The taxonomy and cytology of the genus sisybinchium (iridageae)

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THE TAXONOMY AND CYTOLOGY OF THE GENUS SISYRINCHIUM (IRIDACEAE)

BEING A THESIS SUBMITTED IN CANDIDATURE FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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INTRODUCTION

The genus Sisyrinchium (Iridaceae) has its main area of distribution in the continents of North and South America. Neither the distribution of the various species, nor even that of the genus itself is known in detail in South America, while in North America, though the distribution of the genus is quite well documented, the distribution of the species is confused by nomenclatural inconsistency. In other parts of the world, members of the genus have been recorded as indigenous in the Falkland Islands (Hooker, 1859), Greenland (Bocker, 1948), Ireland (Lynam, 1845) and the Carpathian Mountains (Lauterborn, 1927). Introduced species have been recorded from most European countries, and also Australia and New Zealand.

The presence of an apparently indigenous species of *Sisyrinchium* in Ireland, has given rise to much discussion, and there is no doubt that the origin of the 'North American Element' in the British flora is an interesting problem in plant geography. However, little attempt has hitherto been made to examine the species against a background of their American relatives, from a taxonomic and evolutionary viewpoint. It was felt that such a study might indicate whether the Irish species of *Sisyrinchium* could be regarded as a separate taxon, or whether it was merely a remote colony of a taxon having a wide trans-Atlantic distribution. Such information would give some basic facts on which to base an historical and geographical discussion of the Irish records of the species.

In order to assess the status of the Irish species, a background of knowledge about the American species was necessary. Much of the work for this dissertation has been the accumulation of this information.

The outstanding feature of the North American and European Sisyrinchia is their remarkable morphological uniformity. Previous American research showed that this apparent uniformity concealed an interesting evolutionary situation. Scattered cytological records, collected by Darlington and Wylie (1945) showed a polyploid series on the base number of 8. Unfortunately, the actual species named mean little or nothing, due to the confused state of the nomenclature. The taxonomic and nomenclatural history of the species with which this study is involved will be discussed. The records showed the existence of very high polyploids, but little was known about the distribution of the different levels of polyploidy either in Europe or North America.

A survey of Sisyrinchium over the whole of North America was obviously beyond the scope of this work, and it was decided to limit the study to Europe and the Eastern Seaboard of the United States of America. Even with this restriction, it was not possible in the time available to do more than sample a few habitats in each part of the area, in order to gain some idea of the distributions and relationships of the various species. However, even with this incomplete information, it has been possible to place the Irish species in its proper perspective in the genus as a whole.

Breeding experiments have been carried out with the object of working out some of the evolutionary relationships in the species concerned. The parentage of the high polyploids, has however, proved to be too complex to trace in detail, and only the general pattern of evolution in the genus has emerged.

CHAPTER 1

Nomenclature in the Section Bermudiana

The nomenclature of the species is very confused, due to the broad morphological uniformity which makes many species difficult to diagnose. The present work has shown that at least in some cases, the picture is also confused by interspecific hybridisation. However, the system of nomenclature adopted by Fernald (1950) gives a relatively stable foundation for further study, at least in the Atlantic States. There is some doubt as to whether some of the names used by Fernald can be considered in this chapter. However, in order to avoid confusion Fernald's nomenclature will be used in the rest of this dissertation.

Nine species of *Sisyrinchium* are described by Fernald, and this study includes seven of them. These are:-

S. albidum Rafinesque
S. capillare Bicknell
S. mucronatum Michaux
S. montanum Green *var*. *crebrum* Fernald
S. arenicola Bicknell
S. angustifolium Miller
S. atlanticum Bicknell

The nomenclature used in the cases of *S. albidum* Raf., *S. capillare* Bick., *S. arenicola* Bick., and *S. atlanticum* Bick. is undisputed, and these are distinct and easily identifiable species. The confusion arises in the case of the other three. The three species as described by Fernald are clear enough.

S. angustifolium Mill. is immediately separable, as having a stem which is usually branched, the two branches being subtended by a foliaceous bract. The plant is spreading, lax, and loosely tufted. The perianth is invariably light blue, 1.5-2.5 cms. in diameter.

S. mucronatum Mich. is a predominantly simple stemmed species, with stems 0.5-1.5 mms. and a stiff. upright habit. The perianth is pale blue, violet, or white, 2-3 cms. in diameter. Although branching stems occasionally occur, there is no danger of confusion with S. angustifolium Mill. since the width of the stem and the general habit of the plant are completely diagnostic.

The real difficulty is S. montanum Greene var crebrum Fernald. This species has a habit similar to S. mucronatum Mich. but is a much more robust plant with stems 1-3 mms. wide, and violet-blue flowers. 3-3.5 cms. in diameter. Though predominantly.simple stemmed, branched stems do occur, It is possible to confuse it with both of the former species, particularly if diagnosed in the absence of flowers. However, when flowering, the large violet-blue flowers of this species cannot be confused with the small pale blue flowers of S. angustifolium Mill. even if the specimen of S. montanum Greene var. crebrum Fernald includes branched stems. However, the similarity between these species has been a source of considerable difficulty.

S. montanum Greene var. crebrum Fernald can be separated very easily from S. mucronatum Mich. when both are fruiting. The capsules of the former species are characteristically dark brown or purple-brown in colour, and 3-6 mm. (usually about 4mm.) in height. Those of S. mucronatum Mich. are straw coloured, from 2-4 mm. (usually about 3 mm.) in height. Other characters can also be used to separate them, such as the degree of exsertion of the pedicels, and the length of the junction of the spathe bracts, but it requires experience to diagnose these two species with certainty, though they are undoubtedly distinct.

The three species are illustrated in fig. (1. 1)

The earliest reference to Sisyrinchium is by Plukenet (1696) who describes a plant as:

"Sisyrinchium parvum, gladiato caule Virginianum"

His illustration shows a plant which is easily recognisable as being what Fernald called S. mucronatum Mich.

Later, Dillenius illustrates a species of Sisyrinchium which he describes as:

"Bermudiana graminea, flore minore caeruleo"

His illustration shows a plant of a doubtful species. The vegetative parts suggest that it was S. angustifolium Mill. sensu Fernald, but the shape of the perianth segments does not resemble that of any plant in the genus.

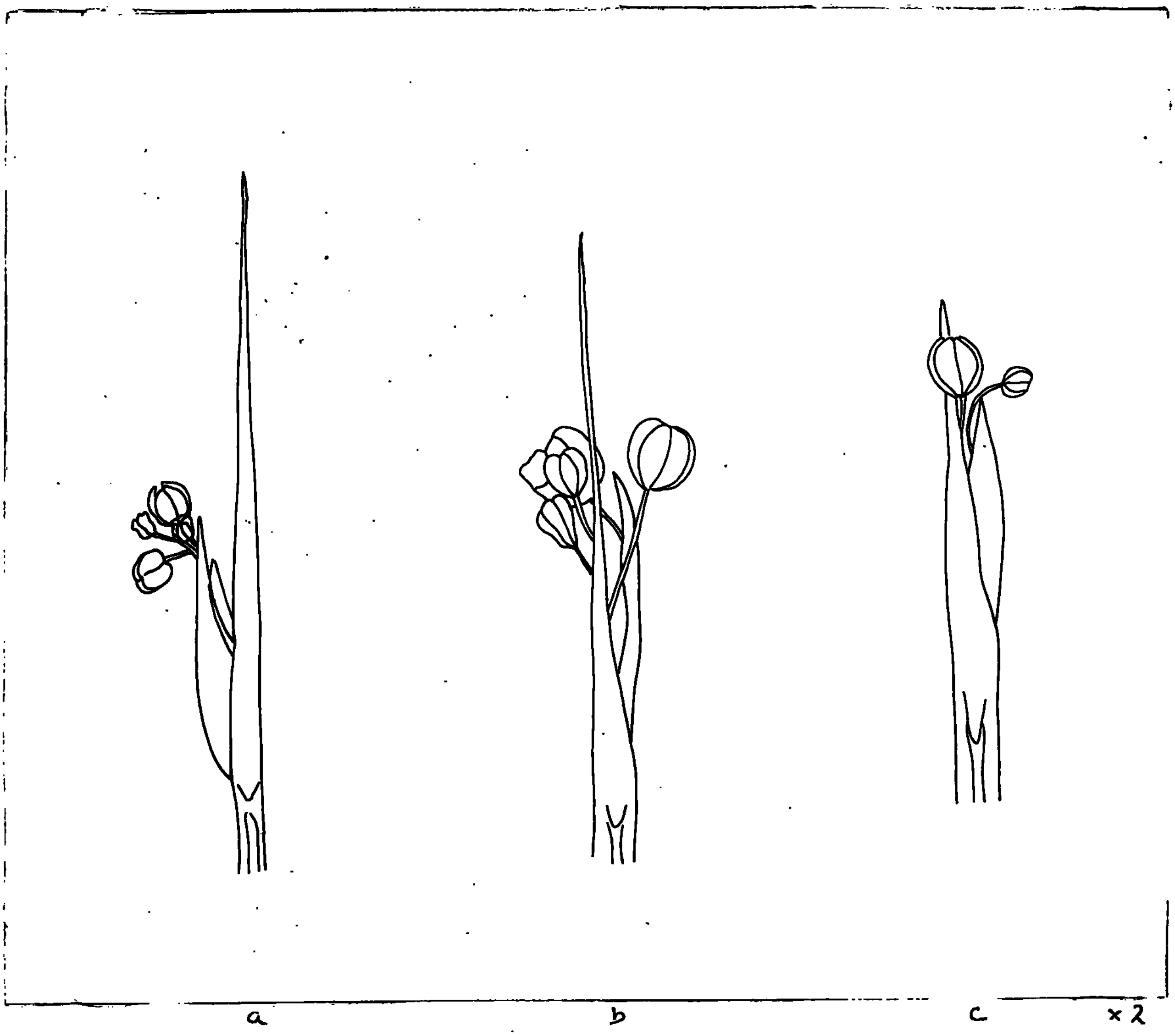


Fig. (1. 1).

Spathe and capsule form in

a) *S. mucronatum* Mich.

b) *S. montanum* Greene *var. crebrum* Fernald

c) *S. angustifolium* Mill.

Linnaeus (1753) divided *Sisyrinchium* into two varieties. The first (var. alpha) he called *S. bermudiana*, thus combining the epithets of Plukenet and Dillenius, but distinctly said that the plant was a native of Virginia, and the Bermudan plant (var. beta) he called *S. bermudiense*. In his description of *S. bermudiana*, it is unfortunate that he cites both Plukenet's and Dillenius' illustrations. Linnaeus' description in 'Species Plantarum' is taken from his previous work on the 'Hortus Cliffortianus', but there is no specimen in the Herb. Cliff. Therefore, according to the 'International Code of Botanical Nomenclature' either Plukenet's or Dillenius' illustration ought to be regarded as the lectotype, and the name *S. bermudiana* L. used for that species.

In the Linnaean Herbarium there is a specimen labelled '*Sisyrinchium bermudiana*', which is undoubtedly *S. mucronatum* Mich. sensu Fernald, and under these circumstances, it would have seemed reasonable to adopt Plukenet's illustration as the lectotype, discarding the name *S. mucronatum* Mich. However, Shinners (1957) has already discussed the identity of *S. bermudiana* L. and has decided that the specific epithet *bermudiana* should be applied to the plant illustrated by Dillenius. He reached this decision without making reference to the Linnaean specimen, and one concludes that he was unaware of its existance. However, even if he had known of it, from a practical viewpoint, his decision has much to recommend it, as will appear below.

At this point it is necessary to examine the origin and use of the name *S. angustifolium* Mill.

Miller described two species under the generic name *Sisyrinchium*.
1) "*S. bermudiana* foliis gladiolatis, complexicaulibus pedunculis brevioribus. *Sisyrinchium* with sword shaped leaves and shorter footstalks to the flower."

Miller goes on to state that this plant grows in Bermuda. The name *S. bermudiana* as used by Miller is thus illegitimate since it is a later homonym for a previously validly published species. However, Miller thus began the misconception of the use of the specific epithet *Bermudiana* which was to persist for two centuries.
2) "*S. angustifolium* foliis lineari - gladiolatus pedunculis longioribus. *Sisyrinchium* with sword shaped leaves and longer footstalks to the flower. Bermudiana graminea; flore minore caeruleo. Hort.Elth.49. Grass-leaved Bermudiana with a smaller blueflower ...... This second sort grows naturally in Virginia."

Thus Miller clearly cites the Dillenian illustration referred to above in his description of *S. angustifolium*. There is, therefore, some doubt as to whether it can now be considered a legitimate name at all, since the legitimate name, *S. bermudiana* L, had already been published for Dillenius' plant. It can be argued that Miller's

S. angustifolium is S. bermudiana L. only in part, and can, therefore, legitimately be used. Fernald (1946) obviously took this view since he quoted S. angustifolium Mill. as being equivalent to S. bermudiana L. 'at least as to citation of Dillenius'. However, under these circumstances, the name S. bemudiana L. ought to have been retained for the species illustrated by Plukenet. Fernald did not do this.

Assuming for the moment that the name itself can be considered legitimate, there is still considerable doubt as to which species Miller intended to describe. The difficulty is due to Miller's translation of 'pedunculis' by the word 'footstalk'. There is then considerable difficulty in deciding whether the word means peduncles or pedicels. If the latter was intended, then Miller's description could equally well be applied to a simple stemmed or to a branched species. The only simple stemmed species with which Dillenius' illustration could be confused is S. montanum Greene var. crebrum Fernald. In fact the name S. angustifolium Mill. was used by Bicknell for this species, and then consistently used until the nomenclature was revised by Fernald in 1946. Fernald, however, translates the word 'pedunculis' as 'peduncles' but then proceeds to interpret Miller's description of S. angustifolium as though it referred to a plant having both long peduncles and long pedicels. He thus appears unwarrantably to have translated 'pedunculis' twice in different senses. (Northfield, 1961).

Miller's epithet angustifolium is thus surrounded by a haze of uncertainty, and it would, therefore, seem of great practical value, and fully in accordance with Article 69 of the International Code, to adopt Shinner's plan of discarding the epithet, and retaining the name S. bermudiana L. for this species, and giving the name S. mucronatum Mich. to the plant illustrated by Plukenet.

Other names have confused the literature at different periods. In historical order these are:-

S. gramineum Lamarck
S. anceps Cavanilles
S. graminoides Bicknell

Both Lamarck (1873) and Cavanilles (1788) give a long list of synonomy. Fernald points out that in fact Cavanilles quotes Lamarck's species as synonomous with his own, so that the name S. anceps Cav. must now be considered illegitimate on those grounds alone. Both authors quote Miller's S. angustifolium and S. bermudiana L., as well as the descriptions and illustrations of both Plukenet and Dillenius. Thus, in the synonomy listed by Lamarck and Cavanilles, S. bermudiana L. would now be considered legitimate, according to modern taxonomic procedure, so that there is no need to discuss which species Lamarck and Cavanilles really meant in their rather ambiguous descriptions.

Bicknell (1894) proposed the specific epithet graminoides to describe *S. angustifolium* Mill. sensu Fernald, and used this latter name for *S. montanum* Greene *var*. *crebrum* Fernald. Since Shinners has now limited the name *S. bermudiana* L. to *S. graminoides* Bick., i.e. *S. angustifolium* Mill. sensu Fernald, Bicknell's name is now superfluous.

Clearly then, the legitimate nomenclature for the three species concerned is as follows:-

1.) *S. bermudiana* L.
  S. angustifolium Mill. sensu Fernald
  S. gramineum Lam. in part
  S. anceps Cav. in part
  S. graminoides Bick.
2.) *S. montanum* Greene *var*. *crebrum* Fernald
  S. angustifolium Mill. sensu Bicknell
3.) *S. mucronatum* Mich.

Despite the fact that *S. angustifolium* Mill, is thus shown to be illegitimate, in order to avoid further confusion, it is proposed to use Fernald's nomenclature in this study - in accordance with the general American usage.

CHAPTER 11

PART 1

Geographical Background of the American Species

The area of study comprised the Atlantic States of America from New Hampshire to North Carolina. It was obviously impossible to survey this whole area in detail, and a system of sampling was adopted, in the course of which, selected areas were examined quite thoroughly. In order to make this study meaningful, it was necessary to assume that the areas selected gave a good indication of the district as a whole. It is admitted that such an assumption is unjustified in some cases where physical features vary within a small area. However, a consistent pattern has emerged which is a useful basis for this study, and this was all that could be expected in the time available.

The selection of study areas

The dominant physical features of the Atlantic States from North Carolina to Maine, is the Appalachian Mountain system. A consideration of the effects of this system on topography is necessary.

The system runs in a North-East South-West direction. In the South, it is bordered on the East by a broad coastal plain, which becomes narrower towards the North, and eventually ends at New York

City. Inland, i.e. Westwards from this coastal plain, is a plateau generally known as the Piedmont plateau. This is an ancient peneplain which in the South forms low rolling country, while in the North, mountains of considerable height still survive, particularly the Presidential Range in New Hampshire which reaches 6293'. This area will be termed (A).

To the West of the Piedmont plateau, the land rises to form a mountain system, called throughout its length, the Blue Ridge. This range reaches its greatest development in North Carolina and Tennessee, where it is locally known as the Smoky Mountains. Further North in the Virginian Blue Ridge, and its continuation in Pennsylvania and New England, the formation is represented by low, worn down mountains. This is area (B).

To the West of the Blue Ridge is the Valley and Ridge province of the Recent, or Sedimentary Appalachians, the underlying rocks of which are dolomites and limestones, succeeded stratigraphically by sandstones and shales. Wherever the limestones have reached the surface, there has been rapid erosion, whilst the sandstones were weathered less rapidly. The result has been a series of valleys with limestone bedrock, divided from one another by sandstone ridges. The broadest of these valleys is the most easterly, and is called the Great Appalachian Valley. This has been termed area (C).

Thus a section A B C at any point would give a cross-section of the effects of altitude and bedrock in that latitude. Sisyrinchia in three such cross-sections were examined in this study; one in North Carolina and Tennessee (1), one in Virginia (2) and one in New England (3). In addition, two special areas will be mentioned. These are:- (2a) - the coastal plain of Virginia

(3d) - the edge of the Great Plain in the West of New York State

Area (2a) gave the opportunity to study the Sisyrinchia on the low-lying coastal plain, and the area (3d) gave some indication of the trend in the genus to the west of the mountains of New England.

All these areas are indicated on the map, Fig. (2. 1).

