	0.10	0.01
Peduncle Length	(P)	12.0 to 18.0	14.3	1.51	0.19
	(Y)	11.8 to 19.0	15.2	2.16	0.31
Leaf Length	(P)	14.1 to 19.7	16.5	1.48	0.18
	(Y)	12.9 to 19.5	16.3	1.87	0.26
Fruit Length-Width Ratio	(P)	1.7 to 2.5	2.1	0.19	0.02
	(Y)	1.8 to 2.5	2.1	0.16	0.02

*All measurements were made in centimeters.

the two entities investigated. Significant differences (greater than 95 per cent level of confidence) were found in the mean values of petal width, sepal width, and peduncle length of the two anther forms.

A total of 56 fruiting capsules of the yellow anthered form and 47 capsules of the purple anthered form of Erythronium americanum were collected and weighed from a large population in Stewart County, Tennessee. These capsules were placed in plastic bags to prevent excessive dessication. Within three hours of collection they were removed from the plastic bags and immediately weighed. Three categories, erect, pendent, and resting on ground were used in respect to the orientation of the capsule. Mean erect values were .202 gms. and .190 gms., pendent .317 and .316 gms., on ground .389 and .401 gms. for the yellow and purple forms, respectively.

Percentage of ovule abortion was calculated by dividing the mean number of ovules per capsule into the mean number of seeds per capsule and subtracting from 100. Ovules from 29 capsules and seeds from 33 capsules of the purple anthered form were counted. Ovules from 22 capsules and seeds from 47 capsules of the yellow anthered were also counted. Results indicate 76.04 per cent ovule abortion of the yellow anthered form and 77.33 per cent ovule abortion in the purple anthered form.

According to Parks and Hardin (1963), Erythronium americanum subsp. americanum and subsp. harperi are morphologically similar with the exception of the fruiting capsule. Subspecies harperi has a distinct apiculus at the fruit apex after the style withers and in subsp. americanum this apiculus is infrequently found and if so much reduced. From collections made in Tennessee, Alabama, and Georgia, this characteristic was found to vary considerably between and within populations, rather than being ecologically or geographically isolated as would be expected in a subspecies. Two populations were observed in Alabama and Georgia, well within the range of subsp. harperi, in which not a single fruit had an apiculate apex. An even more interesting observation was made from a large population in Stewart County, Tennessee. These plants were marked for anther color prior to flowering and were observed until capsule maturity. It was found that the purple anthered forms had a distinct apiculus at the fruit apex (Figure 7), with few exceptions, while the yellow anthered forms were always devoid of this structure (Figure 8). This observation is in contradiction to the description of subsp. harperi by Parks and Hardin (1963) since they report only yellow anthers in subsp. harperi.

Another interesting observation was made from a

Figure 7. Fruiting Capsule of the Purple Anthered Form of *Erythronium americanum*.

Figure 8. Fruiting Capsule of the Yellow Anthered Form of *Erythronium americanum*.

large population in Montgomery County, Tennessee. The capsule of these plants were obovoid and indented at the apex with a distinct apiculus. These plants may well represent the most typical hybrid form since the obovoid, indented capsule resembles Erythronium umbilicatum and the distinct apiculus is similar to that of Erythronium rostratum.

Variations in the stamens of Erythronium have long been of interest to botanists. Meads (1893) work with Erythronium americanum indicates that the stamens are heteromorphic, the pollen sacs of the shorter ones open before the longer ones, and that individuals with yellow anthers are frequently found to be sterile while the dark brown or brownish-red ones are fertile. Graff (1916) states that Erythronium americanum has stamens of two distinct lengths with the shorter having a slight tendency toward later pollen maturation. Pickett (1917) also found the stamens of Erythronium grandiflorum to be dimorphic in all cases. Smith (1966) recently reported a variant of Erythronium americanum that had orchid colored anthers that were very narrow and completely sterile.

Field observations from several populations in Montgomery and Stewart Counties, Tennessee, indicate that the stamens of both anther color forms of Erythronium americanum are heteromorphic in two sets of three with the

stamens opposite the sepals being shorter. Although few measurements were made, it is quite evident that this difference is due to the filament length since there is no apparent visible difference in anther length. It was also noticed that the anthers opposite the sepals mature earlier than the ones opposite the petals. Earlier reports of pollen sterility by Meads (1893) and Smith (1966) seems to be associated with these early maturing anthers since they become coriaceous and much shorter. One to three days later, however, the anthers opposite the petals dehisce and again resemble in size and texture the ones opposite the sepals.

Leaf coloration was also found to be quite variable in Erythronium. Very pale leaves completely unmottled at all stages of growth were occasionally found in association with plants of "normal" leaf coloration. This form of Erythronium americanum was previously described by Moldenke (1953) as forma Oswaldi, in honor of the collector, and is deposited in the Britton Herbarium at the New York Botanical Garden. Plants with deep purple leaves, also appearing unmottled, were infrequently found. To the authors knowledge, these variants have not been given any previous taxonomic consideration.

The extent of petal auricle development was found to be quite variable in Erythronium americanum. Variations

from absent or much reduced to those that practically encircle the filament were observed in both anther color forms. These variations indicate the apparent intermediacy of Erythronium americanum to Erythronium rostratum and Erythronium umbilicatum as advocated by Parks and Hardin (1963) since the auricles of Erythronium rostratum are well developed and encircle the filament while they are absent in Erythronium umbilicatum.

Variations in capsule shape in cross section, tepal anthocyanin, and flecking of perianth parts are extensive and are of no apparent taxonomic importance in Erythronium americanum.

VIII. KEY TO THE SPECIES OF *ERYTHRONIUM*

1. Perianth white . . . . . *E. albidum*

1. Perianth yellow 2

2. Ovary and capsule beaked, rounded, truncate or apiculate at apex; stolons three to five; petal auricles reduced to well developed and encircling a filament 3

2. Ovary and capsule indented at the apex; stolons absent or one; petal auricles absent . . . . . *E. umbilicatum*

3. Ovary with a well developed beak, the style persistent; mature capsules held well above the ground by an upturned peduncle; petal auricles well developed and encircling a filament . . . . . *E. rostratum*

3. Ovary usually without a beak, if so only the base of the style persistent; mature capsules resting on ground or slightly above by distal upturn of peduncle; petal auricles present, rarely absent, never encircling a filament . . . . . *E. americanum*

IX. SYSTEMATIC TREATMENT

ERYTHRONIUM AMERICANUM Ker.

SYNONYMY

Erythronium aquatile Salisb. trans. Hort. Soc. (1812)

Erythronium aureum Hort. Angl. ex Loisel. Herb Amat. i. sub t. 51; Link, Enum Hort. Berol.

Erythronium carolinianum Walt, Fl. Carol.

Erythronium Dens-canis Michx, Fl. Bor. Am.

Erythronium flavescens Loisel. Herb. Amat.

