The Species Problem in Iris

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THE SPECIES PROBLEM IN IRIS

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I. INTRODUCTION

As a biological phenomenon the species problem is worthy of serious study as an end in itself, and not as a mere corollary to work in some other field. It is, to be sure, a problem so fundamentally important that it touches many such fields. Workers in any one of these are humanly prone to regard the evidence from that field as all important and its techniques as all sufficient (particularly if they are themselves unacquainted with other aspects of the problem). When, however, one takes up the problem, *as a problem,* and studies it from the diverse viewpoints of genetics, taxonomy, cytology, and biometry, he realizes that he not only needs most of the existing techniques but that he must devise new ones as well.

Iris versicolor and *Iris virginica* were chosen for such a study since they customarily grow in colonies containing many individual plants; a peculiarity which facilitates the location and study of large numbers of individuals. A preliminary analysis of the problem ('28) and a discussion of certain points connected with the distribution of these species ('33) have already appeared. The following series of papers constitutes a final comprehensive report. The central core of information is an analysis of a precise morphological census of the two species (section IV). For the interpretation of this morphological data it has been necessary to undertake correlated investigations in cytology, taxonomy, glacial geology, and genetics. A technical taxonomic treatment of these irises, together with the related *Iris setosa,* is assembled in section II, although material indirectly of taxonomic interest will be found in sections III and V. The phylogenetic relationship of *Iris versicolor* to *Iris virginica* has proved to be somewhat ex-

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ceptional though it is by no means unique among the higher plants. The case has been presented in detail in section III as an example of reticulate relationship. Finally in section V the general problem of evolution in the genus *Iris* is discussed in the light of all the above information.

The major portion of these investigations has been carried out at the Missouri Botanical Garden and at the Arnold Arboretum of Harvard University. A fellowship from the National Research Council enabled me to acquire cytological and statistical techniques for continuing the work. During this time I was a guest of the John Innes Horticultural Institution and of the Rothamsted Experiment Station. A two-months' leave of absence from Harvard University in 1932 made it possible to study with Dr. Sewall Wright at the University of Chicago. Dr. Wright, Prof. J. B. S. Haldane, and Dr. R. A. Fisher have greatly furthered the final analysis of the data, though they are in no way responsible for the imperfections of the work or of its presentation. To the above individuals and institutions grateful acknowledgment is made for these exceptional opportunities. I am indebted to the University of Chicago Press and to Dr. J. Paul Goode for the base maps used in the second paper of this series and to Mr. Fred A. Barkley for figs. 2 and 13.

Bibliography

Anderson, Edgar ('28). The problem of species in the northern blue flags, *Iris versicolor* L. and *Iris virginica* L. Ann. Mo. Bot. Gard. **15**: 241–332.

————, ('33). The distribution of *Iris versicolor* in relation to the post-glacial Great Lakes. Rhodora **35**: 154–160.

II. THE TAXONOMY OF THE NORTHERN BLUE FLAGS

As a matter of convenience the essential facts in regard to the nomenclature, classification, and distribution of *Iris versicolor* and its relatives are summarized below. Such a segregation of the taxonomic aspects of the problem is essentially artificial and is dictated largely by practical considerations. Material of considerable taxonomic significance will be found throughout the other papers of this series. Attention is called in particular to pages 476 to 480, 495 to 496, and 501 to 506.

For the loan of material for study the author is indebted to the Curators of the following herbaria: Gray Herbarium, University of Wisconsin, Missouri Botanical Garden, United States National Herbarium, University of Pennsylvania, and the Canadian National Herbarium.

KEY TO THE NORTHERN AND SUB-ARCTIC BLUE FLAGS

A. Seeds with a conspicuous raphe; petals setose, less than 2 cm. long.
  B. Stem short, usually unbranched; natives of eastern North America.. *I. setosa* var. *canadensis*
  BB. Stem various, often branched; natives of Asia and western North America.
    C. Bracts often exceeded by the pedicels; stem usually branched; natives of central Alaska.............................. *I. setosa* var. *interior*
    CC. Bracts exceeding the pedicels; natives of Asia and the northwestern coast of North America.................................. *I. setosa*
AA. Seeds with an inconspicuous raphe or none; petals laminate, more than 2.5 cm. long.
  B. Seeds D-shaped, sometimes with an inconspicuous raphe; surface of seed vernicose, regularly pitted; valves of the mature seed capsule reflexed but slightly, if at all; sepals minutely papillate at base of blade; outermost bracts of the inflorescence darker and somewhat vernicose along their margins............................ *I. versicolor*
  BB. Seeds round or D-shaped, without a raphe; surface of seed not vernicose, pitting irregular; valves of the mature seed capsule strongly reflexed; sepals macroscopically pubescent at base of blade; outermost bracts of the inflorescence with undifferentiated margins.
    C. Seed capsules globose or subglobose; natives of the Atlantic seaboard... *I. virginica*
    CC. Seed capsules at least twice as long as broad; natives of the Mississippi Valley.............................. *I. virginica* var. *Shrevei*

Issued August 10, 1936.

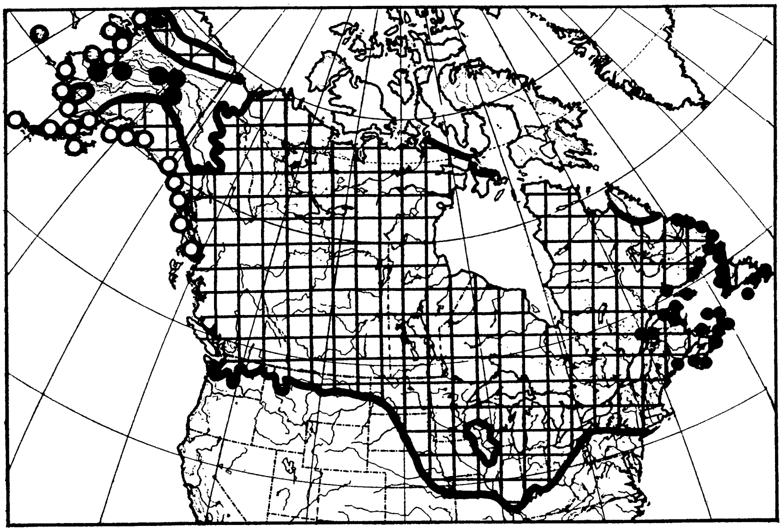
IRIS SETOSA

Iris setosa Pall. ex Link in Spreng., Schrad. u. Link, Jahrb. d. Gewächskunde **1**^{3}: 71. 1820.

Iris arctica Eastwood in Bot. Gaz. **33**: 132, *fig. 2.* 1902.

From coastal Alaska to the Lower Lena River and southwards to Japan.

Perennial from a superficial or underground rhizome; rhizome stout, thickly clothed with the fibrous remains of old leaves; leaves narrowly ensiform to linear, 9–65 cm. long, 0.5–1.7 cm. wide; stem slender to stout, 1–5 dm. high, unbranched or with one or two secondary branches, the latter *not exceeding the main axis;* upper cauline leaves *seldom equalling the inflorescence;* inflorescence a compact, 1–3-, mostly 2-, flowered fascicle; bracts of the inflorescence foliaceous to scarious, 3.5–8 cm. long; pedicels slender, exceeding the bracts or exceeded by them; sepals 4–6 cm. long; haft broad, the margin undulate; blade 3–5 cm. wide, *glabrous even at the base,* dark blue-violet (occasionally wine-colored) with *dark veins on a lighter ground-color;* petals small, *setose;* ovary short, 1–2 cm. long in



Map. 1. Range of *Iris setosa* (open circles), *I. setosa* var. *canadensis* (small solid circles), and *I. setosa* var. *interior* (large solid circles). Cross hatching shows extent of maximum Pleistocene glaciation.

the flower, *conspicuously three-angled, inflated at anthesis;* capsule short-cylindric to ovate, *symmetrical, highly vernicose* within, often persisting on the plant for one or two years; seeds small, D-shaped, *with a conspicuous raphe, highly vernicose* over minute *regular pitting; chromosomes 38* (2n).

IRIS SETOSA VAR. CANADENSIS

Iris setosa Pall. var. **canadensis** Foster in Rhodora **5**: 158. 1903.

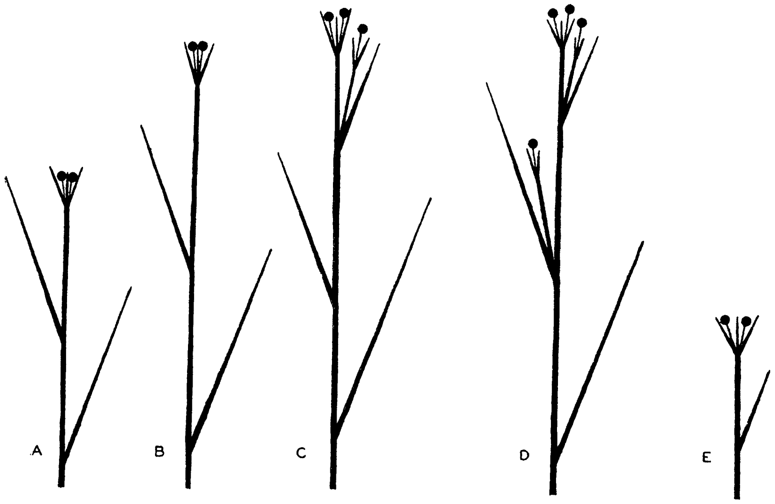


Fig. 1. Diagrams to scale of (a) *Iris setosa* from the Aleutian Islands and Alaskan peninsula; (b) *I. setosa* from northern coastal Alaska; (c) *I. setosa* from southern coastal Alaska; (d) *I. setosa* var. *interior;* (e) *I. setosa* var. *canadensis.* The diagrams are drawn to scale (× 1/10) from measurements and enumerations of the herbarium material. Each drawing represents average numbers and sizes for all the available material.

Iris Hookeri Penny in Steud. Nomencl. ed. 2, **1**: 822. 1840.

From Labrador, around the Gulf of St. Lawrence; up the St. Lawrence River to Riviere du Loup, Quebec, and along the coast to Washington Co., Maine.

This typical preglacial relict differs from the type only in its generally smaller size and lesser variability. As was pointed out by Dykes,[1] the smallest of the Alaskan specimens are indistinguishable morphologically from *I. setosa* var. *canadensis.*

[1] Dykes, W. R., The genus Iris. p. 94. 1913.

The averages of the plants of the two regions are strikingly different, however, as can be seen from fig. 1. Its greater conservatism (i.e. its lesser variability) is a general characteristic of the relict species and varieties around the Gulf of the St. Lawrence, as has been pointed out by Fernald.[2] This point is discussed at greater length in another section of the paper (see below, pp. 495–496).

IRIS SETOSA VAR. INTERIOR

Iris setosa Pall. var. **interior**, var. nov.

Ab specie bracteis scariaceis vel crasse chartaceis non foliaceis rubicundiusculis saepe minoribus quam pedicellis differt.

Bracts scarious to thickly chartaceous, not foliaceous, somewhat florid, often exceeded by the pedicels.

Upper Yukon valley of Alaska, merging into the type in the lower valley and along the western coast.

ALASKA: Fort Gibbon, frequent throughout the Yukon and Tanana valleys in lakes and along small streams, July 4, 1905, *Heideman 62* (US TYPE); same locality, Aug. 10, 1905, *Heideman 98* (US); well-drained gully, Tolstoi, July 4, 1917, *Harrington 37* (US); Rampart, July 24, 1901 [fruit], *Jones 63* (US); alt. 150 m., vicinity of Fairbanks, Aug. 31, 1928 [fruit], *Mexia 2302* (MBG); Fairbanks, July 25, 1931, *Anderson 1221* (US); Fairbanks, June, 1927, *Palmer 1783* (US).

The characters which distinguish *Iris setosa* var. *interior* from the type have been found to characterize all the available herbarium material from interior Alaska. Transitional forms are to be found in the region where this great interior valley meets the coast. The following specimens represent such transitional forms:

ALASKA: Ft. St. Michaels, Norton Sound, 1865–66, *Bannister s.n.* (US); moist grassy places, shade of alders, 16 miles west of Nome City, Aug. 5, 1900 [fruit], *Flett 1560* (US); on the Yukon River, between Andreafski and Anvik, July 16–18, 1889, *Russell s.n.* (US).

According to glacial geologists,[3] this large region remained unglaciated during the Pleistocene, and there, if anywhere, we

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[2] Fernald, M. L. Persistence of plants in unglaciated areas of boreal America. Mem. Am. Acad. Arts & Sci. **15**: 244. 1925.

[3] Capps, S. R. Glaciation in Alaska. U. S. Dept. Inter., Geol. Surv. Prof. Paper 170–A. 1931.

might hope to find living irises most similar to those *Iris setosae* which must in preglacial times have extended across northern North America. A number of facts have been found which support this hypothesis and they are discussed below (p. 480).