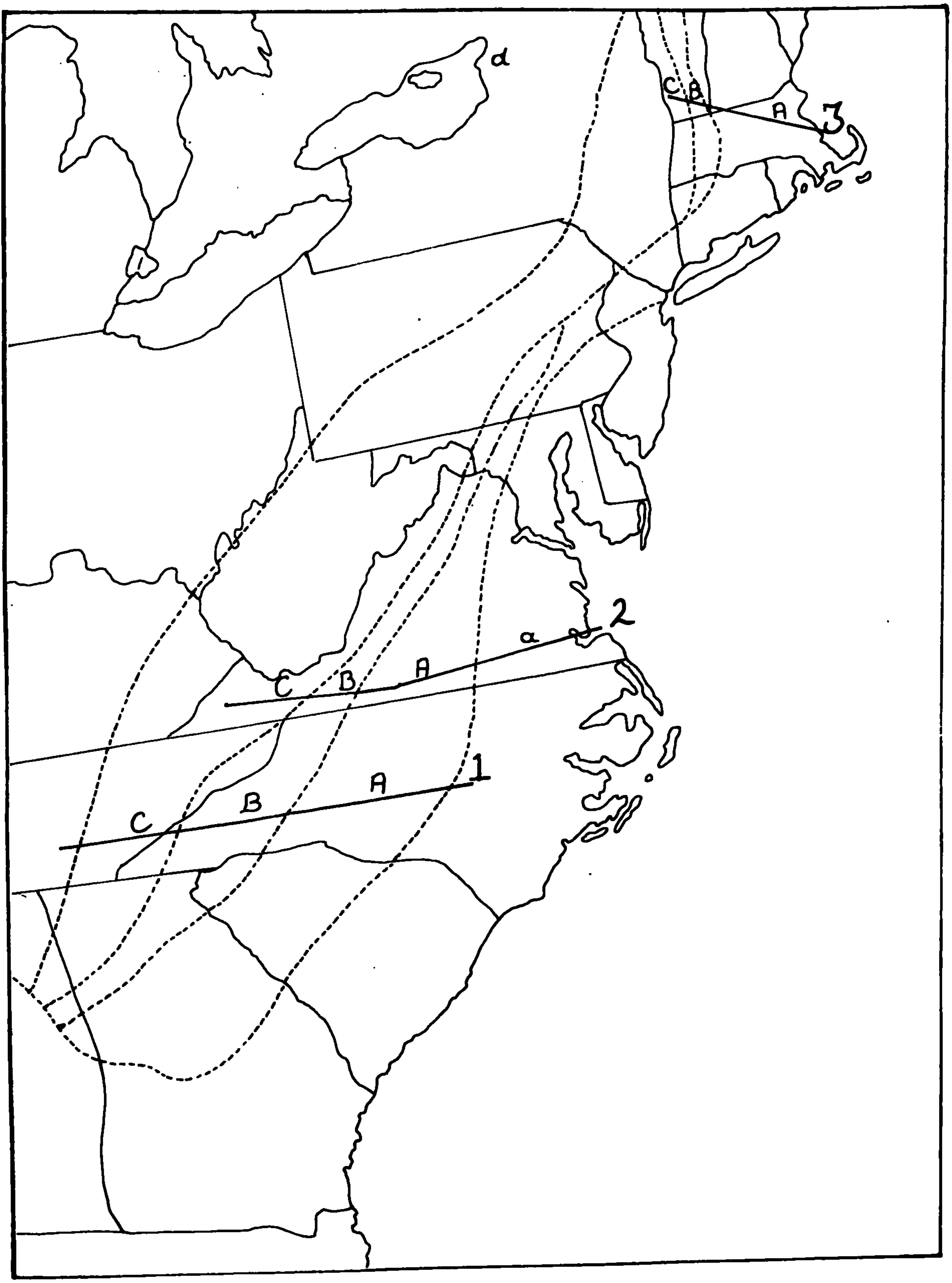


Fig. (2. 1).
Sketch map of the Appalachian System, showing the transects studied.
(see chapter 2, part 1).

PART 2 - Description and Distribution of the American species S. angustifolium Mill.

This is by far the most widespread of the species studied. Fig. (2. 2) map b. gives an indication of the extent of its distribution, though its density in the various areas is difficult to estimate due to the inadequacy of herbarium records. The density of records is shown on the map, but the areas of greatest density correspond to the areas which were studied in detail during myvisit and those areas studied by the New England Botanical Club. However, this is not the only difficulty when trying to form a true idea of the distribution of the species. There is also the difficulty of taking into account the full range of morphological variation which the taxon embraces.

Fernald (1950) describes this species as,

> "Loosely tufted, ascending to geniculate and spreading, the leaves and stem mostly deep green, (glaucescent) and drying blackish; leaves submembranaceous, 1.5-6 mm. wide, shorter than to exceeding the flowering stems; stems broadly winged (except in young or overcrowded individuals), flexuous or even geniculate, 1-5 dm. high, mostly forking; peduncles 2-5 cm., loosely ascending, winged 2-15 cm. long; lowest foliaceous bract slightly shorter than to overtopping the flowering spathes, the latter 1.5-2 cm. long with mostly subequal bracts; perianth pale blue changing to violet; fruiting pedicels long slender, outwardly arching or recurving, much overtopping their spathes, few (mostly 1-5); capsules dark or blackish in drying, 4-6 mm. long. .... Meadows or low woods and thickets or damp shores. Florida to E. Texas. North to S.E. Newfoundland, S. Quebec, S. Ontario, Ohio, Indiana, Illinois, Montana and E. Kansas. May - July."

Thus Fernald indicates that the species varies greatly, particularly in quantitative characters. Actually, it also varies in qualitative characters, but these are more obvious in living plants than in herbarium material. Thus some of the plants are light green in colour, but this does not seem to vary consistently with the habitat. However, both quantitative and qualitative variation in the species is certainly not random. In order to demonstrate this, a number of characters were measured from herbarium material. The results are tabulated in Appendix 1.

It can be seen that plants from group 2a have a higher average stem width and leaf width than those inland from the Blue Ridge and the Tennessee valley. Similarly the plants from New England show a trend towards narrower stems and leaves when compared with those from S.E. Virginia and North Carolina, but are not significantly different to those from Tennessee. The plants from Tennessee also tend to be light green compared to the dark green plants of Virginia and New England.

In making these comparisons plants of similar age were measured. In fact any plant that is flowering is likely to have reached its maximum dimensions in the characters measured, since the plant does not flower in the first year after germination. It flowers in its second year, and thereafter 'tillers', the individual tillers consisting only of a single stem and a number of leaves which enclose the stem at its base. (See Appendix 2). Thus the figures quoted are comparable.

The plants have been compared as distinct groups from different areas, but this is probably an inaccurate picture of what is actually happening. It seems more likely that there is a cline in the directions noted. It was unfortunately impossible to spend the time in America that would have been necessary to check this, though herbarium specimens indicate that this is so. It is important to realise that the plants at the extremes of these gradations are very different in appearance. It was impossible in the time available to study Sisyrinchium in Florida and Texas, though herbarium material indicates that the situation is potentially very interesting. There is material from these areas which broadly resembles S. angustifolium Mill. having a branched stem and pale blue flowers. However, judging from the few specimens that have been examined, the plants are much more robust even than those from S.E. Virginia or North Carolina. Whether or not these are the southern representatives of this complex, is a problem that requires investigation from morphological and cytological aspects.

In North Carolina, Tennessee and Virginia, this plant has a wide range of habitats, a list of which is given in Appendix 3. The habitats vary from extremely dry, rocky conditions, to conditions of permanently standing water. However, one common feature of all these habitats, with two exceptions, is a lack of competition. In the two exceptional habitats, the plants were growing in what appeared to be stable communities, but in all the other habitats such as disturbed roadsides or woodland edges, the plants were growing on disturbed ground. However, the plant was not noticeably a weed of cultivated ground. This is easily explained, because the plant does not flower in its first year, so that even if seedlings occurred, they would be unlikely to become established or reach flowering stage. These considerations of habitat are very important when the North American species is compared with the Irish plant.

Further north in New England, where the species decreases in frequency, it is found in only a limited range of habitats. In the present survey, it was found chiefly in damp and shaded conditions in this area, but herbarium labels show that it also occurs on the shingle of lake shores. This is important, because this normal restriction of habitat towards the edge of the species range is not noticeable in Ireland.

S. montanum var. crebrum Fernald

S. montanum Greene and S. montanum Greene var. crebrum Fernald are described by Fernald as follows:

"S. montanum Greene - Tufted, erect, pale green, not often blackening in drying; leaves 1-3 mm. wide, erect or ascending; stem simple (rarely slightly forked), pale 1-6dm. high, distinctly flattened and wing margined; spathes pale green or stramineous, the outer bract with margins united 2-6 mm. above the base, 2-8 cm. long, the inner 1.5-3.6 cm. long; perianth blue-violet; fruiting pedicels erect or strongly ascending, shorter than to slightly overtopping the inner bract; capsules whitish green to straw coloured or pale brown 3.6 mm, high. - Shores, meadows and damp open soil. W. Newfoundland and Anticosti I., Quebec to Mackenzie and North British Columbia, South to S. Quebec, S. Ontario, W. New York, N. Indiana, N. Illinois, N. Iowa, Nebraska, Colorado etc.

Var. crebrum Fernald - Greener, mostly darkening in drying; spathes deeper green mostly purple tinged, the inner bract mostly 2-6.5 cm. long; capsule green, dull brown, drab or purple tinged when ripe dark to blackish. - Dry to moist open soil, Newfoundland to Ontario, south to Nova Scotia, New England, Pennsylvania, and mountains of W. Virginia."

It will be seen from the distribution of S. montanum Greene given by Fernald, that this species does not occur in the study area, but as Fernald made a common New England taxon a variety of this species, thereby implying at least a taxonomic relationship to it, it seems necessary to at least give a little consideration to this very interesting species. S. montanum Greene affords another problem in plant geography which is as fascinating as the Irish problem. This species was described by Greene from Colorado, and is widely spread in the Cordilleran region. Apart from this area it is recorded only in South East Canada, and is a member of the famous 'Cordilleran' element in the flora of this region (Fernald, 1925). In Fernald's original paper he suggested that these members of the flora had persisted in unglaciated areas in Newfoundland and around the Gulf of St. Lawrence. He marshals a considerable body of evidence in support of this theory, though more recent geological work has disproved some of his basic geological assumptions.

However, it is not within the scope of this work to approach critically the geological aspects of Fernald's theory, but it is necessary to question a few botanical assumptions made in the many theories which explain unusual geographical distributions in this way. A more detailed discussion of these points will be given in the chapter devoted to the Irish species of Sisyrinchium, but a brief resume is appropriate here.

1) It is not necessary to assume a common origin for discontinuous geographical distributions which are similar, though it is very tempting to do so.

2) At least in some genera, morphological similarity is not always a good guide to evolutionary relationships. This point will be discussed in the section relating to the experimental work on the origin of the high polyploids in the genus Sisyrinchium.

3) The present distribution of a species is not necessarily any guide to its former range, which may have been curtailed or modified by a climatic accident, such as an exceptionally hard winter, which reduced the populations throughout its range. If a sufficiently low level was reached, recovery or anhilation in various parts of the range could be largely fortuitous.

Applying this criticism to the case of S. montanum Greene, although from a morphological viewpoint, the taxon can be said to have this peculiar distribution there is no evidence that the two geographical branches of the morphological taxon have the same evolutionary origin, or, if they have, that the present disjunct distribution necessarily implies anything except that the species has died out in the continental interior.

S. montanum Greene var. crebrum Fernald is morphologically very similar to S. montanum Greene. The main differences are given in Fernald's description. However, it must not be assumed that this similarity has any evolutionary significance. S. montanum var. crebrum has a restricted range latitudinally. It occurs in New England in great profusion, but only sparsely in S.W. Canada. Southwards it extends through the mountains of Pennsylvania, and is reported also from the mountains of Virginia, but there is only one record from this region. Morphologically it is very constant, though there is a higher percentage of branched stems in the Green Mountains than in the rest of the area.

Ecologically, it varies with latitude. In Massachusetts it is a plant of dry habitats such as roadside verges, and is not common in damp situations. However, in Vermont it is the only commonly occurring species, and it occurs in a wide variety of habitats.

This is the most common species grown as a rock plant in European gardens, from whence it has escaped to grow wild in many parts of Europe.

S. atlanticum Bick.

Fernald describes this species as follows:

"More slender than S. angustifolium Mill., pale green or glaucous, hardly blackening in drying, ascending to erect, leaves firm, very pale, 1-3 mm. wide, mostly overtopped by the flowering stems; stems wiry, slender narrowly margined, 1-3 mm. wide, 2-7 dm. high, usually forking into 2-4 often geniculate filiform peduncles; spathes pale green or purple tinged, 1-1.5 cm. long; perianth blue violet; fruiting pedicels ascending; exserted; the mostly 3-10 brown or finally dark capsules 3-4.5 mm. high. - Damp to dry meadows, swales marshes and low wood. Florida to Miss., north to W. NOva Scotia., S. Maine, S-central N.H., Vt and S.E. New York., and in the interior, north to Ohio and S. Michegan."

In vegetative characters this species resembles S. angustifolium Mill. but is separable from the more slender forms of this by having a geniculate or potentially geniculate node. In flower it is easily separable from S. angustifolium Mill. since its flowers are larger and violet-blue. The flowers of S. angustifolium Mill. are invariably light blue.

From the present study there is no evidence that the species is very plentiful anywhere, though herbarium records indicate that it is very widespread. It seems likely that it is more common in habitats near the coast, which were not thoroughly studied, and inland in the 'swales' or wet meadows, which would mean that it was less accessible to the collector. All herbarium records show it to be a plant of wet places. In the course of this work it was found in the following localities:-

1) In wet, low-lying woods on James River Island, S.E. Virginia (2a)

2) On rock faces, constantly sprayed by spring water, in the Blue Ridge of North Carolina (1B)

3) On damp woodlands on Mt. Tom, Massachusetts (3A)

4) In meadowland near the coast of Massachusetts (3A)

Over most of its range the species seems to be morphologically surprisingly uniform. However, there are two specimens in the National Herbarium at Washington, which are referred to this species, but are much more robust than the rest of the specimens. These are from Fordin Co. Texas. However, since so few of these peculiar specimens were available, they must for the present remain as forms of S. atlanticum.

S. mucronatum Mich.

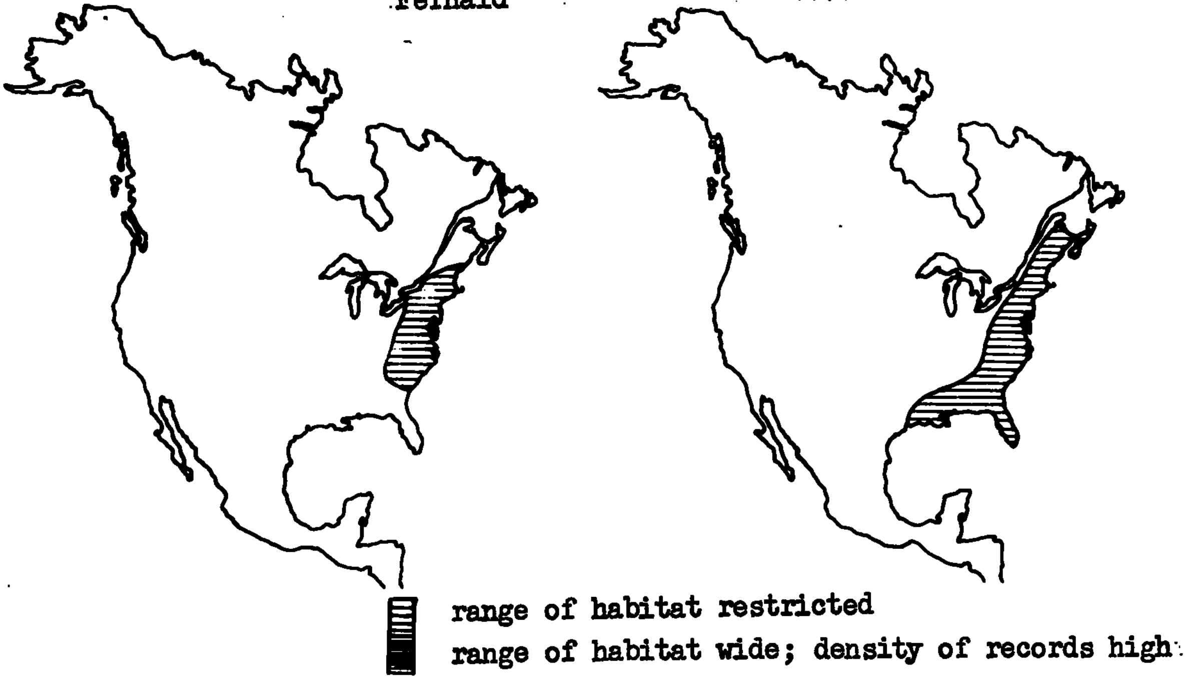
Despite the controversial role that this species has played in nomenclatural history, this is not a common species of Sisyrinchium. Locally it is abundant, but it occurs only spasmodically, and it seems surprising that of all the species in the genus, some of which, like S. angustifolium Mill., are very common, it should have been this species which was first described in Europe. Fig. (2. 2) map c shows the known distribution of the species. All these distribution maps are drawn with the same reservations concerning the use of herbarium records, as were discussed under S. angustifolium Mill.

Like S. angustifolium Mill. this species again comprises great but non-random morphological variability. Fernald's description is as follows:-

> "S. mucronatum Mich. - Tufted, green, 1.5-4.5 dm. high, erect stems flat 0.5-1.5 mm. broad, barely margined; leaves 1-2 mm. broad; outer foliaceous bract with margins united only slightly above base; spathe usually purple or purple-tinged, 1-2 cm. long; pedicels

a. **S.montanum** Greene **var. crebrum** Fernald

b. **S.angustifolium** Mill.



c. **S.mucronatum** Mich.

d. **S. atlanticum** Bick.

Fig. (2. 2)
Distribution maps of the most widespread species of **Sisyrinchium**

soon strongly spreading to recurving, much exceeding inner bract; perianth violet (rarely white); capsules straw colour to yellow-green, 2-4 mm. high. - Meadows, fields and open woods. N.E. Maine to Wisconsin, south to North Carolina.

The species was found in four areas, 1A, 2a, 2A, and 3B, but since only one station was found in each of the areas 2a and 2A, and these plants were very similar to those in 1A, they will be considered with the latter group. A comparison of groups 1A and 3B, in regard to some quantitative characters is given in Appendix 1.

It will be seen that the Southern plants are significantly more robust in all respects, but that the relative sizes of the parts of the plant in the two areas are not significantly different. There are also a few marked qualitative differences between the groups. The most striking is that the southern plants have light blue flowers, or occasionally white flowers, whereas the Northern plants all have bright purple-blue flowers. This character is very clear cut; - there are no light blue flowers among the purple-blue northern plants, and vice-versa. If these two groups are in fact the same species, then these colour forms must merge or overlap somewhere between Virginia and New England. Unfortunately time did not permit an investigation of this. However, the presence of these colour forms may not be very important taxonomically. The pale blue colour has not previously been reported in the species but there is a tendency for workers other than Fernald to describe all the species in the genus as 'blue' which leaves much scope for interpretation. Nevertheless, all the 'blue' species in the genus are divisable into only two shades 'light blue' and 'violet blue'. Hybrids between S. angustifolium Mill. (light-blue) and S. montanum Greene var. crebrum Fernald (violet-blue) show that the character 'light-blue' is completely dominant. Variation in colour could therefore, be due to a single gene, and until further evidence is available, should not be taken too seriously, especially if both alternatives occur in what is otherwise a relatively uniform taxon.

The northern plants are also characterised by a red-purple colouration of the spathe bracts, however, though, this feature is absent in plants from North Carolina, the S.E. Virginian plant did show it. It seems possible that this pigmentation of the bracts is largely a response to light intensity or temperature. If it is permissable to argue from S. montanum Greene var. crebrum Fernald, it was observed that plants of the same population grown in shaded conditions in a greenhouse had no pigmentation of the bracts, whilst those grown in the garden had. It may, therefore, be significant that plants of S. mucronatum Mich. collected in North Caroline were all from shaded woodland conditions, whereas those from Virginia and New England were all from unshaded habitats.