Erythronium flavum Sm, in Rees, Cyclop. xiii. n. 2

Erythronium lanceolatum Pursh, Fl. Am. Sept. i 230

Erythronium Nuttallianum Schult, f. Syst. vii (1681)

Erythronium americanum Ker. Bot Mag. t. 1113 (1808)

Erythronium americanum var. Bachii Farwell, Rep. Mich. Acad. Sci. 21: 363 (1920)

Erythronium americanum forma carolinianum (Walt) Voss, Vilmorin, Blumengartnerei ed. 3 1:1115 (1895)

Erythronium americanum forma castaneum L. B. Smith, Rhodora, 31: 36 (1929)

Erythronium americanum forma Bachii (Farwell) Dole, Dole, Fl. Vermon, ed. 3: 84 (1937)

Erythronium americanum forma Nuttallianum (Schult.) Voss, Vilmorin, Blumengartnerei, ed. 3 1:1115 (1895)

Erythronium americanum forma Oswaldi (Moldenke) Phytologia, 4: 291 (1953)

Erythronium americanum var. rubrum Farwell, Papers Mich. Acad. Sci. 23: 128 (1938)

Erythronium Harperi Wolf, Castanea 6: 24 (1941)

Erythronium americanum subsp. Harperi (Wolf) Parks & Hardin Brittonia 15: 252 (1963)

Perennial herb. Rootstock a deep seated corm, sending out three to five elongate propogating stolons 1-40 cm. in length. Stem slender, 1-2 dm. tall, about half subterranean. Leaves alternate, cauline, borne near the middle of stem and therefore appearing basal, lanceolate to oblanceolate or elliptical, largest leaf 2-6 cm. broad, 12-25 cm. long. Leaves variously mottled with brown ranging from pale green to deep chestnut brown. Peduncle averaging 14-16 cm. long (11 to 19 cm.), arching at summit. Flowers one, regular, bisexual, yellow. Tepals separate to base but connivent, lanceolate, at anthesis spreading and eventually recurved. Petals 1.8 to 4.1 cm. long, 5 to 12 mm. wide; variously mottled with purple or brown spots and flecks on inner surface, darker colored on outside occasionally with a dark streak along the midrib; lateral veins forking and outward arched. Petal bearing a glandular spot or auricle on each margin near the base, sometimes much reduced. Sepals 2.5 to 4.2 cm. long, 6 to 13 mm. wide, variously mottled, usually darker on outside. Stamens six, hypogynous. Filaments distinct, elongate, flattened below, the ones opposite the sepals shorter. Anthers

linear-oblong, basifix, two celled, dehiscing by vertical slits; yellow or various shades of brown or purple, the ones opposite the sepals maturing early. Pistil one. Ovary superior, trilocular with axile placentation. Ovules few to many, bitegmic, biseriate. Style one, slender below, thickened above to three short stigmas. Mature capsule obovoid, oblong, oblong-obovate or ellipsoid; indented, truncate or rounded, with or without a distinct apiculus at the apex; rounded to distinctly triangular in cross section.

Moist ravines, floodplains and slopes of creeks from southeast Canada, Minnesota to Maine, south to North Carolina, Georgia, Mississippi and Missouri.

Type: Unknown

Figure 9 shows the distribution of Erythronium americanum in the southeastern United States. All herbarium material examined from north of the area shown in Figure 9 was found to be morphologically similar to the southeastern United States specimens.

Systematic treatments of Erythronium umbilicatum and Erythronium rostratum were not included in the study. However, distributions of these two entities are shown in Figure 9 for the purpose of presenting possible evolutionary relationships among the yellow flowering species of Erythronium in the southeastern United States.

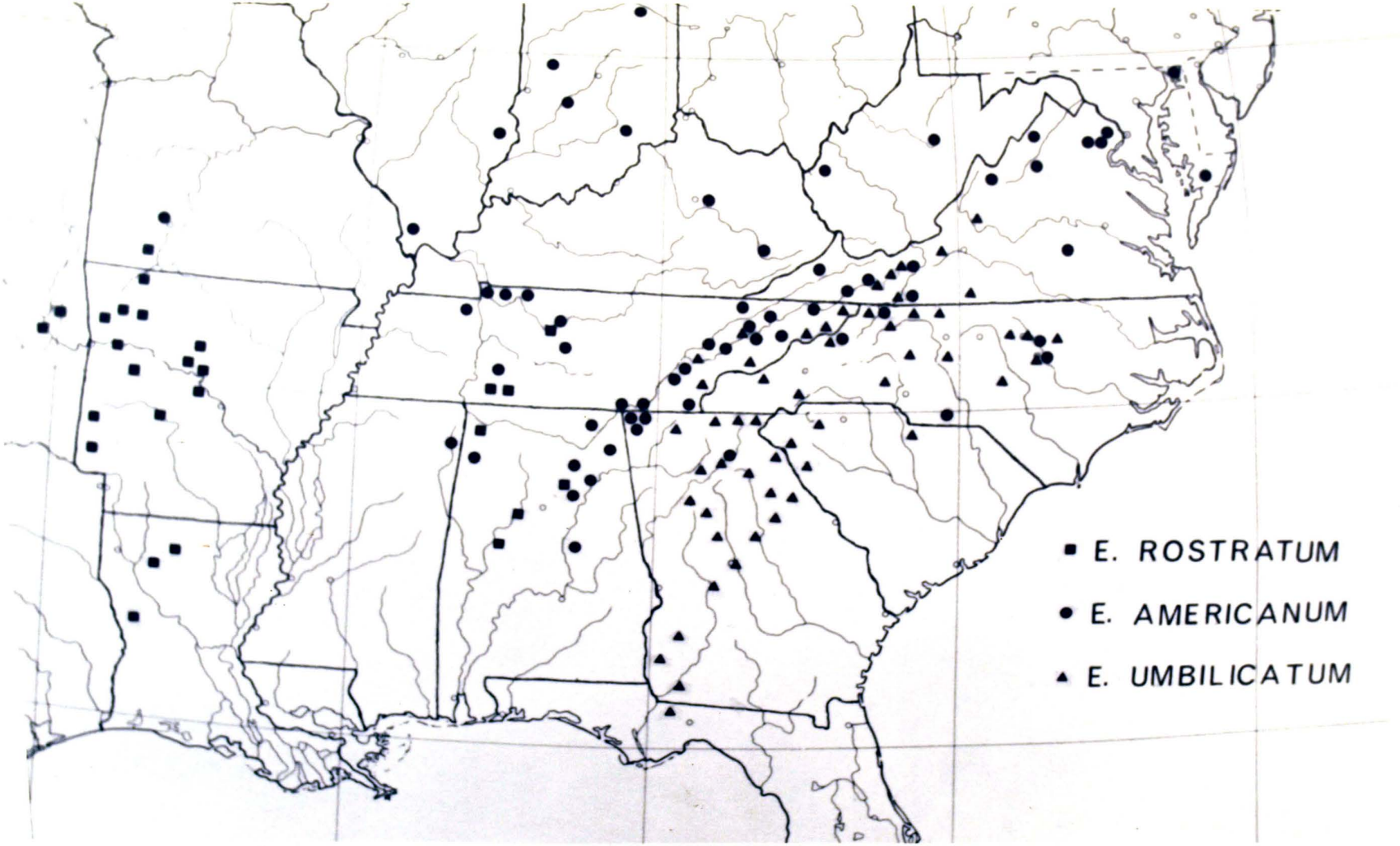

Figure 9. Distribution of the Yellow Flowering Species of Erythronium in the Southeastern United States.

X. SPECIMENS EXAMINED

ERYTHRONIUM AMERICANUM

UNITED STATES

ALABAMA. Blount Co.: Bangor, 20 Mar 1956, George R. Cooley 4465 (NSC); locality not specified, 20 Mar 1956, G. R. Cooley 4465 (NSC). DeKalb Co.: w of Collinsville, 23 Mar 1956, George R. Cooley and Francis Harper 4522 (USF); locality not specified 23 Mar 1956, Cooley and Harper 4522 (NSC). Etowah Co.: small stream on I-59N, ca. 10 mi ne of Gadsden, 29 Mar 1967, B. Eugene Wofford and Lloyd Scott 10158 - 10164 (APSC). Hamilton Co.: locality not specified, 22 Mar 1956, Cooley and Harper 4506 (NSC). Jackson Co.: locality not specified, 21 Mar 1956, Cooley and Harper 4504 (NSC). Marion Co.: 2 to 3 mi n of Guin in bottom lands of Beaver Creek, 17 Mar 1956, George R. Cooley et al. 4460 (USF); locality not specified, 17 Mar 1956, Roland Harper et al. 4460 (NSC); Buttahatchie River nr Hamilton, 28 Mar 1967, B. Eugene Wofford and Lloyd Scott 10137 - 10139 (APSC). Marshall Co.: Grant Fletcher's Hollow, 21 Mar 1956, George R. Cooley and Francis Harper 4496 (USF); locality not specified, 21 Mar 1956, Harper and Cooley 4496 (NSC).

