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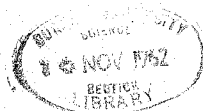
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THE CYTOTAXONOMY  
OF SOME ROSTRATE VIOLETS

Michael John Harvey B.Sc. (Dunelm)

A thesis submitted in candidature for the degree of  
Doctor of Philosophy in the University of Durham.

1962



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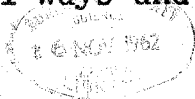
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## ACKNOWLEDGMENTS

First, thanks are due to Professor D.H.Valentine for initially suggesting that I work on violets and for collecting and growing a very extensive range of the European and North American species. It is always a difficulty when studying fairly slow growing plants in that, if parental stocks have to be collected or grown from seed, there may be insufficient time available to complete the production and examination of hybrids, and I was very fortunate in having mature stocks of violets provided so that the work of hybridisation could commence in the spring of my first year at Durham. In addition, thanks are also due to Professor Valentine for allowing the examination of the eight hybrids listed on p.57, for providing plants and slides of triploid and tetraploid Viola reichenbachiana, for a great deal of advice and help, and for listening with patience to my ideas and pointing out the errors in many of them.

Thanks are due to Dr.M.M.Moore for producing and allowing me to use the two hybrids of V.lactea mentioned on p.57, to Dr.P.H.Davis for collecting V.sieheana in Turkey and to the many other people mentioned on pp.34 and 85 who have sent seed or plants to Professor Valentine or myself. In addition I would also like to thank my friends at Durham who helped in numerous small ways and, finally, I am greatly



indebted to D.S.I.R. for the provision of a maintenance grant for the three years, 1958-1961, during which the research was carried out.

Viola is a large, cosmopolitan genus consisting of about 400 mainly herbaceous species found from the subarctic to tropical mountains but with the majority of species in the temperate regions.

Wilhelm Becker's classification of Viola in Engler and Prantl (1925), may be taken as a convenient starting point for this account. In it he classified the genus into 14 Sections of which the first and largest is Section Nomimum, itself divided into 17 Subsections and of these Subsection Rostratae forms the subject of this thesis and is further divided as below:-

Viola Section Nomimum Ging.

Subsection Rostratae Kupffer

- a) Mirabiles Nym.
- b) Rosulantes Borb.
- c) Arosulatae Borb.

These constitute the scentless, blue-flowered dog violets of the North Temperate zone and are frequently referred to in this thesis as the 'rostrate violets'.

The three ultimate divisions are based on life form: the Mirabiles have erect, leafless, almost woody overwintering shoots covered with dead leaf bases and stipules; the

Rosulantes have an overwintering rosette of leaves; and the Arosulatae have dormant buds at ground level. An additional difference which will be commented on later is that the Mirabiles and Rosulantes have rounded leaves whereas Arosulatae tend to have elongated leaves.

Mirabiles is the smallest group, containing only two or three species; Rosulantes consists of over twenty species and Arosulatae about ten. This is the present state of knowledge; as the Asiatic violets become better known it is anticipated that the numbers will rise.

The aim of the research was to find out the evolutionary relationships between the species in the Rostratae and to see if the divisions, Mirabiles, Arosulatae and Rosulantes are valid categories when examined from a biosystematic view.

The method of investigation consisted largely of synthesising hybrids, studying their fertility and where possible obtaining an  $F_2$ , and, above all, studying the behaviour of their chromosomes at meiosis in pollen-mother cells. Conventional taxonomy from herbarium specimens and geographical distributions were also considered but efforts to deduce relationships from these alone have met with little success. Several early predictions of relationships from morphology were later found not to be supported by the



cytological results. For example the connection between V.riviniana and V.sieheana was eventually found to be very much less close than originally expected.

The work of hybridisation is not complete at the time of writing. It is being continued and extended, and in addition the hybrids already made are being treated with colchicine with the aim of inducing polyploids whose fertility, cytology and stability it is hoped to study in the future.

Table 1 is a list of the rostrate violets which have been cultivated at Durham. Five of them- V.bellidifolia, V.elatior, V.faurieana, V.grypoceras and V.jordani -were obtained too late to be included in the first two years' hybridisation programme.

Table 1. SPECIES STUDIED IN CULTIVATION AT DURHAM

<u>MIRABILES</u>	<u>ROSULANTES</u>	<u>AROSULATAE</u>	<u>PLOIDY</u>
V.mirabilis L.	V.adunca Sm. bellidifolia Greene conspersa L. faurieana Bckr. grypoceras Gray labradorica Schrank reichenbachiana Jord. rostrata Pursh rupestris Sch. striata Ait.	V.stagnina Kit.	2x
	V.riviniana Rchb.	V.canina L. elatior Fr. jordani Hanry pumila Chaix	4x
	V.sieheana Bckr.	V.lactea Sm.	6x

Other species which will be mentioned but which have not yet been obtained as living plants are:-

Rosulantes: V.austro-ussuriensis Kom., V.fedtschenkoana Bck.  
V.howellii Gray (8x), V.isopetala Juz., V.mauritii Teplouch.  
V.ovato-oblonga Makino (2x), V.sacchalinensis De Boiss (2x),  
V.silvestriformis Bckr., V.tanaitica Gross., V.walteri  
 House (2x).

Arosulatae: V.acuminata Ldb., V.raddeana Rgl., V.thibaudieri  
 Fries.

Other subsections of Nomimium which will be mentioned are:-

Uncinatae: Kupffer; V.odorata L., V.hirta L., (the sweet violet group).

Stolonosae Kupffer; V.palustris L. (the marsh violet).

### CHROMOSOME NUMBERS

The rostrate violets form a polyploid series based on 10 and the majority of species whose chromosome number is known have  $2n=20$ , (14 species). A further 5 species are known to be tetraploid,  $2n=40$ : These latter all occur in Europe but extend into Asia and include the two most widely distributed species in Britain (V.canina and V.riviniana). Two species are known to be hexaploid, V.sieheana,  $2n=60$  of S.W.Asia and probably the Balkans, and V.lactea,  $2n=58$  in Atlantic Europe. One species, V.howellii of W.North America, was recorded by Gershoy (1934) as having  $2n=C.80$ , a count which

it would be interesting to confirm.

### SUPERNUMERARY CHROMOSOMES

A complication in the cytological study of violets is that many plants of V.riviniana and apparently some of V.canina have more than 40 chromosomes per somatic cell and these extra ones have been called supernumerary or B chromosomes. They are also reported by A.Schmidt (1961), from plants in a single colony of V.rupestris.

Early on in this investigation it was decided not to study these in detail but the subject must be mentioned as some stocks with supernumerary chromosomes (the only ones available at the time) were used for making some of the hybrids which will be reported on later. Such hybrids received one or more of the supernumeraries and their general effect, with one exception, was to raise the number of univalents seen at meiosis. However, having observed supernumerary chromosomes so often while studying violet cytology a few notes will not be out of place.

Their size as seen at meiosis varies from being quite indistinguishable from those of the normal complement to about half the normal length. For this reason the expression 'B' chromosome is not always appropriate since it has sometimes been used to imply chromosomes smaller than normal which is not invariably the case here.

Their behaviour at meiosis shows that they are not

devoid of pairing ability though they are most frequently unpaired. Occasionally two supernumeraries form a bivalent; this has been reported by A.Schmidt (1961) in V.rupestris and observed by myself in V.riviniiana (photograph p.149). Further evidence of their pairing abilities was also found, quite unexpectedly, in the hybrid V.canina × stagnina  $2n=34$ , apparently formed from V.canina  $2n=40$  + supernumeraries, and V.stagnina  $2n=20$ . On the basis of the published genomic relationship between V.canina and V.stagnina (Valentine, 1950) an average of 10 bivalents per pollen-mother-cell at first metaphase of meiosis was expected in their hybrid, but the average turned out to be 11. (photo. p.153).

Valentine postulated that the chromosome sets in V.stagnina, V.canina and their hybrid could be represented as CC, BBCC and BCC respectively, the bivalents observed at meiosis in the hybrid being formed from the two C sets of chromosomes and the univalents from the B set. The most likely origin for the eleventh bivalent is from one of the B set and one of the supernumeraries. In other words the V.canina plant used to form the hybrid was probably trisomic for one particular chromosome belonging to the B set.

It would of course be interesting for comparison to have the hybrid without supernumeraries, but it is not expected that it would show meiotic behaviour different from that of V.riviniiana × reichenbachiana  $2n=30$  represented by the histogram on p.81 and which may be compared with that

of V.canina x stagnina adjacent to it in the diagram.

It has in fact become clear that the origin and perpetuation of supernumerary chromosomes in natural violet populations forms one of the most interesting problems in violet cytology and it is regretted that these chromosomes were not studied in more detail.

#### DESCRIPTION OF SPECIES

It was originally intended to give only a drawing of each species in order that any reader unfamiliar with these violets might have some slight acquaintance with their vegetative and floral morphology, but later it was thought that it would be as well to add a brief description of each in words and these are given below. The descriptions are not intended to provide a complete account of each species but merely note the more conspicuous and contrasting features. More details can be found by reference to floras such as Komarov (1949); Clapham, Tutin and Warburg (1952); Hegi (1925); Fernald (1950); Abrams (1923-51).

The drawings are from what I hope would be considered typical specimens, with the exception of those of V.bellidifolia and V.rupestris which illustrate vigorous shoots from first year plants (older plants have shorter internodes), and that of V.adunca which is a composite picture from plants from several different localities.

Mirabiles: Overwintering as leafless, erect, above-ground shoots; chasogamous flower-buds developed in the autumn.

1. Viola mirabilis L. Stems hirsute or glabrous; leaves eventually large, the lower rounded, upper acute; stipules broad, entire, ciliate; sepals with large appendages; open flowers large, pale to mid blue, spur white, lateral petals bearded, style glabrous. Found in woods and open areas, Europe, Central Asia to Japan.  $2n=20$ . Fig.1 p.15.

Map.p.38.

Rosulantes: Possess two types of shoots, one, a perennial, non-flowering stem is central and grows erect with very short internodes, producing a rosette of leaves which persists through the winter, the other shoots are borne in the axils of the rosette leaves and are of one year's duration only, dying down in the autumn but bearing both the open and closed flowers. The production of the open (chasogamous) flowers is initiated in the autumn.

2. Viola rupestris Schmidt. Stems, leaves, petioles and capsules covered with a fine pubescence although glabrous and glabrescent plants occur. The pubescence on the capsule is the best distinction from V.adunca. Basal leaves rounded, upper tending to cordate; stipules broad, entire or with only a few teeth; flowers a fairly uniform pale to mid blue with not such conspicuous veins as V.riviniana,

spur short, coloured, rounded; lateral petals bearded and style papillose. In base-rich, open habitats, grassland or rocks. Eurasia.  $2n=20$ . Fig.2,p.16 Map p.38.

3. Viola adunca J.E.Smith. Stems, leaves, petioles covered with fine pubescence; differs from V.rupestris in that plants with pubescent capsules have not been reported but glabrous and glabrescent plants occur in many populations. Stipules fimbriate; flowers of many colours occur in different populations, very pale blue to very intense violet-purple; spur coloured, variable length usually long, blunt or pointed, sometimes with hook-like appendage near tip, lateral petals bearded; style papillose. Found in open often sandy places, open woodland or rocks; widely distributed in North America.  $2n=20$ . Fig.3,p.17, Map p.40.

4. Viola bellidifolia Greene. Whole plant glabrous, dwarf, stipules small, toothed; leaves rounded, base cuneate; flowers mid to deep blue, spur very long, pointed. Found in damp, mossy patches along with other dwarf alpines, central Rocky Mountains, North America, 8-12,000 feet.  $2n=20$ . Fig.4,p.18. Map p.40.

5. Viola conspersa Reichenbach. Plants glabrous; leaves thin, lower rounded, upper obtusely pointed; stipules toothed; flowers a uniform pale blue, rather small, spur blue, short, blunt; lateral petals and style bearded. After

fertilisation the peduncle bends downward to hide the rather small capsule on or near the ground, but on the capsule ripening, further growth of the part of the peduncle above the bracteoles raises it again. This feature is shared with V.rostrata. Found in woods and meadows in north-east North America.  $2n=20$ . Fig.5,p.19. Map p.40.

6. Viola rostrata Pursh. Glabrous or nearly so, lower leaves rounded, upper long acuminate; stipules fimbriate; peduncles bending downward while fruit ripens as in Viola conspersa; flowers lilac-blue ie. a more pinkish shade of blue than V.conspersa; spur very long, up to 3 cm., narrow pointed, same colour as petals, lateral petals not bearded; style not papillose, long, narrow; Found in woods usually on calcareous strata, north-east North America and Japan.  $2n=20$ . Fig.6,p.20. Map p.40.

7. Viola labradorica Schrank. Glabrous, plants generally small; leaves rounded; stipules small, entire or with only a few teeth; flowers mid-blue, lip with white zone at base veins fairly strongly marked, extending about half way to edge; lateral petals bearded. Open areas in subarctic regions of Greenland and north-east Canada and mountains in New England.  $2n=20$ . Fig.7. Map p.40.

8. Viola striata Ait. Glabrous or nearly so; plants usually tall; leaves large, basal rounded, upper acute;



stipules very broad with abundant long fimbriations; flowers white with a few dark veins, petals narrow, the laterals bearded; style papillose, spur short, white, blunt. Found in woods and damp meadows, south-east United States of America.  $2n=20$ . Fig.8,p.22. Map p.40.

9. Viola faurieana Becker. Glabrous, leaves sub-acute, shiny yellowish green, thick texture and lasting through the winter well; stipules fimbriate; flowers deep blue except for white base to petals, petals very broad, veins short, laterals not bearded; spur short blunt white; style not papillose. Found by sandy sea shores and probably other places, Japan.  $2n=20$ . Fig.9,p.23. Map p.40.

This species was obtained from the Royal Botanic Gardens Edinburgh under the above name, which has been retained here for convenience although it may not be the correct one. The exact application of the names V.faurieana, V.senamiensis Nakai, and V.grayi Franch and Savat. is not clear at the moment.

10. Viola grypoceras Gray. Subglabrous; lower leaves obtusely pointed, upper acuminate, thin textured; stipules long fimbriate; (flowers not yet seen fresh); Woods, Japan,  $2n=20$ . Fig.10,p.24. Map p.40.

11. Viola reichenbachiana Jordan. Glabrous or with a few scattered hairs, upper leaves more pointed than lower;

stipules fimbriate; sepal appendages very short; petals narrow, dull blue, lip darker towards centre, veins short, spur dark narrow and tapering, lateral petals bearded; style papillose. Woods, often on base-rich soil. Europe, north Africa and Canary Islands.  $2n=20$ . Fig.11,p. 25.

Map p.38.

12. Viola riviniana Reichenbach. .Glabrous or with a few scattered hairs; leaves rounded at base of plant, sub-acute on branches; stipules fimbriate; sepal appendages large; flowers blue, lip petal conspicuously veined, fairly broad, spur fat, blunt, furrowed at apex, white or dark blue, lateral petals bearded; style papillose. Found in woods and grassland. Europe, north African mountains and Canary Islands;  $2n=40$  with supernumerary chromosomes in some plants. Fig.12, p.26. Map p.38.

13. Viola sieheana Becker. Glabrous or nearly so; stipules fimbriate; leaves similar to those of V.riviniana; flowers blue, petals very broad, the lip cupped so that it cannot be flattened without splitting, veins conspicuous and extending almost to edge of lip, spur white, fat, slightly upturned at apex, lateral petals bearded; sepal appendages large; style papillose. Woods from extreme south-east Europe to Persia;  $2n=60$ . Fig.13,p. 27. Map p.39.

Arosulatae: No dwarf, central, non-flowering stem; overwintering as small leafless buds at ground-level; all shoots have equal potential for producing flowers which are formed in spring, hence this group flowers later in general than the other two groups. The leaves tend to be elongated.

14. Viola stagnina Kitaibel. Subglabrous, leaves triangular-lanceolate, stipules fairly small with few teeth; flowers white or pale blue, petals broad with dark veins, spur white, very short, blunt, lateral petals bearded. Found in fens from Europe to central Asia.  $2n=20$ . Fig.14, p.28. Map p.38.

15. Viola canina L. Glabrous or with a few hairs; leaves like those of V.riviniana but slightly more elongate especially the upper; stipules with a few large teeth; sepal appendages large; flowers blue, petals broad, many veins on lip, lateral petals bearded, spur whitish, blunt, medium length. Wide range of habitats from dry sandy areas, limestone rocks, to wet fen peat. Greenland, Iceland, Europe, Asia probably to Pacific coast.  $2n=40$ , supernumerary chromosomes known. Fig.15, p.29. Map p.38.

16. Viola lactea J.E.Smith. Glabrous or very sparingly hairy; stipules medium sized, deeply toothed; leaves lanceolate with cuneate base; sepals with large appendages;

flowers pale blue, lip with fewer veins than V.canina, lateral petals bearded, spur blunt; style papillose. Found on heaths in extreme atlantic Europe from Portugal to England.  $2n=58$ . Fig.16,p.30. Map p.38.

17. Viola pumila Chaix. Glabrous, stipules large with a few large teeth; leaves lanceolate; petals broad, rounded, darker to the centre, laterals bearded, spur short, blunt. Found in fens, meadows and dryer areas, slightly more xerophytic tendency than the next species, V.elatior. Europe to central Asia.  $2n=40$ . Fig.17,p.31. Map p.38.

18. Viola elatior Fries. Similar to V.pumila, differs in being pubescent and generally very much taller with large, elongate-lanceolate leaves. Found in fens, Europe and central Asia.  $2n=40$ . Fig.18,p.32. Map p.38.

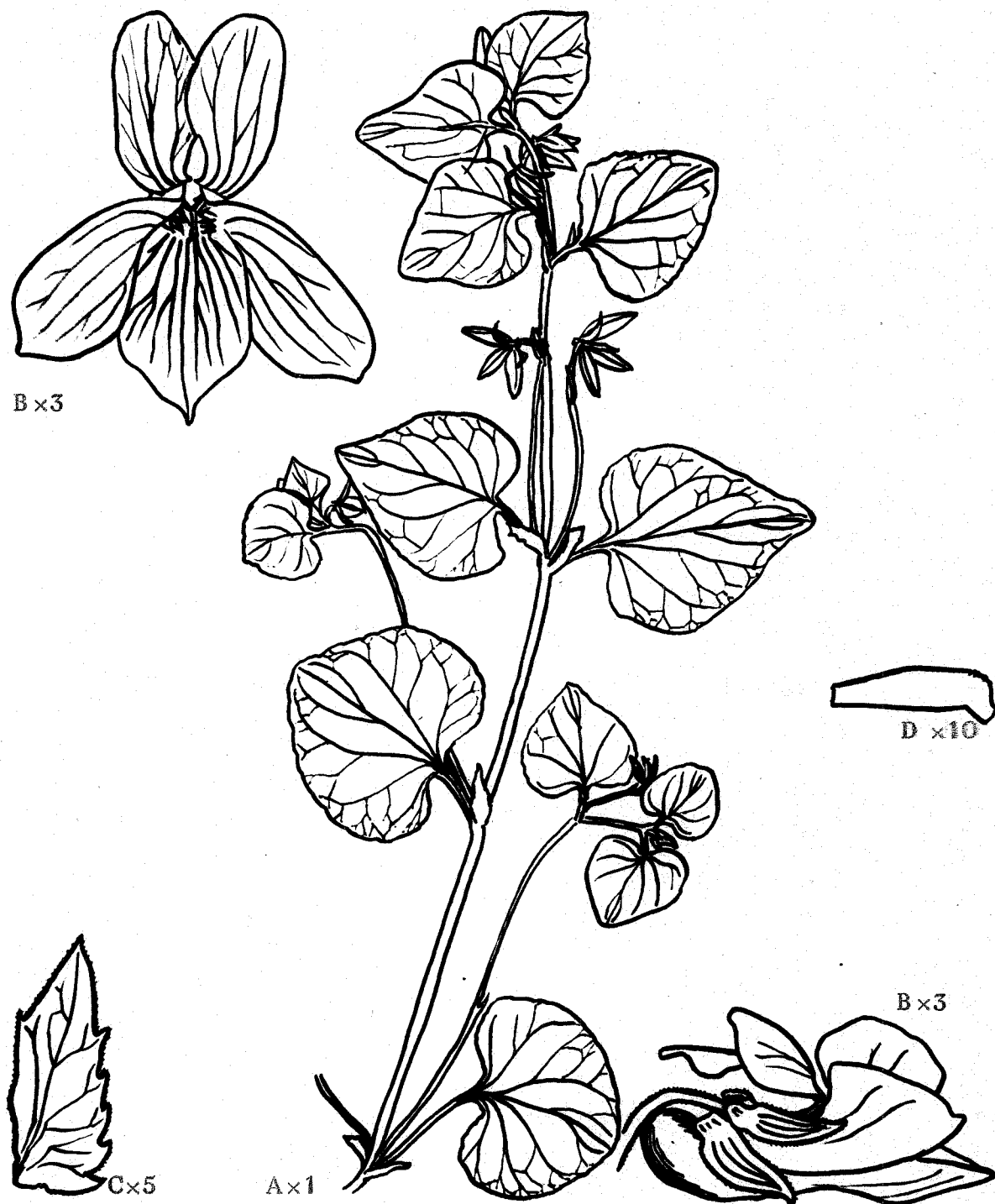
19. Viola jordani Hanry. Sparingly hairy, stipules very large (the largest of any known Rostrate violet), with a few large basal teeth; basal leaves about as long as broad, upper to about 3 times as long as broad; flowers blue or white, petals fairly narrow, lip slightly cupped with a few prominent veins, laterals bearded, spur long, upturned; style papillose. Fens, S.Europe (rare) to Himalayas.  $2n=40$ . Fig.19,p.33. Map.p.38.