It would, therefore, seem that there are no very good grounds for proposing that the species S. mucronatum Mich. comprises more than one taxon. The variability it displays can be interpreted as being no more than would be expected in a wide ranging species. It would be interesting to interbreed plants from different parts of the range, but unfortunately only plants from North Carolina and Virginia have survived the rigours of the journey.

Throughout its range, S. mucronatum Mich. is a plant of dry, well drained situations. In North Carolina its stations are shaded, but the woodland is well drained. In Virginia, its occurrence on a dry railway cutting shows that it can survive extremely parched conditions. In New England it was found in permanent grassland.

S. arenicola Bick.

Fernald describes this species as follows:-

> "Tufted from a fibrous base, the old leaf bases persisting as crowded erect, bristles; leaves and stems green but slightly glaucous, inclined to darken in drying; leaves erect, firm, 1-3.5 mm wide; stems erect, mostly with 2-8 forks or peduncles, 1.5-5.2 dm high, the main axis flattened or winged, 1.5-3.5 cm. wide; peduncles 2-11 cm. long, slender, arched ascending, simple or forking; spathes 1.3-2 cm. long, pale brown; perianths blue-violet; fruiting pedicels erect or ascending, exserted; capsules pale to deep brown drying dark, 3-5 mm. high .... Dry mostly sandy soil on or near the coastal plain. Florida and Ala, north to Mass., and W. Nova Scotia also S. Michegan as the scarcely separable S. farwellii Bick."

Morphologically this species resembles S. atlanticum Bick. and S. angustifolium Mill. However, it has a characteristic tuft of old leaves or leaf bases and is darker green with a tendancy to a greater degree of branching. It has a later flowering time than either of the other two species. In the study area, specimens were found in two areas; 1A, but only in a special area of sandy soil known as the 'Sand Hills' which must have conditions rather like the more coastal habitat, and 2a, where it occurred on a dry railway embankment.

This species, together with S. capillare Bick. belongs to a group of infrequent species which are found mostly in Florida. The only common feature of this group is the tuft of persistant leaf bases. These often appear to be broken off at apparent abscission points occurring at the same height on each leaf. However, closer examination shows that these leaf bases or 'bristles' appear to be due to burning., Since the plant occurs in very dry, sandy habitats, it seems possible that burning may often cause the 'bristle' appearance, and the doubtful diagnoses of some of the herbarium specimens of this species may be due to the persistance of old leaves instead of only the bases.

S. capillare Bick.

Like S. arenicola Bick., this species has at its base the twisted fibrous remains of the previous year's leaves. Apart from this, this species is entirely unlike any other member of the genus.

Fernald describes the species as follows:-

"Excessively slender, erect; old leaves persisting as basal fibres; stems filiform, 1.5-5.25 dm. high, iridescent when fresh and with tight prolonged spiralling leaves capillary, glaucous or iridescent, at most 0.5 mm. thick; spathes two, rarely 1 or 3, closely subtended by an erect setaceous bract 2.5-8 cm. long; the spathes 1-1.3 cm. long with hyaline margined bracts; pedicels exserted, erect or with arching tips; perianth blue-violet, 6-8 mm. long; capsules pale 2-3 mm. high. - Flat pinelands, bogs and wet pine barrens, local Florida to S.E. Virginia."

The specific epithet describes the species very well. The most outstanding feature of the species is the 'twin spathed' condition which occurs also in S. albidum Raf. The feature seems to be due to the extreme reduction of two peduncles, so that a single leafy bract subtends two spathes, Fig. (2. 3).

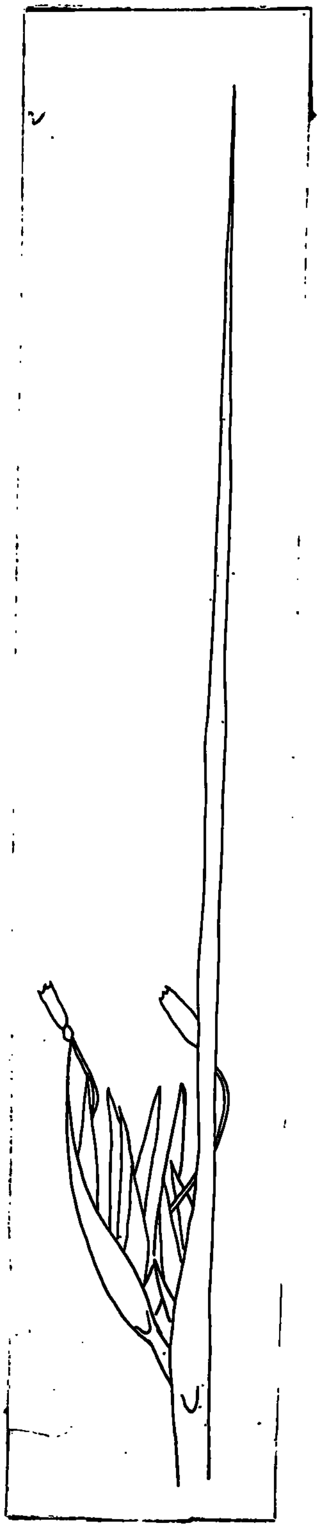
This is an extremely local species, occupying habitats similar to those described for S. arenicola Bick., but having its most northerly record in S.E. Virginia. This species was again found in the Sandhill region of area 1A, and in area 2a in some cleared pineland.

S. albidum Raf.

This species was described by Rafinesque from a white flowered plant, but in fact the albino is rarer than the blue flowered form.

Fernald's description is as follows:-

> "Erect, pale green or glaucous, 1.5-4.5 dm. high, stems 1-3 mm. wide, flattened and slightly winged usually twice exceeding flat leaves; spathes 2 (rarely 1 or 3) with lance-acuminate pale or purple tinged bracts 1.3-2.3 cm. long usually twice exceeded by the erect outer foliaceous bract; pedicels ascending to outwardly arching with exserted tips; flowers about 1 cm. long, whitish or pale violet ... ... Prairies and often dry open soil or thin woodland, Ga. and Ala, to La., north to N.C., S. Ontario, Ohio, S. Michegan, S. Wisconsin and Mo., Casual north-eastwards."



x4

Fig. (2. 3).

S. capillare Bick.

diagram showing the setaceous bract subtending twin spathes.

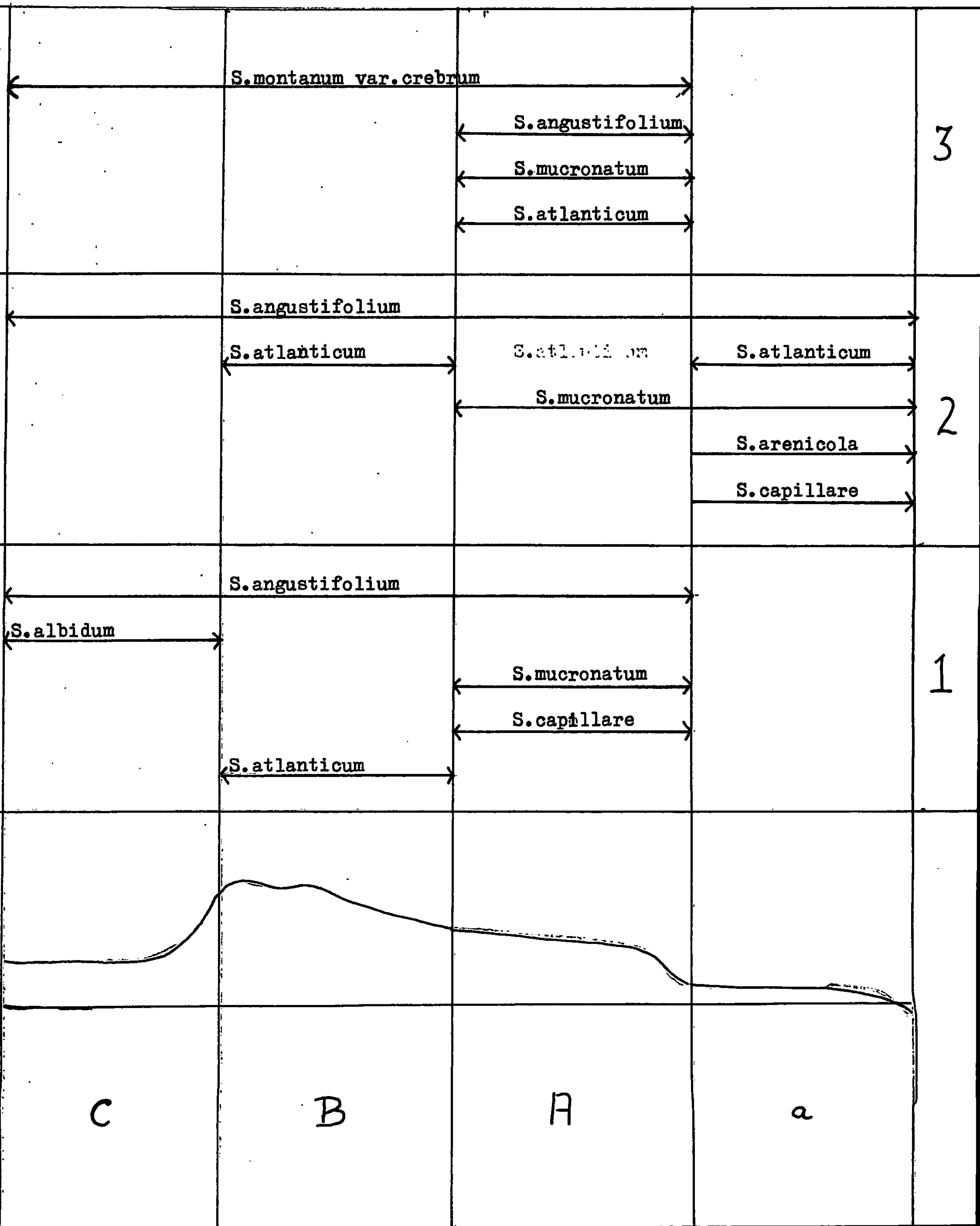
Again this plant has 'twin spathes' as in S. capillare Bick., but this is the only point of morphological similarity between them. S. albidum Raf. is characteristically a plant of the continental interior and in the study area it was found only in area 3C. However, it was important from the point of view of this work, because Bocher (1948) suggested that the Greenland species of Sisyrinchium might be related to it. This will be discussed in a later chapter.

Again this is a very distinct species, easily distinguishable from all the others with which this study is concerned.

This completes the description of the species of Sisyrinchium in the study area. Fig. (2. 4) shows clearly that there are two distinct types of distribution in this group of species. Type 1 is represented by S. angustifolium Mill. and S. montanum Greene var. crebrum Fernald. These species have a very general distribution within their respective geographical ranges, S. angustifolium Mill. being replaced by S. montanum var. crebrum northwards. Both species seem to be relatively unaffected by the climatic effects of altitude, since they occur in areas B and C, as well as in the more climatically favourable area A. These two species also occur in a wide variety of habitats, at least at the 'centre of frequency' (Cain, 1944) of their respective ranges. Towards the edge of their ranges, both tend to be more restricted in habitat. Thus in Massachusetts, S. angustifolium Mill. occurs

Distribution of species

Fig. (2. 4).



exclusively in damp, shaded habitats, and is much less frequent than in Virginia, where it occurs in a wide variety of habitats.

By way of contrast with these species, type 2 distribution is represented by _S. mucronatum_ Mich., _S. atlanticum_ Bick., _S. capillare_ Bick. and _S. arenicola_ Bick. These species are all very limited to a certain type of habitat, even though they have a wide latitudinal range, and all are limited to the Coastal Plain and Piedmont Plateau.

The meaning of this phytogeographical data will be considered after the cytology of the species has been discussed.

CHAPTER III

Cytology of the American species of *Sisyrinchium* in the study area

The following counts have been made by other authors, either previous to or during the present work.

S. angustifolium Mill. (probably *S. montanum* Greene *var. crebrum* Fernald, since this was written before Fernald's revision of the genus)

2n = 96 Bowden (1945) material from 'North America'

S. albidum Raf.

2n = 32 Oliver and Lewis (1962) material from Ontario, Canada.

S. atlanticum Bick.

2n = 96 Oliver and Lewis (1962) material from Texas

S. bermudiana L. (*S. angustifolium* Mill. sensu Fernald)

2n = 96 Oliver and Lewis (1962) material from Louisiana.

Cytology of *S. angustifolium* Mill.

Cytologically this is a difficult species to deal with. It is a very high polyploid, and unambiguous counts could only be made from late diakenesis, or very early anaphase. The bivalents at metaphase usually clumped together. It is not surprising, therefore, that much of the meiotic material brought back from America was at the wrong stage, and gave only approximate counts. Root tip counts are unreliable, as the section on the Botanic Garden material will show.

The following unambiguous counts have been obtained:-

2n = 82	Chapel Hill, North Carolina
2n = 88	Clinch River Valley, Tennessee
2n = 88	Campus of Lynchburg University, Virginia
2n = 90	Near Wartburg, Morgan Co. Tennessee
2n = 90	Near Soddy, Hamilton Co. Tennessee
2n = 90	Near Chickahominy River, Charles City Co. Virginia
2n = 90	Near Courtland, Suffolk Co. Virginia

Each chromosome number is based on at least three counts.

In all the other locations for this plant, counts were obtained, but not without at least one point of doubt. In no case was 2n = 96 observed. However, it seems probable that this might occur somewhere within the range of the species, and the count of Oliver and Lewis shows that it occurs in Louisiana. Since only one collection, from Tennessee gave any evidence of meiotic irregularity, it seems fair to assume that all plants in any breeding population have the same chromosome number, since the species is predominantly outbreeding. The chromosome counts recorded for this species appear, therefore, to be parts of an aneuploid series on the dodecaploid number 2n = 96.

This aneuploid series does not appear to be correlated with the observed morphological variability, and the plants from the Piedmont of North Carolina closely resemble those from the coastal plain of S. E. Virginia, although they differ in chromosome number. Two explanations of this phenomenon must be considered. First of all, it is possible that the aneuploid plants have always been exactly like the dodecaploid plant, due to the compensatory effect of the other chromosome complements present in the polyploid. The only way to test this would be to produce nullisomics. This has been impossible in the time available and few experiments of this kind have ever been performed. However, there are two outstanding examples, which suggest that nullisomics of a polyploid are usually morphologically recognisable. Thus Sears (1944) produced a series of plants nullisomic for each of the 21 pairs of Triticum aestivum, and found that each nullisomic was morphologically recognisable. Similarly, nullisomics in Nicotiana tabacum were studied by Clausen and Cameron (1944), and again, each was morphologically distinguishable. No work has been carried out on polyploids as high as 2n = 96, but it would seem likely that plants lacking not one but three chromosome pairs ought to be recognisable.

The second explanation is that the aneuploidy in this species has its origin from the time when the polyploid was first formed. Assuming that this is an allopolyploid, and the lack of multivalent configurations suggests, though does not prove this, then two of the parent species may have had sufficient chromosomal homology to cause multivalent configurations at meiosis. Stebbins (1950) points out that this situation is the rule in most synthesised allopolyploids. Aneuploidy could easily arise under these circumstances due to lagging chromosomes, and if this had no effect on viability or fertility, different members of an aneuploid series could easily become established in different parts of the range of the species. Even if at the beginning these aneuploid plants were morphologically distinguishable, long periods of natural selection under habitats exerting similar selective pressures could lead to a morphological convergence of different members of the series, particularly in those characters such as size and colour, which have a direct relationship to the environment. Thus the climate in the Eastern part of the Piedmont of North Carolina closely resembles that in S.E. Virginia, whereas the climate in the Tennessee Valley is much more continental. Therefore, the resemblance between the two races having $2n = 82$ and $2n = 90$ in North Carolina and S.E.Virginia respectively, may be due to evolutionary convergence rather than original morphological similarity.

However, there is no evidence at all that all the plants having $2n = 90$ all lack the same three pairs of chromosomes. It seems likely, if the hypothesis put forward is correct, that plants in the same area will lack the same chromosomes, as there are no meiotic irregularities to suggest otherwise, but plants from different areas e.g. from Tennessee and S.E. Virginia, could well lack different chromosome pairs. Some evidence about this is being collected, but is not yet available. Plants from different areas have been crossed, and seeds have been produced. However, since the plants were crossed in 1963, and it takes the plants two years to reach maturity (Appendix 2), the evidence from the presence or absence of meiotic irregularities will not be available until 1965. Nor is it yet known whether the aneuploid races are separated by breeding barriers. Again this information will be available in 1965. All that can be deduced at the moment is that if there are breeding barriers, these do not operate at the stages of pollination and seed production. However, this situation is common in the genus, and breeding barriers do not usually become noticeable until meiosis in the hybrid.

Cytology of S. montanum var. crebrum Fernald

The chromosomes of this species were much less difficult to count than those of S. angustifolium Mill., because there was much less clumping of the chromosomes at metaphase. However, the species was unfortunately in full flower in New England when it was collected which meant that most of the cytological material taken was in too late a stage to permit counts to be made. Few plants survived the journey to England, and of those that did, only three flowered in 1963. This difficulty was unexpected, because mature plants of this species usually flower freely in this country, and the species is widely grown as a European garden plant. However, the following counts were obtained:

$2n = 96$ Springfield Reservoir, Mass.

$2n = 96$ Near Bondville, Vermont

$2n = 96$ South of Lake Catherine, Vermont.

This constancy in chromosome number is characteristic of material of this species obtained from Botanic Gardens in various parts of Europe, and also agrees with material in cultivation from Newfoundland and Gaspe. Bowden (1945) also reported 2n = 96 for this species.

No meiotic irregularities were observed in any preparation. Although this species appears to be uniform in every respect, this does not mean that the species is cytologically uniform. Experiments performed with Botanic Garden material suggest a great diversity. This work will be discussed in another chapter.

Cytology of S. mucronatum Mich.

Counts were obtained as follows:

2n = 32 Chapel Hill, N. Carolina
2n = 30 E. of Chyde, Haywood Co. N. Carolina
2n = 32 Magruder, Virginia
2n = 32 Campus of Lynchburg University, Virginia.

The material from New England was again in full flower, and did not survive the journey to England. However, Lewis and Oliver record 2n = 32 for material from Canada, so that the chromosome number appears to be fairly constant throughout the range of the species. The aneuploid plant found in North Carolina was morphologically slightly different to the rest of the species, having a greatly attenuated spathe bract, but there is no evidence that aneuploidy is a common feature of the species.

Cytology of S. atlanticum Bick.

Again material from only four locations was counted, as most of the New England material did not survive.

$2n = 32$ Ococee River Valley, Tennessee

$2n = 32$ James River Island, S.E. Virginia

$2n = 32$ Two localities on Mt. Tom, Mass.

The only other record for this species is that given by Lewis and Oliver (1962) from Texas $2n = 96$. In the morphological description of the species, it was noted that some herbarium specimens from Texas resembled this species, but were more robust. The explanation may be that these have $2n = 96$, and that the more widespread $2n = 32$ plant is one of its parental species.

Cytology of S. capillare Bick.,

Material from two localities was counted.

$2n = 32$ North of Holly Springs, Wake Co. N. Carolina

$2n = 32$ N.W. of Waverley, Sussex Co. Virginia.

This rare species is again a tetraploid.

Cytology of S. arenicola Bick.

It was unfortunate that none of the material preserved yielded a count of this species, and no specimen has survived in England.

Cytology of S. albidum Bick.

In this case only one specimen was found, but a chromosome count proved to be possible.

$2n = 32$ Scott Co. Tennessee.

Cytology of Sisyrinchium in other parts of America

The following counts of species in other parts of America have been reported.