While the irises of coastal Alaska are probably not varietally distinct from the type[4] (which is from Asia) there are minor geographical differences to be noted, when one compiles careful averages for such regions as the Arctic coast, the Alaskan peninsula and Aleutian Islands, and the southern Alaskan coast. Such averages have been prepared from all the available herbarium material and the results are presented graphically, to scale, in fig. 1, along with similar averages for *Iris setosa* var. *canadensis* and *Iris setosa* var. *interior.*

IRIS VERSICOLOR

Iris versicolor L. Sp. Pl. ed. 1, 39. 1753.

Perennial from a superficial or underground rhizome; rhizome stout, clothed with the fibrous remains of old leaves; leaves narrowly ensiform, 1–8 dm. long, 1–3 cm. wide, green to grayish-green; stem stout to slender, 2–6 dm. high, with one or two secondary branches, the latter *seldom equalling the main axis;* upper cauline leaves *seldom equalling the inflorescence;* inflorescence a compact, 2–4-flowered fascicle; bracts of the inflorescence thickly chartaceous to scarious, 3–6 cm. long, the margins *so heavily vernicose as to be much darker in color;* pedicels slender, some of those in each fascicle usually *longer than the subtending* bracts; sepals 4–7 cm. long, mostly *1.4 times the length of the petals* in living material; blade 2–4 cm. wide, variable in color in different plants, mostly violet-blue to blue-violet, the veins *slightly darker than the ground-color, minutely papillate at the base,* forming at most a dull greenish-yellow spot in living material; petals 2–5 cm. long, 0.5–2 cm. wide; ovary *1–2 cm. long in the flower, obscurely three-sided, slightly inflated at anthesis;* capsule short-cylindric, mostly symmetrical, somewhat verrucose without, *delicately vernicose*

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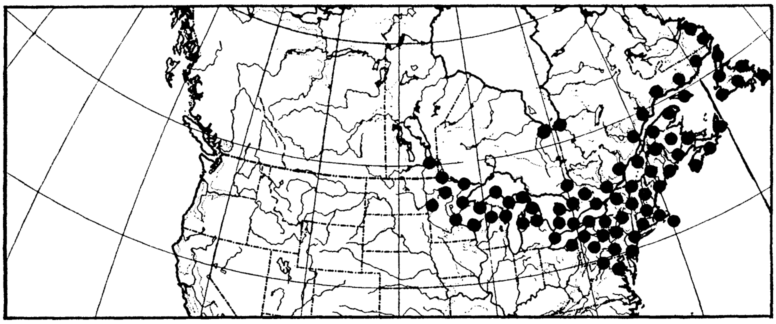
[4] Hultén, Eric. Flora of Kamtchatka and the adjacent islands. Kungl. Svenska Vetenskapsakad. Handl. III. 5^1: 255–256. 1927.

within, usually persisting into the first winter; seeds D-shaped, often showing a *poorly developed raphe,* surface *regularly pitted, vernicose; chromosomes 106–108* (2n).

From Labrador to Winnipeg and southward to central Wisconsin, northeastern Ohio, and northern Virginia.

Morphologically, *Iris versicolor* is much closer to *Iris virginica* than to *Iris setosa,* though in every character by which it differs from *Iris virginica* it departs in the direction of *Iris setosa.* This peculiar intermediacy is discussed at length in the following section of this paper (pp. 478–480).



Map 2. Range of *Iris versicolor.*

Iris virginica

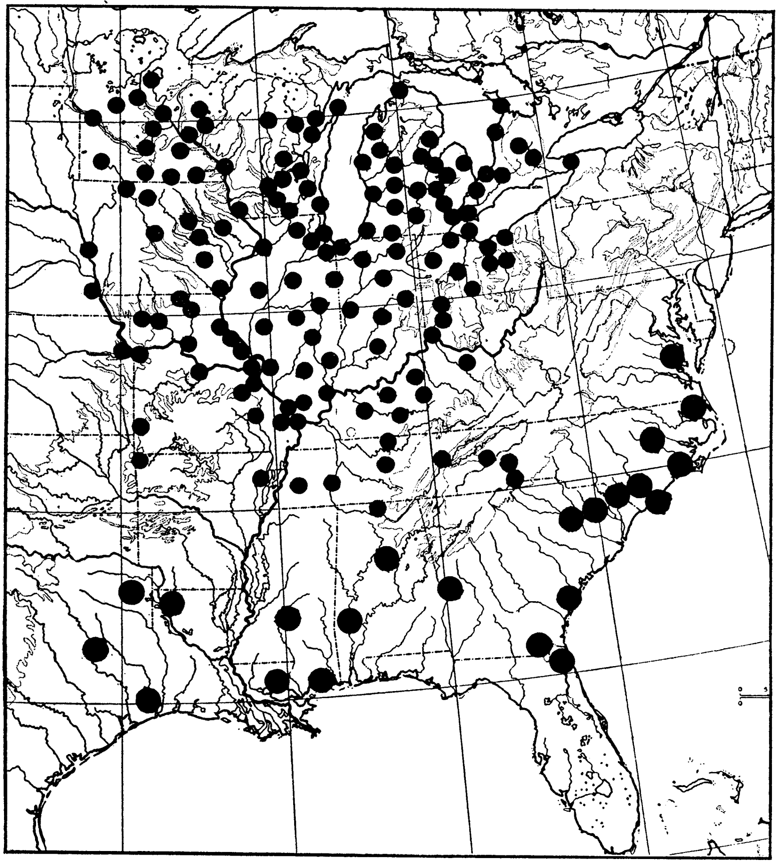
Iris virginica L. Sp. Pl. ed. 1, 39. 1753.

Iris carolina Radius, Naturforsch. Ges. Leipzig Schrift. **1:** 158. *pl. 3.* 1822.

Iris caroliniana Wats. in Gray's Manual, ed. 6, 514. 1890.

Iris georgiana Britton, in Britton & Brown, Illust. Fl., ed. 2, **1:** 537, *pl. 1330.* 1913.

Perennial from a superficial or underground rhizome; rhizome stout, clothed with the fibrous remains of old leaves; leaves ensiform, 2–9 dm. long, 1–6 cm. wide, green; stem stout, coarse, 3–10 dm. high, mostly with one secondary branch, the latter *usually subequal to the main axis;* upper cauline leaves *usually exceeding the inflorescence;* inflorescence a compact, 1–4-flowered fascicle; bracts of the inflorescence *coarsely* and *thickly chartaceous,* 4–8 cm. long, *margins undifferentiated;* pedicels stout, *shorter than the bracts;* sepals 4–8 cm. long, mostly 1.2 times the length of the petals in living material; haft narrow with a *straight margin;* blade 1.5–4 cm. wide, blue to violet-blue and violet, veins *scarcely darker than the ground-color, thick pubescence of fine hairs* at base, forming a *bright yellow signal patch* in living specimens; petals 3–7 cm. long, 1–3 cm. wide; ovary *2–4 cm. long in the flower,* terete or obscurely three-sided, *not inflated;* capsule spherical to long-



Map 3. Range of *Iris virginica* (large circles), and of *I. virginica* var. *Shrevei* (small circles).

cylindric, mostly *asymmetrical, suberose* and *coarsely verrucose* without, *never vernicose* within, seldom persisting until the seeds are fully ripe, valves of the capsule reflexed in well-ripened specimens; seeds *round* or D-shaped, *without a trace of a raphe,* surface *suberose, irregularly pitted; chromosomes 70–72* (2n).

From Virginia southward along the Atlantic coast.

Although well provided with distinguishing characteristics, *Iris versicolor* and *Iris virginica* seem to be under a special curse so far as their recognition in the herbarium is concerned. The shapes of the petals and sepals, the interior glandulosity of the calyx-tube and its shape—any one of these characters is sufficient for accurate specific delimitation. Unfortunately, *Iris virginica* differs also in texture and substance; its flowers, though larger, have less permanency. As a result they wilt very rapidly; even though carefully pressed when fresh, they have so little substance that the resulting specimens are too thin and fragile to be preserved intact. While the pressed flowers of *Iris versicolor* are none too accurate in their reflection of the original condition of the perianth, they are far superior to those of *Iris virginica.* The latter are so badly preserved that it is almost impossible to use them, even by boiling them up. Perianth dimensions from herbarium material are completely unreliable in these species, and for that reason have been largely omitted from the keys and descriptions.

The second most useful set of characters are those provided by the seed capsule and the seed. Here again the characteristic lack of permanency in the capsular walls of *Iris virginica* has been a great hindrance. With the exception of special collections made by the writer and by other recent students of American irises, herbarium specimens of *Iris virginica* seed capsules simply do not exist. The reason is not far to seek. The coarse stems of this species are neither durable nor stout, and they are not held above the leaves as in *Iris versicolor. Iris virginica* furthermore prefers slightly damper situations. In nature, therefore, the less lignified stems and seed-pods of *Iris virginica,* choked by iris leaves and other rank swamp vegetation, begin to rot long before the seeds are mature, particularly in the southern part of its range. By September it is not at all unusual to find the black, deliquescent, half-rotten seed-pods lying flat upon the ground, their corky brown seeds spilling out from the capsular remnants. A special collection of seeds and seed-pods has accordingly been brought together in the herbarium of the Missouri Botanical Garden as a permanent record, and the writer will be grateful for further material of either seeds or seed capsules, no matter how unattractive the partly decayed state of the latter.

It is unfortunate that mature capsular material is so difficult to obtain since it displays a curious and striking character, to which Small and Alexander[5] have called attention. The valves of the capsule in *Iris virginica* are strongly reflexed as in the related European species, *Iris pseudacorus* L.

Fortunately other characters can be found. Of these the most generally useful in the herbarium are the bracts of the inflorescence (the spathe-valves). Those of *Iris virginica* have the texture of coarse paper or thin cardboard. They may or may not be streaked with the fine chestnut lines caused by resinous deposits, but if so the streaking will be uniform throughout the bracts. In *Iris versicolor,* however, the bracts not only are of a finer, yet more durable texture, but the lignification is intensified towards the edge so that the margins are often deep chestnut and are distinctly vernicose.

In well-preserved specimens the pubescence at the base of the blade of the sepal is a useful character. In specimens of *Iris versicolor* it appears under the hand-lens as a minutely papillate area. In *Iris virginica* the hairs are larger, more overlapping, and are often conspicuously straw-colored.

In the field *Iris virginica* is readily identified by the larger, broader petals, the bright yellow pubescent spot on the sepal, and the spongy glandular inner surface of the calyx-tube with its sickish sweet fragrance.

Reasons for attaching the Linnean name *Iris virginica* to

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[5] Small, J. K. and Alexander E. J. Botanical interpretation of the Iridaceous plants of the Gulf States. N. Y. Bot. Gard. Contr. 327: 356. 1931.

this species have been detailed elsewhere[6] and need not be repeated here, other than to state that the type is in existence and has been examined. The suggestion has since been made[7] that material from the presumable type locality may throw some doubt on this opinion. Through the kindness of Dr. T. W. Whitaker, I was able to obtain irises from Nesting, Gloucester County, Virginia, which is in the same general vicinity. Of these plants one or two answered the description given above; others showed signs of hybridization with *Iris versicolor* and were practically sterile. Reference to the distribution maps of *Iris versicolor* and *Iris virginica* (maps 2 and 3) will show that very region as the actual boundary zone of the two species. Because of this fact collections from or near the probable type locality are not so definitive as they otherwise might be.

Iris virginica is centered upon the Ozark-Appalachian landmass, an area which has been available for continuous plant occupancy since very ancient times. It would be strange indeed if no geographical differences were to be found within such a species, particularly in the case of the area along the Atlantic seaboard. Such differences are, however, rather difficult to find. At flowering time, I have been able to detect for the plants of the Atlantic seaboard only slightly narrower perianth segments, a larger average flower size (fig. 12), and an inflorescence which is characteristically somewhat less branched. The seed capsules, however, though variable, are distinct. Well-developed capsules from the upper Mississippi Valley are much longer than broad, while those from the coastal plain are practically spherical, as well as possessing larger, corkier seeds. Unfortunately the difficulty of collecting fruiting specimens of iris in the southern swamps (see above, pages 466–467) makes exact delimitation of the areas occupied by these capsular types a matter of the future. Throughout the upper Mississippi Valley and Great Lakes region it has been a comparatively simple matter to determine, and I am accordingly

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[6] Anderson, Edgar. The problem of species in the northern blue flag, *Iris versicolor* L. and *Iris virginica* L. Ann. Mo. Bot. Gard. **15**: 241–332. 1928.

[7] Small and Alexander. loc. cit. p. 356.