*Viola mirabilis* L.

A, part of plant bearing cleistogamous flowers, "Öland stock"; B, flower;  
 C, stipule; D, style.  
 Petals mid-blue, spur white.

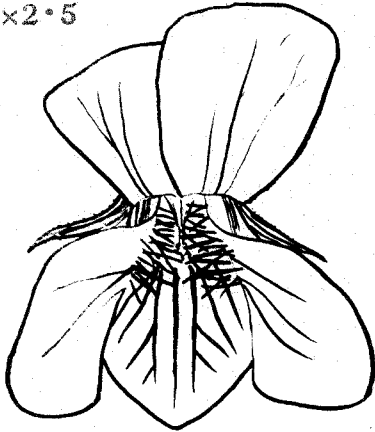
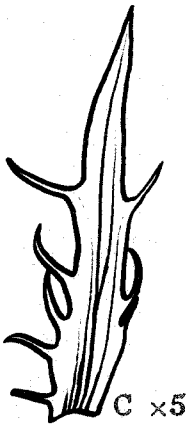
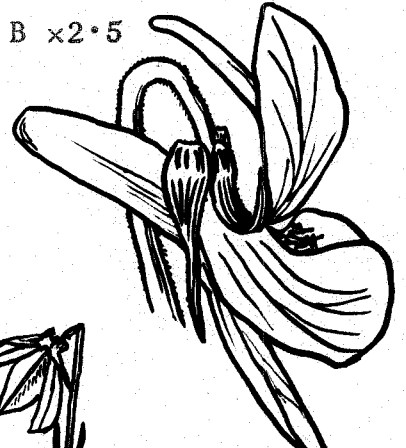
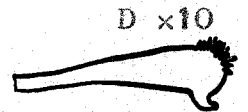
Fig.1



*Viola rupestris* Schmidt

A, shoot from a young vigorous plant, Czechoslovakian stock; B, flowers;  
 C, stipule; D, style.  
 Petals mid-blue, spur coloured.

Fig. 2

B  $\times 2.5$ B  $\times 2.5$ C  $\times 5$ D  $\times 10$ 

*Viola adunca* Smith

A, plant collected in Springfield, Manitoba; B, flowers, Burlington, Vermont;  
 C, stipule, Mather, California stock; D, style, Mather stock.  
 Petals mid-blue in Burlington plants.

Fig. 3

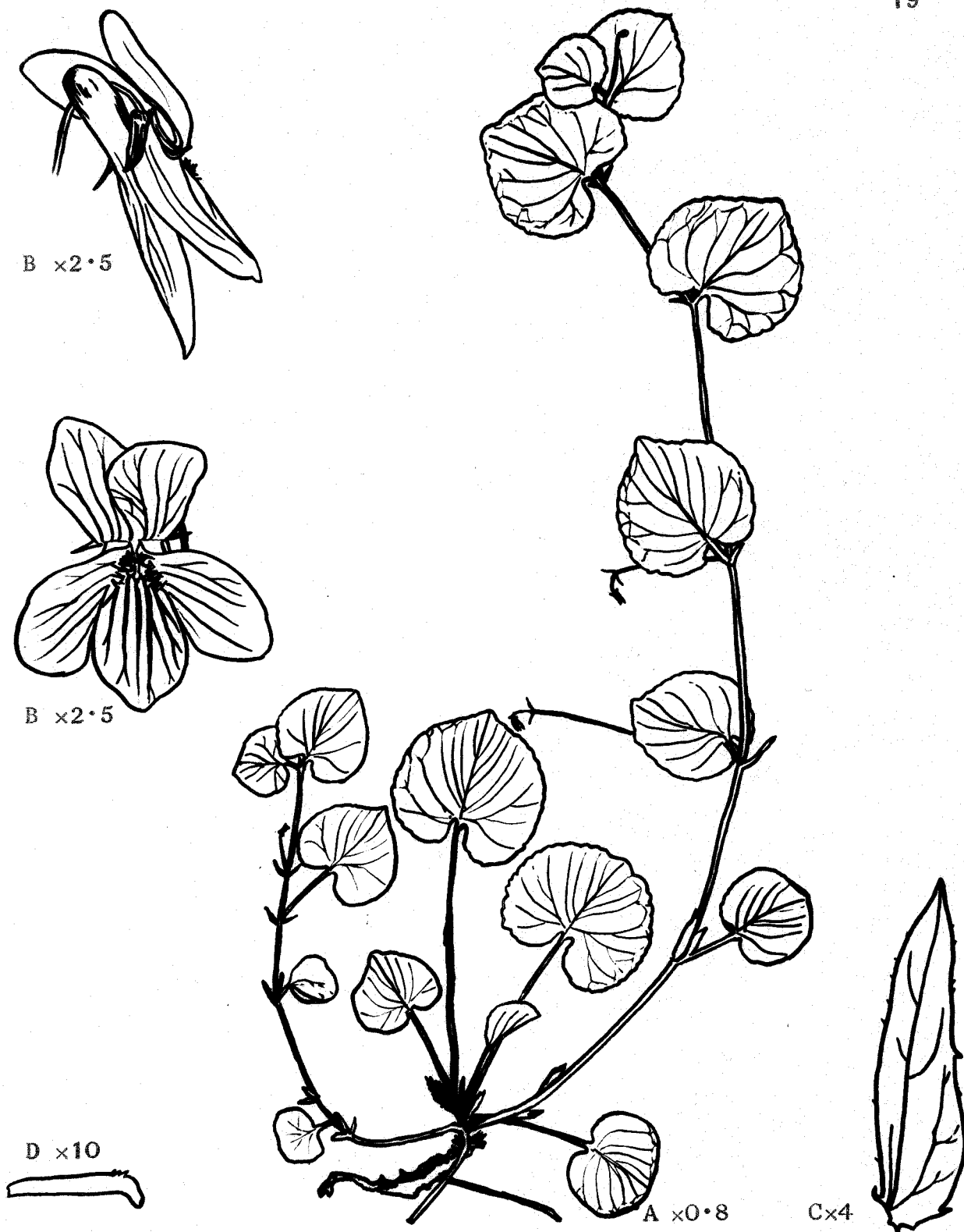


*Viola bellidifolia* Greene

A, shoot from a very vigorous first year plant, stock from California; B, stipule.  
Petals mid-blue, spur dark.

Fig. 4

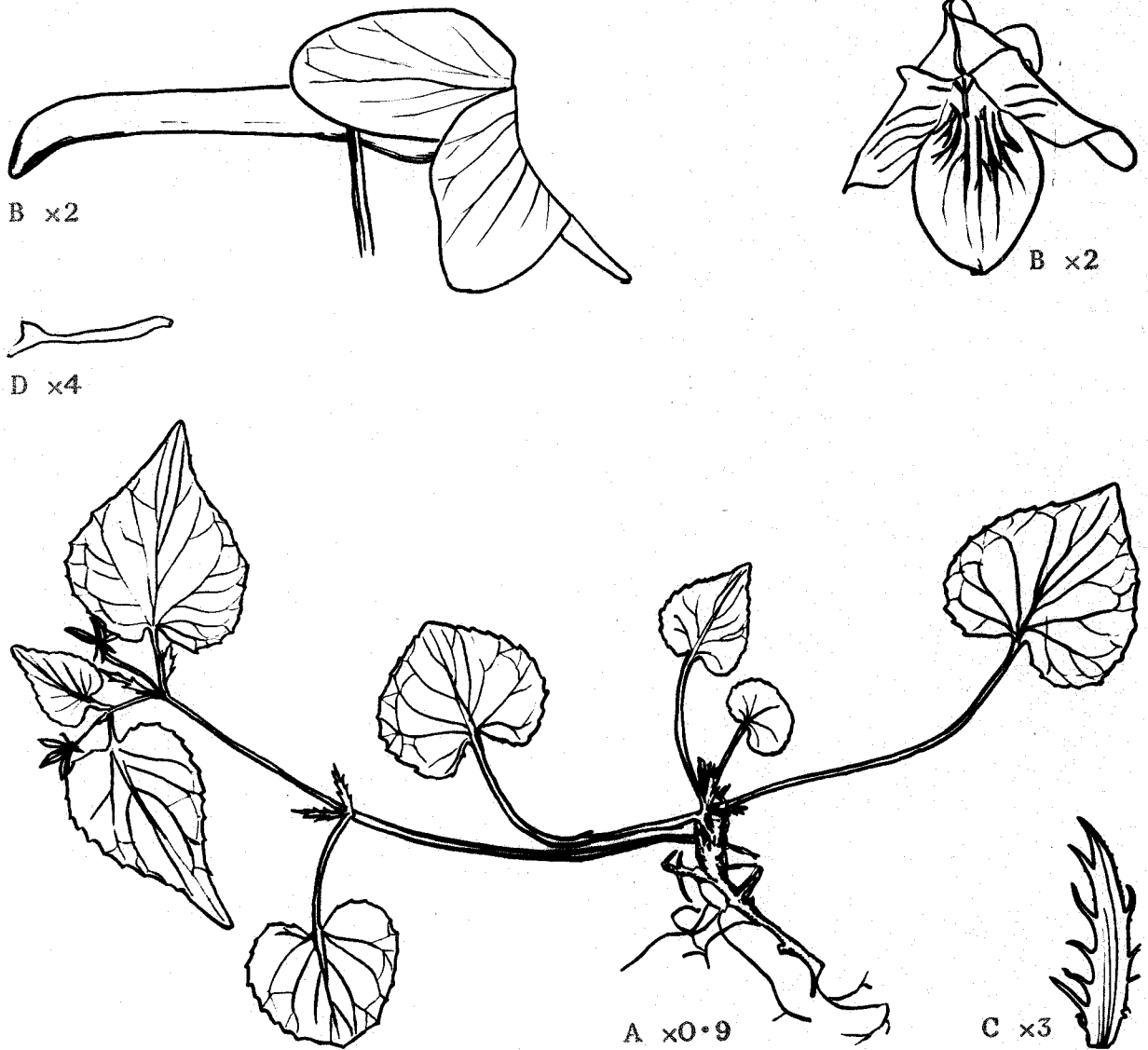




*Viola conspersa* Rchb.

A, part of a plant bearing cleistogamous flowers; B, flowers; C, stipule; D, style.  
Petals mid-blue, spur coloured.

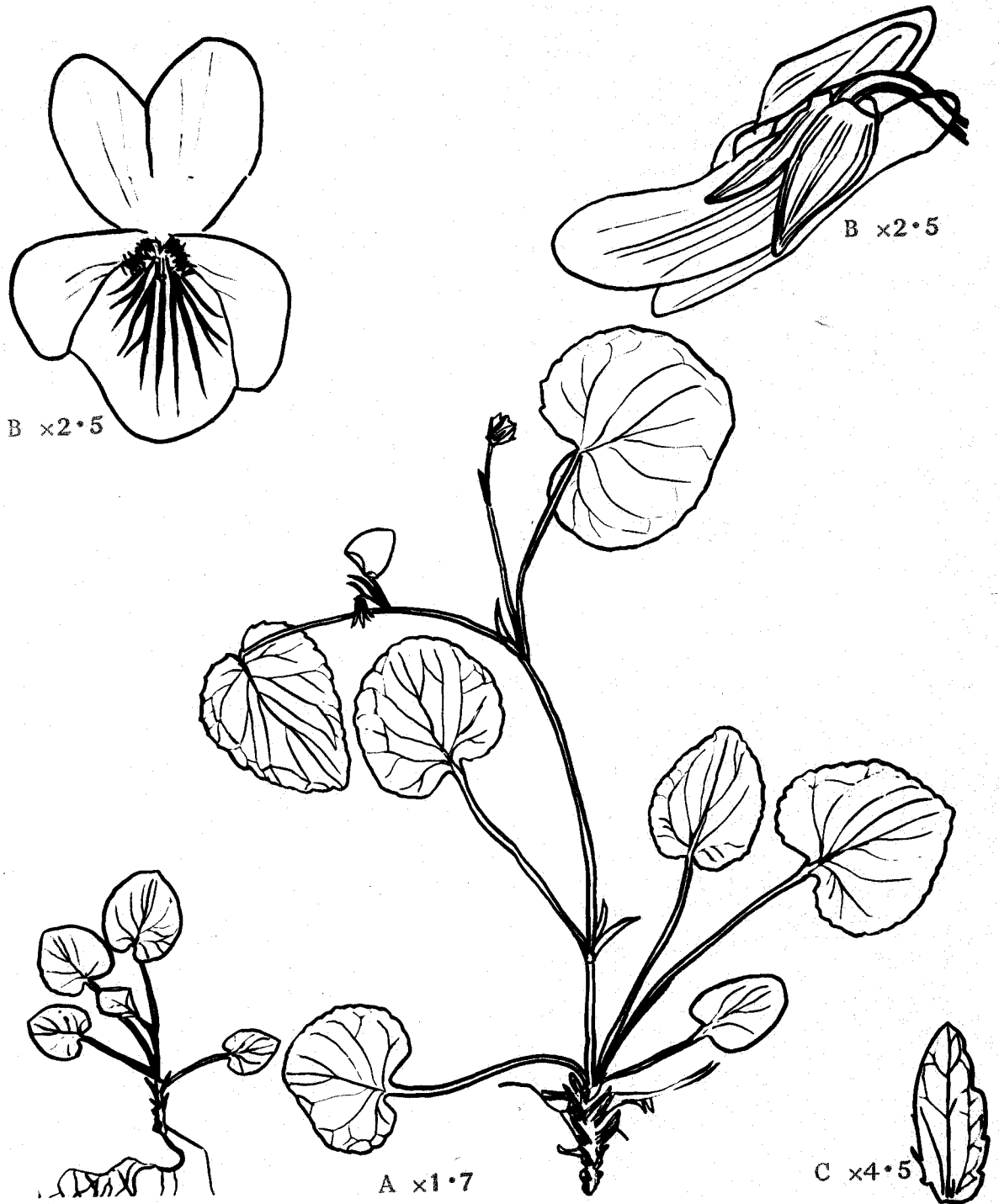
Fig. 5



*Viola rostrata* Pursh

A, plant; B, flowers; C, stipule; D, style.  
 Petals a pinkish-blue, spur similar.

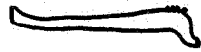
Fig. 6



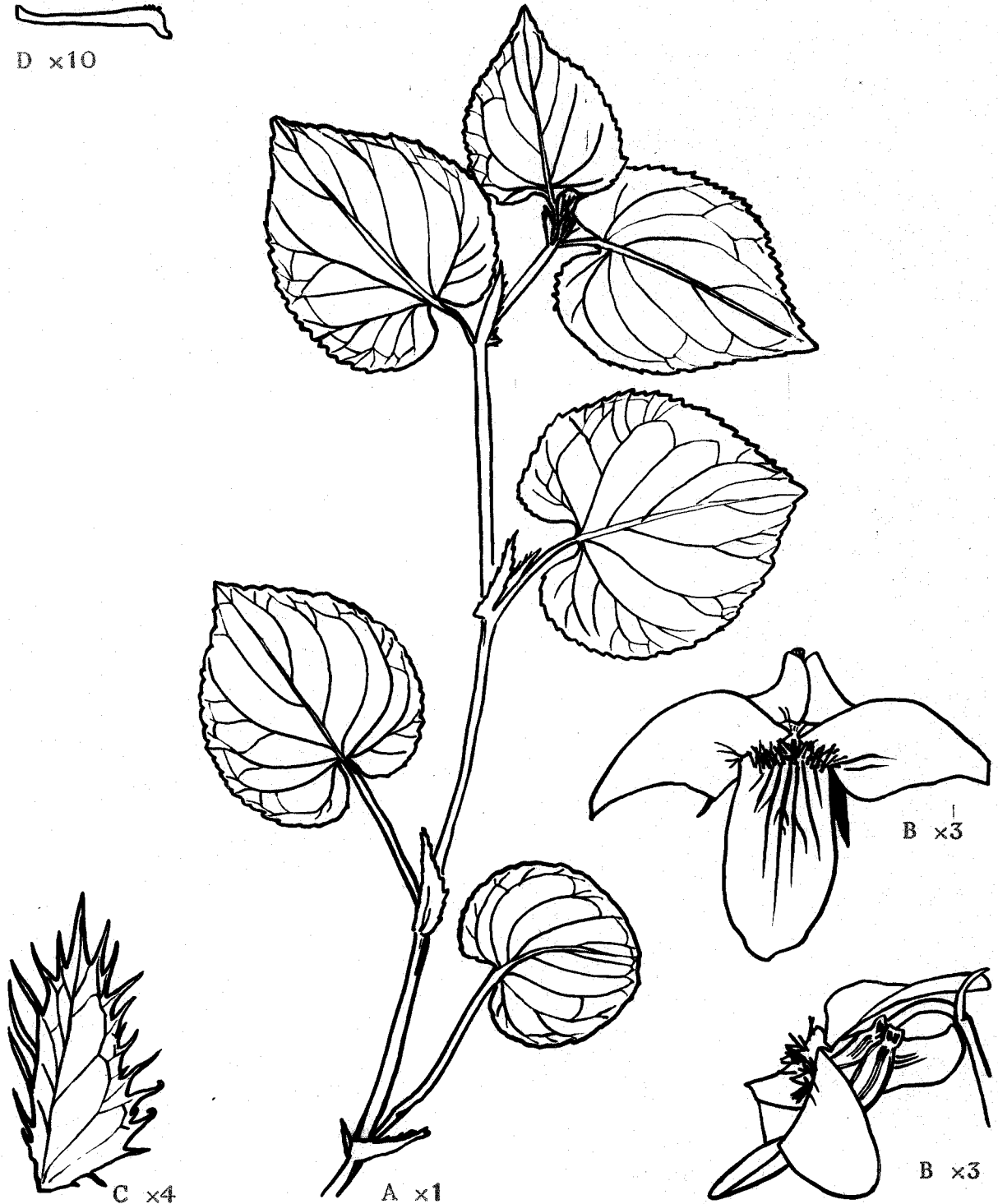
*Viola labradorica* Schrank

A, plant collected on Mount Washington; B, flowers from Mount Albert stock; C, stipule  
 Petals mid-blue, lip petal with white ground colour in centre.

Fig. 7



D x10



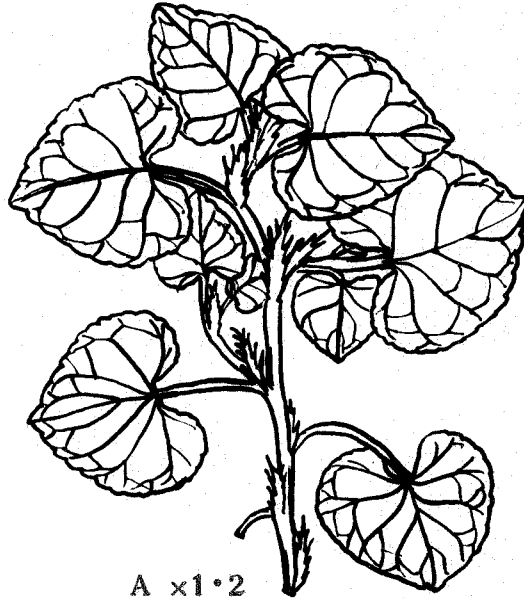
*Viola striata* Ait.

A, single shoot, eastern USA.; B, flowers; C, stipule; D, style.  
 Petals white with dark veins, spur white.