GEORGIA. Catoosa Co.: base of n facing cliff at water gap thru Taylor Ridge 0.7 mi sse of Ringgold, 31 Mar 1951, Wilbur H. Duncan and G. W. McDowell 12223 (GA).

Dade Co.: Sitton's Gulch, Lookout Mountain, 2 Apr 1939, Harold C. Jones 499 (GA); nw facing slope 1.5 mi from Georgia-Alabama state line, Hwy. 11, 29 Mar 1967, B. Eugene Wofford and Lloyd Scott 10147 - 10157. Hall Co.: n facing slopes overlooking Chattahoochee River, 5.6 mi w6°n of Flowery Branch, 9 Apr 1955, Wilbur H. Duncan 18397 (GA). Walker Co.: e facing slopes on bend in Chicamauga Creek, 2.2 mi of Kingston, Wilbur H. Duncan et al. 12131 (GA); small bluff on n side of East Armuchee Creek 1.7 mi s of Villanow, 1 Apr 1951, Wilbur H. Duncan and G. W. McDowell 12259 (GA); 1.7 mi s of Villanow, 29 Mar 1967, B. Eugene Wofford and Lloyd Scott 10143 - 10146 (APSC).

INDIANA. Huntington Co.: along the Wabash River, 6 mi w of Huntington, 18 Apr 1959, N. C. Henderson s.n. (FSU). Jennings Co.: in the Guthrie Memorial Tract, 16 Apr 1937, Scott McCoy 4533 (FSU). Monroe Co.: Cedar Cliff, 10 mi s of Bloomington 18 Apr 1936, Ray C. Friesner 9542 (DUKE); Cedar Cliffs, sw corner section 18, Clear Creek Twsp., 13 Apr 1960, Andre F. Clewell 385 - 386 (FSU). Putnam Co.: sw corner of D.P.U. Arboretum, Greencastle, 12 Apr 1935, R. Dawson 1063 (DUKE).

ILLINOIS. Lawrence Co.: 1½ mi e of Sumner, 15 Apr 1952, Harry E. Ahles 5820 (MISSA). Union Co.: Cobden, date not specified, A. B. Seymour, s.n. (DUKE).

KENTUCKY. Clark Co.: along Boohe's Creek, 29 Mar 1961, Gleen B. Collins 49 (USF). Perry Co.: Trace Branch nr Buckhorn, 10 Apr 1940, R. O. Dedmon s.n. (DUKE).

MAINE. York Co.: Kennebunkport, 17 May 1919, Amy M. Pier s.n. (DUKE).

MARYLAND. Cecil Co.: along Susquehanna River, 18 Apr 1913, Harold St. John and Bayard Long 1008 (DUKE).

MASSACHUSETTS. Hampden Co.: bank of Chicopee River, 12 May 1926, F. C. Seymour 568 (DUKE). Hampshire Co.: M.A.C. farm, May 1885, Minnie A. Locke s.n. (DUKE). County not specified: Swampscott, 30 May 1888, A. B. Seymour s.n. (DUKE).

MICHIGAN. Washtenaw Co.: e of Fleming Creek under Cathedral Elms, 4 May 1963, A. C. Elmore 17 (DUKE). County not specified: about Alma, 26 Apr 1895, Charles A. Davis s.n. (DUKE).

MINNESOTA. Chemmy Co.: low woods, 4 May 1896, T. F. Lucy 10376 (DUKE). Houston Co.: Beaver Creek Valley State Park, w of Caledonia, 8 May 1949, R. M. and O. M. Schuster s.n. (DUKE).

MISSISSIPPI. Itawamba Co.: 6 mi ssw Red Bay, 17 Apr 1965, R. Kral 23649 (DUKE, FSU).

MISSOURI. Greene Co.: ca. 200 yds s of junction county road DC and NN, 4 Apr 1958, Paul L. Redfearn, Jr. 3435 (FSU).

NEW HAMPSHIRE. County not specified: Hammer, 10 May 1931, John F. Reer s.n. (DUKE).

NEW JERSEY. Somerset Co.: Raritan R. n of Raritan, 25 Apr 1936, M. A. Chrysler s.n. (DUKE).

NEW YORK. Madison Co.: White Pine plantation, e of Cazenovia, 13 May 1950, Walter S. Riley 17 (DUKE). Tompkins Co.: s side of six mi creek ravine, s of Ithaca, 3 May 1944, R. M. Schuster A7632 (DUKE); six mi creek ravine, 1 May 1900, H. S. Jackson s.n. (DUKE); around Beebee Lake, 5 May 1944, R. M. Schuster A7633 (DUKE). Ulster Co.: locality not specified, 24 Apr 1889, collector not specified s.n. (DUKE). County not specified: nr Catskill Aqueduct, 20 Apr 1934, J. A. Gleason, Jr. 1319 (DUKE).

NORTH CAROLINA. Anson Co.: Brown Creek, 1 mi e of Hwy 54, 25 May 1948, Steve Boyce s.n. (NSC). Ashe Co.: nr N. C. 88 0.7 mi s of Warrensville, 3 May 1958, A. E. Radford 32783 (NCU, GA). Avery Co.: nr intersection of Highways 184 and 105, 28 Apr 1960, Gwynn W. Ramsey 380 (TENN). Durham Co.: bluffs on Eno River about two miles from Catsburg and 4 mi ne of Durham, 4 Apr 1963, W. R. Anderson (DUKE, USF, FSU, GA); ca 2 mi n of Weaver on Eno River, 16 Apr 1966, Eugene Wofford, H. E. Ahles and R. Riggins 10101-10111 (APSC). Wake Co.: floodplain of Crabtree Creek, 7 mi nw of Raleigh, 11 Apr 1963, James W. Hardin 2571 (NSC);

floodplain of Crabtree Creek of Ebenezer Rd. nw of NCSC, 15 Apr 1966, B. Eugene Wofford 10094 - 10010 (APSC).

OKLAHOMA. County not specified: 3 mi ne of Bernice, 1 Apr 1933, Ramon Reno 28 (DUKE).

PENNSYLVANIA. Sullivan Co.: 1½ mi w of Shunk, 30 Apr 1960, H. A. Wahl 19608 (FSU).

RHODE ISLAND. Bristol Co.: Barrington, 26 Apr 1939, W. V. Brown and J. O. Young s.n. (DUKE).

TENNESSEE. Anderson Co.: above Beech Grove Community, 23 Apr 1961, A. J. Sharp and Dan Brown 27926 (TENN); clear creek, 8 Apr 1939, J. C. McDaniel 509 (TENN); Oak Ridge, 10 Apr 1950, James E. Addison s.n. (TENN); Clear Creek, Norris, 29 Mar 1937, W. D. Varnell s.n. (TENN); Savage Garden, Coal Creek, 12 Apr 1934, H. M. Jennison 471 (TENN); Coal Creek, 12 Apr 1937, H. M. Jennison 3547 (TENN). Clairborne Co.: 7.9 mi e of Speedwell, 22 Apr 1961, A. J. Sharp et al. 27888 (GA, TENN). Davidson Co.: Percy Warner Park, Nashville, 18 Mar 1945, Elsie Quarterman, 110 (DUKE). Grangier Co.: Bean Gap in Clinch Mountain between Bean Gap and Thornhill, 5 Apr 1953, A. J. Sharp 17197 (TENN). Greene Co.: 4,500 ft on top of Bald Mountain, 20 May 1962. A. J. Sharp et al. 31156 (FSU). Hamblen Co.: locality not specified, Apr 1937, D. L. Morgan s.n. (TENN). Hawkins Co.: nr Dodson Creek, 20 Apr 1955, Jim Wolfe 221 (TENN).