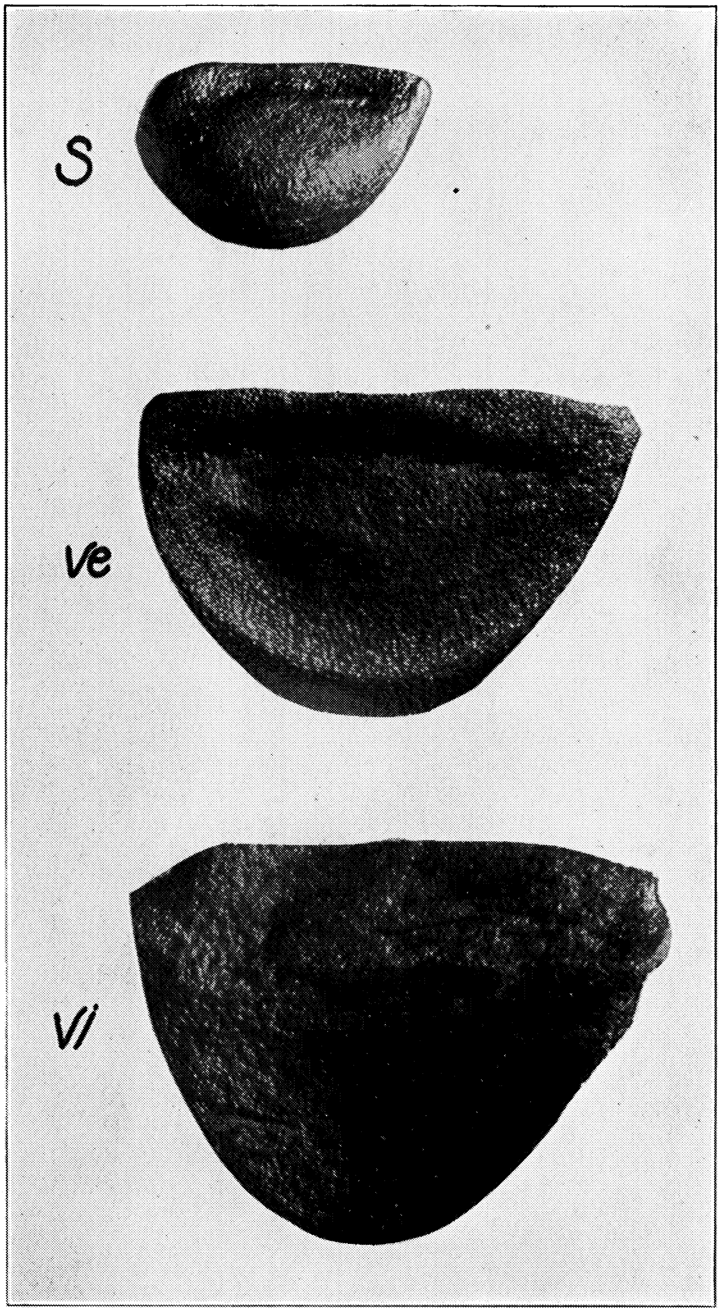
using Dr. Small's name *Iris Shrevei* in a varietal sense for these elongate-capsuled *Iris virginicae.* When adequate material is available for study it will be possible to determine the exact geographical relationships of this and probable other varieties of *Iris virginica.* Until such a time it has seemed prudent to recognize only this one variety, and to postpone for the present the precise delimitation of the typical and other possible varieties.

IRIS VIRGINICA VAR. SHREVEI

Iris virginica L. var. **Shrevei** (Small), comb. nov.

Iris Shrevei Small, Addisonia **12:** 13–14, *pl. 391.* 1927.

Mississippi Valley and Great Lakes region from southern Minnesota and southern Ontario, southwards to Texas and Alabama. The exact boundaries of its junction with the type as yet unknown and perhaps complex.



Seeds × 10: s, *Iris setosa;* ve, *I. versicolor;* vi, *I. virginica* var. *Shrevei.*

III. THE PHYLOGENETIC RELATIONSHIP OF IRIS VERSICOLOR AND IRIS VIRGINICA

The northern blue flags, *Iris versicolor* and *Iris virginica,* were originally chosen for study because they were known to be closely related and preliminary investigation had shown that in any one locality they varied markedly from plant to plant. It was accordingly planned to study the minutae of variation so intensively in these two species that one might demonstrate the way in which one species had evolved from the other, or from some common ancestor. It seemed at the beginning of the work that here was splendid material for illustrating the way in which individual differences merge into racial, racial into varietal, and varietal into specific. A confident beginning was made with this end in view: five years of hard work showed that *Iris versicolor* might vary greatly and that *Iris virginica* might vary greatly but that each remained itself. They were of different fabrics. One might compare them to two old English villages, one in a sandstone region and the other in limestone. In each village there would be no two houses alike but all the houses in one village would be made of limestone, all those in the other made of sandstone. The conclusion was reached that closely related though these irises might be, variation within either species was of quite another order of magnitude from the hiatus between them (Anderson, '28). The variation *within* could never be compounded into the variation *between.* The two species were made of two different materials.

If one of these species was *not* derived from the other through the slow accumulation of minor differences, in what other manner could it have originated? Fortunately, at about the same time that these detailed studies of variation came to an *impasse* there were published a number of accounts dealing with another way in which species might originate in the higher plants: amphidiploidy. This phenomenon may well be illustrated by the case of *Primula kewensis* (Newton and Pellew, '29). *Primula kewensis* originated as a highly sterile hybrid between *P. floribunda* and *P. verticillata* (fig. 2). Kept alive by

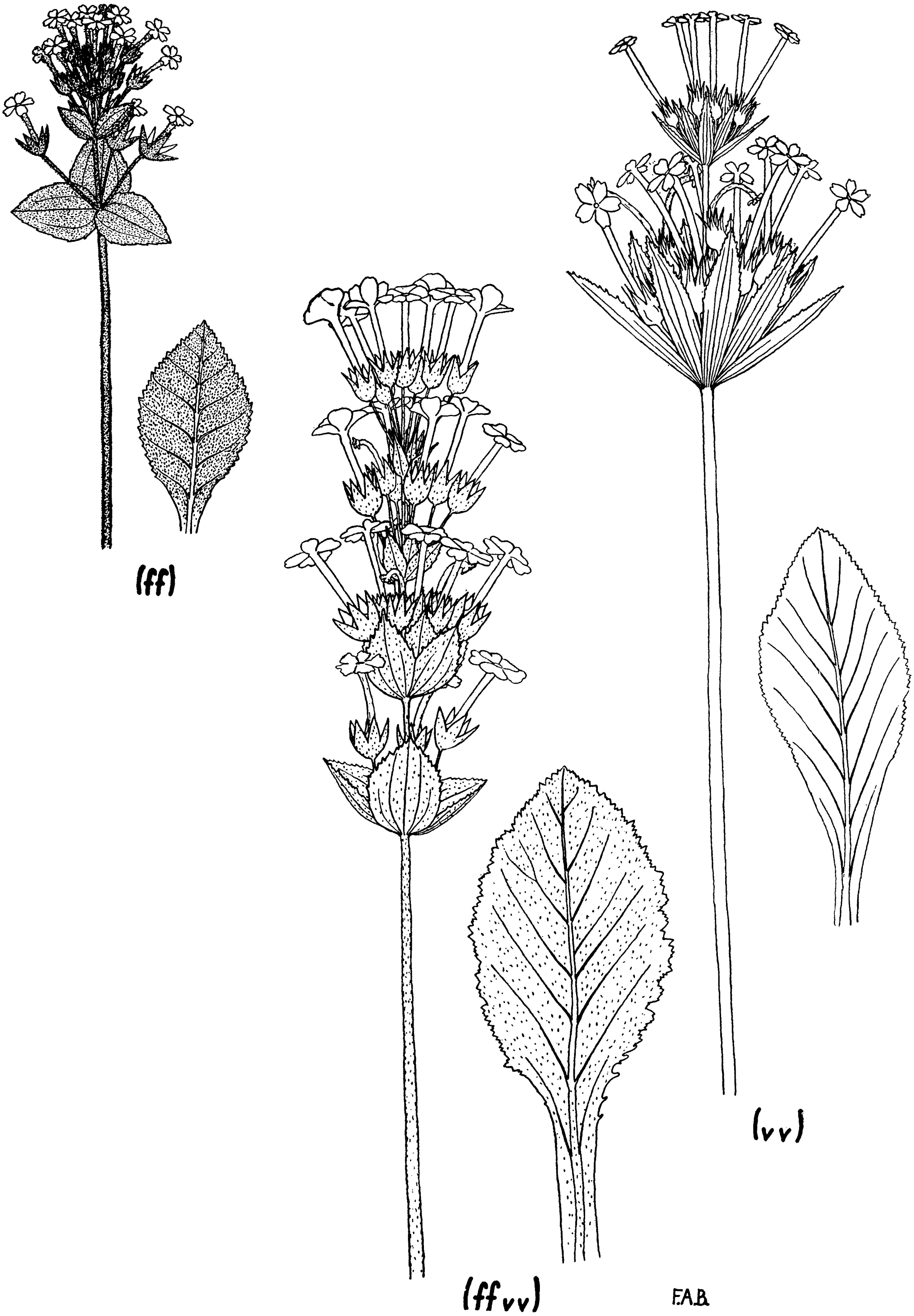


Fig. 2. *Primula floribunda* (ff), *P. verticillata* (vv), and their amphidiploid hybrid *P. kewensis* (ffvv). Drawn from herbarium specimens collected in the greenhouses of the John Innes Horticultural Institution.

vegetative reproduction, it has on several occasions produced fertile flowers. The progeny from these exceptional flowers have furthermore bred true, or substantially so, and the hybrids are today grown commercially by means of this fertile strain. Cytological examination has demonstrated that this fertile, true-breeding hybrid has 18 pairs of chromosomes, while the sterile hybrid and each of the parental species has 9 pairs. The fertile hybrid evidently originated when an exceptional nuclear division in the inflorescence of the sterile hybrid was not accompanied by a cell division and a sector arose in which the entire chromosome complement had been duplicated. On this hypothesis *Primula floribunda* might be diagrammed as 9F + 9F; *P. verticillata* as 9V + 9V; the sterile hybrid as 9V + 9F; and the fertile hybrid as 9V + 9V + 9F + 9F. The original hybrid was sterile because the two sets of chromosomes (V and F) were too unlike to pair and produce fertile gametes. Doubling the number resulted in two sets of V's and two sets of F's so that pairing could proceed regularly, producing a fertile, true-breeding hybrid, or amphidiploid.

Amphidiploidy, the production of fertile, true-breeding hybrids by doubling of the chromosome number, is now known to be a fairly common phenomenon among the higher plants (Winge, '32). It has occurred under controlled conditions in the experimental plots of many investigators. More than 24 such cases are now on record including several among floristically indigenous species (Müntzing, '30, '32; Clausen, '33). It has apparently occurred in the development of the cultivated irises (Randolph, '34). Amphidiploidy is largely confined to the flowering plants and is foremost among several factors which make specific relationships among the higher plants more intricate and more various than they are among the higher animals (Anderson, '31).

If our two blue flags did not originate by the slow accumulation of individual differences, the most likely explanation of their fundamental divergence is that one or both of them came into existence suddenly through amphidiploidy. A simple hypothesis immediately suggested itself.

Iris versicolor is geographically and morphologically intermediate between *Iris virginica* and the Arctic blue flag, *Iris setosa.* Might it be possible that *Iris versicolor* is an amphidiploid hybrid between these two species, a hybrid which occurred in pre-glacial or inter-glacial time? Startling as such an hypothesis seemed, it found confirmation in facts from such diverse fields as geographical distribution, cytology, morphology, and genetics. The hypothesis was used, with complete success, to predict the presence in central Alaska of a previously unrecognized variety of *Iris setosa.* It orients a number of facts which are either puzzling or meaningless on any other hypothesis. For all practical purposes it may be taken as proved though it is capable of still further tests.

The facts which support this hypothesis may be grouped under several different heads:

1. *Genetics.*—Although they have been placed in different sub-sections of the genus, *Iris virginica* and *Iris setosa* are at least partially fertile *inter se.* It is difficult in this latitude to bring both species into flower at the same time, but on one occasion it was possible to do so and two pollinations were made. From these two crosses of *Iris virginica* × *Iris setosa* were obtained two seed-pods well-filled with seeds, but with shrunken endosperms. None of them germinated, but it seems likely that if the cross could be repeated in quantity a few viable seeds could be obtained. It should be pointed out in passing that the most successful amphidiploids so far obtained have been between plants which are ordinarily quite sterile with one another. As was first pointed out by Darlington ('28, pp. 244–245), the more inter-sterile the two parents of an amphidiploid, the more fertile and true-breeding is the resulting progeny.