S. America

2n = 16 (species unspecified) Chile Bowden 1945

2n = 64 (species unspecified) Chile Bowden 1945

Mexico and Texas

2n = 32 *S. ensigerum* Bick. Lewis & Oliver 1961

2n = 32 *S. micranthus* Cav. " "

2n = 32 *S. langloisii* Greene " "

2n = 32 *S. pruinosum* Bick. " "

2n = 32 *S. quadrangulatum* Bick. " "

2n = 32 *S. laxum* Otto " "

Georgia

2n = 16 *S. fibrosum* Bick. " " 1962

Arkansas

2n = 32 *S. campestre* Bick. " "

Tennessee

2n = 32 *S. intermedium* Bick. " "

California

2n = 32 *S. bellum* Wats. Bowden 1945

W. Indies

2n = 32 *S. labidum* "

Chromosome counts of members of the genus which are not in the section Bermudiana have been omitted from this list.

Taking into consideration all available data, it is seen that with one exception, all the species in North America are polyploids. S. fibrosum Bick. is alone recorded as diploid from Georgia. Among these North American polyploids, only tetraploids and duodecaploids have been recorded.

Of course, this absence of diploids and other levels of polyploidy may be more apparent than real. Bicknell (1901) described 24 new species of Sisyrinchium from Texas alone, and in the same year some 10 species from the Pacific North West. Although later taxonomists have not agreed with Bicknell in giving all these taxa specific status, the immense variation which Bicknell's splitting reflects, could easily be due to a multiplicity of chromosome races. However, the evidence available does not point to this explanation, since Oliver and Lewis have now counted eight species from Texas all having 2n = 32. However, there are enormous areas of the United States from which nothing is known about the cytology of the genus. Thus, apart from two counts from California for S. bellum Wats. 2n = 32 Bowden (1945) and Clausen Keck and Hiesey (1940), nothing at all is known about the species in the Cordilleran region. A similar ignorance exists about the Central Plains, Canada, Florida and most of the Southern States. Indeed, diploids and octoploids may even exist within the area of this study. The one diploid count recorded in North America is from S. fibrosum Bick., which Fernald considered to be morphologically indistinguishable from S. arenicola Bick. In the study area, lack of material precluded a clear count for this latter species.

As well as this ignorance about North America, nothing is known of the distribution or cytology of species of the section Bermudiana in South America. Thus it is not really necessary to assume that the diploid ancestors of the polyploid species no longer exist, or that the octoploid level is very uncommon compared with the tetraploid or duodecaploid.

The most likely hypothesis seems to be that the diploid species existed and may still exist somewhere in South America or the Southern states of North America, and that allopolyploidy occurred between them, to produce plants capable of spreading over North America during climatically disturbed conditions. This could explain why there are many tetraploid species in the Southern States of North America, and why most of these decrease in frequency farther North, until at the latitudes of North Carolina and Virginia, they are showing a feature which is characteristic of species at the edge of their ranges i.e. a rigid restriction to a certain type of habitat. In the study area this feature was shown by S. arenicola Bick., S. capillare Bick., S. mucronatum Mich., and to a lesser extent by S. atlanticum Bick.

This may be the case in most of the tetraploid species of Texas and the Southern States, but unfortunately, no data are available on the subject. It does seem as if at least two tetraploid species are in fact very widely spread, namely, S. campestre Bick. and S. bellum Wats. However, chromosome counts of these species are very few indeed, and there is no evidence that they are cytologically constant throughout their ranges.

The distribution of the dodecaploid species in the study area is in marked contrast to that of the tetraploid species, and the wide tolerance of the two dodecaploid species in the study area would suggest that this is their area of maximum distribution. This would imply that these species have originated in North America. However, there is still the curious absence of any report of an octoploid species or race, but as previously emphasised, this does not necessarily mean that such a species or race does not exist.

Any other hypothesis to explain the cytological situation in North America must involve the wholesale extinction of the parental diploid and octoploid species, and although some species may have become extinct, it seems unlikely that no remnant should survive.

CHAPTER 1V

Breeding systems in the species studied

The species S. capillare Bick., S. mucronatum Mich. and S. bellum Wats., are self incompatible. Plants were selfed and produced no seed, but produced seed when pollinated by another plant of the same species, or a plant of a different species with which it would cross (at least to give seeds). These plants are all tetraploids, and it is probably therefore, fair to assume that their ancestral species were also self incompatible, since no instance has been recorded of self incompatibility arising spontaneously at the tetraploid level. A survey of the other tetraploid species of the genus from this point of view would be very interesting.

However, the system of self incompatibility breaks down at the higher levels of polyploidy, and S. angustifolium Mill. ($2n = 96$) and S. montanum var. crebrum ($2n = 96$) are completely self compatible. There is evidence that the breakdown of self incompatibility is complete at the octoploid level, because octoploid material obtained from a Botanic Garden is completely self compatible. This breakdown of self incompatibility in polyploids has been discussed by Lewis (1943). It has been impossible to examine the question of incompatibility in the tetraploid species very thoroughly, because only a few plants of each species were available for investigation, and the flowers were required for other experiments.

The floral morphology of the various species is interesting considered in regard to the breeding system. In the tetraploid species, the style projects well above the level of the filament tube and anthers, and there is little chance of self pollination because the style and anthers are not in close proximity (see Fig. (4. 1) A similar situation exists in the unnamed octoploid species among the Botanic Garden material, though in this case of course, the material is self compatible. However, in nature this separation of ant~~l~~ers h. and stigma must be fairly effective in preventing self pollination, because when the plants of the octoploid species having been kept in an insect-proof greenhouse were examined at the end of the flowering period, of those flowers which were not used for experimental purposes, only three had produced capsules, and at least 100 had not. This then gives an indication of the minimum self fertilisation which this type of floral morphology allows in nature. Of course, it is likely that much more self pollination occurs in nature, because of accidental self pollination by insects.

In S. montanum var. crebrum the situation is very different. The style projects scarcely or not at all above the level of the anthers, and when the stigmatic lobes are turned outwards, they actually project between the tops of the anthers, Fig. (4. 2). The stigmatic lobes of this species open before the flower opens. ~~Self~~ Cross pollination appears to be very unlikely. The possibility that pollen

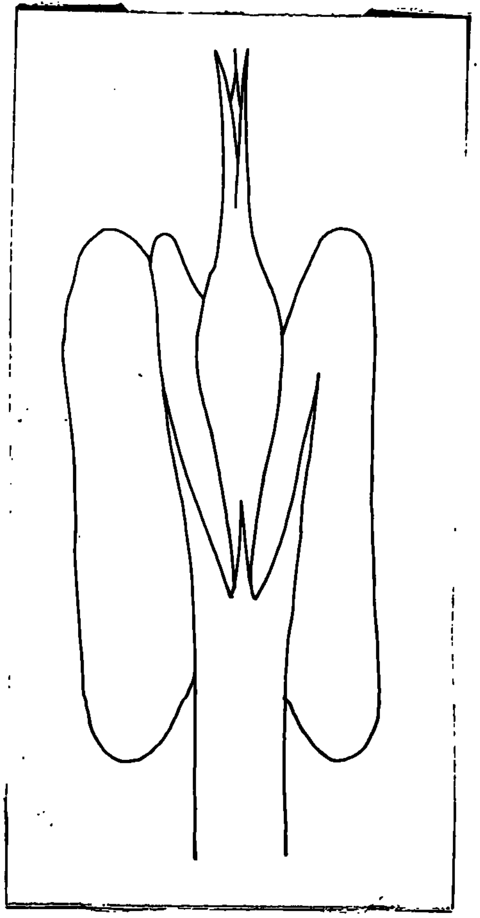


Fig.(4. 1).

Relationship of anthers and style in the tetraploid species of <u>Sisyrinchium</u>
(Diagram drawn from <u>S.mucronatum</u> Mich.)

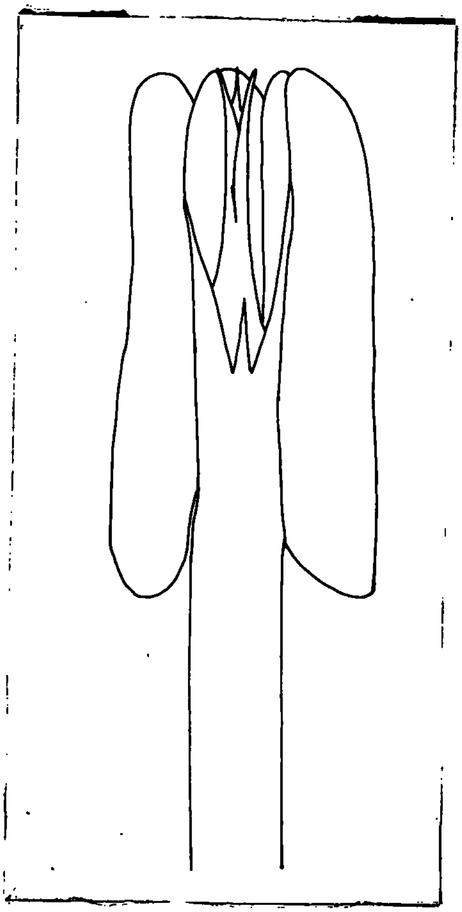


Fig. (4. 2).

Relationship of anthers and style in <u>S.montanum</u> Greene <u>var crebrum</u> Fernald.

from other plants may be at an advantage over pollen from the same plant, i.e. take a shorter time to grow down the style must be considered, but when plants of the same family were pollinated artificially with their own and other pollen, there was no difference in the lengths of the pollen tubes that had grown after eight hours. In eight hours they had reached the middle of the style. The pollen tubes were examined in the style by staining for about two minutes with cotton blue in lacto-phenol, and then mounting in glycerine.

In periods of bad weather when the flowers never open, they produce seed cleistogamously. It is not surprising, therefore, that every flower of this species produces a capsule. Each flowering scape will produce 6-10 capsules, each containing about 40 seeds.

However, it may be possible for outbreeding to occur under special conditions e.g. if the filament tube was retarded in growth, then the stigmatic lobes could open without touching the pollen, and cross fertilisation would be possible. The importance of this type of occurrence will be considered in the discussion of S. angustifolium Mill.

The type of breeding system described for S. montanum var. crebrum is much more like that usually associated with short lived annual plants, rather than that associated with perennials. It is a well known theory that in annuals the chief selective pressure is towards immediate fitness, and this is most easily achieved by the maintenance of favourable gene combinations by self fertilisation. S. montanum var. crebrum, however, is perennial, and the situation is probably best explained by the hypothesis put forward by Stebbins (1950) to explain similar phenomena reported in the genus Stipa etc. The hypothesis is that such perennial species either live in habitats with wide climatic variations, in which case they could easily be eradicated by a particularly severe season, or else they live in a 'pioneer association', in which case a rapid change in the habitat could lead to a dependance on seed to maintain the species in that area. Both situations may apply to S. montanum var. crebrum to some extent. Some of the habitats of the species were certainly extremely dry, and must have been completely parched later in the Summer. The effect of this must be very similar to being in an extreme climate. Perhaps in these habitats, the species acted as an annual. No evidence is available on this point, but under such extreme conditions it seems likely. In other places such as roadsides and lake shores, the species can be said to belong to 'pioneer' associations which are constantly changing their composition, and if the mature plant can no longer compete in any part of the association, it is essential that the species be maintained by seed.

Despite the apparent success of the species, there is no doubt that cross-fertilisation, and genetic recombination are rare, if they occur at all, and it may be that this species has reached an evolutionary cul-de-sac because of its breeding system.

S. angustifolium Mill.

This species is again self-compatible, but has achieved a balance between cross-fertilisation and self-fertilisation. This is because of a morphological plasticity which occurs within a single plant. The mechanism is simply that the length of the filament tube is variable. If the filament tube is long, then the situation is the same as that described for the previous species, i.e. the stigmatic th/ lobes turn outwards, and lie between the ant~~l~~ers, and this automatically causes self pollination. If the filament tube is short, n/ the~~y~~ the stigmatic lobes do not touch the pollen when they turn back and cross-fertilisation is possible. There is, however, a complete gradation between short and long filament tubes, which may be observed even within a single spathe, and this does not appear to be correlated with the sequence of flowering, or with any other obvious circumstance, such as whether it is early or late in the flowering season. The variation appears to be completely random. It may be due to something as intangible as metabolic rate of the plant at the time when the filament tube was elongating. It would be necessary to grow the plants under carefully controlled environmental conditions in order to find out which environmental factor caused this variation.

A liability to produce this variation in the length of the filament tube is inherited in hybrids between this species and <u>S. montanum var. crebrum</u>. It was noted in the discussion of the latter species, that variation in the length of the filament tube is very rare if it occurs at all in that species.

From a random sample of 69 flowers from 25 plants of <u>S. angustifolium</u> Mill. the following figures were obtained:

Long filament tube	11%
Short filament tube	66%
Intermediate filament tube	22%

However, these are the figures from only one population. The proportions may vary in other cases, but in no other cases were large enough samples of populations available.

When this population sample was left in an insect-proof greenhouse, 30% of the flowers produced capsules, which means that almost all the intermediates must have been self-pollinated.

It would obviously be interesting to know more about this phenomenon over the range of the species. However, large samples are necessary, and much space and time would be spent on cultivation. Clearly the results of this variation could play an important part in the evolutionary potentialities of the species, by maintaining a balance between inbreeding and outbreeding.

CHAPTER V

Sisyrinchium in Ireland

The genus Sisyrinchium was first recorded in Ireland in 1845. It was discovered by Rev. J. Lynam near Woodford in Co. Galway. The next records appear to be in the 1880's and 1890's, and many of them are the work of the great Irish botanist Scully, who appears to have concentrated his efforts on Co. Kerry. Sisyrinchium was found to grow extensively in Kerry, extending down into Cork. It is interesting, however, that the earliest records from the North West also date from this period. R. M. Barrington collected a specimen from the Ben Bulben range in Sligo in 1904, which suggests that the stations in the North West are not due to recent expansion.

Since this initial period of discovery, there have been more records from Donegal, most of the localities being discussed by Preager (1934). Other scattered localities have also been reported, but there remain three important groups of localities for the species, namely:

Kerry and Co. Cork
Around Waterford in Co. Galway
In the glens of Sligo and Donegal.

Preager (1934) was firmly of the opinion that Sisyrinchium was native in Ireland. He points out that the plant has quite a wide range in the West, and always occurs on 'wild' ground. Scully was of the same opinion. Preager points out that a frequent argument used against the native status of the plant, is that it spreads freely from gardens and that there are many reports of such escapes from England and the Continent. The present work shows that such arguments can not be used since they are based on the assumption that the native Irish plant is the same species that is commonly cultivated as a rockery plant. This is not the case. The garden plant is *S. montanum* var. *crebrum*, which has showy less transient flowers. The Irish plants are clearly *S. angustifolium* Mill. sensu Fernald. This is not a commonly cultivated species, and indeed has little to recommend it from a horticultural point of view. As far as can be ascertained from the specimens preserved in the British Museum this species does not occur as a garden escape in England. However, it is obviously impossible to say that the species was never grown as a horticultural plant, but its origin in Ireland as a horticultural escape seems unlikely. Its habitats are often far removed from any horticulture and in any case its ecological preferences render it unsuitable for cultivation.

If then, the species is native in Ireland, it is clearly a member of the 'North American' Element in the British flora. This group of species is generally accepted as being genuinely native, but their closest relatives are in North America. The list is as follows:-

r/ *Eriocaulon septangulate* With. Eriocaulaceae. Western Ireland, Sky, Hebrides.

Najas flexilis (Willd.) Riske and Schmidt. Najadaceae. Western Ireland, Hebrides and two localities in Scotland and England.

Spiranthes romanzoffiana Cham. Orchidacea. Ireland and Dartmoor.

Potamogeton epihydrus Raf. var. ramosus Peck. Potamogetonaceae. Hebrides.

Limosella subulata Ives Scrophulariaceae Wales.

Sisyrinchium angustifolium Mill. Iridaceae, Western Ireland.

Two other species are sometimes added to the list, Juncus tenuis Willd. and Juncus dudleyi Wieg, but their native status is very doubtful as they are extending their ranges in the manner of recent introductions.

For the rest, there has been considerable argument as to whether they are recent introductions e.g. by birds (Heslop-Harrison, 1952), or whether they are preglacial survivals. Heslop-Harrison points out that most of the species listed above are species preferring aquatic, or at least damp habitats, and are therefore, particularly suitable to dissemination by birds. There are species of birds which by virtue of their migration routes could possibly do this. However, none of the species constituting the American Element are acting like recent introductions.

The best evidence for their native status would be a fossil record, but a fossil record is available only for Eriocaulon septangulare and Najas flexilis. Eriocaulon was undoubtedly present in Ireland in the Boreal period, which rules out introduction by man. The most frequent records for Najas flexilis are also in the Boreal period but it is also recorded from the Histon Road (Cambridge) interglacial. There is then, good evidence that these two species are not recent introductions.

The most widely accepted view at present, is that the fossil record of Najas flexilis gives the key to the whole problem of the North American element in the British flora. This view was expressed by Deevey (1949), and summarised by Godwin (1956). Najas flexilis now occurs in Western Ireland, the Lake District, Western Scotland, and Western Scandinavia. However, it was formerly much more widespread, particularly in Boreal time when it extended over much of Europe. Its distribution contracts sharply in Atlantic time, and this contraction is continued to the present day, when there is such a marked asymmetry in its distribution on the two sides of the Atlantic. Deevey points out that this is obviously a circum-polar species which has shown marked contraction, and there is no need to postulate survival through the last glaciation in situ.

This could well be the origin of the North American element. The real problem concerning these species is probably not their origin in Europe, but the cause of their asymmetrical contraction.

It is, however, dangerous to argue from the fossil record of one species to an explanation of the distribution of another which has no fossil record, and there is another method of investigation, which has rarely been applied.

If the North American and Irish populations of these species have indeed been separated for an enormous length of time, then unless there has been parallel evolution in the two populations, one would expect to find genetical divergence in the two populations, which may show morphologically, cytologically, or in some more subtle physiological alteration. Some work on Eriocaulon septangulare by Löve and Löve (1961) suggests that the Irish population is probably a polyploid race of the common American species. However, the American plants were taken from only one locality, and little can be concluded from this work until a thorough study of the American species has been carried out. Some work on Sisyrinchium (Löve and Löve, 1961) suggested that a similar situation might exist in this species. It was reported that material collected at Clooney in Co. Kerry gave a chromosome number of $2n = 64$, which differed from any chromosome count then recorded for any species in North America. The material from Clooney was also described as differing in morphological features from any species in North America.

It was considered that an investigation into the precise cytological and morphological relationship of the Irish population of Sisyrinchium to its nearest American relative, would yield interesting information concerning the origin and evolution of the two populations.

It was, therefore, necessary to determine the nearest American relative of the Irish plants, and to investigate its morphological and cytological variation. A similar investigation was necessary for the Irish population. A comparison of herbarium material showed that the Irish species was very similar to the North American species S. angustifolium Mill. sensu Fernald. The morphology and cytology of this species in America has already been described.

It was very difficult to gain the necessary information about the Irish population from herbarium material. Many of the herbarium specimens are scrappy and inadequate, giving insufficient information about habitats, particularly the presence or absence of grazing, which seems to affect the degree of branching of the scape. It was, therefore, necessary to visit as many of the Irish localities as possible. It was unfortunately impossible to visit every recorded site in Ireland in the time available, but all three major areas were adequately sampled. A list of habitats and associated species is given in Appendix 5.