Fig. 8

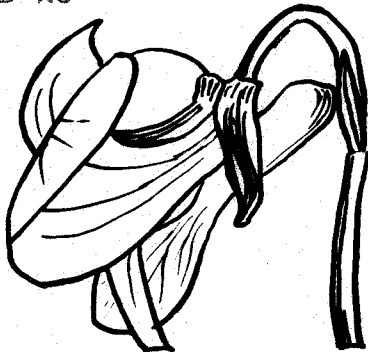


C x5

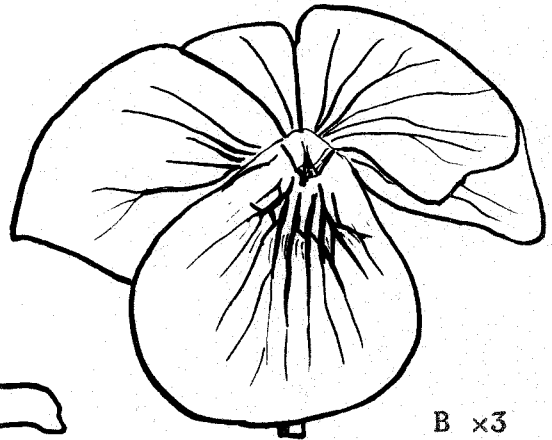
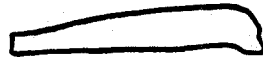


A x1.2

B x3



D x10



B x3

*Viola faurieana* Bckr. (probably *V. senamiensis* Nakai = *V. gravi* F+S.)

A, single shoot, leaves thick-textured, shiny; B, flowers; C, stipule;  
D, style.

Obtained from Royal Botanic Garden, Edinburgh.

Petals deep blue, spur white.

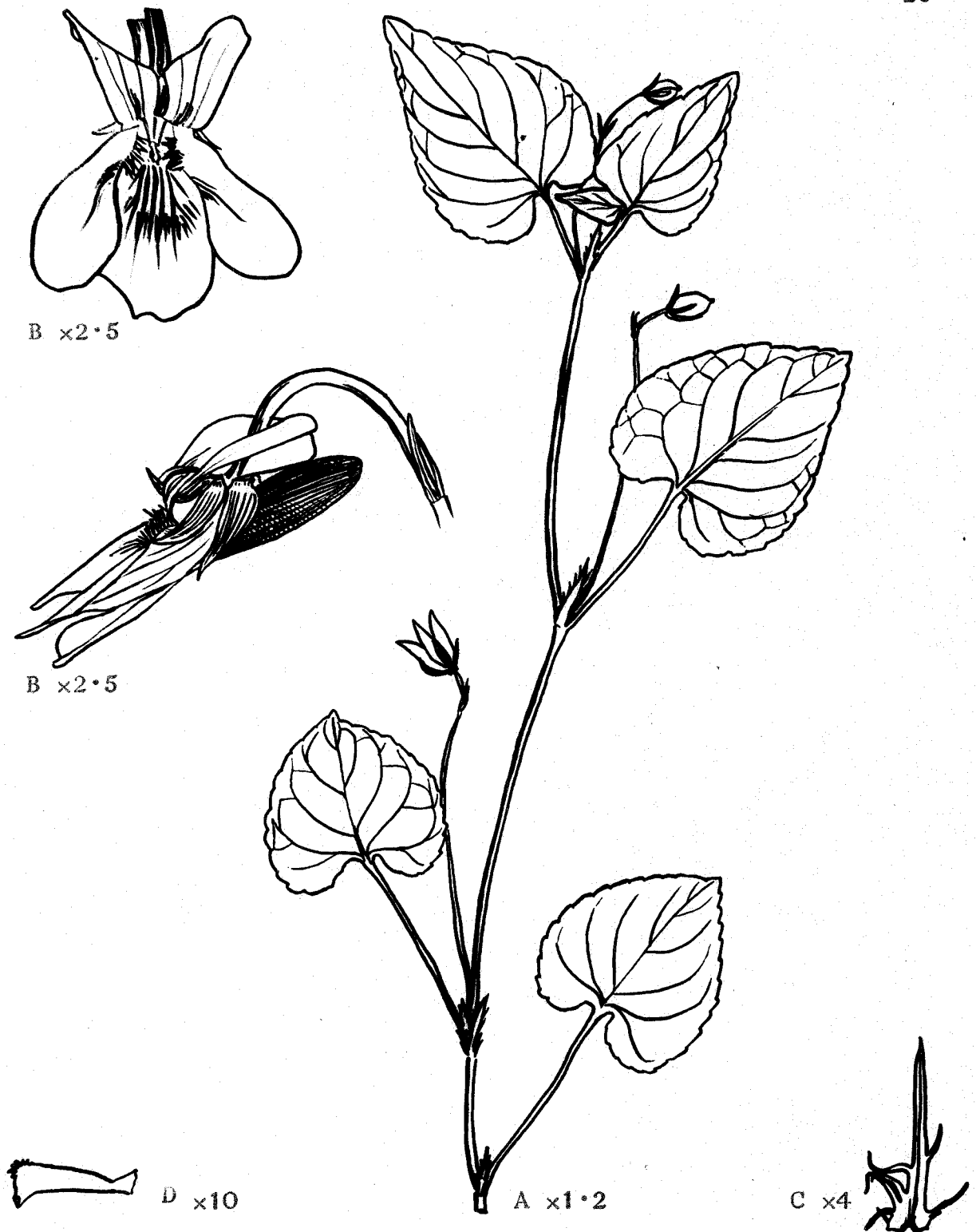
Fig. 9



*Viola grypoceras* Gray

A, single shoot, stock from Sendai, Japan; B, stipule.

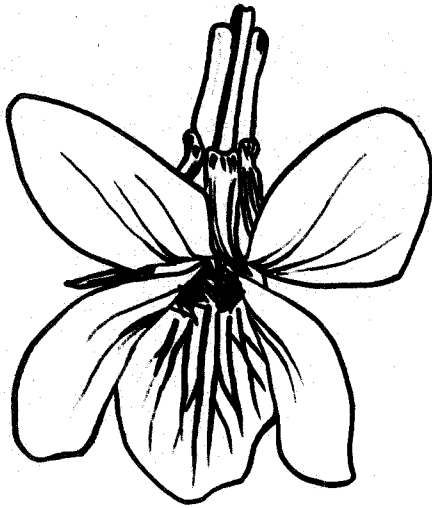
Fig. 10



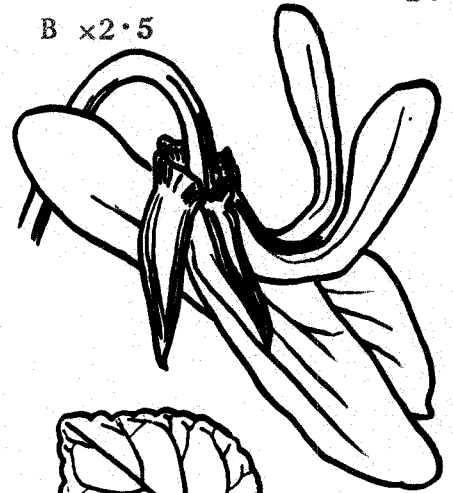
*Viola reichenbachiana* Jord.

A, single shoot, English stock; B, flowers; C, stipule; D, style.  
 Petals dull-blue, darkest towards the centre, spur dark.

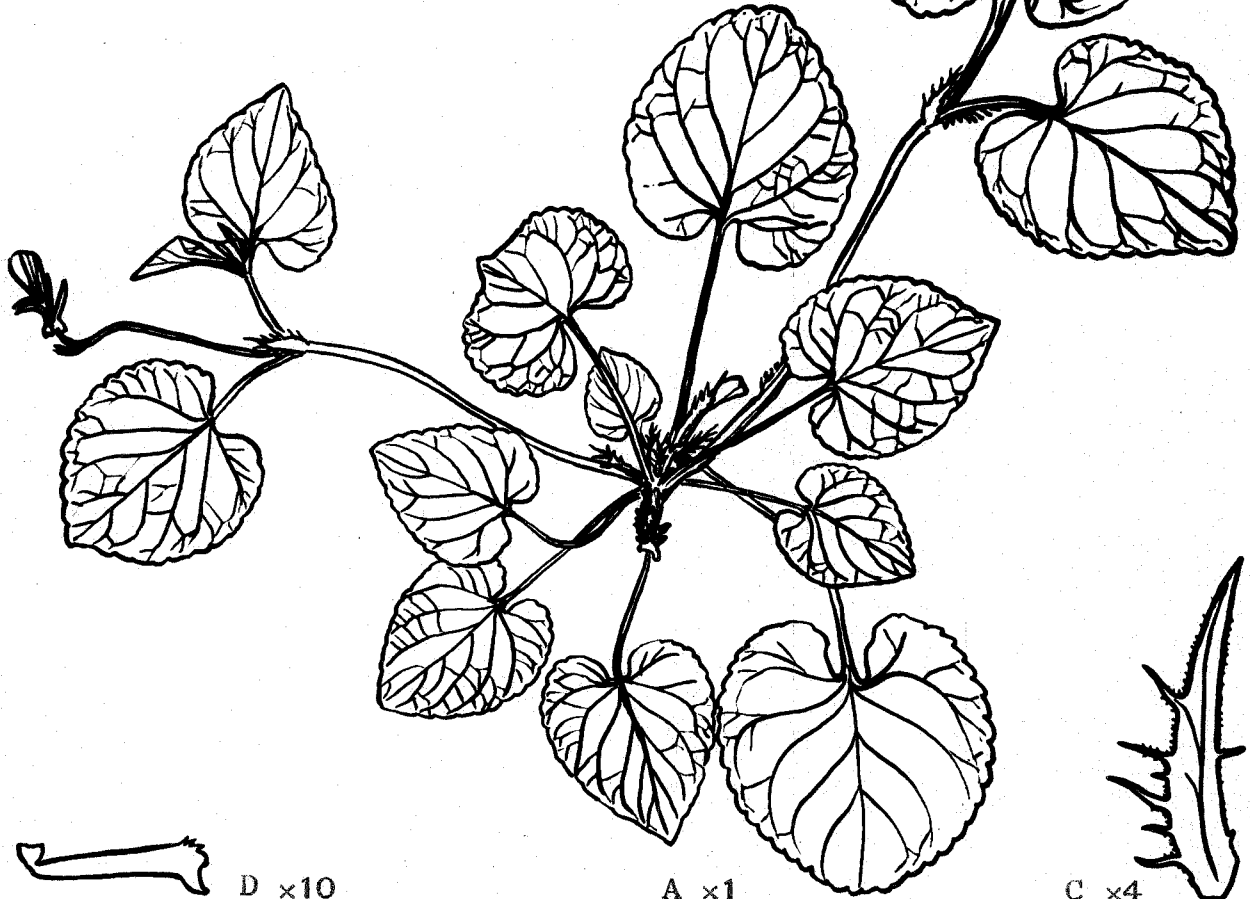
Fig. 11



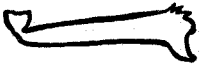
B x 2.5



B x 2.5



A x 1



D x 10



C x 4

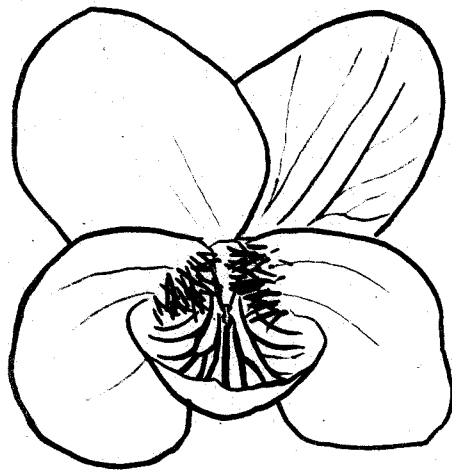
*Viola riviniana* Rchb.

A, hybrid plant, Czechoslovakia x Madeira; B, flowers, England; C, stipule, England; D, style England.

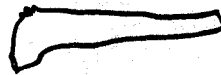
Petals mid-blue, spur white, sometimes dark.

Fig. 12

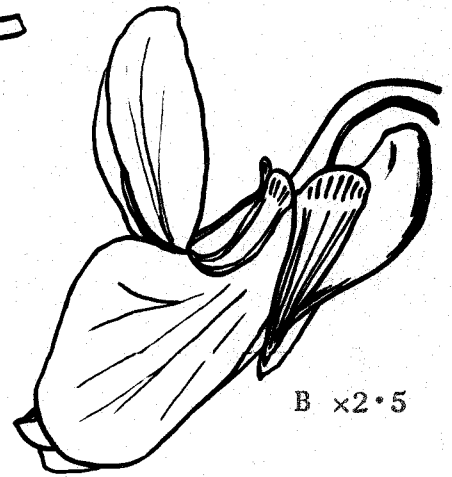




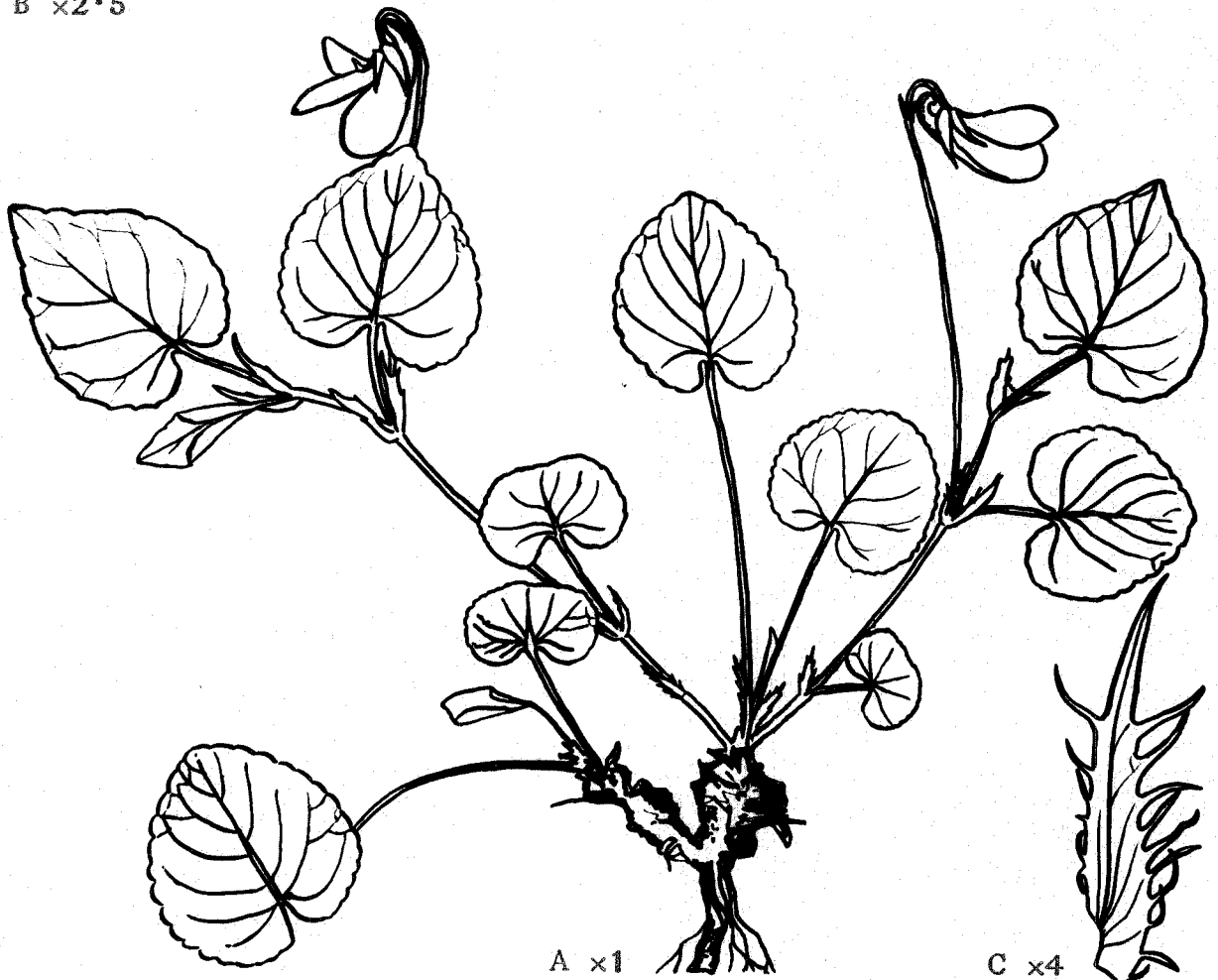
B x2.5



D x10



B x2.5



A x1

C x4

*Viola sieheana* Bckr.

A, plant of Turkish stock from Rizé province; B, flowers; C, stipule; D, style.  
Petals pale bright blue, spur white.



*Viola stagnina* Kitaibel

A, single shoot, Bavarian stock; B, flowers, England; C, stipule; D, style.  
 Petals white, turning pale blue with age, veins dark, spur white.

Fig. 14



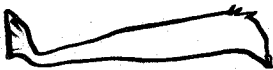
*Viola canina* L.

A, single shoot, Novosibirsk, USSR. stock; B, flowers, Tentsmuir, Scotland; C, stipule. Petals mid-blue, spur white.

Fig. 15



D x 10

*Viola lactea* Smith

A, single shoot, English stock; B, flowers; C, stipule; D, style.  
 Petals pale blue, spur white.

Fig. 16



*Viola pumila* Chaix

A, single shoot, Czechoslovakian stock; B, flowers; C, stipule.  
 Petals mid-blue, paler to base, laterals with darker patch in middle, spur pale.



Viola elatior Fries

A, single shoot, stock obtained from Uppsala Botanic Garden; B, flower; C, stipule.  
Petals mid-blue, paler towards the base.



*Viola jordani* Harry

A, single shoot, Aix stock; B, flowers; C, stipule; D, style.  
 Petals white with dark veins, spur white.

Fig. 19

ORIGINS OF THE PLANTS USED

V.adunca, see separate section p.85.

V.bellidifolia, Tevin Lakes, Wasatch Mts. Utah, 9,500 ft.  
coll. Prof.Cottam and R.K.Vickery.

V.canina, Tentsmuir, Fife, coll.D.H.Valentine; Ross Links,  
Northumberland, coll.M.J.Harvey; north Bohemia, Hora,  
Šebestiána, 850m.; central Bohemia, Vlkov, Vesek, Luz; Plys  
Spisske, Tomasova, Czechoslovakia, coll.D.H.Valentine;  
Uppsala B.G.; Nish, Sharáp, Ordynski, Novosibirsk, USSR.  
(As V.montana L.); Comba Dão, prov. Beira Alta, coll. P.  
Silva.

V.conspersa, Rosemeag and Ile Bizard near Montreal, coll.  
D.H.Valentine.

V.elatior, Donanworth, Bavaria, coll.A.Schmidt; Uppsala B.G.

V.faurieana, Edinburgh, Royal Botanic Gardens (no locality).

V.grypoceras, Sendai, Japan, coll.K.Sohma and K.Munakata.

V.jordani, Aix, south France, coll. Merxmuller.

V.labradorica, Mt.Jacques Cartier, Mt.Albert and Matapédia,  
all on the Gaspé Peninsula, Quebec, Canada, coll.D.H.Valentine.

V.lactea, Fareham, Hants., Coll.S.M.Walters; Lizard, Cornwall,  
coll.E.F.Warburg.

V.mirabilis, Hungary, coll.Papp; Savyalavo, Novosibirsk;  
Mauer, Vienna, coll.A.Schmidt; Kiyose by Seibu railway,  
Tokyo.

V.pumila, Öland, Sweden, coll.D.H.Valentine; Donanworth,  
Bavaria, coll.A.Schmidt; Všetaty, central Bohemia.



V.reichenbachiana, Anston Stones Wood, Yorkshire, coll.D.H. Valentine; Wentbridge, Yorkshire, coll.M.J.Harvey; Levitt Hagg, Sprotborough, Yorkshire, coll.M.J.Harvey; Roche Abbey, Yorkshire, coll.M.J.Harvey; Near Brno, Czechoslovakia, coll. D.H.Valentine.

V.riviniana, Ancaster, Lincs. coll. E.J.Gibbons; Bei Zvolen, and near Brno, Czechoslovakia, coll.D.H.Valentine; Cronkley Fell<sup>Yorks.</sup> and Butterby, Durham, coll.D.H.Valentine; Madeira, coll. D.M.Moore; Reykjavik, Iceland, coll.F.Oskarsson, Sante Comba Beira Alta, Portugal, coll.P.Silva; Lamego, Portugal, coll. Teles and B.Rainha; near Picos de Europa, Puerto Ponton, dist.Leon, Spain, and Sierra de Covadanga, near Picos de Europa, Lago de la Encina, dist.Oriedo, Spain, coll. D.W. Dresser.