2. *Cytology.*—The cytological investigation has been hampered by the high chromosome numbers of *Iris virginica* and *Iris versicolor,* the highest known in the genus. This makes the determination of chromosome number and configuration rather difficult, and I am happy to report that my own counts have been completely confirmed by several other investigators,

principally by Randolph ('34). *Iris virginica* has 70 to 72 chromosomes [2n], *Iris setosa* has 38, and *Iris versicolor* has just what we would expect if it is an amphidiploid hybrid of the

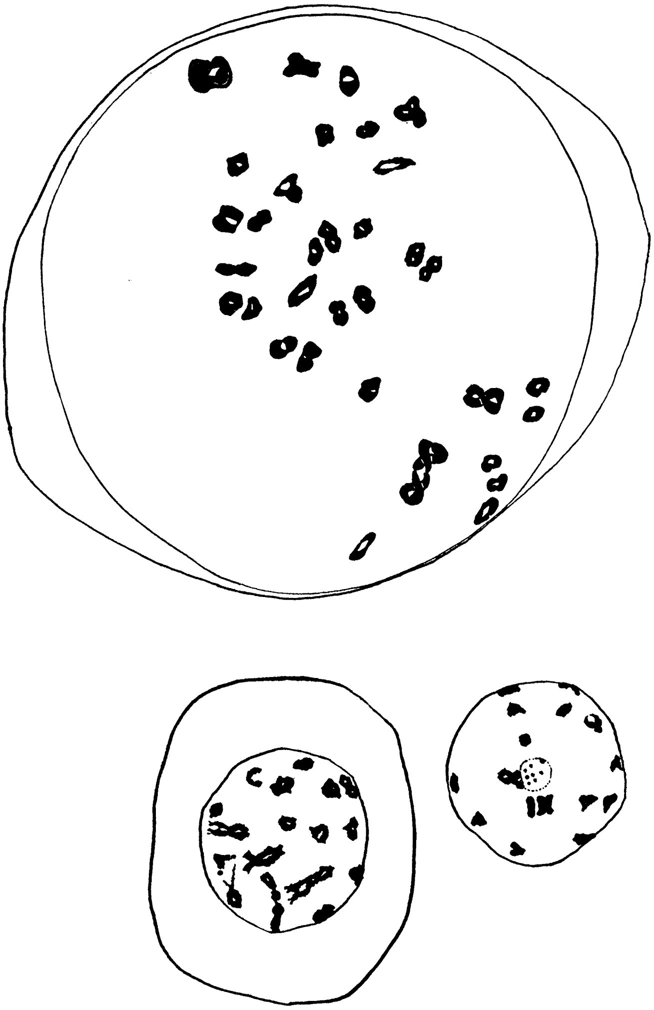


Fig. 3. Above: smear of early metaphase PMC. of *Iris virginica* var. *Shrevei* from Frankenmuth, Michigan, somewhat distorted by pressure. Camera-lucida drawing (made at bench level at × 2280, reduced to × 1140).

Below: smear of PMC. of *Iris virginica* var. *Shrevei* from Frankenmuth, Michigan. Late diakinesis, upper and lower hemispheres drawn separately.

two, 108 chromosomes. *Iris virginica* shows occasional multivalent association, mainly in fours, and very strong secondary association (fig. 3). These facts would suggest that it is itself an ancient amphidiploid hybrid of two species each with 36± chromosomes. *Iris versicolor* has occasional multivalents; hexavalents such as the one illustrated in fig. 4 are not uncommon. The cytological facts therefore are in complete agreement with our hypothesis, and they go even farther by suggesting that *Iris virginica* is a set of two genoms and *Iris versicolor* the component of three genoms.

Fig. 4. Aceto-carmine smear of PMC. of *Iris versicolor* from Connecticut Lakes, N. H. Camera-lucida drawing (made at bench level × 2280, reduced to × 1140).

3. *Geographical evidence.*—The three species of irises which we are considering have strikingly different distributions in North America, and the distribution of each is characteristic of many of the plants with which it is found. The significance of these areas has been pointed out by Fernald ('31). Of the region about which the *Iris virginica* is centered he says: "Temperate eastern North America has, then, an extensive area (the southern Appalachian Upland) in which land-plants have had an opportunity to spread since the advent of the Angiosperms."

The other putative parent species, *Iris setosa,* also comes from a region (again we quote Fernald) "which apparently retained [its] present distinctive flora through at least the last glaciation."

Iris versicolor, which we are presuming to derive from these two ancient species, inhabits a more youthful region, one characterized by Fernald (loc. cit., p. 28) as "the vast region of Canada and the Northern States which has become available for wholesale occupation by plants only since the decay of the Wisconsin ice, within the last few thousand years."

The geographical facts, therefore, point to *Iris virginica* as an ancient southern species and to *Iris setosa* and its variety *canadensis* as being certainly pre-glacial. *Iris versicolor,* our putative hybrid, is either late pre-glacial or inter-glacial. The present distributions of the species would suggest that the original hybridization (or hybridizations) took place in the interior of the continent, perhaps in the general region of the present-day Great Lakes. As will be shown below there are morphological reasons for believing that the *Iris setosa* which entered into the cross was not the depauperate remnant which lingered on around the Gulf of St. Lawrence. The actual ancestor is rather to be sought among the hordes of *Iris setosa* which must have occupied the interior of the continent before the glacial period.

4. *Habitat.*—In its habitat preferences *Iris versicolor* is likewise intermediate. It grows in situations more moist than those preferred by *Iris setosa* and a little drier than those in which *Iris virginica* is found. The three species are not found growing together in nature at the present time but *Iris versicolor* is found with each of the others. Around the Gulf of St. Lawrence the marked preference of *Iris setosa* var. *canadensis* for drier situations has been noted by a number of investigators. In Michigan and Ontario, where *Iris versicolor* and *Iris virginica* are growing together, it can be seen that *Iris versicolor* will continue to flower and fruit in spots so dry that *Iris virginica* only persists vegetatively. It is not uncommon to find *Iris virginica* growing luxuriantly in marshes where

there is standing water over its roots for several months during the growing season. *Iris versicolor* may tolerate such a situation but does not welcome it.

5. *Morphology.*—Before considering these data in detail it may be well to point out again that, according to our hypothesis, *Iris versicolor* was formed by the summation of *Iris setosa* with 38 chromosomes and *Iris virginica* with 70. In other words it received two doses of *Iris virginica* but only one of *Iris setosa.* We should expect therefore to find *Iris versicolor* in an intermediate position morphologically but much closer to *Iris virginica* than to *Iris setosa.* Such does actually prove to be the case.

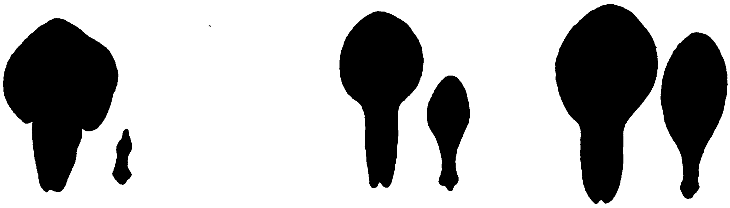
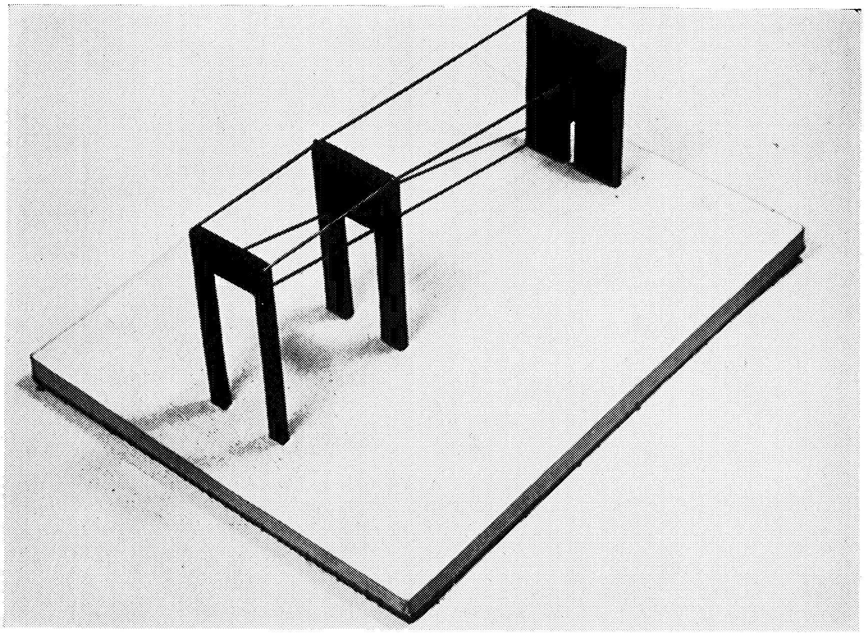


Fig. 5. Outline drawings of petal and sepal from plants of *Iris setosa* (left), *I. versicolor* (center), and *I. virginica* var. *Shrevei* (right).

In the living plant the size and dimensions of the petals and sepals are among the best diagnostic characters for these three species (as indeed for most species of *Iris.*) It will be seen that *Iris setosa* differs from *Iris virginica* in having a shorter, broader sepal and a much smaller and narrower petal. *Iris versicolor,* as our hypothesis demands, has differences in this direction. These significant dimensions are presented diagrammatically in fig. 5. These differences in proportion of sepal and petal are so absolutely in accord with the theoretical demands that, given any two of the three species, it is possible to derive the average proportions of the other by statistical prediction (pl. 23).

The sepals of *Iris virginica* bear a bright yellow pubescent patch, the hairs of which are clearly visible to the naked eye. No such patch exists in *Iris setosa,* and the epidermal cells are



Photograph of three-dimensional model showing the precise geometrical relationship in petal and sepal size and proportions of *Iris virginica* (left), *I. versicolor* (center), and *I. setosa* (right). In the model, *Iris versicolor* is placed two-thirds of the distance between the two putative parents, since their chromosomal contributions to the hybrid are in the approximate ratio of 2 to 1. Measurements combined by method illustrated in figure 8.

seen to be barely papillate when examined with a strong hand-lens. *Iris versicolor* is intermediate; the bearding is clearly visible only with a hand-lens and the color is at best a kind of greenish-yellow. The blade of the sepal in *Iris setosa* is broad and slightly undulate, in *Iris virginica* it is narrow and straight; *Iris versicolor* presents an intermediate condition.

The ovary of *Iris setosa* inflates rapidly after fertilization so that in the ripening capsule there is a wide space between the walls and the seeds. In *Iris virginica* the walls are stretched tightly over the seeds, in *Iris versicolor* the condition is intermediate though nearer to that of *Iris virginica.*

In *Iris setosa* the capsules and stem are so strongly lignified that they sometimes persist for over two years, and it is customary to find last year's seed stalks among this year's flowers. In *Iris virginica* the capsule and stem, though much larger, are poorly lignified. In the humid swamps of the south they fall over and the capsule usually is more or less disintegrated by the time the seed is ripe. Here again *Iris versicolor* is intermediate; the capsules usually persist well into the winter but are seldom found the second season.

Iris setosa bears seeds which are unique in the genus. They are small, heavily vernicose, and with a conspicuous raphe down one side. *Iris virginica* bears large, spongy seeds which may be either round or D-shaped. When the above working hypothesis was first considered, one possible objection seemed to be the fact that *Iris versicolor* was without a raphe. Subsequent examination of the seeds of *Iris versicolor* shows that it does have the shadow of one on nearly every seed and had even been illustrated as having one (though without comment) in Dykes' plate of *Iris* seeds in his monograph of the genus ('13). (See pl. 22.)

When minute comparisons were made in this way, character by character it was found that there were, however, at least three characters in which *Iris versicolor* was not intermediate between *Iris virginica* and *Iris setosa* var. *canadensis.* The theory demanded an *Iris setosa* with several flowering branches, with pedicels longer than the bracts, and with bracts not greenish but brown and subscarious. Since the demands of the theory were met so minutely on all the other characters it seemed possible that these three represented details in which the race of *Iris setosa* entering into the original hybridization differed from *Iris setosa* var. *canadensis.* Such a race might conceivably have present-day relatives living among the *Iris setosa* which is so widely spread in Alaska and Asia, since the species is notoriously variable there. Herbarium material was accordingly consulted. The first few specimens examined proved most disappointing. They were from localities along the arctic coast and they had none of the three desired qualities. Their bracts were long and green, completely eclipsing the pedicels, and the plants were unbranched. Farther down in the pile, however, was a plant which had not only long pedicels, but scarious bracts and a branched inflorescence, the very combination desired. Farther on was another and eight specimens in all were found.

When their distribution was plotted it was found that all came from central interior Alaska and represented, in fact, the only specimens from that region. They have accordingly been described above in the taxonomic section of these papers as a new variety, *Iris setosa* var. *interior.*

Further search unearthed the even more significant fact that *Iris setosa* var. *interior* grows in that part of Alaska which was adjacent to the edge of the continental ice-sheet (map 1). If representatives of the pre-glacial races of *Iris setosa* which must once have covered much of northern Canada are to be sought anywhere today, glacial geologists would suggest this very region (Capps, '31). In other words, we not only found the variety demanded by the theory but we found it in exactly the region which the theory would suggest as most likely.

The comparison of the three species can be closed therefore with the presentation of diagrams to scale of *Iris virginica, Iris versicolor,* and *Iris setosa* var. *interior.* It will be seen that in size, node number, leaf length, number of branches, length of pedicels, and length of bracts the demands of the hypothesis are exactly met (fig. 6).