Phytogeography of Sisyrinchium in Ireland

From observations in Ireland, it seems that Sisyrinchium is much more frequent in Kerry and Galway that it is further north. In Sligo and Donegal the species was represented by fewer individuals in each locality. However, after visiting many of the known localities for the species in Ireland, it seems possible that there may be many other stations not yet recorded. Individual plants are easily overlooked when they are not flowering, and the flowers are so fugaceous that they can easily be missed.

The habitats of the species can be roughly divided into two groups. Most frequent are damp permanent meadows, where the species is often associated with Juncus articulatus, which forms a convenient marker for likely habitats. It was invariably absent from waterlogged meadows. Such conditions were usually marked by the presence of Iris pseudacoris.

The other group of habitats is lake shores and the edges of rivers. Under such conditions the species appeared to be almost sub-aquatic. However, this may be a false impression, because the habitats were visited in Spring and were mainly occupied by seedlings. It is difficult to estimate how many of these would survive later in the season.

The species appears to be absent from the calcarious soils of Co. Clare, even at the edges of the Tulloughs where the water table was very near the surface in Spring. However, there is a very wide fluctuation in water conditions in these localities. The species cannot be said to be strictly calcifuge, because it grew on limestone on Ross Island, but here it was growing in shaded conditions by the edge of a permanent lake.

The plants seem to be able to withstand grazing quite well, but it seems likely that grazed specimens may have led to much of the confusion about the taxonomy of the species. The scape of the species is characteristically branched, but there is a tendency to produce simple shorter scapes under difficult conditions, and also at the end of the flowering season. Many of the herbarium specimens in the National Museum of Ireland show this feature, which could easily be due to grazing. Plants from all localities grown under greenhouse conditions, showed morphology typical of S. angustifolium Mill.

Morphology and Cytology

It is virtually impossible to make a valid statistical comparison of morphological measurements between Irish and American material, because the vegetative features of the American material were measured from plants taken from the wild, but the Irish material was collected in early spring and brought to maturity under greenhouse conditions. A more valid comparison would be between the herbarium specimens of the American material and herbarium material from Ireland, collected when the plants are mature. However, even this is not valid for vegetative characters, which are affected by grazing in many of the Irish specimens. A really valid comparison would be possible only between American and Irish plants grown under the same experimental conditions. This was planned, but was upset by mortality during transit of American plants, and also mortality of American plants during the winter of 1962-3. However, on the basis of appearance only, the Irish population resembles very closely the plants from Virginia. Floral characters are not significantly different in the two populations. Both show a tendency to a variable relationship between anthers and style.

Again on cytological grounds, the Irish population fits in with the American populations. Cytological preparations could not be obtained for many of the specimens brought from Ireland, because the plants refused to flower. This may have been due to the fact that some first year plants had been inadvertantly collected, or because some of the plants had been damaged. However, counts were obtained from material from each of the three major areas for the species:

Cloonee Loughs, Co. Kerry	2n = 88	(confirming Miss Northfield's earlier count. Unpublished M.Sc. Thesis-)
Caragh Lough, Co. Kerry	2n = 88	
Woodford River. Co. Galway	2n = 88	
Killybegs, Co. Donegal	2n = 88	

All counts were based on at least two preparations.

This chromosome number is best explained as being part of the aneuploid series already discussed for the American population. There are thus no morphological or cytological grounds for considering the Irish population to be differentiated from the American population.

There remains the count of 2n = 64 reported by Löve and Löve (1957) from Cloonee Loughs Co. Kerry. However, this count was based on a root tip section and the variability found in root tip counts has already been discussed. (See Appendix 4).

Discussion

There are no morphological or cytological grounds for assuming that Sisyrinchium in Ireland is not a recent introduction. Indeed, cytological evidence would rather support this theory, since only a single chromosome race is present, which it could be argued, would be expected from a chance introduction. On the other hand in any area of comparable size in America only a single chromosome race would be expected.

Ecological evidence, however, suggests that Sisyrinchium is not behaving as a recent introduction, but it occurs in 'closed' habitats. It does not occur particularly near human habitation, and some of its habitats are far removed from habitation. It does not have the same weedy propensities as S. montanum var. crebrum.

The status of the species in Ireland will be further discussed after some experiments have been described.

CHAPTER VI

Sisyrinchium in Greenland

The distribution of the genus *Sisyrinchium* in America and Ireland has already been discussed. The only other area in which *Sisyrinchium* is thought to be native is Greenland. A species was reported from Søndre Strømfjiord by Bøcher (1948). The recent introduction of the plant to this locality by man seems unlikely, since Bøcher reports that there are no settlements. The origin of the plant in Greenland is now open to all the controversy which has raged around the origin of *S. angustifolium* Mill. in Ireland for the past century.

Bøcher describes the Greenland species as being 17-27 cms. tall, with 1-3 flowering shoots in each individual. Capsules 3.0-5.5 mm. high. Leaves 1.0-2.5 mm. broad. Stems 1.0-1.5 mm. wide. Cultivated later in Copenhagen, the leaves were 1.5-3.0 mm. wide. Flowers pale blue, not violet-blue as in the typical *S. montanum* Greene.

Bøcher and Larsen (1952) give the chromosome number 2n = 32 for this species, at the same time classifying the plant tentatively as *S. montanum* Greene. Bøcher (1948) (personal communication 1961) thought that the Greenland plants were atypical of the species, and were more likely to be related to *S. albidum* Raf., this being the only pale-flowered species with this chromosome number then recorded from the United States. However, the present study has revealed that

Bøcher's description of the Greenland plant agrees fairly closely with the pale-flowered form of S. mucronatum Mich. There is no pale-flowered form of S. montanum Greene recorded, whereas S. mucronatum has pale flowers in North Carolina and Virginia. Also forms of S. mucronatum may be quite robust enough to fit Bøcher's description. Most important of all, the chromosome number of S. mucronatum Mich. agrees with that of the Greenland plant. If the Greenland plant is indeed S. mucronatum Mich., it is perhaps strange that the colour form is that of the more Southerly areas of the mainland distribution of the species, and not that of the more northern plants which are characteristically violet-blue. However, the extent of the colour variation in the species as a whole is obscure. It would seem at any rate that the closest relative of the Greenland species is much more likely to be S. mucronatum than S. albidum Raf. The latter species is admittedly pale blue or white, and has a chromosome number of 2n = 32, but it is characteristically twin-spathed, which is a feature entirely absent from the Greenland plant.

From the point of view of the present study, the most important feature of the Greenland species is that it cannot be cytologically of the same taxon as the Irish species. It is definitely not referable to S. angustifolium Mill. sensu Fernald.

Heslop-Harrison (1952) in his discussion of the North American Element in the Irish flora, quotes the migration track of Anser albifrons flavicornii, the Greenland White Fronted Goose, as evidence that the plants of this element, could have been introduced on the feet of birds. This goose breeds in Greenland, in the area in which Sisyrinchium is found, and then part of the flock winters on the west coasts of Ireland, Scotland and Wales, whilst the rest of the flock winters in the St. Lawrence region of Canada. Heslop-Harrison's argument is that some seeds of most plants of damp places are bound to be transported in the course of millions of journeys, and he quotes as a highly significant point,the fact that 'S. angustifolium' reaches its most northerly latitude in Greenland. This really demonstrates the confusion caused by taxonomic uncertainty in this genus. The fact that the Greenland species is not S. angustifolium Mill. sensu Fernald, means that Heslop-Harrison's theory is based on much less convincing evidence.

It would be very interesting indeed to obtain seeds of the Greenland species. Unfortunately they were unobtainable at the time of this investigation.

A wild species of Sisyrinchium has also been recorded from the valley of the River Pruth in the Carpathian Mountains (Lauterborn, 1929). According to Hegi this is S. montanum var. crebrum i.e. the same species that is commonly cultivated in gardens, and which has become naturalised in many parts of Europe. The validity of the claim that the Carpathian species is indigenous thus seems to be doubtful, and will not be discussed further.

CHAPTER V11

Cytology of S. montanum Greene var. crebrum Fernald, and its relationship to S. angustifolium Mill.

The 'Test Material' which will be referred to in this section was a series of stocks of plants from a wide variety of sources, but all referable to the taxonomic unit S. montanum Greene var. crebrum Fernald. Many of the stocks were obtained from Botanic gardens, though some were obtained from wild populations in the U.S.A. A list of the sources of the material will be found in Appendix . The material obtained from wild populations was particularly important, because accurate information concerning the origins of the Botanic garden material was not available, and the material collected from wild populations has, therefore, more relevance to the natural situation.

Three stocks are particularly important in this connection:

Stock 20	Collected wild from Newfoundland (T.T.Elkington)
Stock S.17	Collected wild from Gaspe (D.H.Valentine)
Stock S.55	Collected wild from California (C. Robbins)

S.55 is thus a West coast representative of the taxon, and is probably, therefore, representative of S. idahoense Bick., which is described by Bicknell as being very similar to the East coast species S. montanum Greene var. crebrum Fernald (i.e. S. angustifolium Mill. sensu Bick.) but larger in all its parts. However, since he goes on to say that mountain forms of this species are much more slender than coastal forms, and have smaller flowers, it would seem that there is very little morphological ground for maintaining that the East and

West coast forms represent two different species.

Morphologically the stocks of the test material differed in such characters as bract length, flower size, pollen size and seed weight. The scatter diagram (7.1) shows that these characters are not randomly associated, and on this basis the data fall into two groups. The measurements for these data were made on material which as far as possible had uniform environmental conditions, having been grown in the same greenhouse.

For convenience in description, the group falling to the left of the scatter diagram will be referred to as Test Material Group 1 (T.M.1) and that to the right as Test Material Group 2 (T.M.2). It is important to notice at this stage, that the characters demarkating the two groups are mainly quantitative, and are of the type that would normally be associated with ecotypic differentiation.

In order to test the genetical relationship between the two groups, crosses were made in all possible combinations between the stocks. At the same time, each stock was crossed with stock 10, which was the Irish material of S. angustifolium Mill. sensu Fernald, in order to establish the relationship of this species to the test material. Reciprocal crosses were carried out wherever possible, and each cross was repeated six times wherever material allowed. Unfortunately, some material only allowed three repeats per cross. All repeat crosses were carried out between stocks, and not between the same plants. In second

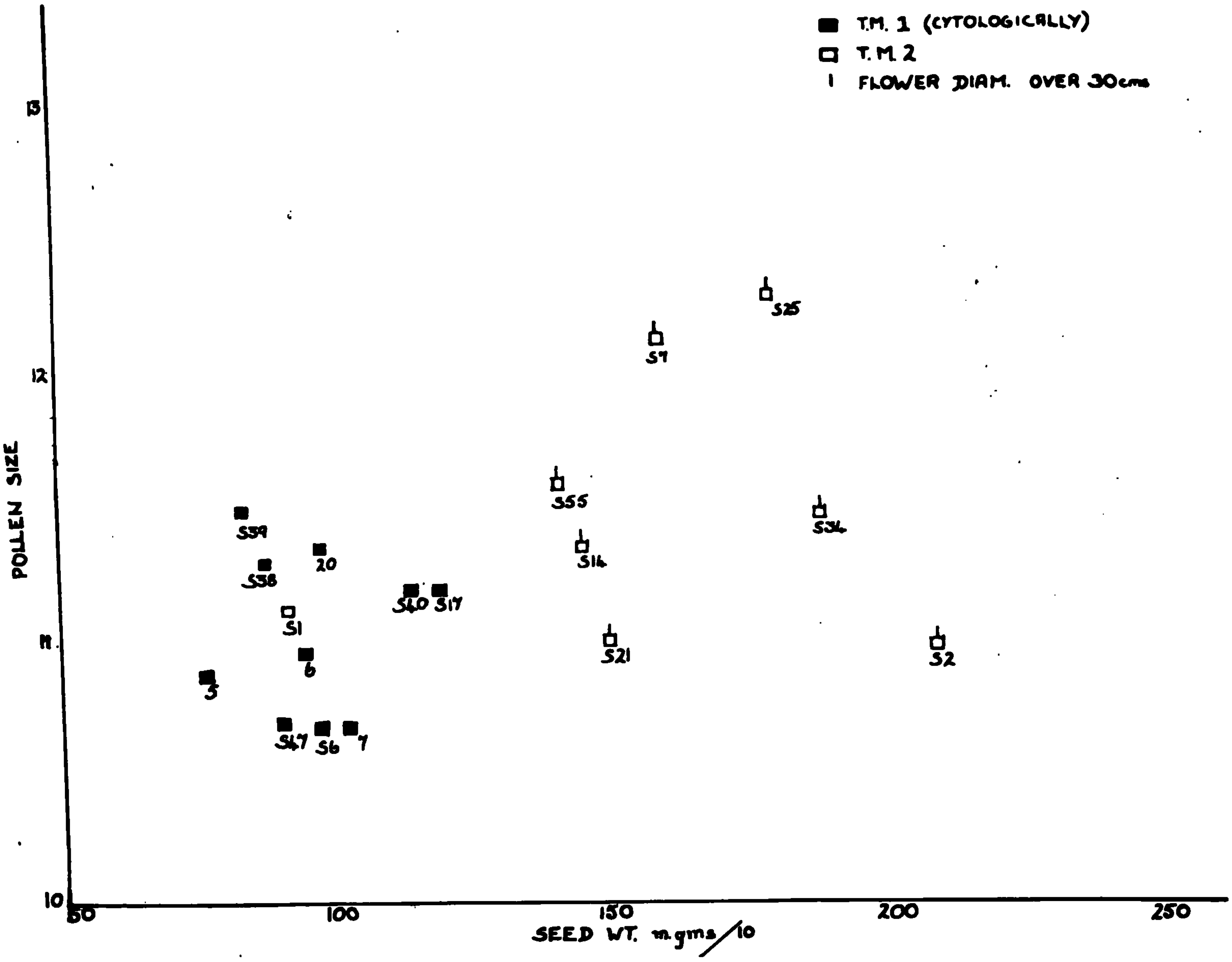


Fig. (7. 1.)
Scatter diagram showing the non-random association of characters within the Test Material.

year plants i.e. plants flowering for the first time, it is unusual to have more than one flower open per day per plant. This necessitated the use of different plants to repeat any one cross on the same day. Later work showed that this decision to carry out repeat crosses on the same day was unfortunate and it would have been more satisfactory to wait for another flower on the same plant, in order to repeat the crosses between the same genotypes. The original reasons for repeating the crosses were, firstly, to avoid the chance effect of using plants which were not completely fertile, and secondly, to allow the production of enough seed to grow an F1 of reasonable size.

There was no reason to suspect that the emasculation technique used in these crosses was not completely satisfactory. The method had previously been used by Miss J. M. Northfield on the same material, and found to give good results, and was also tested as follows by the writer, on stock S.6. Emasculations were carried out on twelve flowers. Six of the stigmas were pollinated with their own pollen, and six were left unpollinated. The six pollinated ones produced seed, but the others failed to do so.

It was therefore, surprising to find that in many cases in which crosses between stocks were replicated, some of the replicated were successful, i.e. produced seed, whilst others failed to do so. Occasionally no seed was produced by any of the replicates. Since all stocks freely produced seed by selfing, failure was not due to intrinsic infertility, and this result could therefore, only have been obained in one of three situations:

1) The emasculation technique was not uniformly successful, and in those failing to produce seed, the stigma had been damaged.

2) Microenvironmental factors might have effected fertilisation in some cases and not in others.

3) There was a difference in reaction between different pairs of geno-types.

All these factors might have had some effect in some cases, and since the analysis of such a situation would be virtually impossible, only those crosses which produced seed were considered to have contributed anything to the results. Selfing was uniformly successful, but unfortunately after the first tests, selfing was carried out without emasculation, so that the information gained from selfing contributes nothing to the solution of this problem, except the fact that the plants are completely self fertile.

It was, therefore, decided to regard failures as accidental, and wherever possible to repeat the cross in the following season. Of the crosses which failed to produce seed, as many as possible were repeated in 1963, the 1962 season having been spent in America. These were crosses between the same plants that had been used in 1961. Some of these replicates were successful in 1963, and on this basis there are no grounds for assuming that the genotype has any effect on the failure of the cross to 'take', but the other two possibilities remain. It was decided, therefore, to regard all crosses as being potentially successful. Enough seed was produced, and of this enough germinated to make the cytological relationship of T.M.1 and T.M. 2 and Stock 10 quite clear.

Preparations of meiosis in pollen mother cells were obtained, and wherever possible, counts were based on at least three preparations, with at least three cells per preparation counted. However, some of the material proved very refractory, and in the diagram (7. 2), an asterisk shows those values based on fewer than 9 counts.

Both groups of the test material had a chromosome number of $2n = 96$ whilst the Irish material of S. angustifolium Mill. had a chromosome number of $2n = 88$, probably being an aneuploid form of $2n = 96$. (See Chapter 3).

It became apparent that the test material differed cytologically from the Irish material of S. angustifolium Mill., and that the test material itself could be divided into two groups based on cytology. A cross between plants which were not cytologically identical always gave a variable number of bivalents (i.e. the number varied from cell to cell). In a hybrid between two species which each have a chromosome number of $n = 48$, a theoretical maximum of 48 bivalents must be postulated, since it cannot be assumed that chromosomes with little homology never pair. However, since in the case under consideration, the two species are both polyploids, and both normally have very regular pairing, in the absence of other evidence, it must be assumed that the species are amphidiploids, of which the genomes are well differentiated.

If two such amphidiploid species are hybridised, pairing would be expected to occur between genomes having substantial homology, which these amphidiploids contained in common. However, there are two factors which would tend to lead to a reduction of pairing in the circumstances postulated.

1) The genomes will have been effectively isolated for a very long period and therefore, some differentiation is likely to have occurred between them.

2) In the hybrid genotype, the timing of the mechanism of meiosis is liable to be upset, and this may mean that fewer chromosomes pair than are cytologically capable of doing so.

In any particular case, it seems probable that the two factors will combine to reduce the bivalent number from the number expected theoretically from substantial homology. For the rest of this argument, this latter value i.e. the theoretical maximum bivalent number in any cross expected from substantial homology, will be termed X.

In any cross, therefore, a mean bivalent number will be obtained which maybe considerably lower than X. Moreover, the mean bivalent number obtained between different individuals of the same two species may be different, not merely due to sampling error, but because of differentiation within the species. Is there, therefore, any way of deriving an estimate of X from the observed bivalent numbers?

On the basis of the theory outlined above, the distribution of values obtained for bivalent number per cell will be basically normal in distribution for any particular hybrid, but there will be a sharp truncation of the distribution at the point X (see Fig. 7. 4). At this point there will be a sharp fall off in the probability of pairing. If there has been marked chromosomal differentiation, the mode may fall a long way below this point. If there has been little differentiation, then the mode may be close to the point of truncation In every case, therefore, no matter what the true mean is, or what the variance is, the best estimate of X will be the maximum bivalent number recorded for that cross. In many cases this will be an under-estimate, particularly in those cases in which the mode falls well short of X. However it is very unlikely to be above X.

The results are recorded in Tables 7.2 and 7.3.

It seems reasonable from these results, to draw the conclusion that crosses between TM.1 and Stock 10 give a maximum bivalent number of 13-15, and crosses between T.M.1 and T.M.2 a maximum number of 29-30. Crosses between T.M.2 and Stock 10 suggest that the true maxima should again be about 30.