Henry Co.: 4 mi n of Paris, 26 Mar 1951, K. Key s.n. (MISSA). Knox Co.: creek n of Kingston Pike at a point opposite Scenic Drive, 6 May 1939, H. M. Jennison 304 (TENN); Little America past Island Home, 7 Apr 1934, H. M. Jennison 175 (TENN); woodland along Tennessee River, 1 Apr 1934, H. M. Jennison 49 (TENN); wooded slope along 3rd Creek, rear Lutz Hill places, 5 Apr 1934, H. M. Jennison 122 (TENN); slopes along 3rd Creek, rear of Lutz Hill places, 5 Apr 1934, H. M. Jennison 121 (TENN); Hewitt's Bluff along Clinch River, 18 Apr 1962, A. J. Sharp and William H. Ellis 26262 (TENN); along Tennessee River nr Cherokee Bluffs, 1 Apr 1934, H. M. Jennison 49 (TENN). Lewis Co.: sw of Hohenwald, bottoms and bluffs of Trace Creek nr bridge on Tenn. Highway 48, 21 Apr 1949, A. J. Sharp et al. 10087 (TENN). Marion Co.: crossing of left Sequatchie River, n of Sequatchie, 22 Apr 1951, R. R. Shanks et al. 15582 (TENN); valley above Orme, 21 Apr 1962, A. J. Sharp et al. 26305 (TENN). Montgomery Co.: APSC farm recreation area nr St. Bethlehem Community, ene facing slopes, 19 Apr 1966, B. Eugene Wofford 10046 - 10064 (APSC); APSC farm, recreation area nr St. Bethlehem community, ene facing slopes, 7 Apr 1966, B. Eugene Wofford and Tommy Bruton 10046 - 10050 (APSC); APSC farm, recreation area nr St. Bethlehem community, ene facing slopes, 30 Mar 1966, B. Eugene Wofford 10031 - 10045 (APSC); n facing slope

adjacent to West Fork Creek, Billy Dunlop Memorial Park, 28 Mar 1966, B. Eugene Wofford 10112 - 10117 (APSC); APSC farm recreation area nr St. Bethlehem community, ene facing slopes, 14 Apr 1967, B. Eugene Wofford 10118 - 10129 (APSC). Polk Co.: Hiwassee Gorge between Wetmore and Reliance, 31 Mar 1949, A. J. Sharp et al. 10521 (TENN); between Junebug and Tieskee Creeks, w of Reliance, 5 Apr 1958, Al Koelling 20130 (TENN). Putnam Co.: Stamps Hollow, Monterey, 18 Apr 1936, A. J. Sharp and S. A. Cain 4388 (TENN). Rhea Co.: Devil's Hole Gorge, 8 Apr 1956, R. E. Shanks 19149 (TENN). Roane Co.: above Harriman, slopes above arm of lake of Emory Lake, 2 May 1962, A. J. Sharp et al. 26473 (TENN). Robertson Co: n facing slope along U. S. 41, 2 mi nw of Adams nr Red River bridge, 12 Apr 1958, Floyd Brown s.n. (TENN). Rutherford Co.: woods along Stone's River, 15 mi nw of Murfreesboro, 16 May 1956, H. R. DeSelm 995 (TENN); Snail Shell Creek nr Murfreesboro, 13 Mar 1934, L. R. Hesler and Harold Bold s.n. (TENN). Stewart Co.: adjacent to Bear Creek, nne facing slopes, .3 mi s of TVA marker 9P1, 22 Mar 1966, B. Eugene Wofford and Billy C. Evans 10008 - 10019 (APSC); adjacent to Bear Creek, nne facing slopes, .3 mi s of TVA marker 9P1, 26 Mar 1966, B. Eugene Wofford 10001 - 10007(APSC); adjacent to Bear Creek, nne facing slopes, .3 mi s of TVA marker 9P1, 10 Apr 1966, B. Eugene

Wofford and Deborah L. Wofford (APSC). Sullivan Co.: nr Kingsport, n facing river bluffs along Holston River, 3 May 1964, A. J. Sharp et al. 32662 (TENN). County no specified: Love's Creek, 6 Apr 1925, S. C. Fain s.n. (TENN).

VERMONT. Chittenden Co.: Burlington, 10 May 1947, Leopold A. Charette 706 (FSU). County not specified: rich woods, Brandon, 5 May 1922, D. Lewis Dutton 207 (DUKE).

VIRGINIA. Amelia Co.: locality not specified, J. B. Lewis and A. B. Massey s.n. (VPI). Augusta Co.: vicinity of Lyndhurst, bank of South River, 25 Apr 1936, Elizabeth S. Rawlinson 23A (VPI). Carroll Co.: ca 2 mi ne of Sylvatus, 22 Apr 1960, R. Kral 9858 (FSU, VPI). Dickerson Co.: 7 mi se of Haysi, 21 Apr 1962, R. Kral 14524 (FSU). Fairfax Co.: along canal, Great Falls, 15 Apr 1934, Virginia Wismer 706 (DUKE); Tripps Run, Sleepy Hollow, 1 mi s of Falls Church, 8 Apr 1945, J. Ewan 17125 (GA); nr Merrifield, 18 Apr 1937, H. A. Allard 2509 (VPI). Fauquier Co.: floodplain Broad Run, below Beverly, 26 Apr 1936, H. A. Allard 1235 (VPI). Montgomery Co.: Ribbs Spring, 17 Apr 1963, Guthrie and Settle s.n. (VPI). Prince William Co.: below Beverly Mill, 19 Apr 1942, H. A. Allard 9845 (VPI); eastern slope of Bull Run Mountain, 27 Apr 1941, H. A. Allard 8470 (VPI). Russell-Washington Co.: summit of Clinch Mountain between Elk Garden and Hayters Gap, 3 May 1965, R. Kral 23933 (DUKE). Russell Co.: exact

sw corner of county ca 3 mi nw of Mendota, Clinch Mountain, 1 May 1965, R. Kral 23835 (DUKE); ca 3 mi nw of Mendota, Clinch Mountain, 1 May 1965, R. Kral 23835 (FSU). Shenandoah Co.: Stonewall Bend, Woodstock, 14 Apr 1933, Lena Artz 326 (VPI). Wythe Co.: at first crossing of Lee Highway and Reed Creek, 21 Apr 1937, A. B. Massey s.n. (VPI). County not specified: White Top Mountain, date not specified, J. H. Roller s.n. (VPI). County not specified: Bridgewater, May 1927, collector not specified, 70 (VPI).

WEST VIRGINIA. Cabell Co.: Milton, 15 Apr 1936, Louis Williams 407 (TENN, DUKE, FSU). Upshur Co.: Buckhammon River nr Tennerton, 14 Apr 1960, Cornelia Ryerson and G. B. Rossenbach s.n. (USF). County not specified: T. Sairs' place nr Fox River, 13 May 1906, P. O. Schallert 207 (DUKE).

ERYTHRONIUM UMBILICATUM

UNITED STATES

FLORIDA. Gadsen Co.: slope above floodplain, Little River, between Havana and Quincy, 14 Feb 1957, R. K. Godfrey et al. 55340 (GH, GA, TENN, FSU, NSC); floodplain, Little River, between Havana and Quincy, 14 Feb 1958, R. K. Godfrey 56230 (GA, FSU, TENN, USF, NSC); along Little River between Havana and Quincy, 10 Mar 1963, R. K. Godfrey 62651 (FSU); 6 mi e of Quincy, 15 Feb 1957, R. Kral 4059B (FSU).