Though it is capable of still more rigorous tests, the theory that *Iris versicolor* is a pre-glacial, or inter-glacial, amphidiploid hybrid between *Iris virginica* and *Iris setosa* var. *interior* would seem to be well established as a working hypothesis. Ultimately it should be possible, as in the case of the European *Galeopsis Tetrahit* (Müntzing, '30), to re-synthesize the species from its two constituents.

Fig. 6. Diagrams to scale of *Iris setosa* var. *interior, I. versicolor,* and *I. virginica* var. *Shrevei.* The diagrams represent precise averages of all the available herbarium material.

The hypothesis also gives an explanation to several curious facts which had previously been most puzzling. The first might be called the one-way relationship between *Iris virginica* and *Iris versicolor,* or so I have attempted to phrase an impression received from long-continued study of variation within these two species. It seemed that the relation of *Iris versicolor* to *Iris virginica* was quite different from that of *Iris virginica* to *Iris versicolor,* or to state it somewhat less mystically, that *Iris versicolor* often reminded one of *Iris virginica,* but *Iris vir-*

ginica never reminded one of *Iris versicolor.* If on the above hypothesis *Iris versicolor* is indeed *Iris virginica* plus something else, then the relationship should be different in one direction from what it is in the other.

Another puzzling fact had been the frequency of albinos. *Iris virginica* and *Iris versicolor* by any ordinary standard were unusually variable species yet pure albinos were exceedingly rare. In spite of prolonged search and inquiry I found only three in *Iris virginica* while in *Iris versicolor* I have found no pure albino without a trace of blue, and only one case has been reported in the literature (Fernald, '36). Yet albinos are common in many species of *Iris,* as, for instance in *Iris missouriensis;* why then should they be absent from our common blue flags? Why should they shun this particular species which by any other standard is peculiarly variable in flower color? On the above hypothesis this is exactly what one might predict. If *Iris virginica* is made up by the summation of two ancient species, albinism, being recessive, cannot appear until it has occurred in each of the constituent sets. In *Iris versicolor* it cannot show itself until it appears in these and also in the set of chromosomes derived from *Iris setosa.* This means that if the original frequency of albinism in the basic species had been, say one in every 5000, that we should find it in *Iris virginica* once in every 25,000,000 and in *Iris versicolor* only once in 125,000,000,000. The infrequency of albinism in *Iris virginica* and its even greater rarity in *Iris versicolor* is therefore in strict accord with theoretical expectations.

SUMMARY

1. The absolute morphological discontinuity previously discovered between the closely related *Iris versicolor* and *Iris virginica* is explained by the following hypothesis: *Iris versicolor* originated suddenly as a fertile, true-breeding hybrid (an amphidiploid) between the southern *Iris virginica* and the subarctic *Iris setosa,* in pre-glacial or inter-glacial times.

2. Since the former has 70 chromosomes and the latter 38 we should expect to find *Iris versicolor* generally intermediate be-

tween these species though much closer to *Iris virginica.* This is found to be the case.

3. A consideration of one or two minor exceptions to this generalization led to the discovery of a previously unrecognized variety from central Alaska, *Iris setosa* var. *interior.*

4. Since it harmonizes so many otherwise incoherent facts from cytology, morphology, geographical distribution, genetics, and geology, the theory is taken to be well established as a working hypothesis.

5. The theory also explains two phenomena which had previously seemed incomprehensible: (1) the "one-way" morphological relationship between *Iris virginica* and *Iris versicolor,* (2) the infrequency of albinos in *Iris virginica* and their even greater rarity in *Iris versicolor,* a species otherwise unusually variable in flower color. It is shown that both of these results are to be expected on the basis of the above hypothesis.

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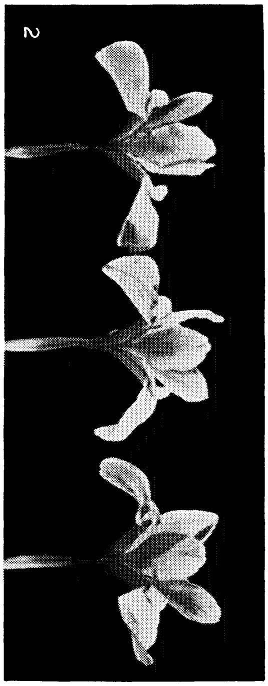
IV. Intra-specific Differentiation in the Northern Blue Flags

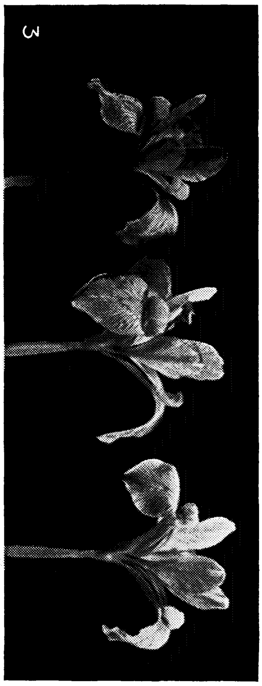
As has been related in the previous paper of this series, detailed studies of variation were made in two species of *Iris* with the aim of demonstrating the way in which one had arisen from the other. Though they failed to produce any such evidence (and have as a matter of fact led to a very different hypothesis of the phylogenetic relationship between these two particular species), the data may still be used to examine the way in which evolution has proceeded and is proceeding to build up differences within these species. Many of the actual data have been presented in full in a previous communication (Anderson, '28). Since that time, however, the interpretation of this evidence has been profoundly affected by information derived from other fields of study.

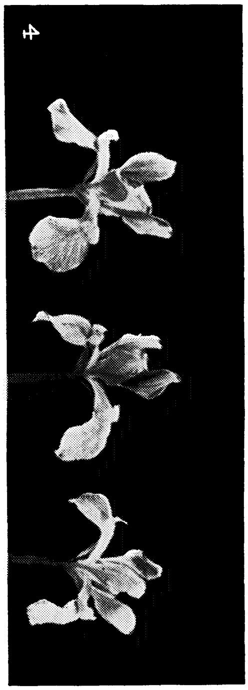
The following paper is largely concerned with the results of a detailed morphological census of two species of *Iris* in eastern North America, *I. versicolor* and *I. virginica;* some attention has also been paid to the related glacial relict, *Iris setosa* var. *canadensis.* The results of such a census may be presented individual by individual or they may be grouped and averaged in various ways. In the following census the colony is recognized as a vegetational and evolutionary unit of major importance, so far as irises are concerned. Throughout most of the region in which they are found today, *I. versicolor* and *I. virginica* grow in small colonies of from one to several thousand individuals. Single individuals usually cover several square feet and send up several flowering stalks each year. In exceptional cases one individual may by vegetative reproduction cover a much larger area, and in rare instances a colony of several acres may be composed genetically of but one plant.

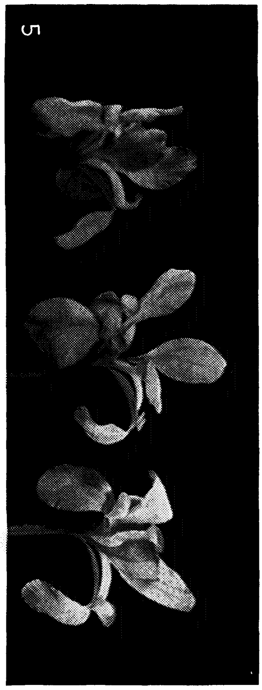
With a little study the recognition of individual plants is not at all difficult. The sea of blue-purple flowers which at first glance seems so uniform resolves itself into a little community with quite as much divergence between the various members as is found in human communities. One plant will have brown spots on the sepals of each flower, the next one will be without the spots but will claim attention by the extraordinary size of its petals. Another will have flowers which are a very light blue, still another will have flowers which are almost wine colored, another will have deep notches in the petals. And just as in a village each man has a hand with characteristically different proportions from all other men yet has his left and right hands built on almost the same pattern, so it is in a swamp full of irises. The petals and sepals of the different flowers on a plant will have substantially the same proportions, but these proportions will vary tremendously from plant to plant. This point is illustrated in pl. 24 where three flowers are shown from each of six plants. These photographs were taken with identical illumination and exposure and were developed and printed uniformly. The differences in shade are due to differences in the flowers themselves; plant no. 2 had flowers of a very light blue and they have photographed almost white; plant no 6 had a great deal of red in with the blue and it is much darker in the picture. It will be noticed that even such a tenuous character as the carriage of the petals and sepals is based upon inherent factors; note, for instance, the floppy aspect of all three flowers of no 5, the contrasted horizontal sepals and upright petals of no. 1, the undulate sepal margins of no. 3.

The number of such colonies is enormous; a rough approximation has been made by observing the numbers to be seen from the highway along various routes and converting these figures into number of colonies per square mile. The method seems to be reasonably accurate, since it yields consistent results when different trips are made through the same territory. According to this method, the average frequency of *Iris* colonies per 100 square miles is 120 in northern Michigan, 350 in southern Michigan, 170 in northern Illinois, 30 in southern Missouri, and 5 in Alabama and Mississippi. Colonies are particularly frequent north of the terminal moraine where an uneven glacial topography produces many small swampy areas favorable for the growth of *Iris*. Figure 7 illustrates a representative area of 50 square miles within this region. It









Three flowers each from six plants of *Iris virginica* var. *Shrevei* from Portage des Sioux, Missouri.

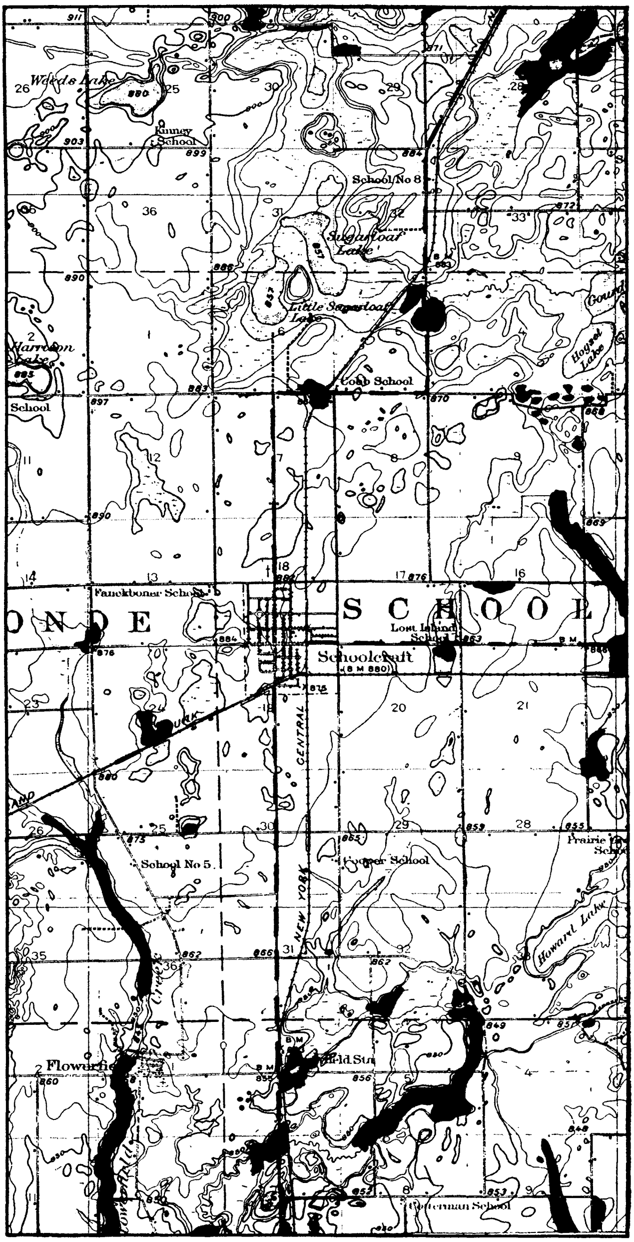


Fig. 7. Representative region of 50 square miles in southern Michigan. Areas occupied by *Iris virginica* var. *Shrevei* shown in solid black.

demonstrates how numerous are the *Iris* colonies and how isolated they are from one another.