The question as to whether or not these results can be interpreted in terms of genomic differences now arises. The maximum numbers of

Bivalent number in hybrids between the Test Material and stock 10(Irish)

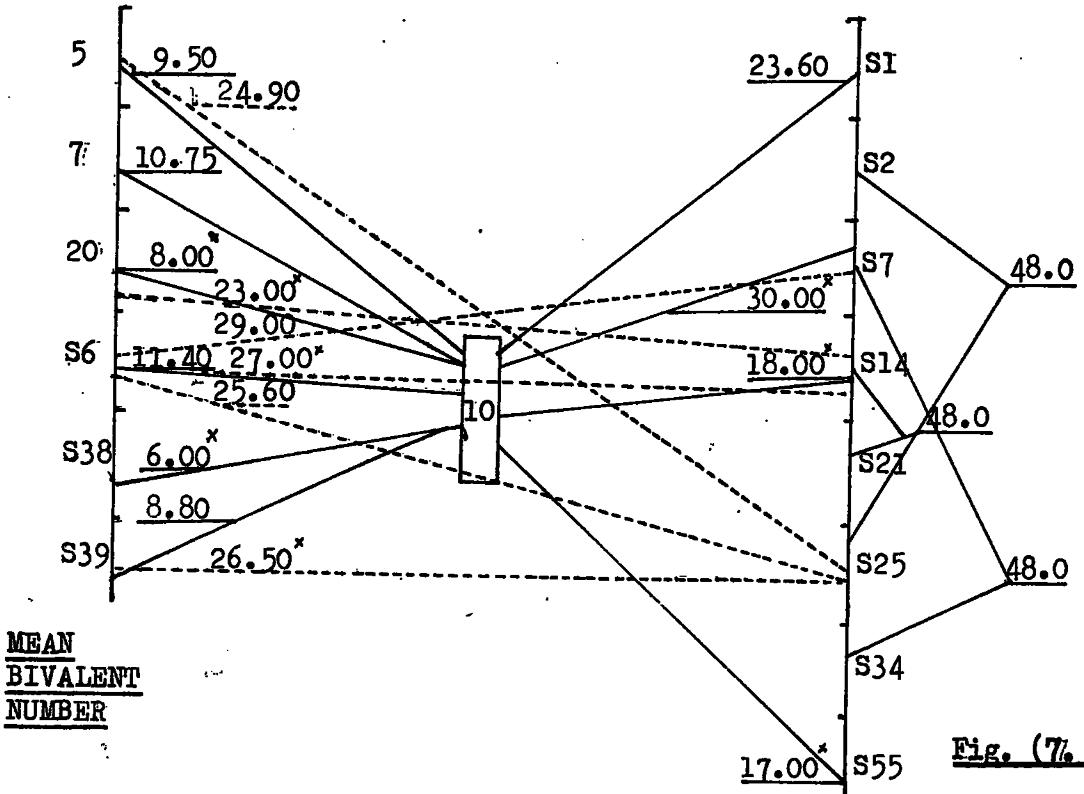


Fig. (7. 2)

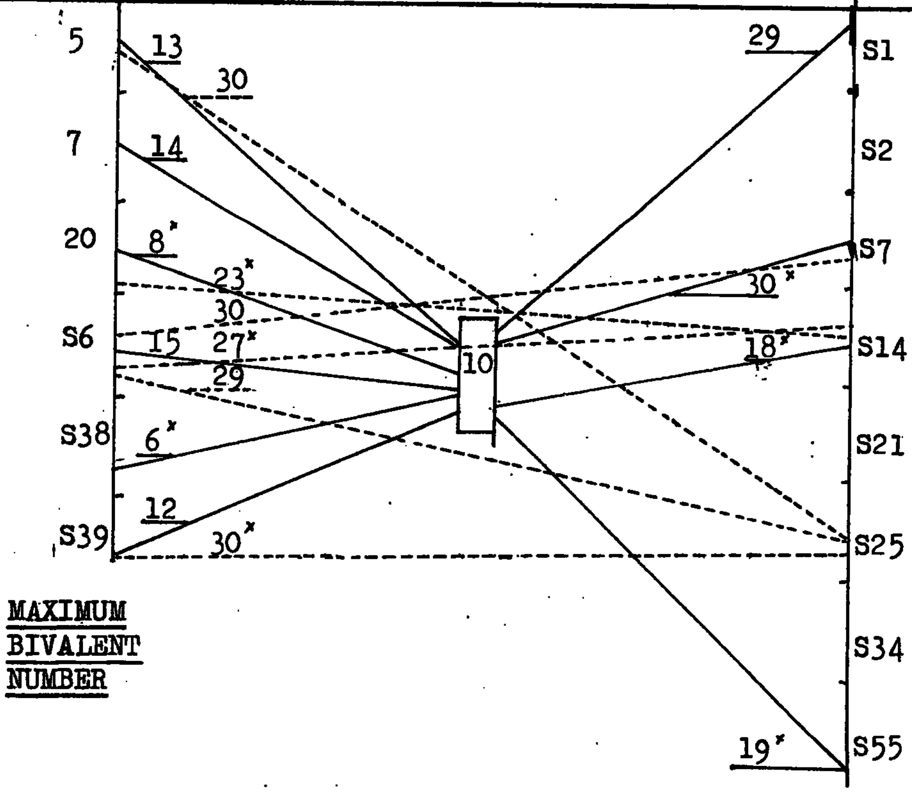


Fig.(7. 3).

The dotted lines indicate crosses between the two groups of Test Material

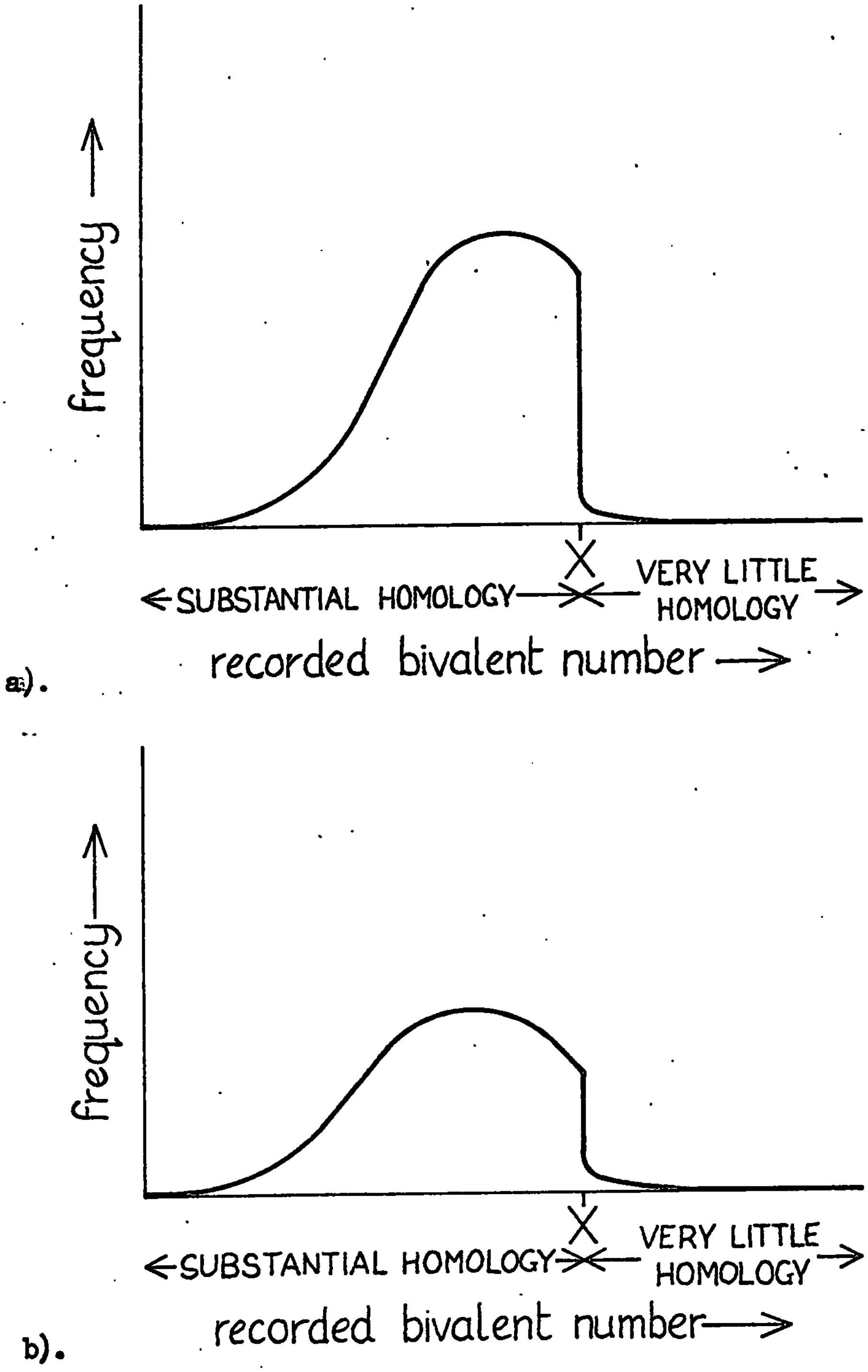


Fig. (7. 4).
Theoretical distribution of values of bivalent number per cell
a) with little chromosomal differentiation
b) with marked chromosomal differentiation.

bivalents in the three types of crosses would suggest that the chromosome number of 96, aneuploid in the case of Stock 10, can be split into three genomes of 32 chromosomes each, and the situation could be simply stated as follows:

Irish material of *S. angustifolium* genomic formula ABC
T.M. 1 " " ADE
T.M. 2 " " ABE

The hypothesis that these three taxa are in fact amphidiploids is supported by the fact that no quadrivalent formation was observed in any preparation. The chromosomes which were not paired as bivalents were seen as univalents scattered throughout the cell.

Even so, the explanation of the situation postulated above is almost certainly an oversimplification. There is some evidence that the species with a diploid number of 96 must be dodecaploids and not hexaploids, since a diploid number of 16 has twice been recorded in this section of the genus. However, since, cytologically, the plants behave as hexaploids they will be treated as such in this work.

With one exception, the morphological grouping of the test material was confirmed by the cytological grouping. The exception is stock S.1 which on morphological grounds was placed in T.M.1, but which is cytologically T.M. 2. The cytological grouping will be used in the rest of this work, since it has greater evolutionary significance.

S. montanum var. *crebrum* from New England must be of the same genomic constitution as T.M.1, i.e. ADE. This was shown clearly from wild hybrids between this species and S. angustifolium found in New England.

The fact that S.55 from California seems to be a member of T.M.2 suggests that the west coast branch of the species S. montanum var. crebrum (S. idahoense Bick.)may differ from the East coast branch in having a different genomic complement, whilst having the same chromosome number. S.55 is the only evidence that T.M.2 indeed corresponds to the west coast branch of the species, and before this cytological difference between the two populations can be accepted as established fact, more material from California must be obtained.

The morphological similarity of the Irish and American plants of S. angustifolium Mill. has already been described, and it was pointed out that they were a continuum from the cytological viewpoint also. There was, therefore, no reason to expect any different reaction when the American plants of S. angustifolium Mill. were crossed with the test material instead of the Irish ones, and indeed no difference was noted until the seeds were harvested. It then became obvious that in most cases of the cross T.M.2 by S. angustifolium Mill. (American) the capsules contained only shrivelled black seed which apparently contained an embryo, but had very little or no endosperm. In the cases of S.1 and S.14, some seed was produced with endosperm, but the seeds were smaller than normal. It is not yet known whether or not these seeds are viable. Reciprocal crosses were not carried out due to lack of American material for use as female parent. It must be emphasised at this point that this cross *never occurs* in nature, because no species having the genomic formula of T.M.2 overlaps with the range of *S. angustifolium* Mill.

There is, then, this very curious difference in reaction to T.M.2 between the Irish and American populations. This reaction occurred with American material from every major sampling area. If then, the Irish plants are a recent introduction from America, they ought to show this same reaction to T.M.2. The fact that they do not, shows that they differ at least in some physiological mechanism.

The situation can thus be summarised as follows:

a) *S. angustifolium* Mill. is represented by two geographically distinct populations.

b) The American population of this species is in contact, on its northern fringe, with *S. montanum* var. *crebrum.* Sterile hybrids between these two species are occasionally formed, but the species are largely separated seasonally and ecologically.

c) The American population of *S. angustifolium* Mill.appears to be separated geographically from any species of the cytological constitution of T.M.2 and is also separated from it by a barrier of hybrid inviability. The Irish population is also separated geographically from any species of the genomic constitution of T.M.2, but is not separated by any barrier of hybrid inviability.

An evolutionary explanation of the situation involves a reappraisal of the concepts put forward to explain the origin of specific isolating barriers.

The phenomena of hybrid sterility and hybrid inviability between distantly related forms are relatively easy to explain in terms of chromosome differentiation and subsequent lack of homology. It is particularly easy in this case, because the species concerned are allopolyploids of different genomic constitution. Thus the hybrid between S. angustifolium Mill. and S. montanum var. crebrum shows lack of pairing between approximately two thirds of its chromosomes, and sterility is easily attributable to this. Similarly, based on the American data alone, it would be easy to explain the hybrid inviability of the hybrid between S. angustifolium and T.M.2 as being due to the great difference in genomic content. However, the Irish data suggests that the situation is not so simple, and it would appear an initial barrier of partial sterility has been deepened to complete inviability. This, of course, assumes that the presence of this inviability barrier follows ~~precedes~~ its absence. The alternative is possible but is not considered to be so probable in this case, because in the cross T.M.1 by T.M.2, which had the same degree of gemonic difference, there was no seed abortion of this type.

The problem is thus the cause of the physiological differentiation between the populations of S. angustifolium.

Without a doubt, if the two populations have been apart a considerable time, they will probably have evolved in response to different ecological factors, and presumably will have adapted themselves physiologically to meet the different conditions. Such alterations may be slight but in the terms of Muller (1942), a new harmonious gene combination will have been formed, which will have new relationships with other populations, and with other species of the same genus. The change may be sufficient to upset the development of the species hybrid, whilst having little or no apparent affect of the inter-population hybrids. This could have occurred in Sisyrinchium, and may explain the situation.

However, this situation in Sisyrinchium has interesting evolutionary implications. A consideration of it suggests a theoretical method whereby species barriers could originate by 'second degree selection'. Dobzhansky (1941) has postulated that species barriers could be the direct result of

> ".......the challenge of hybridisation, leading to the formation of badly adapted genotypes."

He assumes that any mechanism which will prevent the formation of hybrids, will be selected for under such circumstances. These are presumably mechanisms which prevent the gametes coming into contact, such as ecological, seasonal or mechanical isolation. It does not seem possible that barriers of hybrid sterility or inviability could be selected for in this way, since such barriers still lead to a wastage of gametes. However, considering for the moment those barriers which could have originated by the direct action of natural selection, Dobzhansky points out that his hypothesis demands that in order that such barriers may arise, the two incipient species must overlap.

"It is, therefore, not clear how physiologically isolated species could be developed in non-contiguous territories such as oceanic islands, where opportunities for hybridisation are not available".

It would seem that the Mullerian hypothesis of the automatic production of species barriers by the selection of different harmonious gene combinations in different geographical areas could be the only possible explanation of such phenomena.

However, to return again to Sisyrinchium; here there are three basic cytological units, namely:

S. angustifolium Mill, having two geographically separated populations.

S. montanum var. crebrum (T.M.1) which overlaps the distribution of S. angustifolium in New England.

T.M.2. A taxon with an undefined distribution, possibly in Western North America, but not in contact with T.M.1, or S. angustifolium.

S. angustifolium Mill. and T.M.1 i.e. S. montanum var. crebrum are capable of hybridising, but the hybrids are completely sterile, presumably due to their cytological differences. In their area of overlap, however, occasional hybrids do occur, and there will therefore, be some wastage of gametes. The hybrids presumably have S. angustifolium as their female parent, since T.M.1 is almost completely self fertilised. From an evolutionary viewpoint, therefore, there will be selection for any mechanism preventing the formation of these hybrids, with the subsequent raising of the reproductive potential of the pure species. This could occur by an alteration of ecological or seasonal preferences of S. angustifolium. In fact, the two species do differ in both ecological preferences and flowering time, to some extent. If such an alteration has arisen due to 'the challenge of hybridisation', this must have involved the production of new 'harmonious gene combinations', which in this case will presumably have affected only S. angustifolium Mill. but would affect both species if both were outbred. The relationship of these species or other species in the genus, as well as to other populations of the same species, will be altered.

Part of this explanation fits the cases of S. angustifolium quite well. One could postulate that selection has worked on S. angustifolium to produce seasonal and ecological isolation from S. montanum var crebrum. There can have been no similar selective pressure in Ireland. However, the physiological alteration in the American population engendered by this selection, could have been enough to alter the relative reaction between the two geographically separated populations of this species and another quite independant species, represented in this case by

T.M.2. The Irish population is now still able to produce hybrids with T.M.2, whereas the American population shows hybrid inviability. This alteration in the American population would not seem, on the evidence available, to have been enough to alter its relationship with the Irish population, since crosses between the two populations were successful. However, one could postulate that in some circumstances the alteration in physiology of a population due to selection in response to hybridisation with another species, might be enough to cause a barrier with another population geographically separated from it. Barriers of ecological and perhaps mechanical isolation could certainly originate in this way. Whether this could lead to a barrier of hybrid sterility or inviability would depend on the degree of Physiological upset suffered by the hybrid.

This is thus a mechanism which could at least aid the production of species barriers, and could help to explain their existence in cases in which the two populations have never overlapped.

Have these arguments any relevance in the controversy concerning the length of time Sisyrinchium has been present in Ireland? Obviously the answer must be that the two populations have been apart sufficiently long to allow this physiological differentiation. If the alteration has been mainly in the American population, as the selection hypothesis suggests, then they must have been apart a long time, because this gene combination causing seed inviability with T.M.2 seems to occur throughout the large American population. If, however, there has been no such direct or indirect effect of selection following on hybridisation, but merely a random drift in gene content, then any new gene combination having a selective advantage could presumably spread through the relatively small Irish population quite quickly. This is the relative effect of the situations. The actual time scales involved must remain obscure, though they are probably of quite a high order, because the plants are perennial.

CHAPTER V111

Aspects of fertility in the test material

It has previously been explained that only crosses which produce seed have been considered to contribute anything to the results of this work. Seeds were obtained from from replicate crosses between stocks for cytological examination. It was not anticipated that the viability of the seeds themselves would show any interesting features. If such results had been anticipated, then the contents of each capsule resulting from crossing between stocks would have been sown separately.

Table (8. 1) gives results of germination of crosses between stocks, successful germination being represented as a g fraction of the numbers of seeds sown.

The first observation from these results taken as a whole, is that seed viability is generally low. Secondly, it is irregular. It is very difficult to analyse such results, because in many cases, the number of seeds sown was too low to be a reasonable sample of the cross. It was, therefore, decided that the only possible means of analysis was to divide the table up according to the cytological grouping of the stocks. The table, therefore, consists of:

a) Crosses within T.M.1

b) Crosses within T.M.2

c) Crosses between T.M.1 and T.M.2

d) Crosses between T.M.2 and T.M.1

Germination of seeds from crosses within the Test Material Fig.(8. 1).