V.rostrata, Philipsburg, Quebec, Canada, coll.D.H.Valentine.

V.rupestris, Widdy Bank Fell, Durham, coll.D.H.Valentine, T.T.Elkington; Arnside, Westmorland, coll.D.H.Valentine and M.J.Harvey; Long Fell, Westmorland, coll.M.J.Harvey; Radvaň, and Sivá Brada and Pálavske vrchy, Czechoslovakia, coll. D.H.Valentine; Marchfeld near Vienna and Reschenpass, S. Tirol, coll. A.Schmidt; Haute Savoie, Switzerland, coll. T.T.Elkington; Switzerland, coll.M.E.Bradshaw; Poznan; Öland, via Cambridge B.G.; Braunsberg and Neusiedlersee, Austria, coll. D.H.Valentine; Turý promontory, E.Kola peninsula, USSR. (Kirovsh) via Cambridge B.G.

V.sieheana, Turkey, between Orztakoy and Cat, province Rize, district Hemsin, 1700m. coll. P.H.Davis.

V.stagnina, Woodwalten Fen, England, coll.D.H.Valentine;  
Bamberg, Bavaria, coll.A.Schmidt; near Bawtry, Notts and  
Medge Hall Station, Yorks. coll. M.J.Harvey.

V.striata, Gray Summit, Missouri, USA. coll. E.Anderson.

### GEOGRAPHICAL DISTRIBUTIONS

#### Distribution of sub-sections

Mirabiles .....Eurasia

Rosulantes .....Eurasia and N.America

Arosulatae ..... Eurasia

#### Distribution of species

These are given on individual maps on pp.38-40. Most of the maps have been compiled from the information given in the floras listed on p.7 and also Hultén (1944-50). A few of the maps are the result of personal observation of large numbers of herbarium specimens. These are:- V.adunca, V.bellidifolia, V.labradorica, V.riviniana, V.rupestris, although specimens of the latter from Asia are few. My observations agree with the published distributions with the exception that I have seen no specimens of V.reichenbachiana from Kashmir (cf.Clapham, Tutin and Warburg, 1952). The many specimens in Herb.Brit.Mus.(Nat. His.) and Kew which have been referred to this species (as

V.sylvestris Lam.) appear to belong to the V.fedtschenkoana -isopetala group. And in addition specimens of V.jordani collected in Kashmir have been seen at the British Museum but I have not yet seen any reference in the literature to it occurring there.

Added for interest are the distributions of a number of purely Asiatic species because, as will become apparent later, the problems of the evolution of the rostrate violets cannot be <sup>worked out by</sup> considering European and American species only. The Asiatic species must be brought into the programme of hybridisation as soon as living material can be obtained, and indeed a start has already been made with V.sieheana, V.faurieana and V.grypoceras.

V.Thibaudieri Fr.et Savat.and V.Raddeana Regel are given distribution maps but there is some doubt as to their systematic position. Becker put them into Section Bilobatae Bckr. but there is a possibility of their being extremely long-leaved members of Section Rostratae, subsect. Arosulatae. Certainly the herbarium specimens I have examined give that impression, but without knowing their chromosome number, or seeing fresh specimens, it is impossible to give a definite opinion.

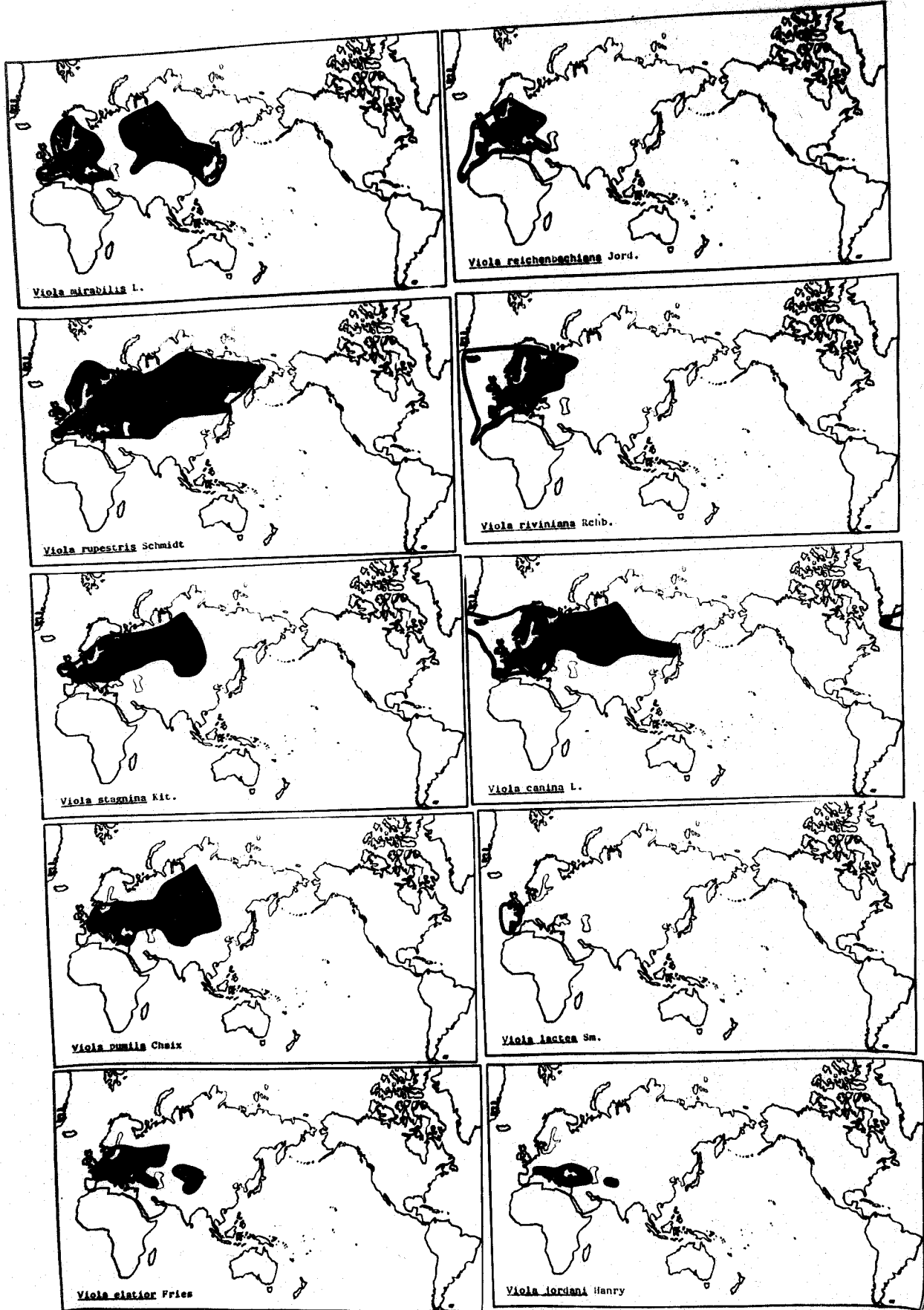


Fig. 20 Geographical distributions

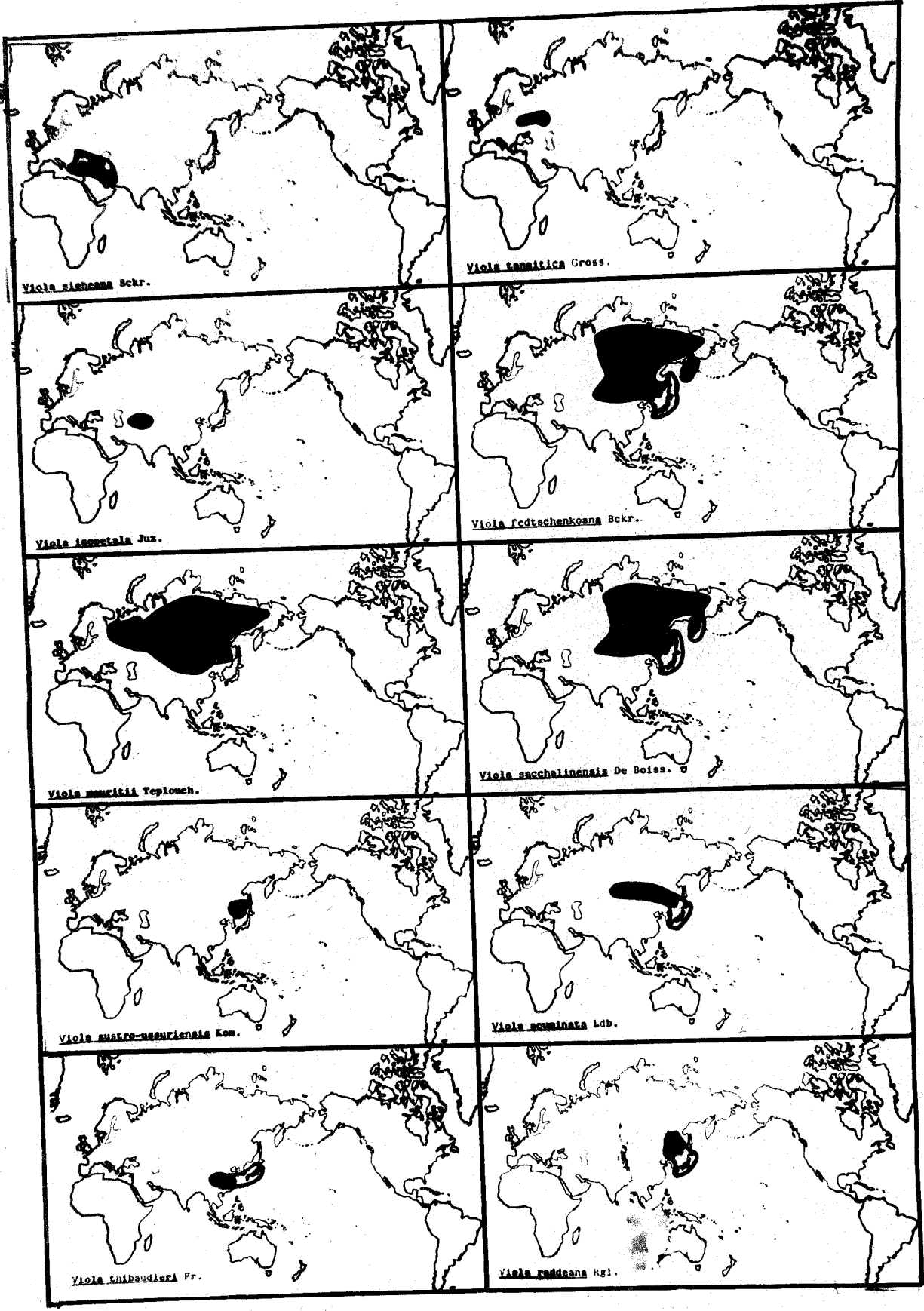


Fig. 21 Geographical distributions

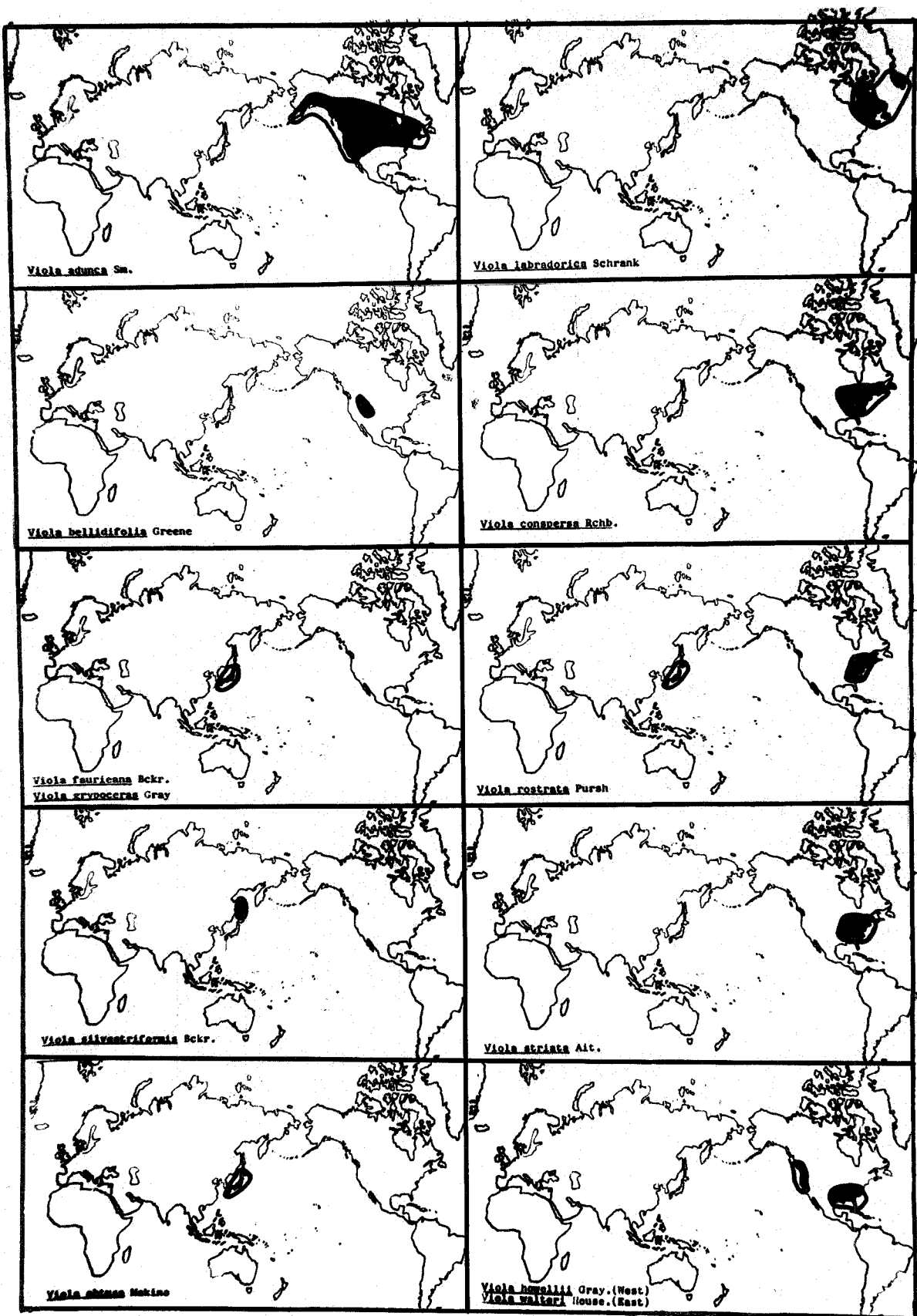


Fig. 22 Geographical distributions

Concerning the distributions of individual species, the information is in many cases not as detailed as could be wished. In Flora URSS. (Komarov, 1949) for instance, Viola mirabilis is not mentioned as occurring in many of the botanical provinces of European Russia, hence the gap in the distribution seen on p.38. There may be a genuine discontinuity or it may be due to lack of knowledge of the regions in question; and it was tempting, when constructing the map, to draw in a continuous distribution. In this particular species added interest is gained from the fact that Becker distinguished two varieties with differing distributions; a sparingly hairy variety in Europe, and a glabrous one in eastern Asia. This is borne out by the specimens cultivated at Durham:-

Viola mirabilis

Hungary .....	Glabrescent
Novosibirsk, central Siberia ....	Glabrous
Tokyo, Japan .....	Glabrous

Viola rostrata has the most outstandingly disjunct distribution among the rostrate violets, (map p.40). The Japanese plants have been given varietal status but close examination of herbarium specimens from Japan and of both pressed and living material from America, has not revealed any difference to my eye; in fact the two sets of plants appear remarkably similar. Plants of Japanese V.rostrata

have not yet been obtained in cultivation in England; seeds sown in autumn 1960 failed to germinate.

Viola riviniana and V.reichenbachiana also have disjunct distributions although to a lesser extent than V.rostrata. Both species occur more or less throughout Europe, in the Atlas Mountains, N.Africa and in the Canary Islands and Madeira. In addition V.riviniana extends further north, reaching Iceland.

#### MEANS OF DISPERSAL OF VIOLETS

##### Short range

The fruit of the rostrate violets efficiently distributes its seeds to a distance of up to several feet from the parent plant. The smooth, hard seeds are squeezed between the valves of the split capsule and shot out in much the same way as is an orange pip when squeezed between the fingers. Horizontal distances of up to 10 feet are probably attained although it must be emphasised that this is an estimate and not the result of measurements.

The only group of violets which does not have its seeds shot out in this way is Section Uncinatae, which includes V.odorata L. Here the seeds simply fall out of the capsule which rests on the ground and is not held erect as in the other sections. The seeds of Uncinatae have a



number of features contrasting with the other groups; they are larger, more angular, rougher coated and have a large fleshy aril which is said to attract ants which appear to be the main dispersal agent. A hybrid between species of sections Rostratae and Uncinatae has been obtained and the seed obtained from it by colchicine treatment has a combination of characters of the two sections, including large size, more or less large aril and a smooth coat.

#### Long-range dispersal

Violets appear to have no regular means of long-range dispersal. The seeds are not regularly eaten by birds or animals, have no means of attachment to fur or feather, and are not adapted to wind or water transport although possibly some of the fen species eg. V.stagnina, may very rarely be water-borne. Even this is not likely to lead to establishment since they are more plants of closed swamp communities than of open places by streams.

Dispersal by man is very unlikely to have been of any importance in enabling the species to attain their present distributions. All are plants of either woodland, fen or natural grassland, and as they are fairly slow-growing perennials, they cannot survive in the disturbed habitats which are the usual result of man's activities. Thus crop-seed, fodder and other materials moved by man are not only unlikely to contain violet seeds but are likely to be deposited in situations unfavourable to the establishment

of violet plants.

One possible exception to the generalisation that the rostrate violets have not had their distributions extended by man, is V.canina which occurs in Greenland and is probably introduced there. Two good specimens of V.canina labelled 'Narsak pr. Julianehaab, legit Dr.L.Kolderup Rosenvinge, a.1888. 16 Jul.', are in Herb.Brit.Mus.(Nat.His.) but there is no indication as to whether the collector considered them native or one of the species which have been either accidentally or deliberately introduced into Greenland. This occurrence is also exceptional in that otherwise members of Arosulatae are absent from the American Continent.

Even in Iceland V.canina and V.riviniana may well belong to the natural vegetation and on other islands, eg. the Mediterranean Islands, the Canaries and Madeira, it has never been questioned that their violets are anything but a natural part of the primitive plant cover.

Thus it can be concluded that, possibly excepting V.canina in Greenland, the observed distributions are natural and have been attained without help from man.

#### CHASMOGAMY - CLEISTOGAMY

Two forms of flowers are produced by violets; large, conspicuous ones in spring called chasmogamous flowers, and small apetalous ones in summer called cleistogamous flowers.

The production of these two types is largely controlled by day length.

In the Mirabiles and Rosulantes, with the exception under Durham conditions of V.striata, the chasmogamous flowers of spring are formed during the shortening days of the previous summer and autumn, but in Arosulatae, which die down completely in winter to small vegetative buds, the open flowers are initiated in spring when growth resumes. Hence Arosulatae flower later than the other two subsections. In all three groups cleistogamous flowers are produced during summer and autumn although some species and many hybrids produce a second flush of open flowers in the autumn under the conditions at Durham City.

A cleistogamous flower contains only two functional anthers, compared with the five in chas<sup>m</sup>ogamous flowers, and in addition is only capable of self-fertilisation. The two anthers contain a very few pollen grains which germinate in situ, the pollen tubes growing down the style and accomplishing fertilisation in the normal manner. This situation was investigated by West (1939), who proved that in V.riviniana cleistogamic fruits were the product of self-fertilisation and not of apomixis. All species, except V.striata produced cleistogamous fruit in great abundance at Durham and the seeds so produced form an important part of the total annual production.

The chasmogamous flowers by contrast have a number of adaptations which aid cross-pollination. The flowers are

conspicuous and attractive to insects with the prominently marked lip-petal forming a landing platform. All five anthers produce abundant pollen which, on being shed, collects on the thin membranous anther appendages which are pressed round the top of the ovary. When these are disturbed, as they are when an insect pushes its proboscis past to obtain nectar from the spur, the accumulated dry pollen is let fall in a sudden shower. The stigma is a small hollow near the end of the projecting style and is held pointing downwards so that it is the first part to contact an insect visitor, while at the same time it is not in a position to allow self-pollination by pollen falling under gravity from the anthers. Should however the flowers be accidentally selfed, good seed is set, since incompatibility is unknown in this group of violets. The breeding system then is a nice balance between inbreeding and outbreeding.