It is a point of some theoretical importance (see below, pp. 495–496) that the colonies are probably distributed in much the same way that they were before the land was cleared but that

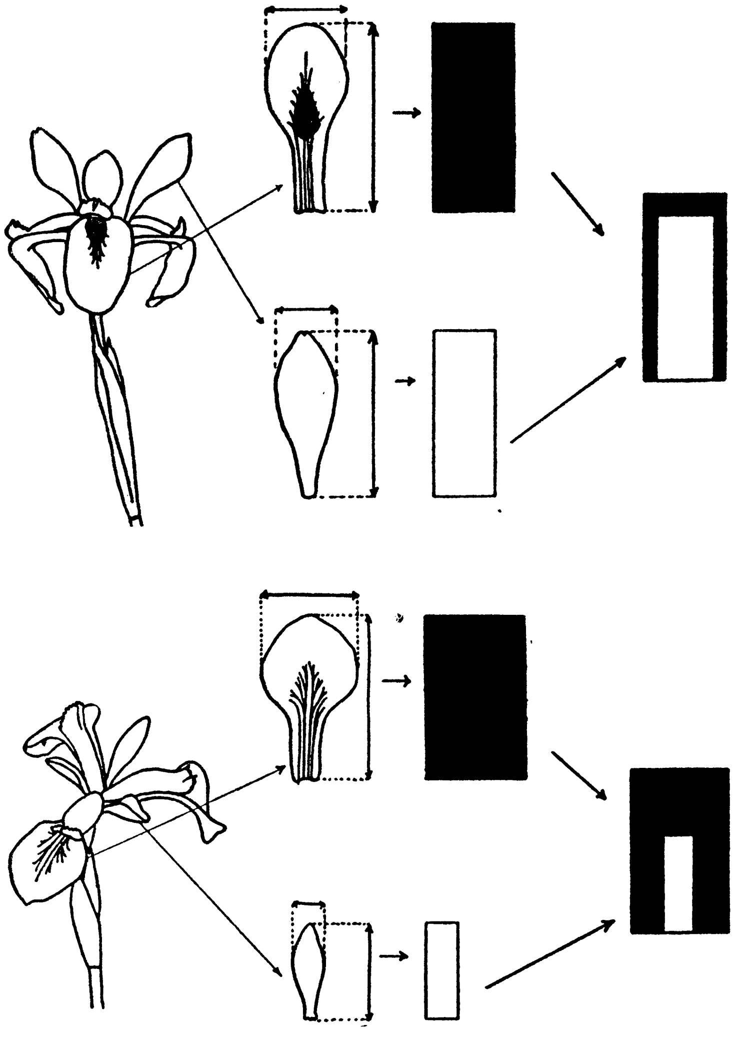


Fig. 8. Diagram illustrating how petal length and width, and sepal length and width are combined to form an ideograph. Above, *Iris virginica;* below, *I. versicolor.*

the numbers of individuals in the colonies are greater. The deforestation and pasturing of swampy areas have increased many fold the area available for these irises throughout the northern states. Turning the land over to pasture is particularly helpful to them since grass, their worst competitor, is kept down by the livestock. In most cases large colonies now numbering thousands of plants are probably the descendants of a much smaller number which were growing in that area before the land was cleared.

The census has to do with four measurements; length and width of sepal, length and width of petal. Since such measurements are of greatest significance when their interrelations with each other are understood, the results are presented graphically in a way which makes it possible to convey these relationships simultaneously. Figure 8 shows how the four measurements of each flower can be built up into a simple black-and-white diagram. This diagram or ''ideograph'' is essentially a white petal superposed upon a diagrammatic black sepal. Figure 9 presents ideographs for 20 plants of *Iris versicolor* and 20 of *Iris setosa* var. *canadensis* which were growing together in a pasture near Île Verte, Quebec (Anderson, '35). It demonstrates how such ideographs may be used to present a large amount of data in a small space. Figure 9 is a graphical summary of four measurements and six proportions on each of 40 plants. It is, in other words, a simultaneous presentation of 400 separate facts. The precise comparison of such colonies can be carried farther by the production of *average* ideographs for the whole colony, utilizing the average petal length, the average petal width, the average sepal length, and average sepal width (fig. 9, central ideographs).

The data for colonies are presented in this way in figs. 10 and 11. The colonies are arranged by species and subspecies and within these categories are placed roughly according to geographical position from south to north. A study of these figures yields the following conclusions:

1. There is little or no regional differentiation in shape within any of the subspecies. No general characteristics can be recognized for the irises from southern Michigan or from

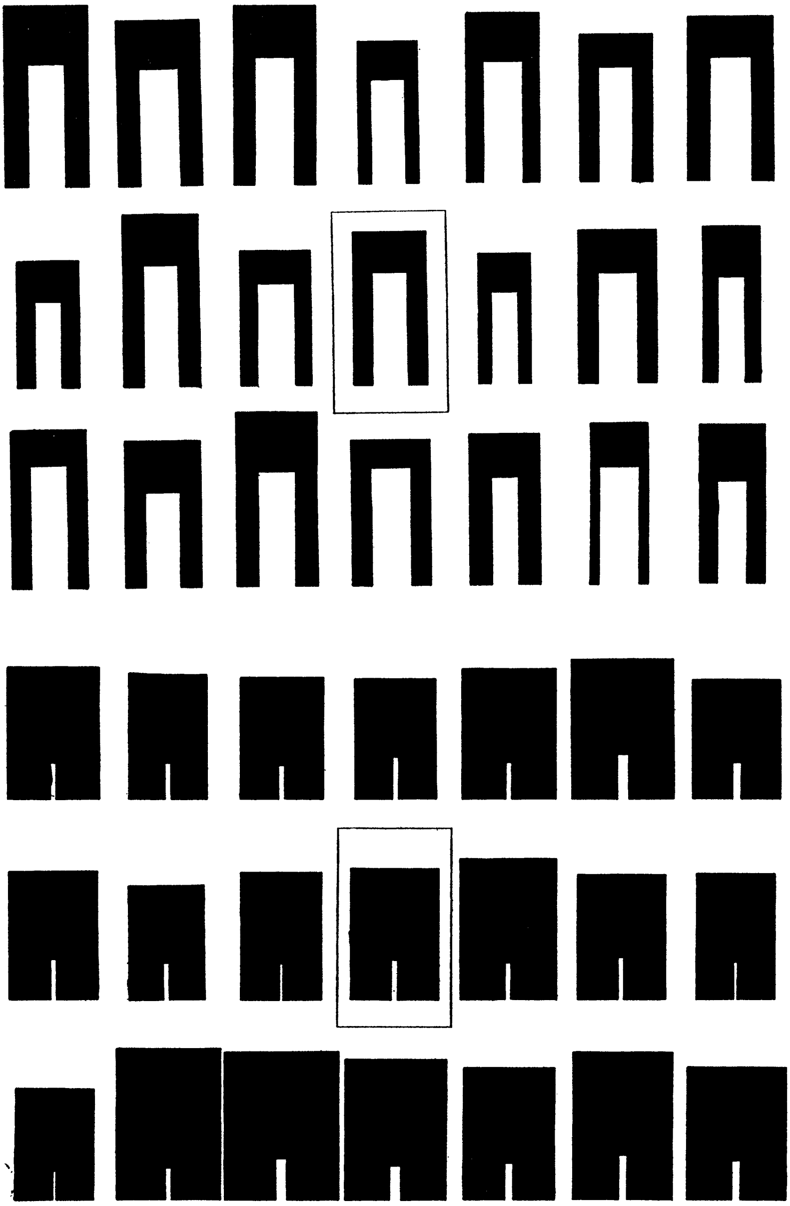


Fig. 9. Ideographs of 20 plants of *Iris versicolor* and 20 of *I. setosa* var. *canadensis* from Île Verte, Quebec. Averages of entire colony (50 for each species) shown in central frames.

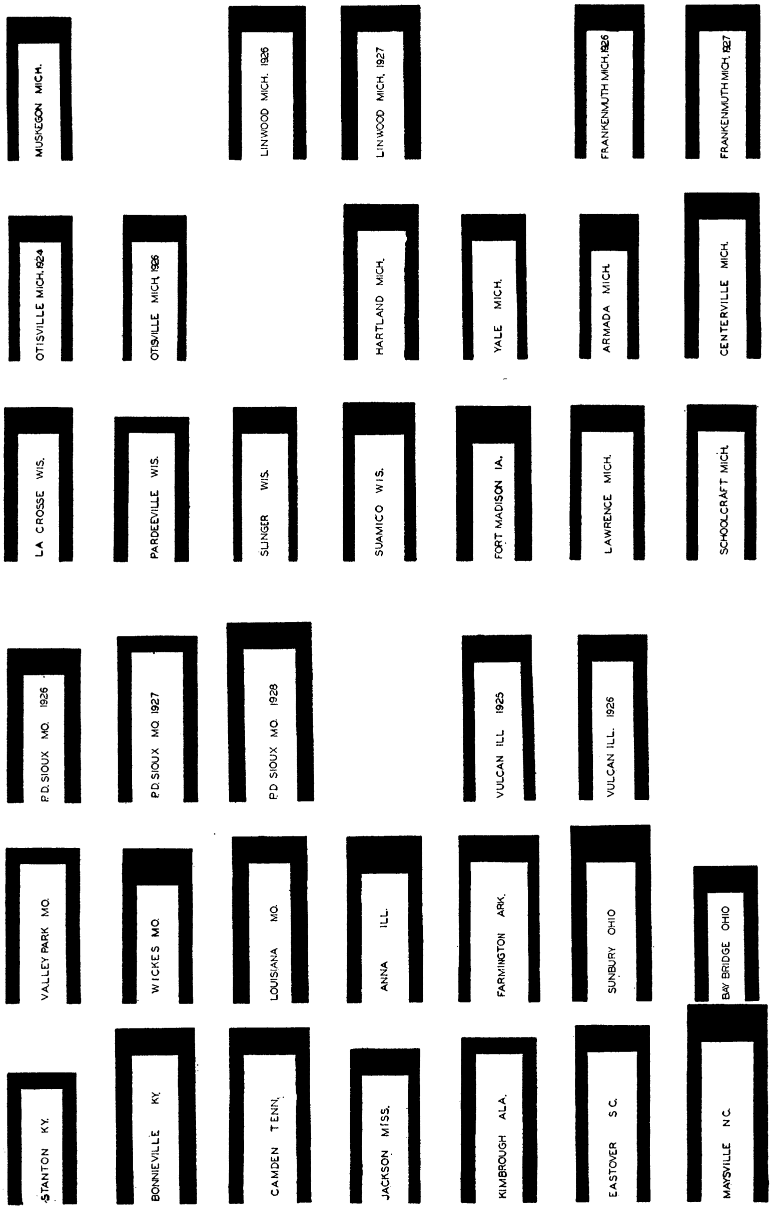


Fig. 10. Average ideographs for 31 colonies of *Iris virginica* and *I. virginica* var. *Shrevei*.

the Mississippi Valley, or from the Cumberlands, or from the prairies. As will be shown below, this conclusion is reinforced by the computation of *regional* averages.

2. There is a very slight trend in size. *Iris virginica* var. *Shrevei* reaches its greatest development in the Cumberlands of Kentucky and Tennessee. Northward or southward it becomes somewhat smaller on the average. *Iris versicolor,* on the contrary, is largest in the north and becomes smaller towards its southern limits. Transplants from these various areas have kept their same relative sizes when grown together in the experimental garden.

Fig. 11. Average ideographs for 18 colonies of *Iris versicolor.*

3. Colony averages are fairly consistent from year to year in those cases where measurements could be made in different years. Allied to this fact is the experimental evidence that the flowers of plants collected and grown together in the garden maintained their characteristic size, shape, color, and color pattern. In several cases divisions of the same plant have been grown and studied in Boston, St. Louis, and Schoolcraft,

Michigan. For these reasons the differences which distinguish the individual plants and thereby create the peculiarities of the colonies are thought to be largely inherent. An even stronger proof is the fact that progeny tests of several individuals produced evidence for the heritability of various individual peculiarities.

4. There are striking differences between colony averages, even for the same region. The colony averages of figs. 10 and 11 are particularly instructive when compared with the regional averages of fig. 12. It will be noted that though there are differences between the regions they are slight, and they have no evident geographical trend other than the slight one in size already referred to. The regional differences are indeed so slight that the variation in these irises might seem to be without any phylogenetic significance. If attention is shifted from the regions to the colonies, the evolutionary significance of the variation is more manifest. Each little colony is a more or less independent evolutionary unit and has evolved a more or less distinctive combination of characters. All that is necessary for the production of a regional variety is the isolation of any *one* of these colonies. Were some succession of droughts and floods to exterminate the great bulk of either species, leaving only two or three colonies persisting in different parts of

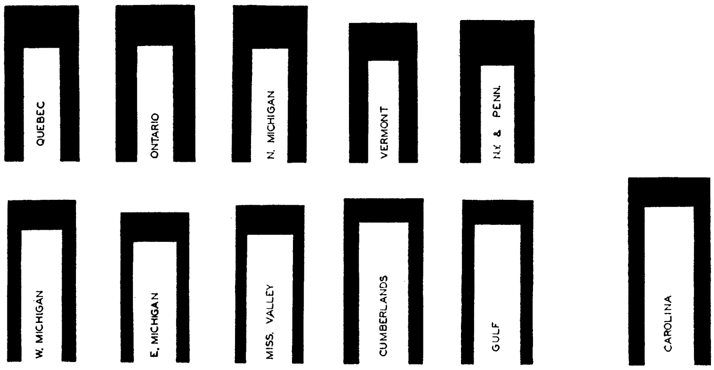


Fig. 12. Regional averages for *Iris versicolor* (above), *I. virginica* var. *Shrevei* (below, left) and *I. virginica* (below, right).

its present range, and were the area to be repopulated from these centers we should then have the formation of geographical varieties. The majority of the colonies as they exist at the present have achieved sufficient individuality to be rated as incipient varieties were they only to reproduce their several types over larger areas. For an actual difference of this magnitude we may compare *Iris virginica* of the Atlantic seaboard and *Iris virginica* var. *Shrevei* of the Mississippi Valley. For an inconceivably long time the irises of the seaboard have been somewhat isolated from their relatives in the Mississippi Valley. It is not surprising then that though we can find no outstanding differences in sepal and petal proportion within the interior of the continent, there is a slight difference between the irises from the interior and those from the seaboard. *Iris virginica* has flowers which are distinctly larger and somewhat narrower. The difference is a minor one as compared to the distinct hiatus between *I. versicolor* and *I. virginica,* but it is reinforced when we study such technical characters as the shape of the seed capsules and the size of the seed.