GEORGIA. Bibb Co.: locality not specified, 5 Feb 1933, Beryl T. Mounts s.n. (DUKE). Butts Co.: slopes on e side of stream 6.5 me w of Jackson, 1 Apr 1953, Wilbur H. Duncan and James W. Hardin 15704 (GA). Decatur Co.: ravine, junction of Flint and Chattachooche Rivers, 31 Mar 1937, Robert F. Thorne and W. C. Muenscher 2591 (GEO). DeKalb Co.: s side of Stone Mountain, 24 Apr 1949, W. J. Dress et al. 606 (GA); Mt. Panola, 6 Apr 1940, J. Mathews s.n. (GEO); base of Stone Mountain, 14 Apr 1941, Brown, Brock and Kelly s.n. (GEO); Stone Mountain, 8 Mar 1934, P. W. Fatting s.n. (GEO); Stone Mountain, 8 Apr 1934, M. L. Smith s.n. (GEO); Stone Mountain, 10 Apr 1941, R. E. Shanks and S. L. Meyer 1198 (TENN); base of Stone Mountain,12 Feb 1937, Don Eyles 1567 (GEO); nr base of Stone Mountain, 9 Mar 1935, Don Eyles 1590 (GEO); bog on sw side of Stone Mountain, 12 Feb 1937, Don Eyles 1567 (GEO); Pine Mountain, Lithonia, 24 Feb 1937, Don Eyles 1620 (GEO); open grassy slopes, Little Stone Mountain, 15 Mar 1936, Joseph H. Pyron and Rogers McVaugh 468 (GA); grassy meadows, Stone Mountain, 15 Mar 1936, Joseph H. Pyron and Rogers McVaugh 479 (GA); grassy meadows, Stone Mountain, 15 Mar 1936, Joseph H. Pyron and Rogers McVaugh 476 (GA, DUKE); Mount Rollaway, 23 Mar 1958, R. Nunan and A. Rebuck 1609 (FSU, TENN, GEO). Early Co.: foot of bluff along Chattahooche River w of Hilton, 7 Apr 1947, Robert F. Thorne and W. C.

Muenscher 2810 (GEO); bluff along Chattahooche River, 12 Apr 1947, W. C. Muenscher and S. J. Smith 2965 (GEO, GA). Forsythe Co.: e base of northern ridge of Sawnee Mountain, 21 Apr 1956, Wilbur H. Duncan 20077 (GA). Greene Co.: 9 mi nnw of Greensboro, 13 Apr 1956, Wilbur H. Duncan et al. 19917 (GA). Hall Co.: n facing slopes overlooking Chattahooche River, 5.6 mi w6°n of Flowery Branch, 9 Apr 1955, Wilbur H. Duncan 18397 (GA). Hart Co.: slopes overlooking Savannah River ene of Hartwell, 16 Apr 1953, Wilbur H. Duncan et al. 15483 (GA). Jackson Co.: Tallassee Shoals, 7 Mar 1936, J. M. Reade s.n. (GA); spring nr dam, Tallassee Shoals, 12 Apr 1933, J. M. Reade E8281 (GA). Murray Co.: mountain slopes about 1 mi e of Grassy Mountain, 26 Apr 1953, Wilbur H. Duncan and Marion B. Duncan 15778 (GA). Oglethorpe Co.: 7 mi se of Lexington, 20 Mar 1937, Joseph H. Pyron and Rogers McVaugh 1533 (GA); Echols Mill, 12 mi ne of Lexington, 4 Apr 1938, Joseph H. Pyron and Rogers McVaugh 2442 (GA); wooded banks of Buggalo Creek e of Lexington, 19 Mar 1942, Wilbur H. Duncan 4646 (GA); along Millstone Creek just below granite outcrop Echols Mill, ne of Lexington, 26 Apr 1952, Wilbur H. Duncan 13448 (GA). Putnam Co.: 13 mi e of Eatonton, granite outcrops, 20 Feb 1938, Joseph H. Pyron and Rogers McVaugh 2071 (GA). Rabun Co.: in bog at edge of North Carolina-Georgia state line, n1°e of tower on Rabun Bald, 4 May 1947, Wilbur H. Duncan

7507 (GA). Randolph Co.: ravine along middle branch of Holanna Creek 1 mi nw of Cuthbert, 3 Apr 1948, Robert F. Thorne and W. C. Muenscher 7951 (GEO, GA); the Rocks, nr Milstead, 23 Mar 1947, Haskell Venard 613 (GEO); along branch, "the Rocks", 12 Feb 1939, Don Eyles 3873 (GEO); 5 km. nne of Conyers, "the Rocks", 5 Mar 1937, Ruth Beall 6 (GA). Taylor Co.: wooded slopes along small tributary of Flint River nw of hwy. bridge, ne of Butler, 28 Mar 1953, Wilbur H. Duncan and James W. Hardin 14850 (GA). Towns Co.: below spring, Enotah Bald-alt. 1350 m, 18 Apr 1937, Joseph H. Pyron and Rogers McVaugh 1566 (GA). Union Co.: w side nr summit of Brasstown Bald, 25 May 1953, Wilbur H. Duncan and James W. Hardin 16326 (GA); n facing ravine about 0.3 mi ese of Wolfpen Gap, 18 Apr 1959, Wilbur H. Duncan 21591 (GA). Wilkes Co.: 5 mi e of Washington, 26 Feb 1939, Joseph H. Pyron 130 (GA).

NORTH CAROLINA. Ashe Co.: 13 mi n of Watanga Co. line, 13 May 1961, James W. Hardin 2366 (NSC). Davidson Co.: along small creek 9 me ssw of Denton, 16 Apr 1953, Wilbur H. Duncan et al. 15507 (GA). Durham Co.: Eno River nr Durham, 29 Feb 1928, P.C.H. s.n. (DUKE); n slopes of Eno River, date not specified, collector not specified s.n. (DUKE); ca. 2 mi n of Weaver on Eno River, 16 Apr 1966, B. Eugene Wofford and Harry E. Ahles 10075 - 10093 (APSC). Henderson Co.: 3 mi n of Mills River, 17 Apr 1952, G. Cooley

982 (USF). Iredell Co.: 9 me w of Statesville off Interstate Hwy 40, 13 May 1961, James W. Hardin 2368 (NSC). Lee Co.: floodplain, Deep River, 22 Apr 1934, H. J. Oosting 3456 (DUKE). Lincoln Co.: floodplain woodland along N. C. Rt. 150, 6 mi e of Lincolnton, 17 Apr 1949, R. K. Godfrey and William B. Fox 49014 (NSC, TENN, DUKE). Mitchell Co.: Roan Mountain, ½ ne above Buladean, 14 Apr 1935, D. M. Brown 176 (DUKE); nr head of Pigeonroost Creek, nw of Poplar, 27 Apr 1963, James W. Hardin 2507 (NSC). Nash Co.: Moccasin Creek, 4 mi se of Middlesex, 11 Mar 1956, Harry E. Ahles and C. R. Bell 10016 (VPI). Orange Co.: bank of New Hope Creek, ca. 3 mi se of Blackwood, 18 Mar 1954, P. A. Kessler s.n. (NCU, USF); tributary Bolin Creek n of Chapel Hill, 18 Feb 1957, P. J. Crutcherfield 4 (TENN); below Piney Mountain, 3 Mar 1933, H. L. B. s.n. (DUKE); 2 mi from Chapel Hill, nw slope overlooking creek, 25 Mar 1959, P. W. Carlton 39 (FSU); Duke forest nature area 7 mi wnw of Duke University, 20 Apr 1960, B. C. Welch s.n. (NSC). Stokes Co.: Hanging Rock State Park, 21 Apr 1956, A. J. Sharp 20469 (TENN); Cascades, 15 Mar 1936, P. O. Schallert s.n. (USF); below the Cascades, Hanging Rock State Park, 23 Apr 1950, R. K. Godfrey et al. 50316 (NSC); banks of Towa Fort Creek outskirts of Walnut Cave, 23 Apr 1950, H. L. Bloomquist et al. 50312 (NSC). Surry Co.: ca. 8 mi sw of Mt. Airy, 22 Apr 1960, R. Kral 9868 (FSU); bank of Fisher River nr bridge