#### VARIATION

Variation in height, leaf size, and flower size is one of the noticeable features of some violets. Such variation can and frequently does occur in natural populations within quite small distances -less than 100 yards- and can make the identification of herbarium specimens extremely difficult, not only among the European violets but even more so among the less familiar Asiatic species. Abnormal and badly pressed fragments of one species can mimic another. This inadequacy of herbarium specimens is of

course a problem in common with many other groups of plants and it is unfortunate that in Viola the flowers, which are in most species the best species-determining feature, are rarely preserved in sufficient detail.

If naturally occurring tall or dwarf plants are dug up and transplanted to uniform conditions in the botanic garden it can be shown that, in the majority of cases, the relative tallness or dwarfness is retained. In other words the variation is genetically determined, just as in Hieracium umbellatum (Turesson, 1922) or Potentilla glandulosa (Clausen, Keck and Hiesey, 1940). Seedlings from seed of cleistogamous capsules usually also reproduce parental characteristics.

It should be added that not all dwarfness is genetically determined and occasionally dwarf plants found in nature have grown to a size comparable with non-dwarf stocks when brought into cultivation. This was the case in one of the collections of V. rupestris from Czechoslovakia reported on by Valentine and Harvey (1961).

The most diverse forms are found in those species which are capable of growing in grassland, possibly because grassland environments can vary so greatly, particularly as to the amount of grazing and wind exposure to which they are subjected. The dwarf plants which may be found in the more heavily grazed or exposed situations are presumably the result of selection by these agencies on originally taller stocks.

Of variation in British species: V. rupestris, found in grassland and among rocks, is quite variable in leaf size and stem length, (Valentine and Harvey, 1961b). V. riviniana, a widely distributed species of woodland and a great variety of natural grasslands, is much more variable than V. rupestris, (Valentine, 1941), but the most variable species is V. canina which ranges in size from minute plants found on grazed, stabilised sand dunes, to very tall plants with large leaves, long internodes and pale flowers, in fens. These two extremes have been given specific names: the dwarf, V. ericetorum Schrader and the fen plant V. montana L. The latter is still distinguished as a species distinct from V. canina in a few modern floras although the status of the various forms may eventually prove to be only that of ecotypes.

The other species are much more restricted in their habitat range, at least in Britain. This is true, for example, of V. stagnina, a very local plant of fens in the southern half of Britain and Ireland, and V. reichenbachiana, also southern but more widespread and a plant of woodland on calcareous soils. These two are conspicuously less variable than the species mentioned previously and this can be attributed to the greater uniformity of their habitats throughout their range.

Flower colour does not vary much in the European violets although it does in the N. American V. adunca (which will be discussed later). White-flowered varieties occur

in the normally blue-flowered V.riviniana, V.rupestris and probably others. Two species at least always have white flowers; V.stagnina and V.striata, although the former may be tinged a very pale blue and both have normal dark-blue veining.

Apart from the occasional albino, several species, especially V.riviniana and V.canina, exhibit minor variations in flower colour. The ground colour may be pale or dark, and the veining may be more or less prominent. In V.riviniana the spur may be white, dark blue or any intermediate colour, but all flowers on any one plant are always of the same type. Dark-spurred forms of V.riviniana have sometimes been confused with V.reichenbachiana, which always has a dark spur.

#### ADVENTITIOUS SHOOTS

Vegetative shoots develop quite normally on the roots of V.stagnina and serve as a means of vegetative propagation. The same phenomenon is also found in some but not all stocks of V.riviniana where it appears to be associated with supernumerary chromosomes, (Valentine, 1949).

During 1959, samples of all the violet species and hybrids in cultivation at Durham were collected for preservation as herbarium material. Where sufficiently large stocks warranted it, complete plants were dug up with a small amount of root. A few months later it was found that round the holes from which plants had been removed

were numerous shoots arising from the cut ends of roots. Thus it appears that under these abnormal conditions many rostrate violets are capable of regeneration from root cuttings and that in some stocks of V.riviniana and all stocks of V.stagnina this capability extends to the intact root system.

GERMINATION of violet seeds only takes place after prolonged exposure to cold damp conditions in soil, (stratification). The method used to germinate seeds was to sow them in soil in pots in the autumn and to keep them moist in an unheated greenhouse through the winter. Good germination was generally obtained in March or April.

Attempts were made to hasten this process by keeping samples, of 50 seeds, at, a)  $-10^{\circ}\text{C}$ ; b)  $0^{\circ}\text{C}$ ; c) about  $3^{\circ}\text{C}$ . The species used was V.riviniana, the seed was freshly ripened, and the time of the treatment for each sample was six weeks. The pots were then placed in a warm greenhouse and kept moist. No germination was obtained from any of the treatments.

Pots in which, as occasionally happened, no germination was obtained after one winter, were dried out for the summer and remoistened for the following winter. In some cases germination took place the following spring. In one case seeds of a violet belonging to the Uncinatae were sown in November 1958 but did not germinate until March 1961; three winters were thus required to break their



dormancy.

This delay in germination, plus the need for a season's growth of the seedlings before they produce chasogamous flower buds suitable for studying meiosis, is the reason why it has only been possible to report on meiosis from the hybrids made in the first season (1959), apart from the hybrids already made by D.M.Moore and D.H.Valentine. The other hybrids, at the time of writing, are either immature plants or seeds undergoing stratification, and will undergo meiosis in spring 1962 and 1963 respectively.

One exception to the need for long stratification is seed of a form of V.adunca originally collected among open grassland and scrub close to the coast near San Francisco, California. This area has little or no frost, the climate being of the Mediterranean type, and the seeds germinate in Durham during the autumn or winter long before any other violets. Moreover this character is transmitted to its hybrids both with other forms of V.adunca and other species, whether used as male or female parent.

The need for stratification under cold conditions is common to a large number of North Temperate plants and is an adaptation ensuring germination at the most favourable time of the year. The coastal V.adunca is stimulated to germinate by moisture alone and not by moisture and cold combined as in all other rostrate violets. This may be regarded as an adaptation to the climatic conditions in the areas concerned.

INTER-SPECIFIC HYBRIDS result from cross-pollination between the chas<sup>m</sup>ogamous flowers of different species and some are fairly frequent in natural mixed populations. The following wild hybrids are known from the British Isles:-

V.riviniana x reichenbachiana

V.riviniana x rupestris

V.riviniana x canina

V.riviniana x lactea

V.canina x stagnina

V.canina x lactea

and many more have been reported from the Continent.

Hybrids were made artificially by transferring pollen from one flower to the stigma of another on a clean needle. The plants were kept in an insect-proof greenhouse in a fairly still atmosphere and under these conditions the great majority of flowers did not become self-pollinated. In most cases well-filled capsules were formed and the seed germinated, often 100%, in the following spring.

FAILURE to obtain vigorous hybrid plants occurred at several stages, ranging from failure to obtain seed-set, to weak, inviable seedlings and these cases are noted below:-

Stage 1. The flowers after pollination withered and did not form capsules. Cases where this was met are:-

♀	♂
<u>V.mirabilis</u>	all pollinations
<u>V.reichenbachiana</u>	large proportion of flowers wither
<u>V.rupestris</u>	pollinated with <u>V.stagnina</u>
<u>V.striata</u>	all pollinations except <u>V.conspersa</u> and <u>V.rostrata</u> when a few seeds set.

Hybrids were successfully obtained when the species in the ♀ column were used as male parent. For example, V.stagnina when pollinated with V.rupestris pollen, set seed in every capsule.

The reason or reasons for these failures have not yet been investigated but the failures have been noted during two or three years work, and Gershoy reports having an identical experience with V.striata, which would not function as female parent when pollinated with V.silvatica Fr. (= V.riviniana Rchb.) or V.elatior, but did function as pollen parent with these two species, (Bamford and Gershoy 1930).

Stage 2. Seed was formed but did not germinate.

♀	♂	<u>No.seeds</u>
<u>V.reichenbachiana</u>	× <u>adunca</u> (Ft.Simpson)	20
<u>V.rostrata</u>	× <u>striata</u>	18
<u>V.conspersa</u>	× <u>reichenbachiana</u>	47
<u>V.sieheana</u>	× <u>rupestris</u>	70

♀	♂	<u>No. seeds</u>
<u>V. riviniana</u>	x <u>hirta</u> ( <u>Uncinatae</u> )	400
<u>V. rupestris</u>	x <u>odorata</u> ( <u>Uncinatae</u> )	119

The remarkable thing in the last three cases is the way in which all flowers pollinated formed well-developed capsules containing normal-sized seeds which however were cream-coloured (normally brown) and hollow. It may be deduced from this that pollen-tube growth and probably fertilisation had occurred but that some stage embryo growth had failed. It may in future attempts be possible to germinate seeds from the first three crosses above since the seed which was obtained was well formed.

Stage 3. Germination to give non-viable seedlings:-

♀	♂	<u>No. of seeds</u>	
		<u>Sown.....</u>	<u>Germinated</u>
<u>V. conspersa</u>	x <u>labradorica</u>	32	28
<u>V. striata</u>	x <u>rostrata</u>	25	4
<u>V. stagnina</u>	x <u>lactea</u>	173	8
<u>V. sieheana</u>	x <u>faurieana</u>	161	5
<u>V. riviniana</u>	x <u>odorata</u>	46	7
<u>V. riviniana</u>	x a species of the <u>Boreali-Americanae</u>	24	20

In the above cases the seedlings emerged above ground, opened their seed leaves, but failed to make any growth

once the reserves in their cotyledons was used up; they remained the same size until the autumn and then died.

Stage 4. Other hybrid families were produced in which some seedlings were vigorous, others weak and inviable.

These are:-

	♀	♂	<u>No. seedlings</u>	
			<u>Vigorous...</u>	<u>Weak</u>
<u>V.adunca (coastal) x rupestris</u>			3	9
<u>V.rostrata</u>		<u>x adunca (Burlington)</u>	16	2
<u>V.conspersa</u>		<u>x adunca (coastal)</u>	5	3
<u>V.conspersa</u>		<u>x adunca (Burlington)</u>	1	7

It seems likely that the reasons for the weak seedlings in this group of crosses are different from those in the previous group. Two possible explanations are, first, segregation of lethal genes which only produce their effect in hybrids, and secondly, production of haploids. The first explanation is unlikely since the parental stocks are probably highly inbred; and by the time it was realised that some of the seedlings, after normal germination, had not grown, they were too moribund to check the second explanation.

Stage 5. Dwarf but viable plants were obtained of V.rostrata ♀ x rupestris and these are still healthy and surviving after two seasons' growth. They are characterised by a very abundant production of small leaves and by the

complete failure of stem elongation, giving crisp little rosettes. The whole progeny of this cross, 20 plants, are identical and no flowers, either chas<sup>m</sup>ogamous or cleistogamous have been produced. A single treatment with gibberellic acid at concentrations of 0.1% and 0.01% in lanoline was tried, but at these concentrations and method of application, it made no alteration to growth or flower production.

#### HYBRID VIGOUR

Despite the few failures noted above, over 45 hybrids were synthesised which showed very marked hybrid vigour and produced abundant cleistogamous and chas<sup>m</sup>ogamous flowers. Their free production of flowers was of immense value in enabling good cytological preparations to be obtained from all but a few of the mature hybrids and the examination of their meiosis has provided the main results of this thesis.

Table 2

## VIGOROUS HYBRIDS IN CULTIVATION, 1961

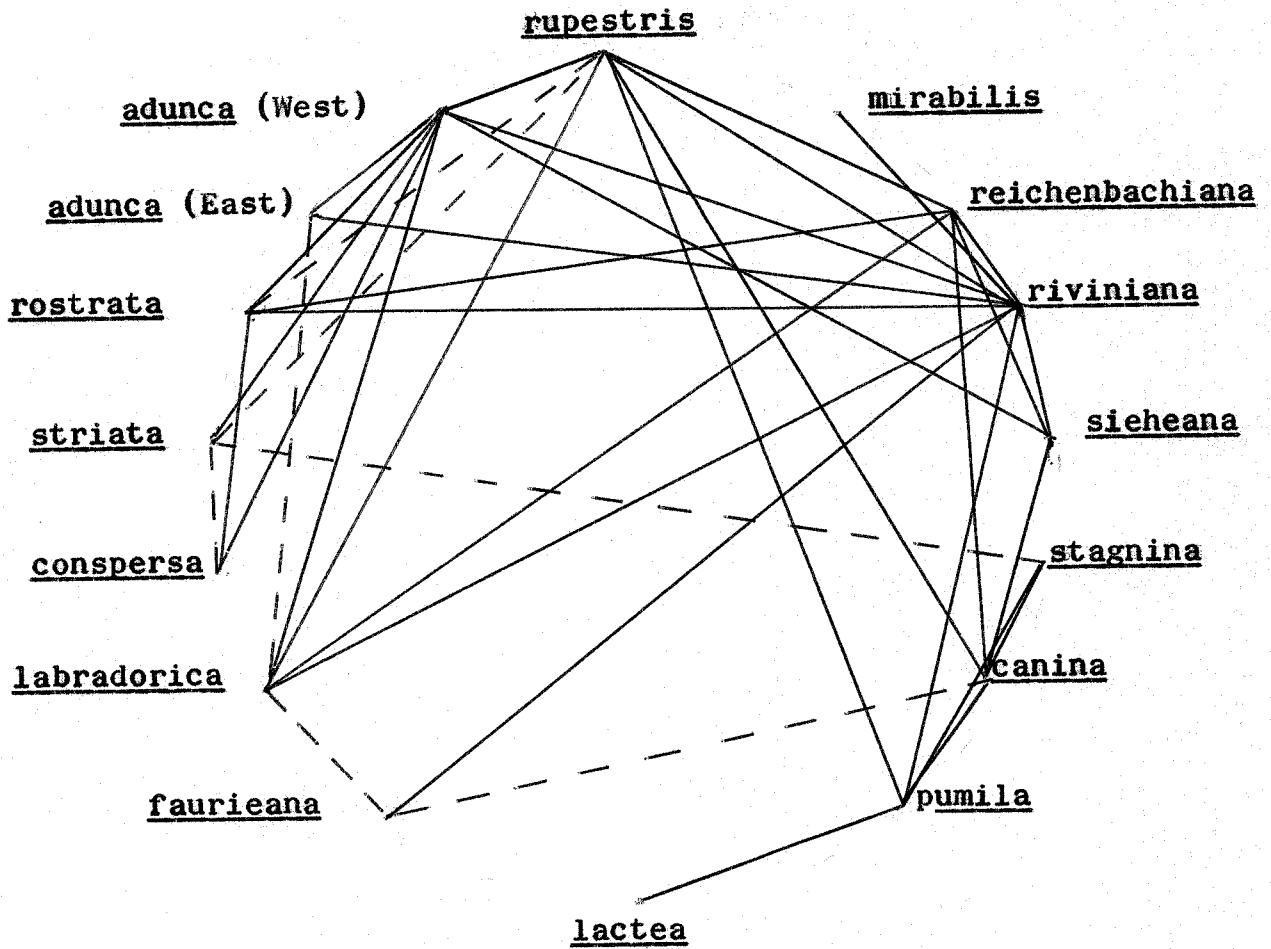
2x	<u>V.adunca (West)</u>	x <u>adunca (East)</u> *
	<u>V.adunca (West)</u>	x <u>rostrata</u> *
	<u>V.adunca (West)</u>	x <u>conspersa</u> *
	<u>V.adunca (West)</u>	x <u>striata</u> *
	<u>V.adunca (West)</u>	x <u>labradorica</u> *
	<u>V.adunca (West)</u>	x <u>faurieana</u>
	<u>V.adunca (East)</u>	x <u>labradorica</u>
	<u>V.adunca (East)</u>	x <u>conspersa</u>
	<u>V.rupestris</u>	x <u>adunca (West)</u> *
	<u>V.rupestris</u>	x <u>labradorica</u> *
	<u>V.rupestris</u>	x <u>striata</u>
	<u>V.rupestris</u>	x <u>reichenbachiana</u> *
	<u>V.rostrata</u>	x <u>conspersa</u> *(D.H.V.)
	<u>V.conspersa</u>	x <u>striata</u>
	<u>V.reichenbachiana</u>	x <u>rostrata</u> *
	<u>V.reichenbachiana</u>	x <u>labradorica</u> *
	<u>V.faurieana</u>	x <u>labradorica</u>
<u>V.stagnina</u>	x <u>striata</u> (D.H.V.)	
3x	<u>V.riviniana</u>	x <u>reichenbachiana</u> *
	<u>V.riviniana</u>	x <u>mirabilis</u> * (D.H.V.)
	<u>V.riviniana</u>	x <u>rupestris</u> * (D.H.V.)
	<u>V.riviniana</u>	x <u>rostrata</u> * (D.H.V.)
	<u>V.riviniana</u>	x <u>labradorica</u> *
	<u>V.riviniana</u>	x <u>adunca (West)</u>
	<u>V.riviniana</u>	x <u>adunca (East)</u>
	<u>V.riviniana</u>	x <u>faurieana</u>
	<u>V.canina</u>	x <u>reichenbachiana</u> * (D.H.V.)
	<u>V.canina</u>	x <u>rupestris</u> *
	<u>V.canina</u>	x <u>stagnina</u> * (D.H.V.)
	<u>V.pumila</u>	x <u>rupestris</u> *
	<u>V.pumila</u>	x <u>stagnina</u> *
4x	<u>V.sieheana</u>	x <u>reichenbachiana</u> *
	<u>V.sieheana</u>	x <u>adunca (West)</u> *
	<u>V.pumila</u>	x <u>riviniana</u> *
	<u>V.pumila</u>	x <u>canina</u> * (D.M.M.)
5x	<u>V.sieheana</u>	x <u>riviniana</u> * (D.H.V.)
	<u>V.sieheana</u>	x <u>canina</u> *
	<u>V.lactea</u>	x <u>pumila</u> * (D.M.M.)
Also	<u>V.riviniana?</u>	x <u>odorata</u> (Uncinatae)
	<u>V.riviniana</u>	x <u>palustris</u> (Stolonosae)

\* Denotes meiosis studied.

D.H.V. = hybrid made by D.H.Valentine.

D.M.M. = hybrid made by D.M.Moore.

Fig. 23

DIAGRAM OF HYBRIDS MADE

————— Meiosis examined

----- Plants in cultivation but meiosis not yet studied



In addition to the foregoing, seed was obtained in 1961 from the following cross pollinations:-

<u>V.reichenbachiana</u>	x	<u>mirabilis</u>
<u>V.reichenbachiana</u>	x	<u>stagnina</u>
<u>V.reichenbachiana</u>	x	<u>lactea</u>
<u>V.sieheana</u>	x	<u>mirabilis</u>
<u>V.sieheana</u>	x	<u>elatior</u>
<u>V.sieheana</u>	x	<u>pumila</u>
<u>V.sieheana</u>	x	<u>lactea</u>
<u>V.elatior</u>	x	<u>pumila</u>
<u>V.elatior</u>	x	<u>lactea</u>
<u>V.lactea</u>	x	<u>stagnina</u>
<u>V.rupestris</u>	x	<u>conspersa</u>
<u>V.rupestris</u>	x	<u>stagnina</u>
<u>V.rupestris</u>	x	<u>lactea</u>
<u>V.rostrata</u>	x	<u>striata</u>
<u>V.adunca</u>	x	<u>stagnina</u>
<u>V.jordani</u>	x	<u>rupestris</u>
<u>V.jordani</u>	x	<u>stagnina</u>
<u>V.jordani</u>	x	<u>lactea</u>
<u>V.jordani</u>	x	<u>elatior</u>
<u>V.jordani</u>	x	<u>pumila</u>
<u>V.jordani</u>	x	<u>canina</u>
<u>V.jordani</u>	x	<u>riviniana</u>

It is hoped that the seed of the above will germinate in spring 1962 and produce hybrids.

RECIPROCAL HYBRIDS have only been made in this investigation in five cases:-

V.riviniana x pumila

V.riviniana x adunca

V.rupestris x reichenbachiana

V.adunca (Mather) x adunca (Queen Charlotte Is.)

V.adunca (coastal) x adunca (Queen Charlotte Is.)

The reciprocals are identical, and there is no trace of any maternal tendency. Other authors have also found reciprocal hybrids to be identical; eg. Valentine, (1950), V.riviniana x reichenbachiana; and Gershoy, (1934), various hybrids.

#### HYBRID FERTILITY

All interspecific hybrids show a reduced, often greatly reduced, seed production compared with the parental species. Many are completely sterile; that is, no capsules have been observed to result from either cleistogamous or chas<sup>m</sup>ogamous flowers during at least two seasons' growth.