	T.M.1										T.M.2							
	5	6	7	20	S6	S17	S38	S39	S40	S47	S1	S2	S7	S14	S21	S25	S34	S55
5			0/19	0/16	0/18						0/24		0/8	0/16	0/1	0/18		
6	1/18		4/34	0/3	0/28	8/22					0/1	1/14		0/19	0/9			
7																		
20					0/8	0/63	0/40			0/27	0/3	0/5	0/31	0/4	0/75	0/59		0/26
S6	11/40	19/20		0/12		1/12	0/2		2/4	8/14			01/21	0/3				1/27
S17				0/20									0/15		0/10	0/7		
S38	6/15	0/11		2/16	0/30				2/30	0/4	2/9	0/35			1/1	0/38	0/7	0/26
S39	0/30			0/15	0/31							0/37					0/3	
S40				0/60		0/21							11/29					7/10
S47	3/37	0/6		5/6	2/6	12/25	0/47	3/21	0/19		4/9	1/3	0/9		0/1	0/1		0/2
S1	16/35	5/7		5/20		0/11	0/1			2/2		0/9	0/7	0/4		0/7	0/38	6/6
S2					2/32		14/23	4/43		0/40	1/8		0/5	1/21	6/22	5/17	0/1	
S7		0/19			12/23		12/18	4/7		8/20	0/17	13/22				7/10	7/8	
S14	28/52	1/17		22/30	11/17	0/5	0/22	0/14	1/3	0/8	2/3	1/4	1/16		0/4	0/12	0/2	0/7
S21	0/19	0/5		0/35		0/4	0/1	0/29						17/30			2/8	
S25	3/6				4/4			1/1	2/8	2/2		1/2	3/4					
S34					0/1								25/32	0/8	6/14	20/22		
S55		0/12		0/12	4/31	1/36	0/29	0/2		31/38	8/38				0/3	0/4	2/3	

Crosses within T.M.1 and T.M.2

Examination of the data suggested that, between the two groups, the seeds produced by crosses within the groups differed in their viability. Overall viability for seeds produced by crosses within T.M.1 was 11.27% and for crosses within T.M.2, 32.12%. However, it was at the same time noted that overall seed production seemed to be lower in crosses within T.M.2.

Table (8. 2) gives the numbers of seeds per capsule which together make up each of the denominators in Table (8. 1). The average seed number per capsule for crosses within T.M.1 is 15.29, and for crosses within T.M.2, 9.26. The difference between these is highly significant (P is less than 0.1%).

Capsules obtained by selfing give no significant difference in seed number per capsule between the two groups, the average being 21.50 seeds per capsule. The reduction to 15.29 recorded for crosses within T.M.1 could perhaps be due to less efficient pollination by hand than that achieved by selfing without emasculation, but this leaves the further reduction for crosses within T.M.2 unaccounted for. There would not appear to be any reason why hand pollination should be less efficient in one group than in the other.

A possible hypothesis is that a proportion of seeds from crosses within T.M.2 aborted. The question that now arises is whether the apparently higher viability of seeds from crosses within T.M.2 could be because a proportion of the seeds aborted, which in crosses within

Seeds per capsule from crosses within the Test Material Fig.(8. 2).

	T.M.1										T.M.2							
	5	6	7	20	S6	S17	S38	S39	S40	S47	S1	S2	S7	S14	S21	S25	S34	S55
5			19	16	18						24		8	16	1	18		
6	18		34	3	28	22					1	14	18 1		8 1			
7																		
20					8	20 32 11	16 24			5 22	3	2 3	31	4	27 18 30	36 23		26
S6	15 25	18 2		12		10 2	2		2 2	12 2	2		23	3				20 7
S17				7									15		10	7		
S38	15	11		8 8	30			22	30	4	9	5 30			1	38	7	26
S39	30			15	31								12 25				3	
S40				25 35		21							20 9		30			10
S47	18 19	6		5 1	6	25	20 27	21	14 4 1		5 4	3	9		1	1		2
S1	19 16	4 3		10 3 7		5 6	1			2		7 2	7	4		7	4	6
S2					12 20		23	18 25		26 14	8		5	21	22	16 1	1	
S7		19			23		18	7		20	17	22				10	8	
S14	20 32	16 1		30	13 4	5	22	12 2	3	8	3	1 3	9 7		3 1	9 3	2	7
S21	19	5		35	4	1	29							30			8	
S25	6				4			1	2 6	2		1 1	3 1					
S34					1								32	8	14	22		
S55		12			12	31	6 20 10	29	2	13 25	16 22				3	4	3	

T.M.1 were apparently normal, though inviable. If it is assumed that the real seed production before abortion is the same as that of crosses within T.M.1, the percentage viability would be 19.47%, which is much nearer that of T.M.1, though still appreciably higher.

It is interesting at this point to examine what data is available concerning normal germination of seeds produced by the two groups. The only data available is the germination data for the seed samples from which the stocks were grown. No specific tests were carried out, because the relevance of such data was not apparent until fairly late in the investigation. The data is only approximate, because the seed pots were kept for only one season (those of the crosses were kept for two seasons, and a few seeds germinated in the second year.). The number of seeds sown was approximately 50 in each case. Of the seedlings produced, 24 were kept to represent each stock. Seedlings in excess of this number were discarded. It can, therefore, be safely stated that the normal germination rate is at least 50% for all the stocks considered, with the exception of stocks 5, 6 and 7, which were passed on to me from a previous investigation by Miss J.M. Northfield. It would obviously have been more satisfactory to have had precise data, and it would also have been useful to have had data on the results of crosses within the stocks.

However, the data as it stands suggest the following points:

1) Self pollination leads to the production of seeds which are at least 50% viable.

2) Crossing between stocks which are apparently cytologically identical leads to a marked reduction in viability.

3) In the case of crosses within T.M.2, pollination between stocks leads to a reduction in number of seeds per capsule.

It is difficult to postulate a genetical situation which would account for this, but the following is a possibility.

In Chapter 3, it was pointed out that in S. montanum var. crebrum self pollination is virtually obligatory, because of the arrangement of the anthers and the style. This is not the cases in any other species of Sisyrinchium discussed in this work. The most similar situation is that in S. angustifolium Mill., in which the relationship between anthers and style, appears to show a considerable degree of developmental plasticity. In all the other species discussed, the style projects well above the filament tube.

Stebbins (1950) in his discussion of alloploidy, points out that most newly formed allopolyploids are only partially fertile. Most of the examples of such infertility described by Stebbins, appear to have been ascribable to chromosomal upsets at meiosis in the hybrid due to heterogametic association, and asynapsis of homologous chromosomes because of physiological disharmonies in factors affecting meiosis. However, these need not be the only factors capable of causing sterility in the 'raw' allopolyploid. It could equally well be argued that the disturbance producing sterility could occur either during endosperm production, or during endosperm-embryo interaction. Thus it is known that in some F 1 hybrids, there is disharmony between endosperm and embryo, which can be overcome by excising the embryo from the endosperm and culturing it (Blakeslee A.F. and Satinas, 1944). There is no reason why seed inviability in a raw allopolyploid should not be of the same nature.

The balance between genomes present in triple dosage in the endosperm, is new and untried, and endosperm production may be unsuccessful, or only partially successful. Alternatively, the genetic relationship of embryo and endosperm is also untried, and may be physiologically incompatible.

It would seem possible then, that such mechanisms could lead to seed inviability in the seed of the 'raw' allopolyploid.

Any genetic changes leading to greater harmony would be at a selective advantage in such a situation. However, if the plants are exclusively self pollinated, then the gene changes producing such an increase in fertility need not be the same in different progenies. It could be postulated that the end result of such a system would be that plants would be reasonably self fertile and reasonably fertile with their ancestors and relatives, but there is no reason why distantly related lines should be interfertile, since a different genetic mechanism achieving the same end may have been selected in different lines.

Such a situation is very similar to the situation described in crosses between stocks of plants which are cytologically identical in the test material.

The whole argument really hinges on whether or not there is any evidence that embryo development is affected by genomic balance in the endosperm, or with the endosperm. The data for reciprocal crosses between the two groups of the test material suggests that there is evidence of such a system.

Crosses between T.M.1 and T.M.2

Average seed germination for crosses T.M.1 by T.M.2 is 4.3%, whilst seed germination for T.M.2 by T.M.1 is 31.90%. The average number of seed per capsule is almost identical in the two groups, i.e. 12.70 and 12.2 so that the difference in seed viability must be real. Obviously, the genomic constitution of the embryos of the two types of cross must be identical, i.e. AABDEE. The only factor which could cause the difference is the endosperm, which in one cross will be AAADDBEEE, and in the other, AAABBDEEE. The former combination appears to be unsuccessful, and the latter relatively successful.

The genomic balance therefore, appaears to be of considerable importance in determining seed viability, which lends support to the theory outlined above.

SYNOPSIS

The work for this dissertation has fallen into three parts:

1) The accumulation of cytological and phytogeographical data for the section Bermudiana of the genus Sisyrinchium, in order to obtain a complete picture of the present day dituation, so that the evolutionary situation might be elucidated as far as possible.

2) The detailed cytological analysis of the Irish and American populations of S. angustifolium Mill. in the attempt to trace any genetical divergence between the populations.

3) An analysis of the breeding structure of S. montanum var. crebrum.

The work for all these sections is necessarily incomplete. An adequate treatment of the first section alone would involve many years work. Interesting situations have been revealed in all sections, which would in themselves offer fruitful projects for further research.

A brief survey of the conclusions reached and the problems raised in each section will now be in order.

1) Cytology and phytogeography of species of Sisyrinchium occurring in the North Eastern States of the U.S.A.

Cytologically there are two groups of species

Group 1 Tetraploid species having 2n = 32 (S. atlanticum, S. capillare S. albidum and S. mucronatum)

Group 2 Dodecaploid species having $2n = 96$ (*S. montanum* var. *crebrum*
S. angustifolium)

The phytogeographical grouping of these six species corresponds to their cytological grouping.

Group 1 Species occurring in specialised habitats over a fairly wide geographical area.

Group 2 Species occurring in a wide range of habitats over a large part of their geographical range, though showing greater habitat specialisation at the edges of their ranges.

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Group 1 in this shows features which suggest either that the species are at the edges of their ranges, in which case their main range must be in South o~~f~~ Central America, or else the situation represents the end of an evolutionary chain of events of which most of the links are missing.

There is evidence for each of these views. In the first place, there are now two counts of $2n = 16$ recorded, one from Chile, and the other from Texas. This suggests that diploid species are still in existence further south, and that the ancestral diploids and tetraploids may still occur. However, the evidence put forward from the study of the Irish population of *S. angustifolium* suggests that this is a relict of a former much wider distribution of this species, and this is a dodecaploid species. If the range of this species has contracted to a great degree, then the polyploidy in the genus must be very old indeed, and there would seem to be every possibility that the parental species of the allopolyploids may be extinct, and the diploids still in existance may be irrelevant from the point of view of tracing ancestries.

It has proved impossible to work out the relationship of the tetraploids and dodecaploids which do occur, because crosses between the two chromosome levels appear to be impossible. It would seem that the missing octoploids would be necessary to carry out studies on the origin of the polyploids.

2) The American and Irish populations of S. angustifolium Mill.

It was shown that cytologically, the American population formed an aneuploid series. Unfortunately, the crosses between the members of the series are not yet mature. The Irish population fits into this aneuploid series, but does not within itself show aneuploidy. This could be used as an argument in favour of the recent introduction of Sisyrinchium into Ireland, as this cytological situation would be expected if only one or few biotypes had been introduced. However, crosses carried out between S. angustifolium Mill, and the complex taxonomic unit S. montanum var. crebrum suggest that there has been some genetic differentiation between the Irish and American populations of S. angustifolium. There is no way of knowing how rapidly this may have occurred.

3) The breeding structure of S. montanum var. crebrum.

It was found that seeds derived from crosses carried out between cytologically identical stocks of this species showed a marked degree of infertility. A theory is put forward to explain this. Further data on crosses carried out within stocks is really essential for any further study of this problem. The problem really arose as a sideline, during tests of the cytological identity of these stocks.

Another sideline which could well be developed, is the interesting range of breeding systems displayed by the species in this section.

The project as a whole has become a series of 'preliminary investigations' many of which deserve further study.

APPENDIX 1

Variation in S. angustifolium Mill. and S. mucronatum Mich.

The measurements tabulated in tables A1.1 to A1.4 are taken from herbarium specimens. The aim is to give an indication of the kind of variation which occurs in nature, and the way this variation is distributed geographically. It has been convenient to treat the samples as discreet units, and to test for significant differences using the 't' test. The results of this proceedure are given in table A1.5.

S. angustifolium Mill.

Table A1.5 shows that the sample from Area 2a has a significantly greater stem width, leaf width, and bract length than samples from the other areas to the North and West.

S. mucronatum Mich.

The measurements again show that the samples differ quantitatively. Bract length only was tested for significance, because the measurements of stem width and leaf width were often so small as to introduce a large error in measurement.

The ratio of inner and outer bract shows no significant difference between the samples. This merely underlines the fact that the difference is purely a matter of overall size, and not of proportion.

Stem Width of S.angustifolium Mill.

Areas IB, IC,&2B	Area 2a	Area 3A
2.75mm.	3.00	2.25
2.75mm.	4.00	2.50
3.00	3.25	3.20
2.50	4.25	2.80
3.00	3.00	2.50
2.25	2.50	2.00
3.25	3.25	3.50
3.25	3.25	2.80
2.75	2.50	3.00
2.50	2.75	3.00
3.00	3.75	2.00
2.00	3.50	2.00
2.75	3.00	2.00
3.50	2.50	2.60
3.00	3.75	1.80
2.25	3.25	2.20
2.00	4.15	3.50
2.50	2.00	2.50
2.25	4.75	3.50
3.25	3.75	2.80
2.25	3.00	3.50
3.50	2.75	3.50
2.75	2.50	2.50
2.50	3.75	3.00
3.00	4.75	2.50
	3.50	3.50
	2.25	3.50
		3.00
	1.50	1.50
		2.00
		5.00
		2.50
		2.80
Mean 2.89	Mean 3.30	Mean 2.76

Fig.(A1. 1).

Leaf width of S.angustifolium Mill.

Areas 1B, 1C & 2B	Area 2A	Area 3A
3.75 mm.	3.75 mm.	2.80 mm
2.50	4.75	4.20
3.00	3.25	2.50
2.50	4.00	2.40
3.30	3.25	2.50
3.50	2.75	3.00
3.25	3.50	3.50
3.25	3.25	3.50
3.25	2.75	3.80
4.25	3.00	3.00
3.50	4.00	3.50
1.75	4.00	1.50
1.50	2.75	1.50
2.75	3.75	3.00
2.75	3.50	2.00
2.75	4.00	4.50
2.75	3.50	4.00
1.75	2.25	3.10
2.50	4.50	3.00
4.75	5.25	3.50
2.25	3.25	3.00
2.75	3.75	2.50
3.50	3.25	2.80
2.00	4.00	3.50
2.75	4.25	3.50
	4.75	3.50
	3.50	1.50
		2.00
		2.50
		2.00
		2.00
Mean 2.91	Mean 3.64	Mean 2.89

Fig. (Al. 2).

Bract Length of S.angustifolium Mill.

Areas 1B, 1C & 2B	Area 2A
9.40cms.	8.00
7.15	11.40
7.25	5.50
7.50	8.70
10.70	10.80
7.55	6.70
9.00	9.15
6.85	7.85
9.75	6.50
8.85	9.50
7.10	12.05
8.35	9.60
7.70	10.10
7.80	9.90
	10.60
	12.15
	13.30
	10.65
Mean 8.21	9.55

Fig.(Al. 3).

S.mucronatum Mich.

Areas 1A, 2a & 2A

Length of outer bract	Length of inner bract	Ratio of bracts	Width of stem
3.60cms.	1.80cms.	2.00	0.20cms.
3.65	1.95	1.87	0.10
3.40	2.00	1.70	0.16
3.25	1.95	1.66	0.10
4.23	2.05	2.06	0.20
4.55	2.35	1.94	0.20
4.90	2.20	2.22	0.15
4.70	1.90	2.47	0.20
4.25	1.95	2.18	0.20
4.80	1.90	2.52	0.15
3.90	2.10	1.85	0.20
3.90	2.25	1.73	0.15
3.80	2.05	1.85	0.30
3.80	2.10	1.80	0.15
4.40	2.25	1.95	0.20
3.60	2.15	1.67	0.20
4.20	2.00	2.10	0.15
3.30	1.75	1.85	0.15
4.10	2.00	2.05	0.20
3.80	2.00	1.90	0.20
3.30	1.90	1.73	0.15
Mean 3.97	2.02	1.96	

Area 3B

Length of outer bract	Length of inner bract	Ratio of bracts	Width of stem
2.80	1.75	1.60	0.10
3.35	1.70	1.97	0.10
2.70	1.80	1.50	0.10
3.65	1.80	2.03	0.05
3.75	1.85	2.03	0.05
3.35	1.90	1.76	0.05
2.80	1.65	1.69	0.10
2.50	1.80	1.38	0.10
2.90	1.65	1.75	0.10
4.40	1.55	2.84	0.10
3.10	1.20	2.58	0.10
Mean 3.21	1.69	1.92	

Fig.(A1. 4).

Comparison of the Means

S.angustifolium Mill.

Character	Area	with Area	P
Stem width	1B,1C & 2B	2a	0.005
	3A	2a	0.001
	1B,1C & 2B	3A	Not Sig.
Leaf width	1B,1C & 2B	2a	0.001
	3A	2a	0.001
	1B,1C & 2B	3A	Not sig.
Bract length	1B,1C & 2B	2a	0.05

S.mucronatum Mich.

Character	Area	with Area	P
Outer bract length	1A,2A & 2a	3B	0.001
Inner bract length	1A,2A & 2a	3B	0.01
ratio	1A,2A & 2a	3B	Not sig.

Fig.(Al. 5).

APPENDIX 2

Taxonomic position and Life History

The genus Sisyrinchium is a member of the herbaceous monocotyledonous family Iridaceae. The genus is characterised by having a fibrous root system with a very short rhizome; narrow leaves; a stem which is always two edged, and usually winged; and a perianth with segments equal.

Pax, in Engler and Prantl (1899) divides the genus into four sections as follows:-

1) Eriphlema

"Flowers campanulate, pink or purple; the upper parts of the stamens free e.g. S. grandiflora Dougl. from California and British Colombia. There are two other species in Chile."

2) Bermudiana

"Flowers patent, blue, purple, or white and purple striated; stamens fused, or nearly so, up to the top. 12-15 species in Tropical and Subtropical America. However, there are several species with a broader distribution, such as S. bermudiana L., which is naturalised in Mauritius, Australia and Ireland. Four species of this section form a group (Cephalanthes Baker) which is native in Brazil and the Andes, having leafless stems and caespitose inflorescences. There are several species of the section Bermudiana in cultivation, especially several varieties of S. bermudiana L. and S. iridifolium H.B.K. (ground colour whitish)".

3). Echthronema

"Flowers patent, yellow, the upper parts of the stamens free. Many species from Brazil and the Andes and several from Chile. S. californicum Dryand. with leafless stem, found in California. There are several species in cultivation e.g. S. convolutum Nocca and S. graminifolium Lindl. Also S. palmifolium L., with a crowded head of spathes, and S. striatum Sm., with a group of spathes arranged in a spike."

4). Nuno

"Stamens free to the base e.g. S. nuno Benth. from Chile and S. filifolium (Hooker) from Tierra del Guego."