e of Dobson, 13 Apr 1963, Jackson and Scarborough s.n. (NSC). Swain Co.: nr Indian Gap, 4 May 1936, H. M. Jennison 2275 (TENN); nr Indian Gap, 5 May 1961, James W. Hardin 2360 (NSC); beech gap, sw of Newfound Gap in GSMNP, 27 Apr 1963, James W. Hardin 2596 (NSC); nr Indian Gap, 6 May 1961, James W. Hardin 2360 (NSC). Wake Co.: "the Hemlocks", w of Cary, 28 Feb 1950, R. K. Godfrey 50301 (NSC, TENN); slopes at Hemlock Bluff about 4 mi s of Cary, 5 Apr 1955, R. L. Wilbur 3638 (NSC, GA); along Crabtree Creek nr Raleigh, North Carolina, 3 Apr 1937, R. K. Godfrey 00240 (NSC); locality not specified, Mar 1938, M. Wilson 02239 (NSC); bluff on Neuse River, 2.5 mi w of Stony Hill Church, 28 May 1951, R. K. Godfrey 51087 (NSC); 4 mi n of Raleigh, 11 Apr 1951, R. K. Godfrey s.n. (NSC); floodplain of Crabtree Creek off Ebenezer Rd. nnw of NCSC, 15 Apr 1966, B. Eugene Wofford 10065 - 10074 (APSC). Wilks Co.: Kilby's Gap, 6 mi s of Moravain Falls, 2 Apr 1941, Catherine Keever 255 (DUKE). County not specified: Biltmore, 13 Apr 1897, Ruth 425B (TENN).

SOUTH CAROLINA. Abbeville Co.: base of wooded slope along Calhoun Creek ene of Calhoun Falls, 23 Apr 1953, Wilbur H. Duncan and James W. Hardin 15740 (GA). Anderson Co.: Gumostil Creek, data not specified, F. Earle 1885 (VPI). Greenville Co.: Blythe Shoals 5½ mi nw of Marietta, 17 Mar 1939, A. E. Radford 8 (GA). Lancaster Co.:

Forty-Acre Rock, 2 mi w of Taxahaw, 25 Mar 1939, Dorothy Huntley 198 (DUKE); locality not specified, 19 Mar 1964, H. W. Cooper 2563 (NSC).

TENNESSEE. Carter Co.: Roan Mountain, 5 May 1934, Stanley A. Cain and Wilbur H. Duncan 213-2 (TENN); Forge H-11, 5 Apr 1956, A. B. Grindstaff s.n. (TENN); Sinking Creek area, 11 May 1956, Jack Pearman s.n. (TENN). Grainger Co.: w of Chesney, 20 Mar 1966, A. J. Sharp et al. 40456 (TENN). Johnson Co.: Headwaters of Stoney Creek, 14 Apr 1934, A. J. Sharp 470 (TENN). Monroe Co.: Strattons Meadow, Cherokee National Forest, 14 Apr 1959, R. E. Shanks 25623 (TENN). Roane Co.: left bank of White Oak Lake, 8 Apr 1953, F. R. Nease 204 (TENN, DUKE). Sevier Co.: Chimney Gaps GSMNP, 8 Apr 1953, Stanley A. Cain s.n. (TENN); Le-Conte, 21 May 1882, John B. Kinsey s.n. (TENN); Jakes Creek, vicinity of Elkmont, 8 Apr 1934, Stanley A. Cain and Wilbur H. Duncan 10-2 (TENN); vicinity of Elkmont, 10 Apr 1934, D. C. Bain s.n. (TENN). Unicoi Co.: Nolichucky River Gorge, Unaka Springs, 100 yds. for spring, 15 Mar 1955, Charlotte Lyle 19281 (TENN).

VIRGINIA. Montgomery Co.: n side of Blacksburg, 5 Apr 1960, R. Kral 9787 (USF); n side of Blacksburg, 5 Apr 1960, R. Kral 9787 (VPI); n of Highland Park, Blacksburg, 6 Apr 1941, J. W. Hodge, Jr. 1 (VPI); e of Ironto, 17 Mar 1938, A. B. Massey s.n. (VPI); Peppers Ferry, 20 Apr 1939,

H. B. Massey s.n. (VPU); Trillium Vale, 30 Mar 1941, A. B. Massey s.n. (VPI). Patrick Co.: locality not specified, 30 Mar 1935, Blanche C. Davis s.n. (VPI). Pittsylvania Co.: Moses Mill Pond on Chereystone Creek nr Chatham, 6 May 1939, A. B. Massey s.n. (VPI). Pulaski Co.: below Newboern, 15 Apr 1939, A. B. Massey s.n. (VPI); nr Newbern, 5 Apr 1938, W. R. Garmo s.n. (VPI). Rockbridge Co.: 5.2 mi above South River, along Irish Creek, 17 May 1965, Ruskin S. Frear 2926 - 2927 (VPI). Wythe Co.: 5 mi w of Wytheville, 20 Apr 1961, R. Kral 12154 (VPI).

ERYTHRONIUM ROSTRATUM

UNITED STATES

ALABAMA. Blount Co.: Blount Co. line, Warrior Fork of Mulberry River, 29 Mar 1967, B. Eugene Wofford and Lloyd Scott 10130 - 10136 (APSC); Blount Springs, May 1941, M. Morgan s.n. (FSU); ca. 1 mi ne of Blount Springs, 3 Apr 1941, Roland M. Harper 3832 (FSU). Colbert Co.: .5 mi from mouth of Spring Creek, ca. 1 mi from Sheffield on Tennessee River, 28 Mar 1967, B. Eugene Wofford and Lloyd Scott 10140 - 10142 (APSC); nr Sheffield, 17 Mar 1956, F. Harper and G. R. Cooley 4454, 4455, 4462 (NSC, USF); s side of Tennessee River just above mouth of Spring Creek, 17 Mar 1956, George R. Cooley and Francis Harper 4458 (USF); 3 mi sw of Tuscumbia, 17 Mar 1956, George R. Cooley and

Francis Harper 4462 (USF). Tuscaloosa Co.: locality not specified, 17 Mar 1951, O. D. McChermock 54 (USF). Walker Co.: locality not specified, 17 Mar 1956, Cooley and Harper 4452 (NSC).