#### COMPLETELY STERILE HYBRIDS

2x V.reichenbachiana x labradorica

V.reichenbachiana x rostrata

V.reichenbachiana x rupestris

V.rupestris x rostrata (dwarf plants)

V.rupestris x striata

V.adunca (Mather) x striata

V.adunca (Mather) x labradorica (abnormal)

V.stagnina x striata

3x V.riviniana x mirabilis

V.riviniana x rupestris

V.riviniana x adunca

V.riviniana x labradorica

V.riviniana x rostrata

V.riviniana x conspersa

V.canina x rupestris

V.pumila x rupestris

V.pumila x stagnina

4x V.riviniana x pumila

V.canina x pumila

V.sieheana x reichenbachiana

V.sieheana x adunca (Mather)

5x V.sieheana x riviniana

V.sieheana x canina

The other hybrids have a low seed production which is however very difficult to measure because fertility varies from season to season. The reason for this lies in the varying size of the anthers throughout the year; this is a day-length response and is part of the changes associated with the different flower forms. In one partly fertile

hybrid (V.riviniana × reichenbachiana), it was particularly noticed that the cleistogamous flowers formed in the first half of the season all aborted, but that those produced later in the year, during late summer and autumn, set increasingly large numbers of seeds. It is quite likely that this can be correlated with increased anther size, and hence greater numbers of pollen grains, in the later formed cleistogamous flowers since this hybrid, and many others, produced in the autumn some flowers which were intermediate between the open and closed forms and then a small crop of perfectly formed open flowers, (in addition to the buds of the spring, open flowers which first become visible in the autumn but remain dormant through the winter). This marked variation in seed production from season to season means that a true measure of fertility would best be done in terms of total number of seeds and total number of flowers per plant per complete growing season. As the seasonal variation in seed production was not noticed until it was too late to collect a complete year's output, no figures have been given of seed production in the partly fertile hybrids. As a substitute, which is by no means the same thing, some measurements are given of the percentage of good pollen as seen stained in aceto-carmin.

In general it may be observed that hybrids between European and between European and American species are completely or nearly completely sterile, (excepting perhaps V.canina × lactea, Moore 1959), but that some hybrids

between American species have an appreciable seed output.

#### COLCHICINE TREATMENT

During spring 1961 it was realised that an essential follow-on from the study of fertility and meiosis in the F1 hybrids was a similar study on their artificially induced polyploids and that the successful production of these would help to solve some of the problems connected with the evolution of the polyploid series found in Viola. Accordingly some of the actively growing shoot tips of all the available hybrids were treated with colchicine solution.

The method used was to apply one drop of an aqueous solution of colchicine to each shoot tip so that the drop was held by surface tension between the youngest leaves. The solution was allowed to dry and not washed off. Strengths of 1% and 0.2% were used, with some success in each case. The 1% solution killed many shoot apices, and in that case subsequent growth of the branch was from an unaffected axillary bud lower down. The 0.2% solution often had no effect; but in a number of sterile hybrids one of the treatments resulted in a resumption of apical growth in about six weeks and cleistogamous capsules full of seed were produced. The seed was of normal appearance, not hollow, and there seems no reason to doubt that it contains embryos with the doubled hybrid chromosome number and that it will germinate in the spring of 1962.

Table 3    SEEDS FROM COLCHICINE TREATMENT

	<u>No. seeds</u>
<u>V. rupestris x reichenbachiana</u>	135
<u>V. rupestris x labradorica</u>	245
<u>V. rupestris x adunca</u> (Mather)	99
<u>V. adunca (M.) x conspersa</u>	5
<u>V. adunca (M.) x striata</u>	50
<u>V. adunca (M.) x adunca</u> (Ft. Simp.)	18
<u>V. rostrata x conspersa</u>	23
<u>V. adunca(Q.C.) x faurieana</u>	3
<u>V. stagnina x striata</u>	20
<u>V. riviniana x adunca</u>	64
<u>V. riviniana x labradorica</u>	21
<u>V. riviniana x pumila</u>	274
<u>V. canina x rupestris</u>	15
<u>V. sieheana x adunca</u> (Mather)	26
<u>V. odorata x ? (riviniana)</u>	6

HYBRIDS EXAMINED BY OTHER WORKERS

During the late 1920s and early 1930s Gershoy, at the Vermont Agricultural Research Station, Burlington, Vermont, USA., collected and grew a large number of North American and some European Viola species and made many hybrids. The results of this work were published in a series of four papers entitled 'Studies in North American Violets'.

Gershoy's experiments embraced a large number of species

covering all North Temperate sub-sections of the genus and hence of much greater scope than the present investigation which is confined to a single sub-section. Gershoy's main aims were, apparently, to find out to what extent hybridisation was possible, and to investigate the vigour fertility and cytology of the hybrids. From the vast amount of information he obtained he drew up a tentative scheme of relationships for all the groups of Viola found in North America and Europe. His investigations were more concerned with the relationships between the sections and subsections than with the relationships between the species within any one subsection.

In only two hybrids between rostrate violets was a study made of chromosome behaviour at meiosis, (Bamford and Gershoy 1930 ); these were V.elatior x striata,  $2n=30$ , and V.silvatica x striata,  $2n=30$ , (V.silvatica Fr.=V.riviniana). Sectioned anthers and ovaries were used but no squashes. The authors reported only univalents at first metaphase of meiosis and although these particular hybrids have not been studied for this thesis, the result is in agreement with other similar observations.

Gershoy also mentions that V.adunca, V.rostrata, V.striata and V.conspersa are a closely related, freely interbreeding group of species.

ROSTRATAE HYBRIDS SYNTHESISED BY GERSHOY

	<u>Comment</u>
<u>V.canina</u> × <u>striata</u>	sterile, vigorous.
<u>V.canina</u> × <u>elator</u>	" "
<u>V.canina</u> × <u>pumila</u>	" "
<u>V.elator</u> × <u>stagnina</u>	" "
<u>V.pumila</u> × <u>elator</u>	" "
<u>V.pumila</u> × <u>riviniana</u>	" "
<u>V.riviniana</u> × <u>elator</u>	" "
<u>V.riviniana</u> × <u>howellii</u>	" "
<u>V.riviniana</u> × <u>striata</u>	" "
<u>V.riviniana</u> × <u>rostrata</u>	" "
<u>V.elator</u> × <u>striata</u>	" "
<u>V.pumila</u> × <u>striata</u>	" "
<u>V.stagnina</u> × <u>rostrata</u>	" "
<u>V.striata</u> × <u>howellii</u>	" "
<u>V.conspersa</u> × <u>rostrata</u>	fertile, vigorous
<u>V.rostrata</u> × <u>striata</u>	" "
Also: <u>V.riviniana</u> × <u>palustris</u>	
<u>V.conspersa</u> × <u>papilionacea</u>	
<u>V.septentrionalis</u> × <u>striata</u>	

D.H.Valentine, (1950), dealt with the experimental taxonomy of V.riviniana,  $2n=40$ , V.reichenbachiana,  $2n=20$ , and their hybrid, showing that at meiosis in the hybrid



there are 10 bivalents and 10 univalents commonly present at first metaphase although there was a good deal of variation. He postulated that V.riviniana is an allotetraploid originating from two species with  $2n=20$  by hybridisation and chromosome doubling and that one of these diploids was V.reichenbachiana or something very similar and the other a species as yet unknown. Also studied was the hybrid V.canina x reichenbachiana, which was found to have 0 to 4 bivalents at meiosis. In a later paper, (1958), the same author included V.canina,  $2n=40$  and V.stagnina,  $2n=20$ , in a genomic system as below:-

<u>V.reichenbachiana</u>	A	<u>V.stagnina</u>	C
<u>V.riviniana</u>	AB	<u>V.canina</u>	BC

Schöfer, (1954), studied several hybrids, mainly wild ones, and gave some meiosis results:-

	2n	Meiosis	
		Bivalents	Univalents
<u>V.riviniana x reichenbachiana</u>	30	10	10
<u>V.rupestris x 'Waldveilchen'</u>	20		
	24		
	28	13	4
		9	10
	34		
	40		
	44		
<u>V.riviniana x montana</u>	44		
	48		
	42	1	40
<u>V.montana x rupestris</u>	30		
	30	7	16
	32		

Of the 'Waldveilchen' hybrids, some are interpreted as V. rupestris x reichenbachiana, others as rupestris x riviniana. From the camera lucida drawings illustrating the paper it is seen that in all preparations the pollen-mother-cell wall is intact, and the cytoplasm not or only slightly spread. This condition is known from personal experience to make an accurate description of the chromosome configurations very difficult, and hence little reliance can be placed on his results. It was explained by one of Schöfer's colleagues that the use of only cells with intact walls derived from the fear that otherwise chromosomes would be lost or, alternatively, broken. My own experience is that in violets loss of cytoplasm is rare and readily recognised and that the chromosomes are too small for even severe squashing to fragment them.

A. Schmidt, (1961), reported that V. jordani was a tetraploid, ( $2n=40$ ), and that the artificial hybrid between V. canina and V. jordani was fully sterile. He studied in detail a number of hybrids within the subsections Uncinatae and Rostratae and in the latter subsection included hybrids both with and without B chromosomes. The hybrids in Rostratae were:-

	<u>2n</u>
<u>V. riviniana</u> x <u>rupestris</u>	30 and 30 + 4 to 9 B
<u>V. canina</u> (or <u>montana</u> ) x <u>rupestris</u>	30 and 30 + c.4 B
<u>V. canina</u> x <u>riviniana</u>	40 and 40 + 10 B

Meiosis was studied in the above and both V.riviniiana × rupestris and V.canina × rupestris were reported as having 7 to 8 bivalents and 16 to 14 bivalents, and V.riviniiana × canina 10 bivalents and 20 univalents. The hybrid between V.canina and V.montana was several times mentioned as being fully fertile.

D.M.Moore, in a study of the cytotaxonomy of V.lactea (Moore and Harvey, 1961), reported the chromosome number of V.lactea as  $2n=58$  (ie. subhexaploid), and gave the results of a study of meiosis in several hybrids whose chromosome numbers and most frequently observed pairing behaviour are reproduced below:-

	<u>2n</u>	<u>II</u>	<u>I</u>
<u>V.lactea</u> × <u>riviniiana</u>	49	10	29
<u>V.canina</u> × <u>lactea</u>	49	20	9
<u>V.canina</u> × <u>stagnina</u>	30	10	10
<u>V.canina</u> × <u>riviniiana</u> or <u>montana</u>	40	10	29

## CYTOTOLOGY RESULTS

### CHROMOSOME BEHAVIOUR IN VIOLET SPECIES

Any cytotaxonomic study of a group of species should normally start with an examination of the chromosomes and chromosome behaviour in the species before the hybrids are dealt with. This order is partly reversed in the present investigation for two reasons. First, many of the species have been reported on by other workers, and secondly, all efforts were directed to obtaining as many hybrids as possible during the first three years of research and removing flower buds and root tips would have interfered with this, especially as some of the more interesting species were shy of flowering. Hence several species have been insufficiently worked on; but so far as chromosome numbers are concerned, none are in doubt since those which have not been counted directly have <sup>been</sup> amply deduced from the numbers of their hybrids, which have been subjected to a very detailed examination.

The only new counts recorded are  $2n=60$  for V.sieheana from Rizé, Turkey, and  $2n=20$  for V.bellidifolia from Utah, USA.

Chromosome size within the complement, as seen at meiosis in pollen-mother-cells, varies little and it was

not possible to distinguish individual pairs or groups with any degree of assurance. Fothergill, (1944), studying root-tip material, was able to divide the chromosomes of several species (V.reichenbachiana, V.riviniana and V.canina), into four size classes. These differences are not so obvious at meiosis and the only comparable observations on root-tip chromosomes in this thesis are of V.rupestris from Long Fell, Westmorland. In this material distinct satellites were seen on two chromosomes in each set. The roots had been pretreated with 8-hydroxyquinoline and were examined as Feulgen stained squashes mounted in 'Euparal', (photo. p.149). Satellites do not appear to have been previously reported for violet chromosomes. Below are listed the chromosome observations made on species:-

Table 4      CHROMOSOME COUNTS OF SPECIES

POLLEN-MOTHER-CELL COUNTS

	<u>2n</u>
<u>V.canina</u>	40
<u>V.reichenbachiana</u>	20
<u>V.riviniana</u>	43 (with supernumeraries)
<u>V.labradorica</u>	20
<u>V.sieheana</u>	60
<u>V.pumila</u>	40

ROOT-TIP COUNTS

	<u>2n</u>
<u>V.rupestris</u>	20
<u>V.adunca</u> (coastal)	20
<u>V.bellidifolia</u>	20

V.labradorica from Mt. Jacques Cartier, Gaspé was the only stock of this species examined and all cells at meiosis had 8 bivalents plus 1 quadrivalent. This discovery was prompted by an abnormality in the hybrid V.adunca × labradorica which was deficient for part of a chromosome, but it is clear from four other hybrids that the gametes of V.labradorica normally carry 10 chromosomes. Meiosis was examined in only one plant and it would be interesting to extend this investigation to other plants and populations. This appears to be the only reported case of quadrivalent formation in a violet species.

V.reichenbachiana was available as normal diploid and as synthetic triploid and tetraploid. The <sup>tetraploid</sup> was formed by colchicine treatment of seedlings of the diploid, and the triploid by crossing the tetraploid and the diploid. Chromosome pairing at meiosis was studied carefully as the information was required to help interpret the configurations seen at meiosis in the various hybrids. The oddity with V.reichenbachiana is that although it has a low chromosome number it was found quite difficult to obtain well-spread pollen-mother-cell squashes since the

chromosomes tended to clump together more than in higher-numbered species. The results of the examination were that the diploid, triploid and tetraploid plants consistently formed bivalents, trivalents and quadrivalents respectively. The diploid and tetraploid were fertile, the latter obviously producing viable gametes with 20 chromosomes, and the triploid had a reduced fertility, only setting seed in a low proportion of cleistogamic flowers. Photographs of meiosis in the triploid and tetraploid are given on p.150.

V.pumila was the only species examined in which failure of bivalent formation at meiosis was observed; 2 cells out of a total of 19 showed 19 bivalents plus 2 univalents, instead of 20 bivalents. Even so this makes quite a small proportion of abnormal cells and V.pumila may be regarded as normally a bivalent forming species since all the hybrids made from it appeared to result from normal gametes.

V.sieheana has normal bivalent formation and regular separation of bivalents at first anaphase. Over 20 cells were examined but no univalents or multivalents were seen.

#### CHROMOSOME BEHAVIOUR IN HYBRIDS

All observations on meiosis were performed on pollen-mother-cells obtained from chas<sup>m</sup>ogamous flowers in early spring and no attempt was made to examine the very few pollen-mother-cells found in cl<sup>a</sup>istogamous flowers later in

the year, (though this is just feasible), or to study meiosis in megaspore-mother-cells.

Fixation was in the commonly used mixture of glacial acetic acid and absolute alcohol (1:3), to which a trace of ferric acetate solution was added and the tubes were stored in the deep freeze at  $-10^{\circ}\text{C}$  until required. It was found that the buds kept in good condition for two years. Single anthers were dissected out from the flower buds, squashed in iron aceto-carmin solution in 45% acetic acid and the slide, if good enough, made permanent by dehydration in alcohol and mounting in 'Euparal'

It was found that the commonest stage of meiosis seen, apart from very early prophase, was first metaphase, with first anaphase only slightly less common. I was of course acutely aware that theoretically the best stage of meiosis for analysis of pairing is diakinesis of first prophase but this stage is very rarely seen in Viola and was come across only about a dozen times during the three years of the study. Fixing buds at 9am. and 7pm. gave results which were no different and whenever diakinesis does occur it would appear to be passed through quickly. It is comforting to note that this peculiarity of violets had been met with by Gershoy, (1934), who failed to find any diakinesis stages.

The result is that practically all meiosis observations were made on first metaphase with a few on early anaphase.



It was found that sufficiently well-spread preparations were readily obtained of the above stages such that the numbers of bivalents and univalents could be counted in the cells of most hybrids at a magnification of 400. In only a few hybrids did extensive numbers of cells have to be drawn under the camera lucida at  $\times 1000$ ; the two most difficult were V.sieheana  $\times$  riviniana and V.sieheana  $\times$  canina, both with  $2n=50$  and an average of 10 bivalents per cell.

Scoring of the results was made easy by the fact that most chromosomes associated either as bivalents or univalents; higher categories were rare and where met with were treated as if composed of the appropriate number of univalents and bivalents, eg. a trivalent = 1 univalent + 1 bivalent; a quadrivalent = 2 bivalents.

Since the amount of pairing observed is quite variable, eg. from 2 to 8 bivalents per cell in V.riviniana  $\times$  rupestris, the examination of a small number of pollen-mother-cells could give a misleading impression. It was therefore decided to analyse a considerable number of cells of each hybrid so that an accurate idea could be gained of the average and variation in numbers of bivalents per cell. For this, a provisional target of an accurate analysis of 100 cells per hybrid was set; in 20 hybrids this was attained from the first slide and in only 10 did lack of buds at the right stage prevent the target being reached.

A considerable amount of thought was given to the

Various possible ways of presenting the results. A method was required which would allow the rapid and easy comparison of meiosis in one hybrid with that in any other, and for this purpose a diagram scores heavily over a detailed table of figures. With over 30 hybrids to deal with (a number to be greatly increased in subsequent years), the method adopted by Valentine (1950) and Moore and Harvey, (1961), of giving for each hybrid a table of the numbers of cells with the various observed combinations of univalents, bivalents, trivalents etc., while undoubtedly enabling the exact and detailed results to be presented, would inevitably lead to a complicated blur of tables when extended to 30 or more hybrids. Such a series of tables would not prevent the comparison of meiosis in one hybrid with that in another, but it would certainly not facilitate it, and would involve a tedious examination of one line of figures and a comparison of this with another line, possibly several pages away.

It was not found possible to devise a diagrammatic representation of the meiosis results which allowed for the representation of trivalents and higher associations and consequently, as stated above, these were reduced to their equivalent numbers of univalents and bivalents and included as such. It was decided that the greater ease of comparison which the diagrammatic presentation of results brought about far outweighed any loss of information which

the omission of higher associations entailed. Except in V.rostrata x conspersa multivalents were very rare and in many hybrids none were seen, so the loss of detail is only slight.

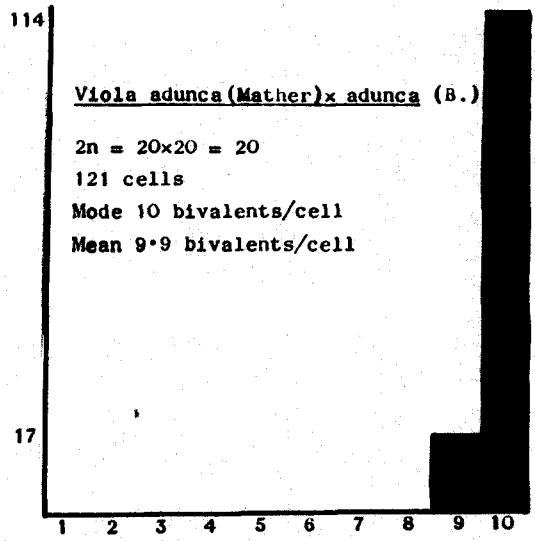
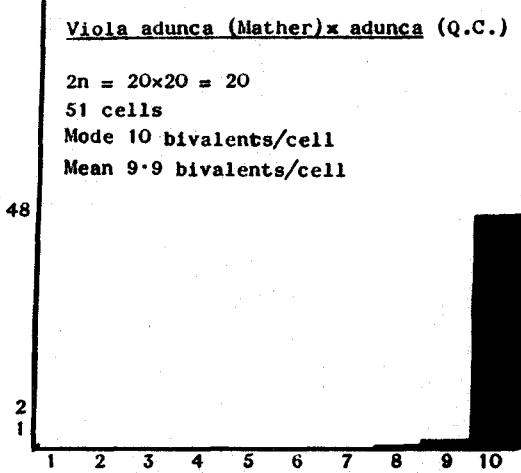
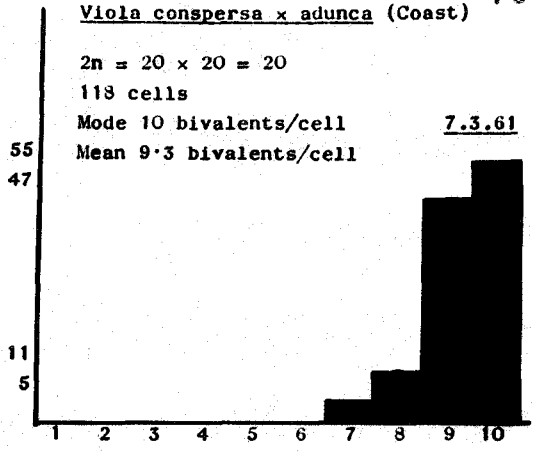
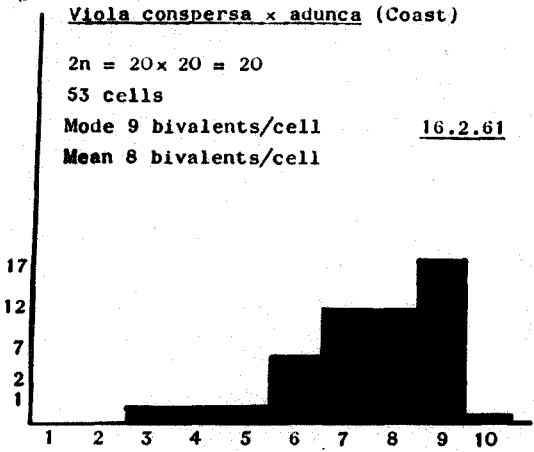
The implication behind the presence of multivalents in hybrids is that the transfer of material between chromosomes within each set has taken place at some time during the evolutionary divergence of the parental species. That this process has been of minor importance in the differentiation of the chromosomes of rostrate violets, at least from the point of view of major translocations, is shown by the scarcity of multivalents in their hybrids, with the one exception mentioned above. Presumably the rare multivalents seen in some hybrids have arisen as a result of chiasma formation involving a small or very small translocation and it may well be that such small transfers have been of cumulative importance in the formation of the chromosome sets of our present violets.