Particularly significant is the fact that the difference between *I. virginica* and *I. virginica* var. *Shrevei* is of about the same order of magnitude as the differences between colonies of *I. virginica* var. *Shrevei.* It would indeed be possible to find two swamps in the same township in southern Michigan whose iris populations have as great an average difference as that between *Iris virginica* of the Atlantic Coastal plain and *Iris virginica* var. *Shrevei.* But in this latter case the difference, slight though it is, characterizes a whole region and has superimposed upon it the varying pattern of colony differences in each region.

An evolutionary factor of basic importance in our common blue flags, therefore, is the rapid accumulation of minor differences in the little colonies into which the species are divided. Nearly every colony carries within itself the potentialities of a variety or a subspecies. The conditions under which these irises exist seldom release these potentialities. Many colonies arise, develop a distinctive type, and pass on with little or no influence on the main evolutionary stream. It should be remembered that the territory in which they are growing possesses very few geographical or climatic barriers. But the potentiality is there and when opportunity allows a colony to play a larger role it is ready to do so. Then the peculiarities evolved in one colony, or a few colonies, might come to characterize all the colonies of a region.

By mathematical deduction from the known facts of genetics, Wright ('31) has produced a generalized theory of evolution. From the standpoint of pure theory he finds that evolution will proceed most effectively neither in a large inter-breeding population nor in a very small one but in a large population "divided and sub-divided into partially isolated local races of small size." Under such conditions he predicts "a continually shifting differentiation among the latter which inevitably brings about an indefinitely continuing, irreversible, adaptive, and much more rapid evolution of the species."

The irises of this study present just such a picture. They are divided into partially isolated small colonies ["Local races"] which before the land was cleared were probably even smaller. These colonies differ from one another and from the mean of the species to a degree which is almost of varietal magnitude. Their differences are inherent and to all appearances are largely non-adaptive. Isolation, in dividing the species up into these smaller units, has made possible their several divergencies. Were isolation to be made complete, as by another glacial period, the phylogenetic potentialities of the survivors would be released and what had been colonial peculiarities might become varietal differences.

The variation within *Iris setosa* var. *canadensis* seems particularly instructive in the light of its recent history. It is typical of those species whose once continuous range across northern North America was reduced to the northeastern and northwestern edges of the continent by the Pleistocene ice. In Alaska a large central region was left unglaciated; around the Gulf of St. Lawrence, on the other hand, the plant refuges in glacial times were little more than rocky nunatacks rising above the ice. The results on the two sets of irises are just what a geneticist might predict. Even from the few specimens which are available in herbaria one can see that the *Iris setosae* of Alaska are a varied assemblage. They include one well-marked variety (described above) and several fairly well-marked regional variants. The irises of eastern Canada present a very different picture. Figure 9 gives some slight indication of their lesser variability from plant to plant. Compared to the millions of irises which might well have continued to live in Alaska during the ice age, those of the St. Lawrence region were a mere handful. From that handful must have descended the millions upon millions of irises which now carpet the meadows and shores of that region in early summer (Anderson, '35). Compared with our other American blue flags they are a singularly invariable lot. Graphical comparisons are made in fig. 9. They are furthermore the smallest, much smaller than any other recognizable type of *Iris setosa* (note fig. 1). They are short, seldom branching, with small leaves and few nodes.

This conservatism of *Iris setosa* var. *canadensis* is distinctive of most of the glacial endemics (or near endemics) of the region around the Gulf of St. Lawrence. In one of his classic contributions to the subject, Fernald ('29) has aptly characterized them as "already waning types, too old, or too conservative to spread into closely adjacent and virgin soils." In the case of *Iris setosa* var. *canadensis* the invariability cannot be a direct effect of time, for the highly variable irises of Alaska are quite as aged. It is more probably, as Professor Fernald has suggested, an innate conservatism; a conservatism founded genetically upon the fact that these irises are descendants of a small and highly selected stock. Hard times removed from the region all the luxuriant types which may once have existed there. When the ice age was over the immediate area was repeopled from the few plucky survivors. Their descendants, *Iris setosa* var. *canadensis,* bear the scars of the glacial period, so to speak, in their conservatism; an innate invariability which, on the one hand, gives them a greater uniformity, and on the other, prevents their adapting themselves readily to other environments.

In the light of its probable history, it is not surprising to find no regional differentiation in *Iris versicolor.* If, as seems probable, it originated in interglacial times, it is a comparatively young species. It is furthermore inhabiting a region which is extremely youthful floristically, most of it not becoming available for plant occupancy until the last retreat of the Pleistocene ice. Having only recently moved into most of the territory it now occupies, *Iris versicolor* has had as yet little or no opportunity to develop geographical races within the species.

Much the same argument can be made for the northern flank of *Iris virginica* var. *Shrevei.* It is in this part of its range, thanks to irregular glacial topography, that it occurs most frequently and is therefore most easily studied. Within this area there is little or no evidence of geographical races. Were it possible to study *Iris virginica* and *Iris virginica* var. *Shrevei* in the same detailed manner on the older lands they occupy in the south, it is more than probable that considerable geographical variation would be found. There are indications of such differentiation in the few samples from these areas which are available in herbaria. A detailed statistical census would probably reveal still more.

SUMMARY

1. Most of the northern blue flags occur in more or less isolated colonies of from a few to many thousand individuals. The average frequency of such colonies per 100 square miles was found to vary from 350 in southern Michigan to 5 in Alabama and Mississippi.

2. From a statistical study of 60 colonies it is shown that there is little regional differentiation within any of the subspecies. Even within the same region, however, there are pronounced differences between the colonies.

3. The colony is an important evolutionary unit in these irises. Through its isolation each colony develops a distinctive type which is of potential phylogenetic importance.

4. It is shown that the differences between geographical varieties in these irises are of about the same order as differences between colonies. Many of the colonies carry the po-

tentialities of a variety or a subspecies, though these are seldom released by the conditions under which they exist.

5. The conservatism of *Iris setosa* var. *canadensis* is discussed in the light of its history as a glacial relict. Its comparative invariability and poor colonizing ability are probably innate as suggested by Fernald. They result genetically from the fact that this subspecies has descended from what in glacial times must have been a small population living under adverse conditions.

6. The lack of geographical differentiation within *Iris versicolor* and the northern colonies of *Iris virginica* var. *Shrevei* is correlated with their occupancy of a floristically youthful territory. *Iris virginica* var. *Shrevei* would probably reveal greater regional differences if it could be studied on floristically older lands in the south.

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V. THE EVOLUTIONARY PATTERNS OF THE GENUS IRIS

Students of the species problem are faced with a curious dilemma. By the nature of that problem they are forced to confine their attention to the details of a few species; yet if they do so exclusively they will be unable to interpret their results in general terms. The details of evolution vary from genus to genus and even from species to species; when one discusses the evolutionary patterns discerned by intensive work on one or two species he must attempt to determine to what extent those patterns are general characteristics of most species and to what extent they are special features of those few. Throughout the following discussion an attempt will be made to apply the information derived from these three species of *Iris* to the entire genus. It is as yet too early to consider the larger problem of speciation in the higher plants in the light of these results, except in the most general way.

In addition to the correction factor for the peculiarities of the germ-plasm one must also allow for the peculiarities of the region in which the studies were made. Speciation is a function of the region under observation. If a region is without pronounced barriers, speciation for most of the organisms in that area will be simpler than in an area with a complex system of barriers and partial barriers. It will also be affected by the age of the region. In one floristically young, geographical differentiation within species will be less intense than in regions which have been continuously available for occupancy for a long period of time. It should therefore be kept in mind during the following discussion that these studies were, for the most part, carried on in an exceedingly youthful region floristically and one in which there are no geographical barriers of any great importance.

A number of processes of evolutionary significance are taking place in these irises, all of which affect the ground-plan of the genus. Three of them, hybridization, amphidiploidy, and colonial differentiation, have produced effects which were readily perceptible by the methods used in this study. A general summary of the results is presented graphically in fig. 13.

The fundamental pattern is composed of the three species, which can be represented as great compound cables, stretched from the past into the future. For long eras these cables are practically parallel; even the detailed methods of this investigation have produced little or no evidence for the ultimate derivation of these separate cables from a common source.

Examining these cables more closely it is apparent that they are made up of smaller cords, the colonies. These cords likewise maintain their individuality for considerable time. Such a division of the species into small colonial units is probably characteristic not only of these three species but of a good part of the genus *Iris*. Certainly the other species with which I am personally acquainted in the field, *I. fulva, I. foliosa, I. prismatica, I. foetidissima, I. pseudacorus,* and *I. missouriensis,* grow in much the same sort of more or less isolated colonies.

Within this small group of irises there are three apparent cases where colonial differences have developed into geographical varieties. Very anciently the *Iris virginicae* of the Atlantic seaboard became slightly differentiated from those of the Mississippi Valley. As has been shown above, this difference is of about the same order of magnitude as that between colonies and is therefore most easily interpreted as due to some ancient geographical change which reduced one or both of these regions to one or a few colonies. By some such process also *Iris setosa* var. *interior* diverged from the *Iris setosae* of coastal Alaska and of northeastern Asia. More recently, during glacial times, *Iris setosa* var. *canadensis* was reduced to a highly inbred remnant (see pp. 495–496).

At the present time, therefore, *Iris virginica* is composed of two slightly divergent sub-cables, *Iris setosa* of three, and the youthful *Iris versicolor* is as yet but a single cable. Supposedly by the successive compounding of such divergencies, subspecific differences might be built up into differences of specific magnitude. There is little evidence in this study to contradict such a theory, but it should be pointed out that there is none to support it. In the opinion of the author the theory that geographical varieties are potential species is a debatable one.

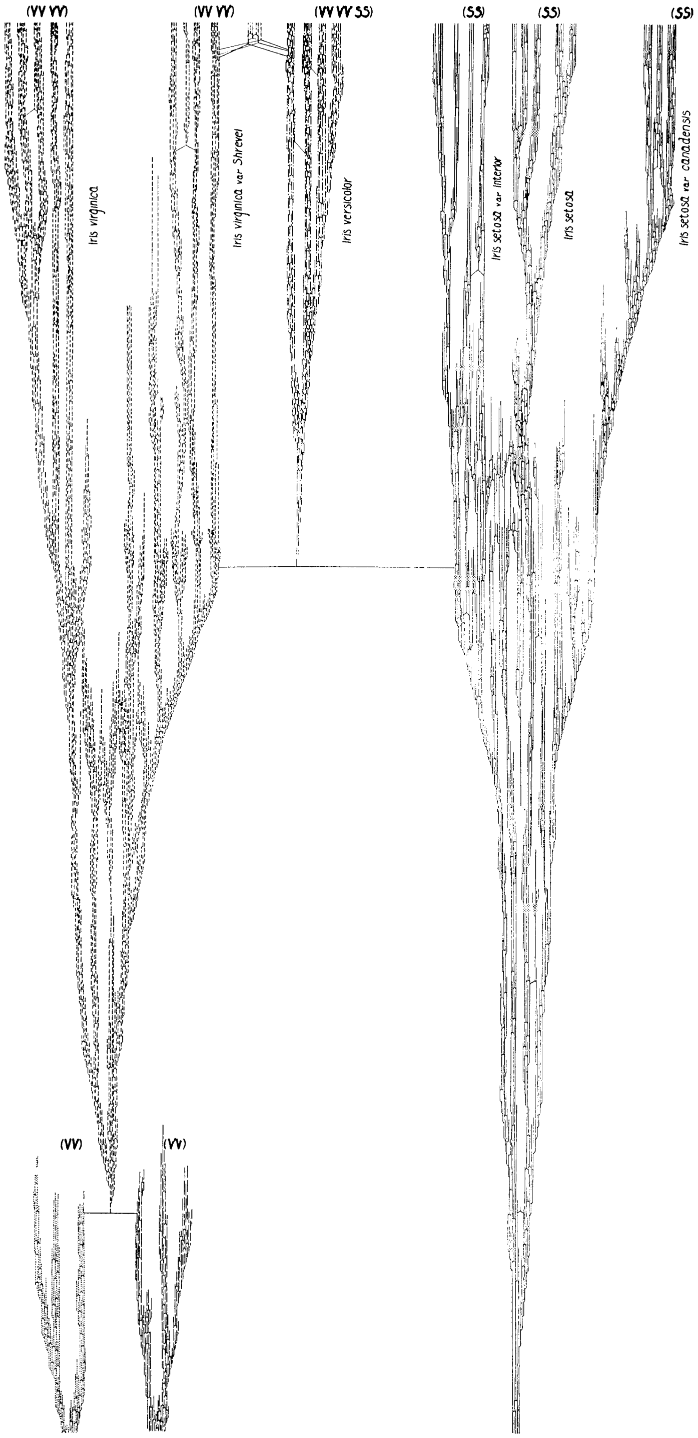

Fig. 13. Diagram showing phylogenetic relationships in the northern blue flags. Further discussion in the text.