ARKANSAS. Carroll Co.: Euraka Springs, 2 Apr 1933, D. M. Moore 330018 (UARK). Conway Co.: Petit Jean Mt., 12 Mar 1955, Jewel E. Moore 326 (UARK). Faulkner Co.: A. A. Lane Farm, 10 mi e of Conway on U. S. hwy 64, 13 Mar 1954, Forrest Lane 167 (UARK). Franklin Co.: n end of Shores Lake, 31 Mar 1967, James R. Aist 35 (UARK). Hot Springs Co.: n of Sleepy Water Springs, 2½ mi ne of Hot Springs, 21 Mar 1934, H. R. Gregg 21 (UARK). Logan Co.: e slope of Magazine Mt., 22 Apr 1933, H. R. Pyle 190 (UARK); e of Buckmann Inn on Flat Bench, 21 Mar 1937, H. R. Pyle 615 (UARK). Madison Co.: roadside to Huntsville, 7 Apr 1924, A. D. Oxley s.n. (UARK). Newton Co.: Jasper, 8 Apr 1939, D. Isley s.n. (UARK). Polk Co.: Ouachita Nat. Forest: small spring on Big Fork Creek, 18 mi s of Mena, 19 Mar 1955, Aileen McWilliam s.n. (UARK). Pulaski Co.: locality not specified, 31 Apr 1937, Ralph J. Bailey s.n. (UARK). Sevier Co.: DeQueen, 1939, Lugilla Denson s.n. (UARK). Van Buren Co.: Shirley, 31 Mar 1928, D. Demaree 4751 (UARK). Washington Co.: 3 mi s of Farmington, 7 mi sw of Fayetteville, 2 Apr 1954, Forrest Lane 190 (UARK); Farmington, Mar 1950, Robert G. French 244 (UARK); along White

R., 5 mi E., 10 Apr 1954, D. M. Moore 54-26 (UARK); Parksdale, 24 Mar 1950, Robert G. French 246 (UARK); Ozark Plateau: foothills of Boston Mt. Cove Creek Valley, ca 15 mi s of Prairie Grove (23 mi sw of Fayetteville), 9 Apr 1960, Bill Bagley 31 (UARK); Camp Neil Martin, 12 Apr 1960, Will Jeffrey 5 (UARK); Cove Creek Valley ca 15 mi s of Prairie Grove, 9 Apr 1960, Frank McHan 31 (UARK). County not specified, near Johnson, Apr 1921, J. T. B. s.n. (UARK).

LOUISIANA. Bienville Parish: along small stream, center of section 22, ca 3¼ mi sw of Arcadia, 2 Apr 1966, John W. Thieret 22158 (FSU, DUKE). Lincoln Parish: 2.5 mi nw of Vienna, 20 Mar 1963, R. Kral 16349 (FSU). Sabine Parish: area to be flooded by Toledo Bend Dam, along Bayou LaNana, 7 mi sw of Many, 20 Mar 1966, John W. Thieret 21957 (FSU, DUKE).

MISSOURI. Barry Co.: Roaring River State Park, 16 Apr 1961, Paul L. Redfearn, Jr. and T. Stombaugh 8047 (FSU). County not specified, Jam Up, 17 Apr 1936, B. F. Bush 15240 (UARK).

OKLAHOMA. Cherokee Co.: base of Keyough Bluffs, 2 mi n of Ft. Gibson, 6 Apr 1958, Charles S. Wallis 6195 (UARK). Muskogee Co. base of hill on Maynard Bayou, 2 mi e of Ft. Gibson on State 10 & 2 S, 7 Apr 1958, Charles S. Wallis 6208 (UARK).

TENNESSEE. Davidson Co.: Percy Warner Park, Nashville, 18 Mar 1945, Elsie Quarterman 1100 (DUKE). Lawrence Co.: w of Barnesville, Buffalo River bottoms, 8 Apr 1949, A. J. Sharp et al. 11007 (TENN). Wayne Co.: e of Leatherwood, bluffs on Beech Creek, 22 Apr 1949, A. J. Sharp et al. 10205 (TENN).

XI. DISCUSSION

Morphological variations found between and within populations of *Erythronium americanum* in the southeastern United States have been of primary concern in this study. It was anticipated that a study of these variations would result in a better understanding of the taxonomic status of this species.

A review of the literature indicates that a number of previous investigators found this species to be highly polymorphic. It is this author's opinion that many of these investigations led to formal taxonomic rankings which are merely continuous segments that may be found throughout the entire range. For example, Smith (1929) described *Erythronium americanum* forma *castaneum* on the basis of flowers that were smaller than the average and inner perianth parts that were deep chestnut-brown. Farwell (1920), elevated forma *Bachii* to variety *Bachii* on the basis of stamens and inner perianth parts that are purple or magenta. Parks and Hardin (1963) proposed that subspecies *harperi* is distinct from subspecies *americanum* on the basis of an apiculate fruit apex.

Possible explanations to the various phenotypic expressions of *Erythronium americanum* which may or may not have been overlooked by previous investigators should be

considered. Stebbins (1960) states that difficulty in the classification of certain plant genera is due largely to the effects of hybridization, accompanied by polyploidy, apomixis, or both. In considering that Erythronium americanum is presumed to be a tetraploid hybrid between the diploids Erythronium rostratum and Erythronium umbilicatum, and that it reproduces by both sexual and asexual means, it can readily be anticipated that numerous polymorphic forms would be found throughout the range. It is this investigator's opinion, therefore, that many formal taxonomic recognitions have been given to local variants without consideration of the total range of variation or the effects of hybridization, polyploidy, and apomixis.

Since anther coloration was found to be the most distinguishing characteristic, it was used as the basis for distinguishing probably subspecific categories of Erythronium americanum.

Biosystematic techniques employed in this study indicate that the two anther color forms of Erythronium americanum are ecologically, geographically and cytologically similar. Both forms are found throughout the entire range and occur sympatrically within the same population. Chromosome counts for both forms were found to be $2n = 48$. Insignificant morphological and anatomical differences were found in petal length, sepal length, leaf length, fruit

length-width ratio, stomata length, stomata width, and pollen grain length.

Similar mean values were also observed for the percentage of ovule abortion and pollen fertility between the two forms. It was anticipated that dissimilar mean values would have been observed since the purple form is more common in Montgomery and Stewart Counties, Tennessee, than the yellow form. Since this was not the case, (Chapters VI & VII), it can only be hypothesized that the purple anthered form is a more successful asexual reproducer than the yellow anthered form or that the seeds of the yellow anthered form have a lower viability rate. Seed viability and/or germination tests would be of much value in future studies.

Significant differences of mean values were found in the number of stomates per unit area of leaf surface, petal width, sepal width, and peduncle length between the two forms. These differences, however, may be the result of selection pressures, polyploidy or other factors. More research in these areas is planned in the future.

Field observations were also of value in distinguishing these two forms of Erythronium americanum. Anther coloration, which served as a primary basis for the study, was found to be distinct. Although purple anther coloration

may range from light purple to deep chestnut-brown, in no instances were intermediate colors found between the two forms. The entire plant body of the purple anthered form was observed in most cases to be slightly larger than the yellow anthered form. This difference, however, was found to be sufficient for identifying the two variants even if the anthers were obscured from view.

The fruiting capsule may also be considered of taxonomic importance. One population was observed in Stewart County, Tennessee, in which a distinct apiculus was found at the fruit apex, with few exceptions, only on the purple anthered forms. Parks and Hardin (1963) state that this structure is characteristic of subsp. harperi, which is reported to have yellow anthers.

Many other variations, e.g., tepal anthocyanin, flecking of perianth parts, auricle development, and capsule shape in cross section were not found to occur with a greater degree of frequency in either form.

Data collected in this investigation indicate that two distinct biotypes of Erythronium americanum exist. It now appears that these suspected biotypes should be placed in the category of forma on the basis of the definitions of this taxonomic unit by Lawrence (1965) and Stebbins (1960). Lawrence (1965) states that some botanists consider the

taxonomic unit of forma as showing no degree of geographic discontinuity and occurring sporadically throughout the population irrespective of the degree of morphological variation or constancy. Stebbins (1960) further states that the category of forma consists of all the individuals which possess in common some conspicuous aberration from the norm of the species. The taxonomic nomenclature of the proposed biotypes was not included in this study since variations throughout the entire range have not yet been sufficiently observed.

Another phase of the study that was not earlier anticipated is that of the evolutionary relationships of the genus. The distribution map (Figure 9) shows the two diploid species, Erythronium rostratum and Erythronium umbilicatum to be geographically isolated at the present time, and the tetraploid species, Erythronium americanum to be widely distributed. It may then be hypothesized, according to Parks and Hardin (1963) that Erythronium americanum has been produced by the hybridization of the diploid species, Erythronium rostratum and Erythronium umbilicatum. The original hybridization possibly occurred in Alabama during the Pleistocene when Erythronium habitats extended farther south. Extant populations in this general area (Figure 9) may represent relicts of a cooler period since sympatric populations have not been observed.