Although some of the geographical distributions mentioned in the above description are vague, incomplete, or even inaccurate, the morphological basis of the sections hold for all the species examined, and it seems that the sections have evolutionary significance also. This study was concerned with species belonging to the section Bermudiana, but as species of the sections Echthronema and Eriphlema occur within the same geographical range, an attempt was made to make crosses between these sections, using S. californicum Dryand. (Echthronema), Sisyrinchium sp. (Eriphlema), and many species of the section Bermudiana. These crosses were completely unsuccessful. No capsules were developed and no seeds were formed. In intrasectional crosses such complete failure is not usually seen. Even if the cross is unsuccessful to the point of producing no seeds, there is usually a very small capsule formed, which shrivels away.

Life History of the species in the section Bermudiana

The plants are grown easily from seed. If the seed is sown in January or February, it will germinate in April and May. The plants will not flower in their first year, but flower freely in their second year. The plants are perennial, and increase by a process of tillering. Each shoot consists of a single flowering stem and its leaves, which enclose the base of the stem. The plants can also reproduce by the production of young plants at the end of a flowering stem

This happened in a number of plants which had been brought over from America. Plants grown from seed in this country showed no sign of this phenomenon. It may therefore be a reaction to abnormal conditions.

The second year plants flower in May and June. Flowering is controlled by light conditions, and flower buds only open in full sunshine. In dull weather the flower buds may not open at all, and in the next sunshine two buds which have emerged sequentially from the spathe bracts may flower on the same day. The flower remains open for a maximum of six hours, and then the perianth shrivels and appears to autolyse, degenerating to a drop of liquid containing a few strands of tissue.

In some species the anthers dehisce before the flower is open, or else immediately after opening. These species, e.g. S. montanum var. crebrum are self compatible and it is necessary to emasculate before the anthers burst. It is possible to do this in the morning (before 9 a.m. B.S.T.) by cutting off the unopened perianth members, and removing the anthers from the filament tube with a pair of sharp forceps. Emasculation is tedious and lengthy, and this limits the number of crosses that can be made in any one day.

The style is very delicate, but broadens slightly at the top just before dividing into the free stigmatic lobes. These lobes remain closed until the flower is about to open. In some species they turn outwards before the flower opens, and in others, remain closed until the perianth opens. The inner receptive surfaces are exposed by this process of bending outwards, and the stigmas are then receptive.

The capsules ripen during late July or in August. The three carpels become papery and then split, and the hard black seeds are exposed. In most species these seeds seem to require a period of dormancy before germination will take place. The seeds will remain viable for a considerable length of time. Seeds 6 years old have germinated readily, so they may remain viable for much greater periods. The seeds were stored in a dark dry cupboard, but were given no other special treatment.

Many species of this section are hardy in the British climate, even if they have been transplanted from a much warmer climate, such as the warm temperate climate of North Carolina and Virginia. However, a few plants were lost in the very severe winter of 1962-3. A few plants were kept in a heated greenhouse during the winter 1960-1 to see if they did better there than in an unheated house. However, despite careful watering, these suffered badly from Botrytis and looked much less healthy than those that remained in the unheated house.

APPENDIX 3

Habitats of S. angustifolium Mill. in North Carolina, and Tennessee

Area	
1A	Moist open woodland
	Woodland on clay soil in standing water
	Well drained shaded roadside
1B	Grazed grassland on bank of stream
	Garden weed on dry shaded soil
	Shaded roadside
1C	Open marshy ground
	Loose soil on river bank
	Disturbed soil on dry roadside
	Well drained open river bank
	Dry, sparse, grazed grassland in limestone area
	Dry shaded ground on the edges of woodland
	Stream bank in deciduous woodland
	Edge of path in pine woodland
	Very damp open ground on lake shore
	Very dry rocky ground in *Thuja* grove

Other habitats similar to those listed were also recorded from areas 2a and 2A. One interesting habitat in area 2a should be mentioned. The species was found growing in Brackish conditions on the estuary of the Chichahominy River in Virginia.

APPENDIX 4

Cytological material and methods

At the beginning of the investigation, chromosome counts were made during mitosis in root-tips. However, when a few successful preparations had been achieved, it was found that the chromosome number obtained from root-tip preparations varied within any one preparation. Two mitotic plates from a root tip of stock S2 are illustrated in Fig. A4.1. The first of these two gives a count of 95 chromosomes, and the second, 86. It is obvious therefore, that in this taxon at least, root tip material is not suitable for making chromosome counts. Similar variability was found in root-tip material of stocks S 1 and S 6. In order to avoid any error, therefore, the use of mitotic material was abandoned, and all the counts recorded in Chapter three and succeeding chapters, were made from preparations of meiosis in pollen mother cells. A metaphase plate of stock S 2. is illustrated in fig. A4.2. This quite clearly gives a count of 2n = 96, and this was quite consistant throughout this preparation. No variation analogous to that occurring in root-tip cells has been recorded for pollen mother cell material.

It is difficult to say whether this variability in chromosome number in root-tip cells is real, or whether it is an artefact due to very small chromosomes overlying one another. Squash preparations, like those illustrated, appear to be quite clear and well spread, which

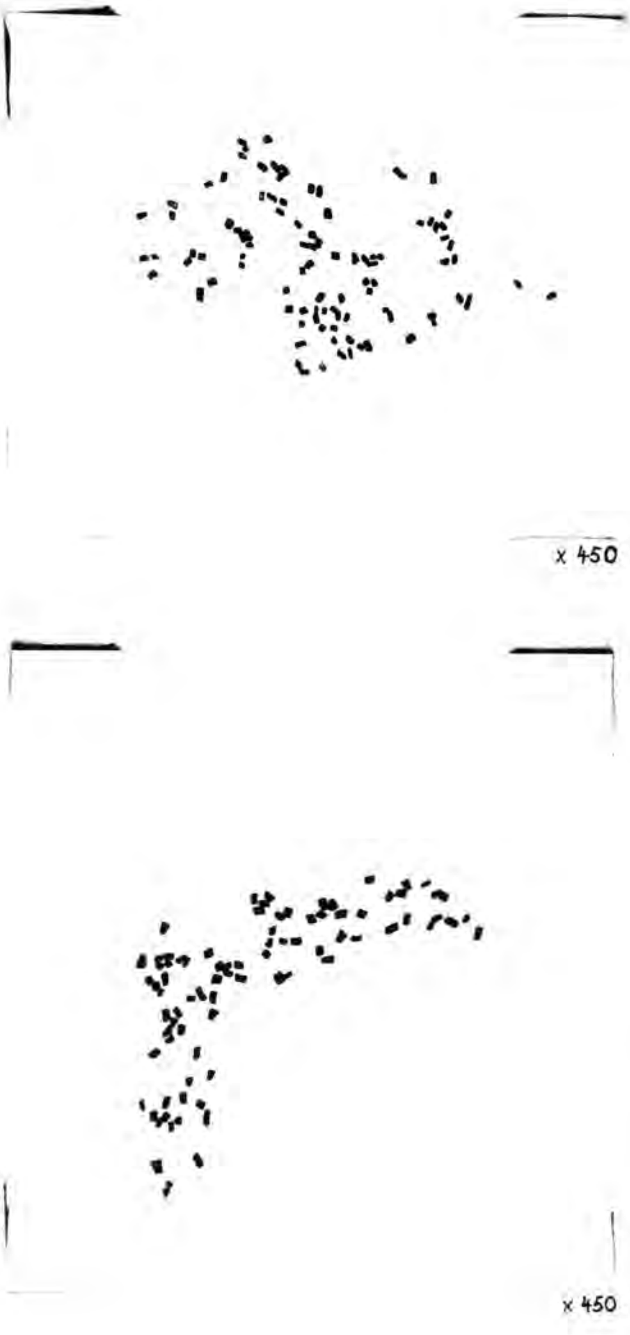

Fig.(A4. 1)
Mitotic plates from a root tip of stock S2, giving counts of 95 and 86 respectively.

Fig. (A4. 2).
Metaphase of meiosis (p.m.c.) in stock S2. This shows clearly that the true diploid number for this stock is 96

a) n = 44

b) n = 42

Fig. (A4. 3).
Diakinesis in aneuploid populations of S. angustifolium Mill.
Populations from a) Clinch R. valley, Tennessee
b) Tolland State Forest, W. Mass.

Fig. (A4. 5).
Meiosis in the hybrid stock 7 x stock 10(Irish)

suggests that the variation might be real.

The decision to abandon root-tip material was confirmed when it became obvious that S. angustifolium Mill. contained aneuploid races in America. Variability in root-tip cells might very well be misleading under these circumstances. Figs. A4.3 and A4.4 illustrate metaphase plates of aneuploid races of S. angustifolium Mill. The theoretical implications of this aneuploidy is discussed in Chapter 3.

Hybrid material was particularly difficult to interpret cytologically. If large numbers of univalents occured, they were difficult to separate. However, satisfactory preparations were obtained. Two such preparations are illustrated in figs. A4.5 and A4.6. The first is a hybrid between stocks having chromosome numbers of $2n = 96$ and $2n = 88$. The hybrid therefore must have $2n = 92$. It is obviously very difficult to pick out the bivalents, though some are obvious. However, by counting the number of discreet bodies present, one can deduce that there must be 12 bivalents. The stock illustrated in fig. A4.6 again has $2n = 92$. A similar process of deduction gives a bivalent number of 30 in this case. The importance of these hybrids is discussed in Chapter 7.

Mitotic material

Somatic chromosome counts were made from root tips, which were obtained from plants grown in pots. An attempt was made to obtain seedling roots by germinating seeds on filter paper. The seeds of only two families germinated under these conditions, and these were collections from California. The other seeds were from Northern latitudes, and perhaps required a period of dormancy. However, this was never proved conclusively, because the experiments were discontinued when it became clear that root tip material was not very satisfactory.

The root tips were pretreated with paradichlorbenzene, and then fixed in acetic alcohol 1:3, and stained by the Feulgen squash method (Darlington and Lacour 1960) using a hydrolysis time of 25 mins., which is very long compared with that necessary for most root tip material. However, maximum staining was achieved with this treatment.

It was found that aceto-carmine was unsuitable for this material because it gave very heavy staining of the cytoplasm. Acetic-orcein failed to stain anything.

The preparations of mitosis and meiosis were made permanent by the quick freeze method (Conger and Fairchild 1953). This method, which is now beginning to come into more general use by British cytologists, has a number of advantages over the floating off method of making squash preparations permanent.

1) It is a much simpler and quicker method, the whole operation being completed in a maximum of seven minutes.

2) The speed of the operation means that when aceto-carmine is used, as it was for meiotic material in this study, the staining of the cytoplasm is reduced.

3) Less material is lost from the preparation.

Material stained by the Feulgen method was taken through Xylene and mounted in Canada Balsam. Slides of the genus prepared by Miss Northfield and left for my benefit, show that slides stained with Feulgen and mounted in Euparal, tend to fade after twelve months. No fading has been observed in Canada Balsam preparations kept for more than two years.

<u>Meiotic Material</u>

Meiosis occurred in pollen mother cells about two weeks before the bud opens, though this time will be altered if weather conditions are unfavourable. The buds mature sequentially in the inflorescence, but by the time that the first flower appears, all the buds have usually undergone meiosis.

Meiosis is regulated by the weather, and occurred only in sunshine, or on warm days with a high light intensity. If the weather is unsuitable, meiosis will be delayed, and the buds will be larger when the pollen mother cells eventually divide. This leads to a considerable wastage of material. In the right weather conditions meiosis usually takes place between 10 and 11 a.m. B.S.T. though this rhythm seems to be upset in hybrid material. However, it was almost impossible to judge at what time meiosis would occur under the different climatic regimes of the United States, and this again led to much wastage. This regularity in division seems easily explicable by assuming that the plant requires a fixed length of time at a certain temperature to stimulate division.

In families where material was scarce, a method devised by Miss Northfield (1961) to obtain buds without damaging the rest of the inflorescence was used. An incision in the spathe bracts was made with a sharp needle. A bud was then selected and removed from the immature inflorescence. If the spathe was then left untouched, the whole of the inflorescence dries out, but if the spathe was covered with a small polythene bag, about 6 cm. x 1.5 cm., there was a chance that the inflorescence would survive and mature normally. About 50% of the inflorescences treated this way survived.

The buds were fixed in acetic alcohol 1:3, containing a few drops of saturated ferric acetate. The aceto-carmine squash method was found to be quite satisfactory for meiotic material. Cytoplasmic staining was not so dense as in root tip material, and the extra time necessary to use the Feulgen technique was considered to be unjustified. Preparations were again made permanent by the Quick Freeze method.

It is clear from this description that Sisyrinchium was not easy to deal with cytologically. Preparations were difficult to obtain, and then often difficult to interpret due to the large numbers of chromosomes present, and the fact that they all varied enormously in size. It was particularly difficult to interpret some of the hybrid material, but interpretation was possible in a reasonable number of cases.

Most of the cytological illustrations have been drawn by camera-lucida, because it was considered that a greater amount of accurate observation could be illustrated by this method than by photographic techniques which are usually only satisfactory when the interpretation of the preparation is completely obvious. The correct interpretation of many preparations could have been illustrated by taking a number of photographs at different levels, but it would then have been necessary to superimpose them mentally. The same effect is achieved much more simply by a camera-lucida drawing.

APPENDIX 5

Irish Material

Co. Kerry

1. 1 Cloonee Loughs, grassy bank

1. 2 Cloonee Loughs, gravel at waters edge

1. 3 Snave Bridge, Ballylikey, tussock in marsh near sea

1. 4 Drominassig Bridge, Kenmare, alluvial level near waters edge.

1. 5 Sheen R. Bridge, damp meadow

1. 6 Sheen R. Bridge, hedgerow

1. 7 Shore of Upper Lake, Killarney, silt

1. 8 Shore of Upper Lake, Killarney gravel

1. 9 Shore of Ross Island, Killarney, limestone shore line

1. 10 Boolteens, Castlemaine, damp meadow

1. 11 East of Boolteens, Castlemaine, damp meadow

1. 12 Outflow from Lough Caragh, grassy river bank

1. 13 Shore of Lough Caragh, grazed grassland

Co. Galway

115 N. bank of Woodford River, damp meadow

116 Edge of Woodford River, 1 mile East of Woodford. Grassy bank

117 Meadow in same locality as above

119 Bank of Woodford River at Rossmore Bridge

Co. Sligo and Donegal

120 Entrance to Glencar, Sligo, damp meadow

121 Killybegs, Donegal, water meadow.

Table A5. 1 gives the associated species at each of these sites.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Equisetum arvense L.	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–
Isoetes lacustris L.	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Achillea ptarmica L.	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Achillea millefolium L.	x	–	–	x	–	–	–	–	–	–	–	–	x	–	–	x	–	–	–	–	–
Alchemilla vulgaris agg.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–
Anemone nemorosa L.	–	–	–	–	–	–	–	–	x	–	⊖	–	–	–	–	–	–	–	–	–	–
Angelica sylvestris L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–
Bellis perennis L.	x	–	–	–	–	–	–	–	–	–	x	–	–	–	x	–	–	–	–	–	–
Cardamine pratemsis L.	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–
Centaurea nigra L.	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x
Cerastium arvense L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–
Cirsium dissectum (L)Scop.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–
Cirsium palustre (L)Scop.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x
Conopodium majus(Gouan)Loret	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x
Filipendula ulmaria(L) Maxim.	x	–	–	–	x	–	–	–	x	–	–	x	–	–	–	x	–	–	x	x	–
Fragaria vesca L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–
Galium saxatile L.	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Geranium sp.	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–
Hedera helix L.	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hieracium pilosella L.	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–
Hypochearis radicata L.	x	–	–	–	–	x	–	–	–	–	x	–	–	–	x	x	–	–	–	–	–
Lotus corniculatus L.	–	–	–	–	–	–	–	–	x	–	–	–	–	–	x	–	–	–	–	–	–
Lobelia dortmanna L.	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lysimachia nemorum L.	x	–	–	x	–	–	–	–	–	⊖	–	–	–	–	x	x	–	–	–	–	–
Medicago lupulina L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–
Mentha aquatica L.	–	x	x	–	–	–	–	–	x	–	x	–	–	–	–	x	–	–	–	–	–
Plantago lanceolata L.	x	–	–	x	–	–	–	–	x	x	–	x	x	–	x	–	–	–	x	x	x
Plantago media L.	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Potentilla anserina L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–
Potentilla erecta (L)Rausch	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–
Potentilla reptans L.	–	–	–	–	–	–	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–
Primula vulgaris Huds.	–	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ranunculus bulbosus L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	x	–	–	–	–	x	–

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Ranunculus ficaria L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Ranunculus repens L.	-	-	-	-	-	-	-	-	-	x	x	x	-	-	-	-	-	-	x	-	-
Rhinanthus minor L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
Rosa sp.	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
Rubus sp.	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rumex acetosa L.	-	-	-	-	-	-	-	-	x	x	-	-	-	-	x	-	-	-	-	-	-
Subularia aquatica L.	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
Taraxacum officinale Weber	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-
Trifolium sp.	x	-	-	x	-	x	-	-	-	x	-	-	-	-	x	-	-	-	-	-	-
Ulex gallii Planch.	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-
Vicia cracca L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
Viola sp.	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
Dactylorchis maculata agg.	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endymion nonscriptum(L)Garche	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Juncus articulatus L.	-	-	x	-	x	-	x	-	-	x	-	-	x	-	-	-	-	-	-	x	x
Juncus effusus L.	-	x	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-	-	-
Luzula campestris(L) D.C.	-	-	-	-	-	x	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
Luzula pilosa(L) Willd.	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Luzula sylvatica(Huds.)Gaud.	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carex flacca Scheb.	-	-	-	-	-	-	-	-	x	-	x	x	-	-	-	x	-	-	-	x	-
Carex panicea L.	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carex sp.	-	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
Agropyron repens(L)Beauv.	-	-	-	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	x	-	-
Agrostis stolonifrea L.	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
Agrostis tenuis Sibth.	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Anthoxanthum odoratum L.	-	-	-	-	-	x	-	-	x	-	-	-	-	-	x	-	-	-	-	-	-
Deschampsia flexuosa(L)Trin.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-
Festuca rubra L.	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molinia caerulea(L)Moench.	-	-	-	-	-	-	x	-	x	-	-	x	x	-	-	-	-	-	-	-	-
Nardus stricta L.	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-
Phleum pratense L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
Poa pratensis L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
Bryophytes	-	-	x	x	-	x	θ	x	x	x	x	x	-	-	-	x	-	-	-	x	x

APPENDIX 6

Sources of Test Material

		Received as:
5	St. Andrews Botanic Garden	S. sp.
6	St. Andrews Botanic Garden	S. sp.
7	St. Andrews Botanic Garden	S. sp.
20	Collected wild. Newfoundland. T. T. Elkington	
S1	Royal Botanic Garden, Edinburgh	S. graminifolium
S2	Royal Botanic Garden, Edinburgh	S. idahoense
S6	Royal Botanic Garden, Edinburgh	S. pachyrhizum
S7	Private Garden. Dr. J. L. Crosby	
S14	Royal Botanic Garden, Kew	S. bermudianum
S17	Collected wild. Gaspe. Prof. D.H. Valentine	
S21	Leicester Botanic Garden	S. bermudianum
S25	Cambridge Botanic Garden	S. angustifolium
S34	Major Howell, Germany	S. angustifolium
S38	Major Howell, Belgium	S. pachyrhizum
S39	Major Howell, Sweden	S. platyphyllum
S40	Major Howell, Austria	S. scardicus
S47	St. Andrews Botanic Garden	Symphyostemma narcissoides
S55	Collected wild, Clyde Robins, California.	

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