Observation and experiment have shown (Anderson, '28) that in *Iris versicolor* and *Iris virginica* the colonies themselves are more or less divided into inbred lines, so that in these species we can speak of the colony "cords" as being made up of pure-line "threads." Since cross-pollination occurs occasionally the threads maintain their identity for only a few generations. This detail of the evolutionary pattern will vary greatly from *Iris* species to *Iris* species. There are very probably species in which inbreeding is more severe; there are certainly self-sterile species in which there is complete outcrossing. In the former the threads would be longer, in the latter there would be no recognizable threads at all within the colony cords (Anderson, loc. cit. pp. 308–310).

The great ground pattern of the cables is a simple one, with two exceptions. Very occasionally by amphidiploidy a single strand runs out from one trunk line to another and at an intermediate point a whole new cable arises. An earlier paper of this series presented detailed evidence for the amphidiploid origin of *Iris versicolor.* The secondary pairing and multiple association characteristic of the pollen mother-cells of *Iris virginica* strongly suggest that it is itself the result of an ancient amphidiploid hybridization, perhaps between a species somewhat like *Iris tripetala* and one related to *Iris hexagona.*

Such occasional interweaving of phylogenetic lines is apparently characteristic of most of the genus *Iris.* Simonet ('34) reports chromosome numbers for the section Pogoniris which suggest amphidiploidic relationships and has presented cytological proof ('35) for the occurrence of amphidiploidy in cultivated irises. As Randolph has shown (*loc. cit.,* p. 65), there is experimental evidence that in *Iris,* species with differing chromosome numbers cross more readily than in many other genera. He also presents some evidence for the functioning of unreduced gametes in *Iris.* Both of these conditions favor amphidiploidy. The presence of even occasional amphidiploidy within a genus will so complicate the phylogenetic relationships that it will be impossible to divide and subdivide it naturally into sections and subsections. We would therefore predict for the genus *Iris* that while there might be evidence of groups of related species, it would be impossible to arrange all these groups in a clear-cut natural system. Such is actually the case. The latest monographer of the genus has the following to say about the difficulties of subdividing the section Apogon, numbering some 50–100 species and native to Europe, Asia, and North America: "It seems unfortunately impossible to select any one character or set of characters to form a guide through the maze of species. . . . The classification given below is therefore admittedly unsatisfactory partly because some of the species seem to stand by themselves and to have little or no affinity to any others" [Dykes, '13]. He then proceeds to divide the section into fifteen sub-groups. On the theory outlined above *Iris versicolor* is an amphidiploid hybrid between *Iris virginica* of his group X and *Iris setosa* of group XIV. A few relationships of this sort would produce exactly the difficulties which Dykes describes.

The other tangle in the evolutionary pattern of these irises is provided by hybridization. Though such tangles are a characteristic feature of the evolutionary pattern in the genus *Iris,* they form in this group of species a small knot of minor consequence. *Iris virginica* and *Iris setosa* are now geographically isolated, though they still in part occupy the same river system. *Iris setosa* var. *canadensis* and *Iris versicolor* grow together throughout the range of the former, but there is very great numerical isolation between them (38 vs. 108 chromosomes). Hybrids are occasionally found, but they are very rare. The only hybridization of any consequence within the group at the present time is between *Iris versicolor* and *Iris virginica.* Even in this case, there is geographical isolation between the bulk of the two species. Along the eastern seaboard, where the two species have apparently been longest in contact, they are both quite rare. It is only around the Great Lakes, where they are both exceedingly common, that hybridization is at all common. Even here there is partial isolation for the blooming periods of the two species barely overlap. At the northern end of the southern peninsula of Michigan, the two species have evidently been closely associated throughout much of post-glacial time (Anderson '33) and their areas of contact, due to the

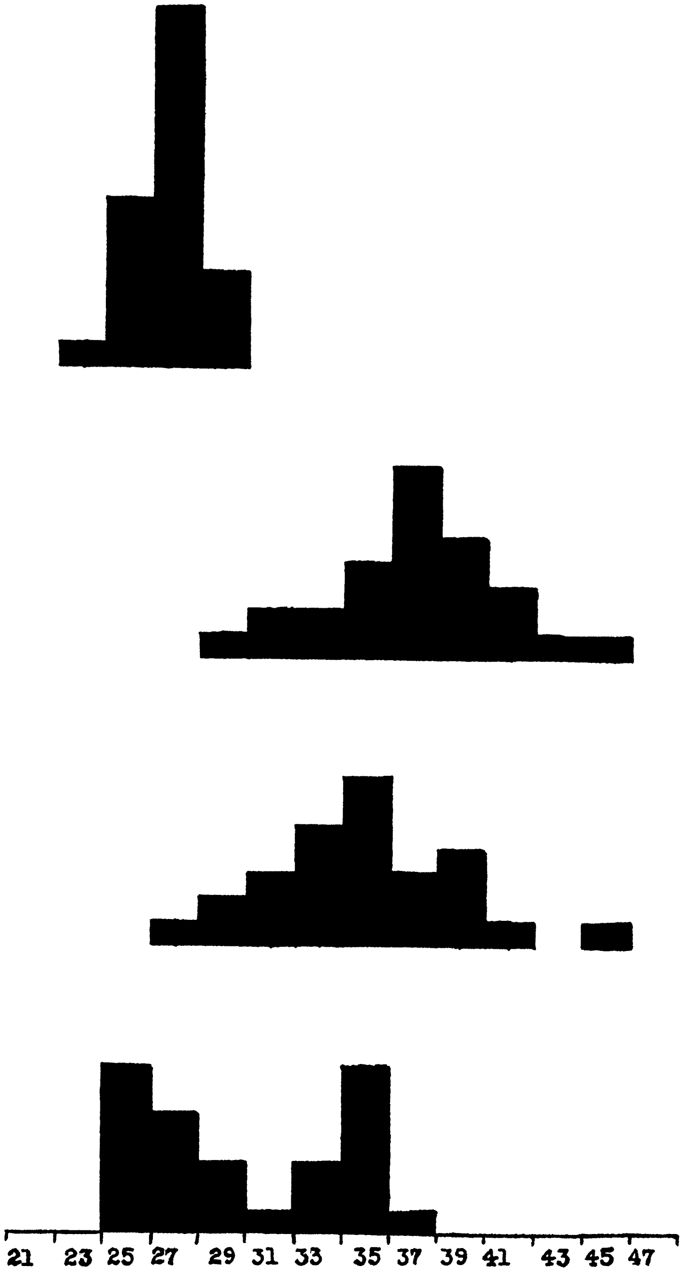

Fig. 14. Frequency distribution for the index, sepal length/petal length + sepal width/petal width, for 27 plants each from the following colonies: Pardeeville, Wisconsin *(Iris virginica* var. *Shrevei)*; Billings Bridge, Ottawa, Canada *(I. versicolor)*; Engadine, Michigan (hybrids); St. Ignace, Michigan (hybrids).

peculiarities of the various post-glacial lakes, are extremely involved. Even in this area the great majority of colonies are composed exclusively of one species or the other.

On the north shore of Lake Michigan, in an area with a similar post-glacial history, two hybrid colonies were studied in some detail. One was located three miles west of St. Ignace and has since been partially obliterated by relocation of the state road. At this location there were to be found apparently normal *Iris versicolor* and *Iris virginica* and a large number of peculiar intermediates. Few of these resembled the first-generation and second-generation hybrids which had been raised in the experimental plots. Most of them, on the other hand, were very similar to back crosses which had been made between the first-generation hybrids and the two species. This is borne out by fig. 14 where representative colonies of each species are contrasted with these two hybrid colonies, using the following index as a basis for comparison: sepal length/petal length + sepal width/petal width. Figure 14 also shows the very different condition which was encountered at the other hybrid colony which was studied in detail. It was located just west of Engadine, Michigan, and was composed entirely of plants which closely resembled the artificial F_1 hybrids of the breeding plot. Since they also presented the characteristic vigor of such hybrids it is probable that they were for the most part such hybrids, the parental species having been exterminated by their vigorous offspring. It is not impossible, however, that they may represent some new balanced combination of chromosomes. In crosses between such complex amphidiploids as *Iris versicolor* and *Iris virginica* various new polyploid types are not at all unlikely. Unfortunately, the large numbers of chromosomes and the very short period during which the reduction division can be studied (only a few days out of the year) render this rather unprofitable material for such examination.

One can summarize the effect of hybridization between *Iris versicolor* and *Iris virginica* by saying that it does occur very occasionally. Its only effect so far has been a slight blurring of the two species along the zone of contact. It does, however, provide a means by which new polyploid hybrid species might eventually arise.

Were there fewer technical difficulties involved a detailed analysis of hybridization in these irises would be well worth while, since hybridization is a characteristic part of the phylogenetic pattern throughout the genus. The remarkable phylogenetic tangle of species and hybrids discovered in the Mississippi delta by Dr. J. K. Small is by no means exceptional. Much the same situation exists among the Pogoniris species around the Mediterranean; the irises of the Spuria group apparently behave in the same way in western Asia; it was perhaps from some such complex that the Japanese irises had their beginnings.

Because hybridization is apparently such an important phylogenetic factor in the genus *Iris,* the hybrids of the Mississippi delta deserve careful analytical study, genetically, taxonomically, and cytologically. It is not enough to prove that hybridization is taking place. Much more important is the determination of its exact role among the Louisiana irises.

The effects of hybridization are various according to the peculiarities of the germ-plasm upon which it is operating and the external conditions under which it takes place. It may result in new amphidiploid species such as *Iris versicolor.* It may produce intermediate swarms which obliterate previous specific boundaries. It may increase the variability of one of the parental species by introducing a small proportion of germplasm from the other (Anderson & Woodson, '35, p. 37).

Possibly all of these processes are taking place among the Louisiana irises. It should not be difficult to determine their relative importance and ultimate phylogenetic effects. These irises possess a number of technical advantages for such a study. They have comparatively low chromosome numbers; they are easy to cultivate in experimental gardens; they possess conspicuous specific differences. They are, as Viosca has said ('35), "uniquely adapted for the study of experimental evolution" and one may join with him in predicting a "bright future for them in the study of biology."

To summarize: the evolutionary patterns of the blue flags are fairly typical of the genus as a whole. Seen in a greatly foreshortened view they can be represented graphically as in fig. 13. They form a somewhat tree-like system of cables, the ground pattern occasionally made more complex by amphidiploid cross-connections. The pattern is complicated at one point by inter-specific hybridization. The cables themselves are divided into distinctive cords, the colonies.

Much the same set of patterns would characterize the entire genus *Iris*. The complex knots due to interspecific hybridization would be larger and more significant in certain other portions of the genus; in certain sections the cross-connections of amphidiploidy would be lacking altogether; in a few sections they might be somewhat commoner. All in all, however, fig. 13 may be taken as a fairly accurate representation of phylogenetic relationships for the whole genus. This general similarity of evolutionary patterns within the genus rests upon the fact that the germ-plasm of any species of the genus *Iris* is not a vague generalized germ-plasm. It is **Iris** germ-plasm. It has a number of inherent characteristics which affect speciation and which cause the details of speciation, to be similar throughout (Anderson, '31). It is a genus in which amphidiploidy occasionally occurs, in which every species has a strong development of vegetative propagation, in which interspecific fertility is the rule. It is, on the other hand, a genus which is ecologically conservative.

Species after species exhibits rather precise demands as to habitat and seems unable to produce variants adapted to more or less moisture, more or less shade, more or less acidity. In the genus *Aquilegia* every species which I have studied extensively is richly provided with ecotypes; sun-forms, shade-forms, types inherently adapted to sour, wet swamps, to sunny, limestone cliffs, to sand dunes, and to woodlands. In *Iris versicolor* and *Iris virginica,* in spite of intensive search, I have been unable to recognize a single ecological variant. Like many genera of the Liliiflorae, the order to which they belong, they are curiously unamenable to ecological differentiation. This conservatism, since it characterizes the whole genus, must rest upon some inherent property of *Iris* germ-plasm. What that property may be we cannot, as yet, even hazard a guess. It may be that the germ-plasm of *Aquilegia* varies in a way that is unknown or uncommon to the germ-plasm of *Iris.* It may be that due to its ontogenetical organization the genus *Iris,* though possessing the same basic kinds of germinal variation as the genus *Aquilegia,* will nevertheless not respond with variants equipped for other habitats.

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