XII. SUMMARY

The yellow flowering species of *Erythronium* in the southeastern United States, with particular emphasis on the purple and yellow anthered forms of *Erythronium americanum*, were studied and inferences were made from geographical, ecological, cytotaxonomical, anatomical and morphological data.

Ecological and geographical data indicate that the three species of *Erythronium* occupy a wide variety of habitats, but occur most frequently in rich woods, ravines, shaded and open slopes of floodplains of creeks. These three species are considered allopatric since sympatry was observed in only one population of *Erythronium umbilicatum* and *Erythronium americanum*. *Erythronium rostratum* was not found to occur sympatrically with either *Erythronium americanum* or *Erythronium umbilicatum* in a single population.

Chromosome counts indicate that *Erythronium rostratum* is a diploid with $2n = 24$ chromosomes and *Erythronium americanum* is a tetraploid with $2n = 48$ chromosomes. Parks and Hardin (1963) state that *Erythronium umbilicatum* is a diploid with $2n = 24$ chromosomes. Preliminary cross-breeding experiments indicate that the yellow and purple anther color forms of *Erythronium americanum* are cross-fertile. More research is needed in this area.

Statistical analysis of anatomical and morphological data indicates no significant differences between the two anther color forms of Erythronium americanum for stomata length and width, pollen grain length, sepal length, petal length, leaf length and fruit length-width ratio. Similar values were also observed for percentage of ovule abortion, mean fruit weights, and pollen fertility. Significant differences of the number of stomates per 0.2 mm^2, sepal width, petal width and peduncle length were found between the two forms.

On the basis of the data assimilated in this study and of the findings of Parks and Hardin (1963), it is this investigator's opinion that Erythronium americanum arose through the hybridization of Erythronium umbilicatum and Erythronium rostratum.

The results of this preliminary investigation indicate that two distinct biotypes of Erythronium americanum exist and are best distinguished on the basis of anther coloration. In the southeastern United States, it now appears that these two biotypes "best fit" into the taxonomic category of forma. Taxonomic nomenclature will not be presented until variations throughout the entire range have been sufficiently biosystematically studied.

LITERATURE CITED

Belling, J. 1926. The iron-acetocarmine method of fixing and staining chromosomes. Biol. Bull. 50: 160-162.

Blodgett, F. H. 1900. Vegetative reproduction and multiplication in *Erythronium*. Bull. Torr. Bot. Club. 27: 305-315.

Buell, M. F. 1946a. Size frequency study of fossil pine pollen compared with herbarium preserved pollen. Amer. Jour. Bot. 33: 510-516.

Cooper, D. C. 1939. Development of megagametophytes in *Erythronium albidum*. Bot. Gaz. 100: 862-867.

Cain, Stanley A. 1940. The identification of species in fossil pollen of *Pinus* by size-frequency determination. Amer. Jour. Bot. 27: 301-308.

Darlington, C. D., and A. P. Wylie. 1955. Atlas of Flowering Plants, 2nd ed. George Allen and Unwin, Ltd., London. xix + 519 pp.

Dice, L. R., and H. J. Leraas. 1936. A graphical method for comparing several sets of measurements. Univ. Mich. Lab. Gen. 3: 1-3.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy: Angiosperms. Chronica Botanica Co., Waltham, Massachusetts. 539 pp.

Farwell, Oliver A. 1920. *Erythronium americanum* var. *Bachii*. Rep. Mich. Acad. Sci. 21: 363.

Graff, Paul W. 1916. The stamens of *Erythronium americanum*. Torreya 16: 180-182.

Haque, Ashraful. 1951. The embryo sac of *Erythronium*. Bot. Gaz. 112: 495-500.

Hardin, James W. 1957. A revision of the American Hippocastanaceae-II. Brittonia 9: 173-195.

Harper, Roland M. 1941. Diversity of *Erythronium* in the eastern United States. Castanea 6: 1-6.

———. 1945. Erythronium albidum in Alabama, and some of its relatives. Castanea 10: 1-7.

———. 1949. A fifth species of Erythronium in Alabama Castanea 14: 49-52.

———. 1952. An undescribed Erythronium in middle Georgia. Jour. Tenn. Acad. 27: 201.

Hauser, E., and J. Morrison. 1964. The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. Amer. Jour. Bot. 51: 748-752.

Hoagland and Arnon. 1938. A water culture method for growing plants without soil. Cal. Agri. Expt. Stat. at Berkeley, Circular 347.

Hubbs, C. L. and A. Perlmutter. 1942. Biometric comparison of several samples with particular reference to racial investigation. Amer. Naturalist 76: 582-592.

King, J. R. 1960. The peroxidase reactor as an indicator of pollen viability. Stain Tech. 36: 225-227.

Kral, Robert. 1966. Observation on the flora of the southwestern United States with special reference to northern Louisiana. Sida 2: 395-408.

Lanjouw, J., and F. A. Stafleu. 1959. Index herbariorum. Part I. The herbaria of the world. 4th ed. Regnum Veg. 15: 1-279.

Lawrence, George H. M. 1965. Taxonomy of Vascular Plants. 10th printing. The Macmillan Co., New York. xiii + 823 pp.

Maneval, W. E. 1936. Lactophenol preparations. Stain Tech. 11: 9-11.

Meads, M. E. 1893. The range of variation in species of Erythronium. Bot Gaz. 18: 134-138.

Moldenke, Horold. 1953. Notes on new and noteworthy plants. Phytologia 4: 291-292.

Owczarzak, Alfred. 1952. A rapid method for mounting pollen grains with special regard to sterility studies. Stain Tech. 27: 249-251.

Parks, C. R., and J. W. Hardin. 1963. Yellow Erythroniums of the eastern United States. Brittonia 15: 245-259.

Pickett, F. L. 1917. The length of *Erythronium* stamens. Torreya. 17: 58-60.

Pohl, Richard W. 1954. A rapid softening agent for dried plant structures. Proc. Iowa. Acad. Sci. 61: 149-150.

Sax and Sax. 1937. Stomata size and distribution in diploid and polyploid plants. Jour. Arn. Arb. 18: 164-172.

Sinclair, C. D., and D. B. Dunn. 1961. Surface printing of plant leaves for phylogenetic studies. Stain Tech. 36: 299-304.

Smith, Frank H. 1955. Megagametophyte development in five species of *Erythronium*. Amer. Jour. Bot. 42: 213-224.

Smith, L. B. 1929. *Erythronium americanum* forma *castaneum*. Rhodora 31: 36.

Smith, Ruth C. 1966. A new variant of *Erythronium americanum*. Mich. Bot. 5: 63.

Stebbins, G. Ledyard. 1960. Variation and Evolution in Plants. 4th ed. Columbia University Press, New York. xix + 643 pp.

Stone, D. E. 1961. Ploidal level and stomatal size in the American hickories. Brittonia 13: 293-302.

Underwood, Benton J., *et al*. 1954. Elementary Statistics. Appleton-Century-Crofts, Inc., New York. 1x + 242 pp.

Whitehead, Donald R. 1963. Pollen morphology in the Junglandaceae, I: Pollen size and pore number variation. Jour. Arn. Arb. 44: 101-110.

_____. 1965. Pollen morphology in the Juglandaceae, II: Survey of the family. Jour. Arn. Arb. 46: 369-410.

Wodehouse, R. P. 1935. Pollen Grains. Hafner Publishing Co., New York. x + 574 pp.

Wolf, W. 1941. *Erythronium*, a neglected genus in Alabama. Castanea 6: 21-27.