The results of the examination of meiosis in the hybrids are presented as a series of histograms, one for each hybrid; the numbers of bivalents are plotted on the horizontal axis and the numbers of cells on the vertical axis. Table 5, p.84, summarises some of the figures in the histograms.

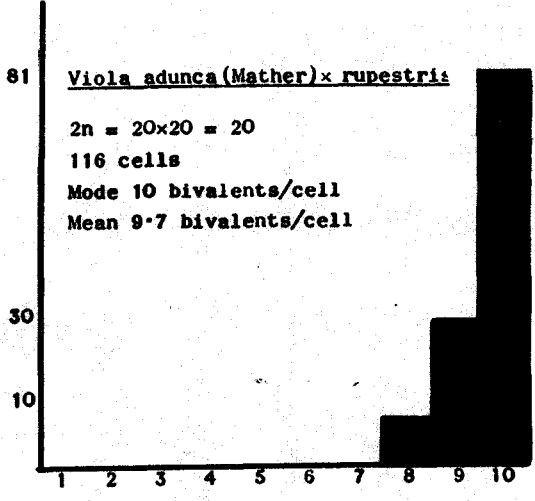
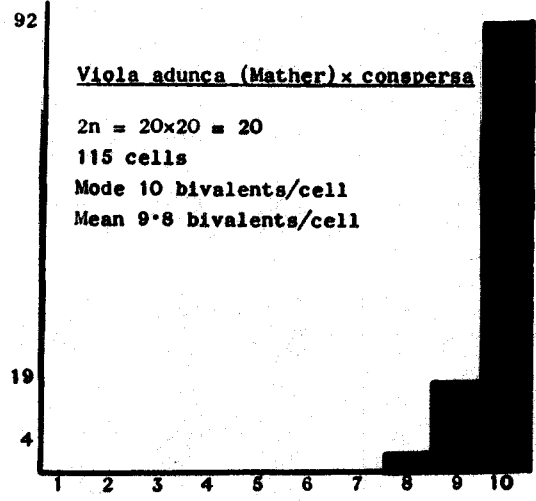
It was chosen to plot the data for bivalents rather than univalents because the presence of supernumerary

chromosomes in some hybrids resulted in higher numbers of univalents being scored than would otherwise have been the case.

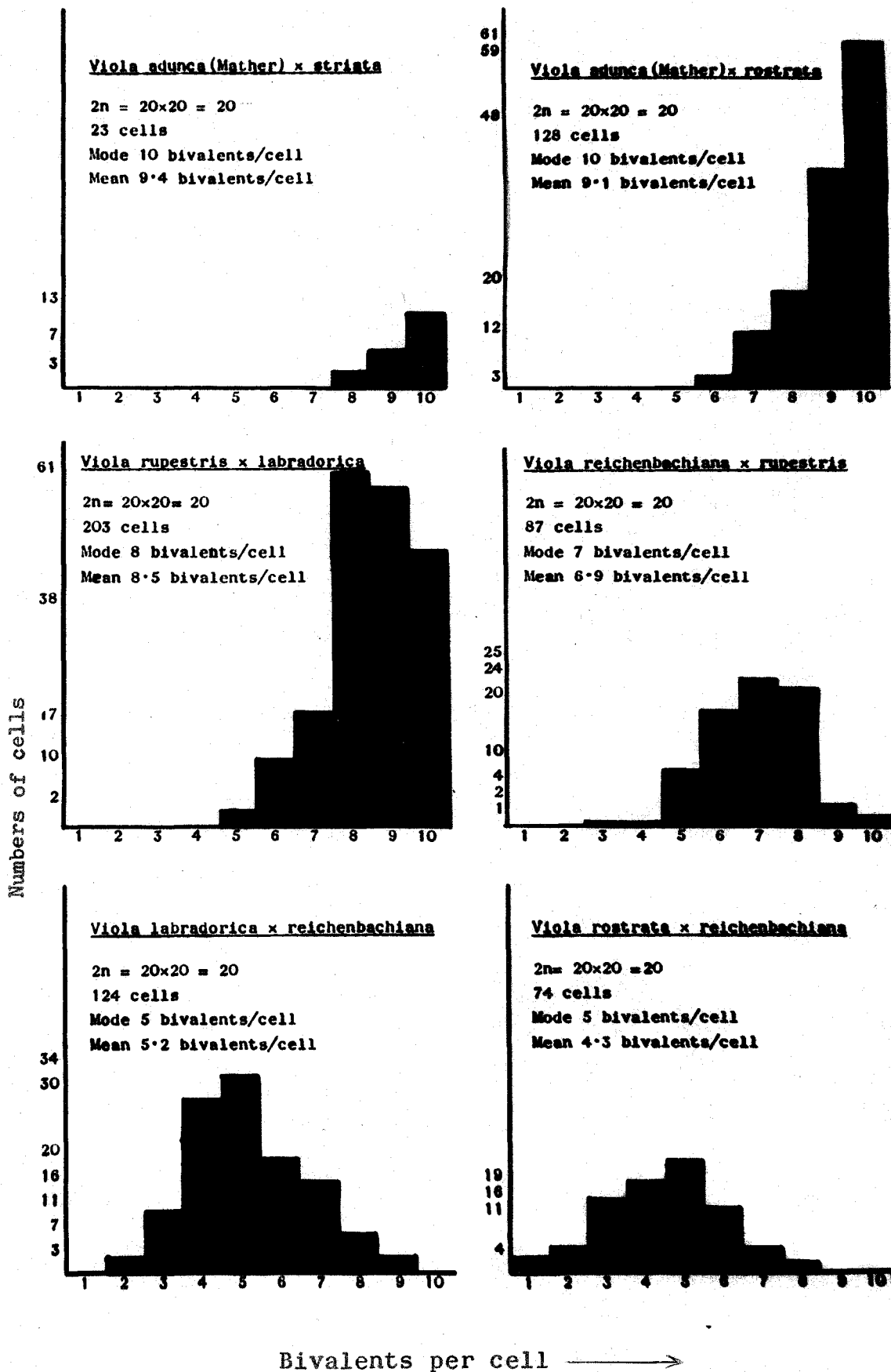
It is possible to tell at a glance from each histogram what is the most frequent number of bivalents seen in the pollen-mother-cells, also the range of variation in the numbers of bivalents, as well as the exact number of cells examined. Furthermore the shape, and hence the sum of the above factors, of any histogram can be readily compared with that of any other.

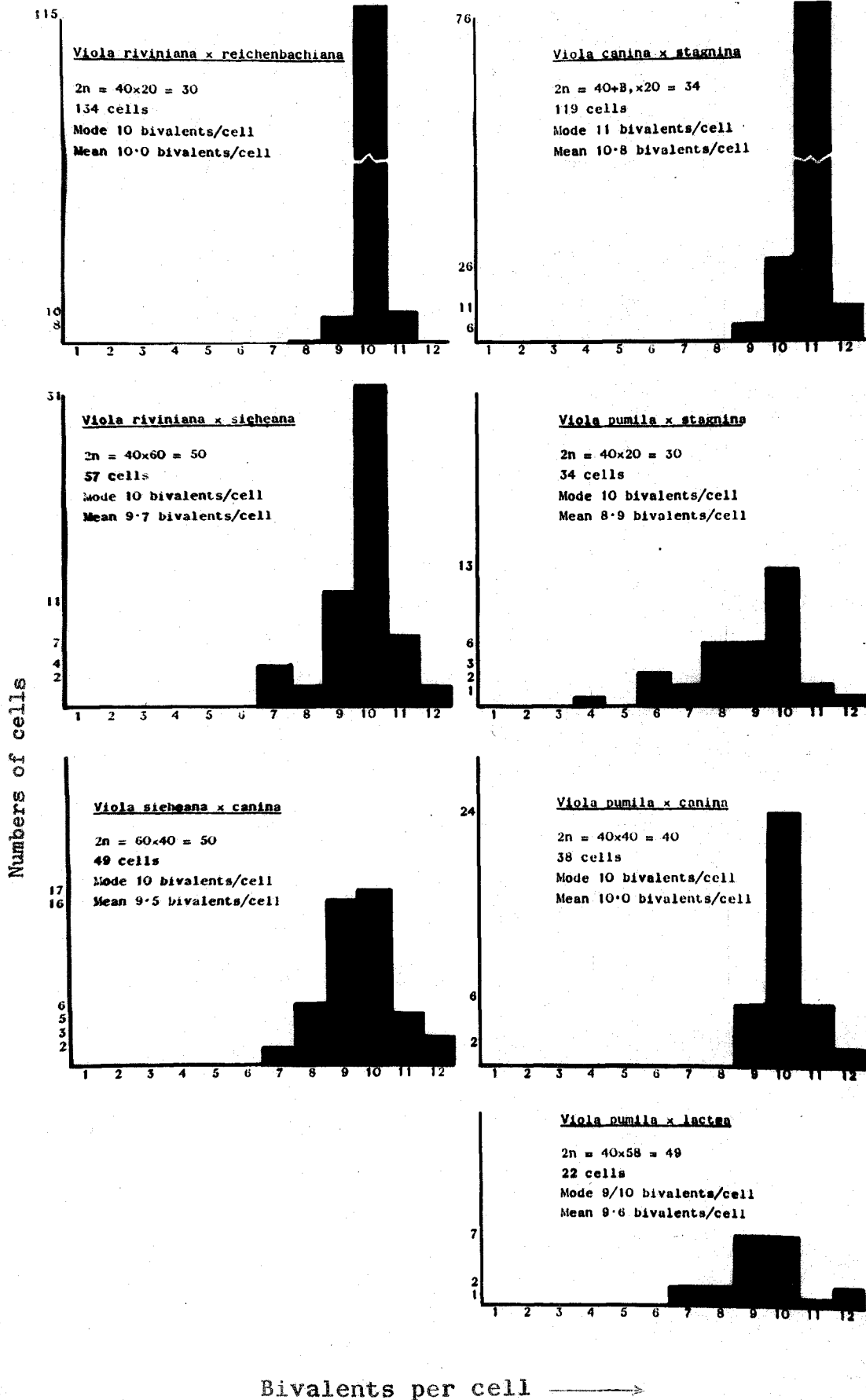


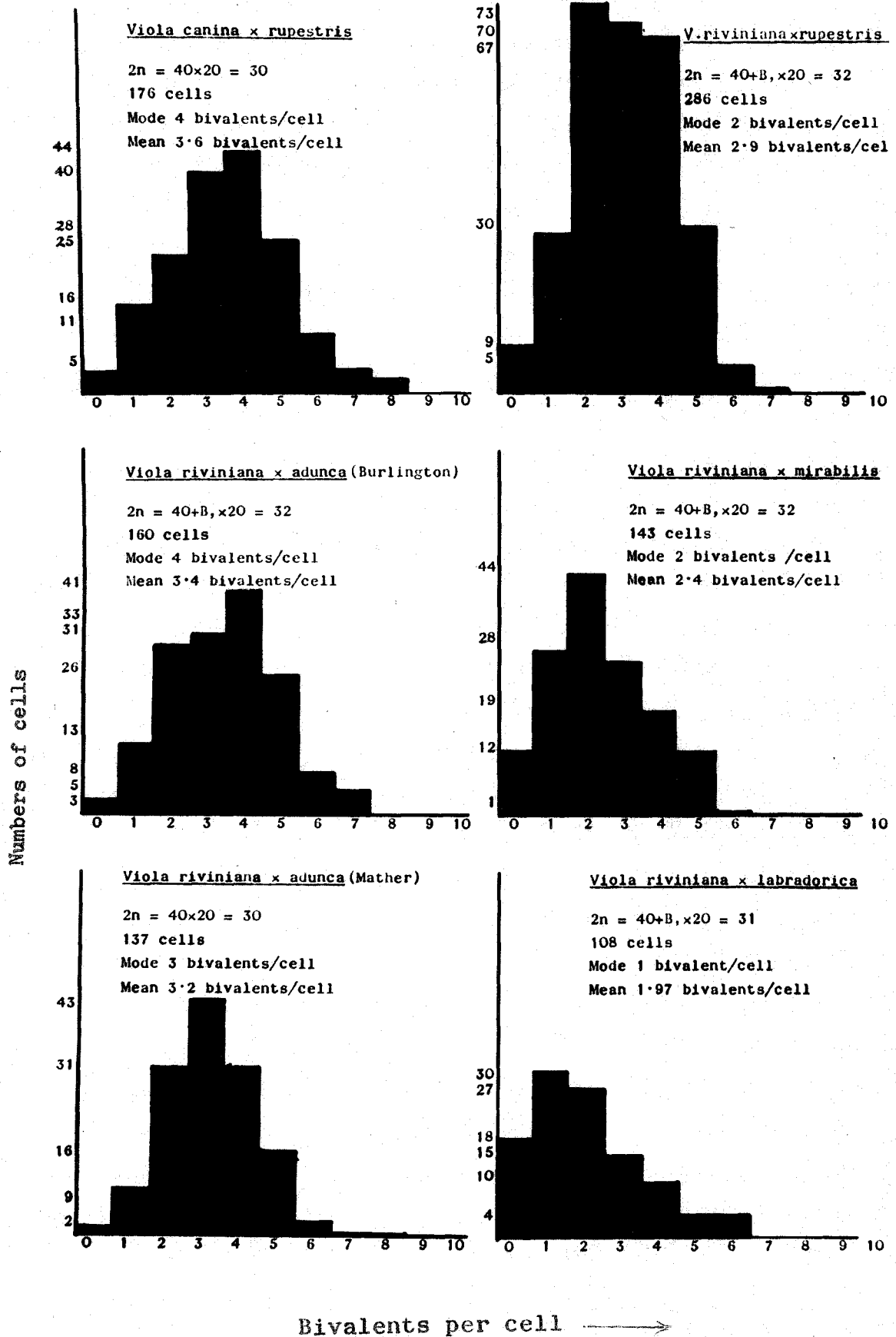
Numbers of cells



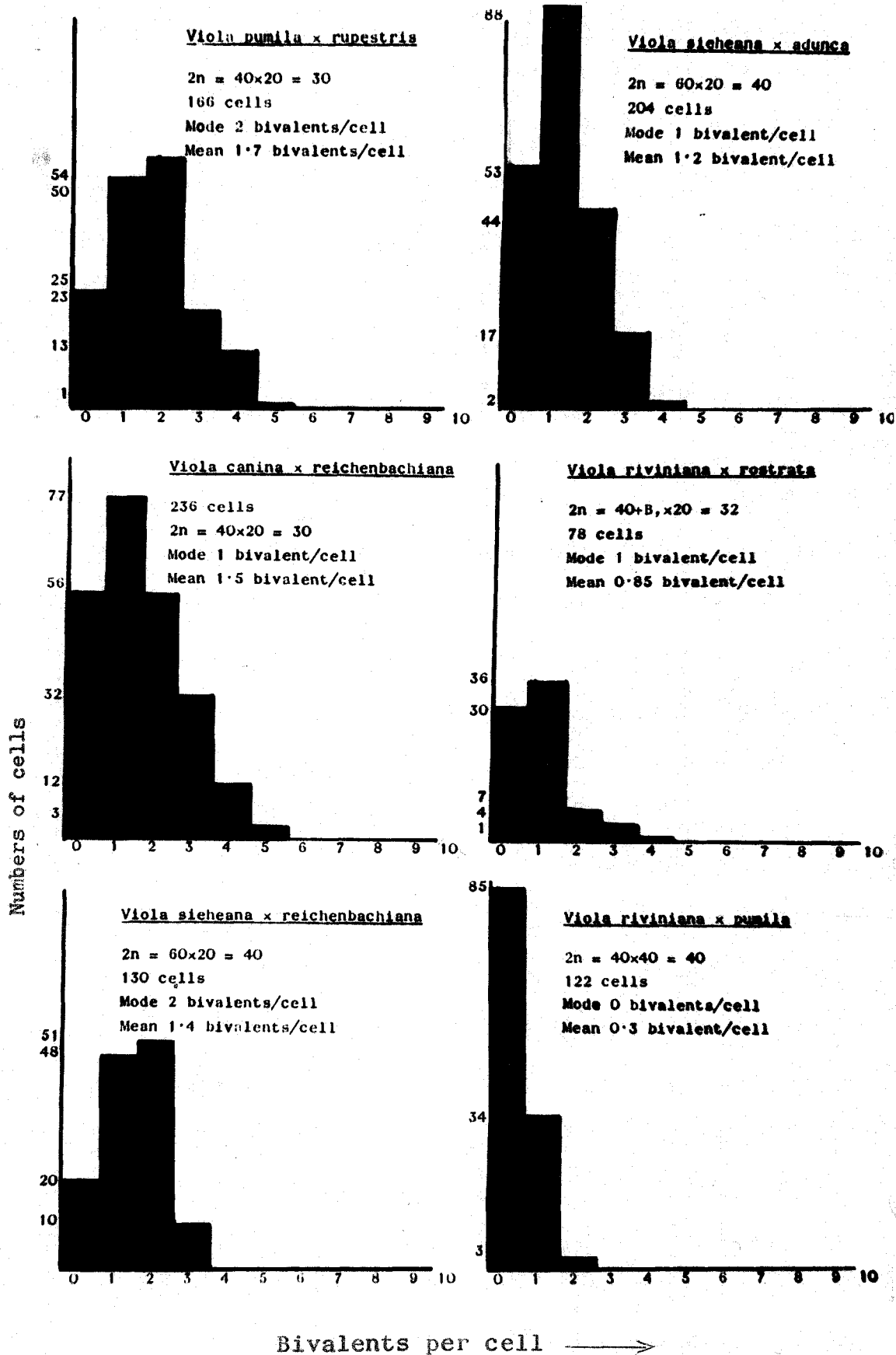
Bivalents per cell →











a) 2x HYBRIDS

	II/cell		No. cells	2n
	Mode	Mean		
<u>V.adunca (Mather) x adunca (Q.C.)</u>	10	9.9	51	20
<u>V.adunca (Mather) x adunca (B.)</u>	10	9.9	121	20
<u>V.adunca (Mather) x conspersa</u>	10	9.8	115	20
<u>V.adunca (Mather) x rupestris</u>	10	9.7	116	20
<u>V.adunca (Mather) x striata</u>	10	9.4	23	20
<u>V.conspersa x adunca (coastal)</u>	10	9.3	118	20
<u>V.adunca (Mather) x rostrata</u>	10	9.1	128	20
<u>V.rupestris x labradorica</u>	8	8.5	203	20
<u>V.reichenbachiana x rupestris</u>	7	6.9	87	20
<u>V.labradorica x reichenbachiana</u>	5	5.2	124	20
<u>V.rostrata x reichenbachiana</u>	5	4.3	74	20

b) 3x, 4x, 5x, HYBRIDS WITH ABOUT 10 BIVALENTS PER CELL

<u>V.canina x stagnina</u>	11	10.8	119	34
<u>V.pumila x canina</u>	10	10.0	38	40
<u>V.riviniana x reichenbachiana</u>	10	10.0	134	40
<u>V.riviniana x sieheana</u>	10	9.7	57	50
<u>V.pumila x lactea</u>	9/10	9.7	22	49
<u>V.sieheana x canina</u>	10	9.5	49	50

c) 3x, 4x HYBRIDS WITH LESS THAN 10 BIVALENTS PER CELL

<u>V.canina x rupestris</u>	4	3.6	176	30
<u>V.riviniana x adunca (B.)</u>	4	3.4	160	32
<u>V.riviniana x adunca (Mather)</u>	3	3.2	137	30
<u>V.riviniana x rupestris</u>	2	2.9	286	32
<u>V.riviniana x mirabilis</u>	2	2.4	143	32
<u>V.riviniana x labradorica</u>	1	2.0	108	30
<u>V.pumila x rupestris</u>	2	1.7	166	30
<u>V.canina x reichenbachiana</u>	1	1.5	236	30
<u>V.sieheana x reichenbachiana</u>	2	1.4	130	40
<u>V.sieheana x adunca</u>	1	1.2	204	40
<u>V.riviniana x rostrata</u>	1	0.9	78	33
<u>V.riviniana x pumila</u>	0	0.3	122	40

Viola adunca Smith

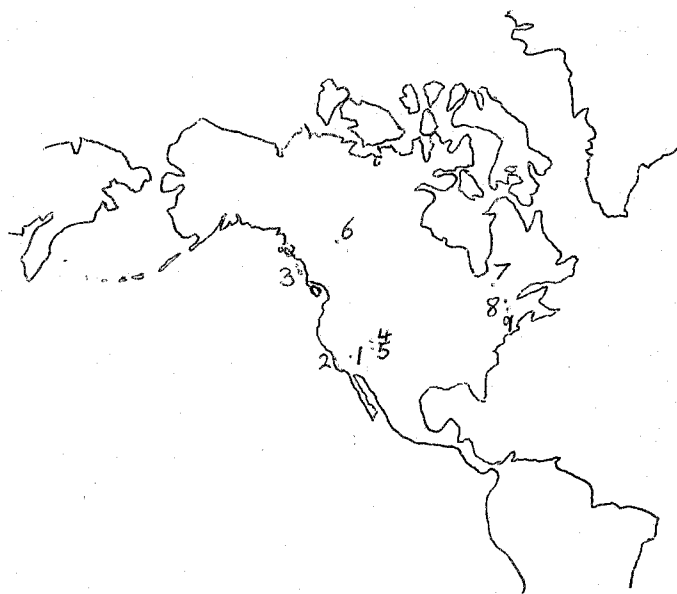
The case of V.adunca is so different from the other violets that it is treated separately.

The species is widely distributed over central North America (map p.40) and plants were in cultivation from the following localities which are shown on the map below. Two different forms were obtained from the Wasatch Mountains but only one from each of the other localities.

ADUNCA LOCALITIES

1. Mather, California, USA. 4000ft. coll.D.H.Valentine.
2. Coastal locality near Pescadero, South of San Francisco, California, USA. sea level, coll. D.H.Valentine.
3. Queen Charlotte Islands, British Columbia, Canada, near mouth of Thell River, also 1.5 miles S. of Jungle Beach, coll. Calder.
4. Murdock Peak, Wasatch Mountains, Utah, USA., 9000ft. coll. R.K.Vickery.
5. Tevin Lakes, Wasatch Mountains, Utah, USA., 9500ft. coll. R.K.Vickery.
6. Fort Simpson, N.W.Territory, Canada, coll. Cody and Matte, No.8658.
7. Senneterre, Quebec Province, Canada, coll. L.Levèsque.
8. Oka, Quebec Province, Canada, coll. D.H.Valentine.
9. Burlington, Vermont, USA., coll. D.H.Valentine.

The position of the V. adunca localities is roughly shown on the map below.



The variation in V. adunca in flower morphology and colour is far greater than that seen in any of the other violets studied and there are in addition variations in habit, leaf shape and size which are probably ecotypic. Examples of the latter are the dwarfness of the Senneterre plants, the absence of a need for stratification in the seeds of the coastal Californian plants and various differences in habit of growth. There are also differences in pubescence and it is <sup>seen</sup> from herbarium collections that many populations contain both pubescent and glabrous individuals.

Using the variation which at first sight appears not to be ecotypic, the plants in cultivation may be split into four groups:-

1. Mather. Flowers of a uniform, very intense dark purple-violet, black at the base of the petals; spur long with a prominent hooked appendage at the end.

2. Coastal California. Petals pale blue at edge, darker in middle; spur a contrasting reddish-purple, blunt, rounded; erect growth. Murdock Peak plants similar.

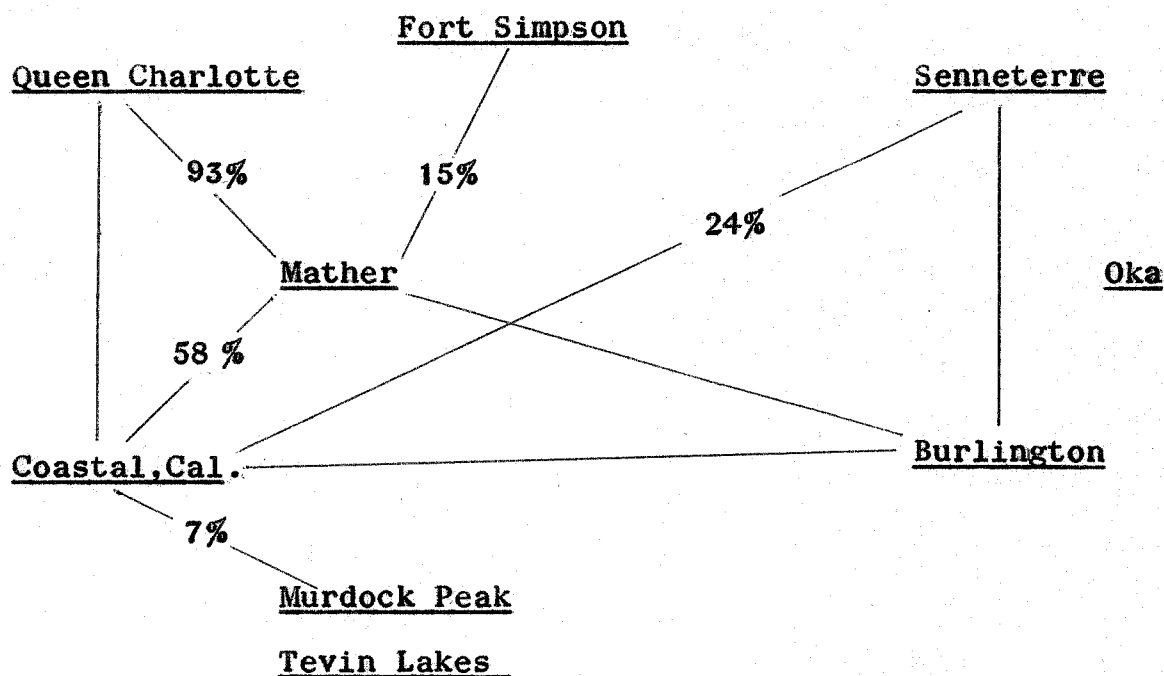
3. Queen Charlotte Island. Petals mid-blue, uniformly coloured, spur not hooked. Tevin Lakes plants similar.

4. Eastern. Flowers pale blue, lip paler in centre; spur not hooked; flowers smaller than in the other types. All the populations east of the Rocky Mountains belonged to this type.

#### HYBRIDS BETWEEN ADUNCA POPULATIONS

To find out more about the basis of the above variation a series of hybridisations was planned which unfortunately was not completed owing to the very poor production of flowers in most stocks except that from Mather, which, by contrast, was among the most floriferous of all the violets grown.

In the following diagram a line indicates the hybrids which have been made. The figures indicate the percentages of well-formed pollen when examined in aceto-carmin. At least 200 grains were counted in each case.

VIOLA ADUNCA HYBRIDSFig.24

That the plants obtained were indeed hybrids was readily seen from their hybrid vigour and intermediacy between parents in leaf and flower type.

The immediate knowledge gained from the first year's hybridisations was that hybrids within the group Mather, Coastal and Queen Charlotte, ie. those west of the Rockies, gave hybrids producing more or less normal capsules as also did the hybrid Senneterre x Burlington, involving two eastern populations. In contrast the two hybrids Mather x Fort Simpson and Mather x Burlington were very largely sterile. On this basis it was reasonable to think that there were two mutually intersterile groups; one comprising those populations to the west of the Rockies, the other

those east. Furthermore, while the floral morphology of the eastern populations was quite uniform, there was a great deal of genetic variation in flower colour and shape between the populations in the west.

This simple picture of east-west intersterility was not supported by hybrids made later. The hybrid Coastal x Murdock Peak, (two morphologically similar types), is sterile. Hence morphological similarity does not necessarily imply interfertility. Conversely the hybrid between Coastal and Burlington is fairly fertile so the idea of a sterility barrier roughly corresponding to the Rocky Mountains does not wholly hold either.

#### MEIOSIS IN V. ADUNCA HYBRIDS

Examination of meiosis in:-

Mather x Queen Charlotte (histogram p.79)

Mather x Burlington (histogram p.68)

Mather x Coastal, Cal.

showed that all the chromosomes normally associated as bivalents and that few univalents and no multivalents were formed. Very rarely bridges and fragments were seen at first anaphase and a bridge was also seen at second anaphase, (photograph p.152). In contrast to this regular behaviour during at least the early stages of meiosis, the pollen in the sterile hybrids contains a large percentage of deformed and shrunken grains.

One thing noticed in the F2 from Mather x coastal, Cal., is that there appeared to be very little segregation back to the parental characters, ie. the F2 resembles the F1. The plants on which this observation was made have only been growing for one season and the only flowers seen were autumnal open flowers but the plants do contrast with the F2 from V.adunca (Mather) x rostrata, in which there was a conspicuous segregation for various parental characteristics.

#### VIOLA ADUNCA x LABRADORICA

This hybrid had such abnormal cytology that the results are given here instead of including them with the other hybrids.

The hybrid was made by pollinating V.adunca (Mather) with pollen from V.labradorica (Mount Albert); only one capsule containing 5 seeds was obtained and of these only 2 germinated. The two plants obtained were identical and obviously of hybrid origin from the intermediate nature of their leaves and flowers, their hybrid vigour and by their complete sterility.

Since the relationship between V.adunca and V.labradorica was one of special interest it was gratifying to see, during winter 1960-61, that both plants bore a large number of flower buds. These buds, which contained large anthers of normal appearance, were examined for meiosis during spring 1961. In squash preparations no signs



of meiosis were seen until late in spring, by which time the flower buds were very much larger than is normal for violets at meiosis. Previously all squashes had yielded nothing but large masses of darkly-staining, actively-dividing cells but no trace of pollen-mother-cells or pollen grains. Then, late in spring when it was thought meiosis had been missed for the year, a few small cells on a slide were seen to have the thick cell wall typical of pollen-mother-cells. The slides were scanned and on the two best, 45 cells were found. This is an exceptionally poor yield, especially from such large anthers.

Examination of several cells at a magnification of  $\times 400$  showed apparently 19 chromosomes per cell. Since this was considered unlikely, (both parents have  $2n=20$ ), a number of cells were drawn under the camera lucida at  $\times 1000$  and it was then noticed that in addition to the 19 normal sized chromosomes there was also a small fragment in all the cells examined. This fragment was also seen among the chromosomes in the dividing cells surrounding the pollen-mother-cells and comprising the main mass of the anther. These latter contained high numbers of chromosomes; one cell was observed to have a count of 293 which, considering that most such counts are too low, probably means that this particular cell was 32-ploid ( $32n=320$ ). Photograph p.154.

Since both plants were of identical morphology and vigour it had not been thought necessary to keep the flower

buds separate when fixing buds for meiosis. Consequently it is not known whether both plants have  $2n=19+$  a fragment, or only one. Both plants showed the abnormal proliferation (for this group of violets) of undifferentiated cells filling the main cavities of the anthers, and in view of their identical morphology and sterility it seems probable that they also have identical chromosome complements.

At the first readily analysable stage of meiosis there is no nucleolus, the chromosomes are fully contracted and 19 univalents + the fragment are visible. There was no bivalent formation and the univalents were scattered irregularly about the cell and not aligned on a spindle. The irregular, thin connecting strands observed between some chromosomes are not of the appearance associated with bivalents. If this stage can be given any name at all then it corresponds to first metaphase with complete failure of bivalent formation.

What is presumed to be the next stage appears to be derived from the above by the division of each chromosome. The resulting 38 chromosomes + 2 fragments are scattered about the cell and not arranged in groups. This corresponds to anaphase and no later stages could be found.

During 1961 plants were grown of another V. adunca x labradorica hybrid, derived this time from V. adunca coastal California and V. labradorica Mt. Jacques Cartier, Gaspé. These plants will not be ready for examination of meiosis

until spring 1962 but from the reasonable number of capsules containing seeds derived from cleistogamous flowers in autumn 1961, it can be deduced that the process of meiosis proceeds fairly regularly in this stock and is expected to resemble meiosis in the majority of the American hybrids and to be in complete contrast to the first V. adunca x labradorica described above.

Thus the result so far obtained for meiosis in V. adunca x labradorica, -zero pairing-, is quite unacceptable as a measure of the affinities of the two species. All other evidence points to the chromosome sets of all the American diploids, including V. labradorica, being substantially identical from a pairing point of view. Why then the abnormal behaviour in this hybrid?

Presumably the missing piece of chromosome has some connection with the abnormal meiosis. The fragment observed is most likely to have arisen from misdivision of the ring quadrivalent known to be present in the V. labradorica parent. One difficulty is to explain why the loss of such a substantial amount of chromatin from a diploid species, a) enabled the gamete of V. labradorica to function at all, and b) still gave a vigorous hybrid. A further difficulty to explain is, since misdivision of a quadrivalent is presumably uncommon, how has it come about that two apparently identical plants were produced at the same time? It is possible for a fragment of a chromosome to be lost

during development but this again is a rare accident to happen twice.

Apart from the various possible ways in which this hybrid could have arisen, the main interest is that the timing of the various stages of meiosis and the cell divisions immediately previous appear to have got completely out of step. The polyploid cells which continue dividing in an active way and fill the main mass of the anther are derived either from pollen-mother-cells which failed to enter meiosis and continued mitosis, or from tapetal cells which continued proliferation long after the stage at which they normally degenerate. Whatever the origin of the extra cells their continued division accounts for the abnormally large size of the anthers. There is a hope that a careful study of anther development, by examination of sections, will reveal where cell differentiation first goes wrong. There is even the possibility that such a study would reveal what it is that triggers off some cells in an anther to proceed on a course in which they develop into pollen-mother-cells, while other cells only divide by mitosis and then stop.

## DISCUSSION and CONCLUSIONS

### AGE OF THE SPECIES

The implication behind the geographical distributions given in the introductory section and in the conclusion that they are the result of natural spread is clearly that, to have attained these by means of their known dispersal mechanism must have taken the more widely distributed species a very long time and that most, if not all, must be very old.

Viola rostrata,  $2n=20$ , map p.40, is outstanding in occurring in two widely separated areas. The two populations concerned must have been separated for a long but unknown time but show extremely little, if any, morphological divergence.

The migration to produce the two populations cannot have occurred since the last glacial maximum since the climatic conditions across the Bering Straits would never have been mild enough to permit the migration, and if the migration had taken place since the last glaciation then it could be expected that other areas with a suitable climate, and geographically between Japan and N.E.America (west North America), would also still harbour populations. That they do not leads to the conclusion that the movements concerned occurred during one of the interglacials or, as

will be suggested below, during the Tertiary era. There is no evidence for or against migration from sub-fossil plant deposits since the different violet species cannot be distinguished from the very few remains (seeds) found.

Analogies can be drawn with other species with similar present-day distributions but whose past history is better known from sub-fossil deposits. There is a whole group of plants with this type of distribution and one example which springs to mind is Liriodendron which has such a characteristic leaf-shape that its remains are readily detected and for which there is hence a comprehensive record. Its present-day native distribution is very similar to that of V.rostrata although not exactly identical. It is found in eastern N.America and S.E.Asia at present but contrasting with this are its fossil records which come from a wide range of the Northern Hemisphere including many from Europe. (Cain, 1944).

This pattern of present-day distribution and past history is not confined to one or two species but applies to what may be termed whole floras. Thus Reid and Chandler, (1926), investigating the Oligocene plant beds at Bembridge in the Isle of Wight, found many types of plants which now occur only in North America and East Asia. This flora they postulated was part of a completely circumpolar flora the greater part of which has since been destroyed leaving only the two widely separated remnants. The later (Pliocene) Reuverian flora also consisted of types now confined to North America

and east Asia.

The fact that V.rostrata shares this very characteristic distribution suggests, but does not prove, that it has had a similar history, which if true implies that it was a well-differentiated species before the ice age.

An experiment is needed in which Japanese and American V.rostrata is hybridised together to see if the resulting progeny have a lessened fertility or show any segregation in the F2 generation. This would give some idea of the rate of divergence of the two geographically isolated populations. In the absence of the necessary Japanese material a similar but smaller-scale experiment has been performed on V.riviniiana,  $2n=40$ , one of the more variable European species.

Plants from Madeira of a fairly dwarf, short-stemmed form which had come true from cleistogamous seed for at least two generations was used as female parent and pollinated from Czechoslovakian plants of a very large-flowered, tall form. The progeny of this cross were much taller than the Madeira plants so they were not the result of accidental self-pollination and the flowers were of intermediate size and markings.

Pollen from these hybrid plants was of perfectly normal appearance, 99% good grains, and the capsules were filled with good seed. Chromosome behaviour was not studied.

Madeira was unglaciated and its flora is thought to have been isolated from the mainland since early or pre-glacial times, (Manton, 1950). Hence we have here evidence

that isolation of this order of time has been insufficient in this species for sterility barriers to develop.

#### REASONS FOR STUDYING MEIOSIS

Before discussing the results of the examination of meiosis in detail, it will be as well to give some of the ideas which lay behind the study.

One of the reasons for studying chromosomes was that by and large, ordinary morphological comparisons of flower, leaf and stipule did not give any clear indication of how one species was related to another. There were one or two exceptions, but the majority of species could not be confidently classified into any order beyond that represented by the groupings Mirabiles, Rosulantes, and Arosulatae.

Now it was known that the rostrate violet species usually formed bivalents at meiosis but that some of their hybrids showed the presence of univalents in addition to bivalents and occasional higher associations. There therefore existed the possibility that the degree of chromosome association at meiosis in hybrids could be used to obtain some information about the relationship between the parental species of the hybrid. The idea was roughly that two closely related species might show a greater bivalent formation in their hybrid than two more-distantly related species. This was the working assumption behind the



study and although it proved, when it came down to details, not to be the entire truth, it did at least provide the impetus for a fairly large scale study of chromosome pairing in hybrids. The contradictions which arose when an attempt was made to force this idea on to some of the results are of interest and will be discussed later.

### VALIDITY OF RESULTS

Most of the individual histograms pp.79-83, are the result of the examination of a fraction of the pollen-mother-cells from a single anther. The aim behind making as complete a study as possible of the cells in a single anther was to try to eliminate possible unconscious selection of any particular class of cell which might have occurred if a coarse survey of a larger number of slides had been made. This introduces the possible danger that the results might be affected by the chance use of an abnormal anther and the whole work needs repeating using a number of anthers from each hybrid to check this. Since the study of a single slide takes a considerable time, and there are over 30 hybrids, nothing has been done towards repeating the work and the results have had to be taken as they stand. A further check needed is to resynthesise the hybrids using different parental stocks since there is the possibility that different geographical stocks might produce hybrids which

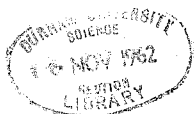
behave differently. This again is work for the future.

What was studied, accidentally, was the effect of frost on meiosis. Ever since the early paper of de Mol (1923), on the action of heat on generative nuclei, it has been known that extremes of heat, cold and drought can upset the normal course of meiosis and violets are no exception to this.

The stock of V.adunca from coastal California which was mentioned earlier in connection with the precocious germination of its seeds, is additionally ill-adapted to the frosty Durham climate and began meiosis one month before other dog violets and this property is also passed on to its hybrids. Some buds of V.conspersa x adunca (coastal) were in metaphase of meiosis as early as 16 February 1961 while the later buds did not reach this stage until 7 March 1961, and by this latter date the majority of the violet hybrids were reaching the same stage.

The interest in the samples obtained on these two dates lies in the weather prior to the collectings. January and early February 1961 were noted for a series of severe frosts but the period late February and the whole of March was exceptionally mild for Durham. Thus the first sample of buds collected had gone through the early stages of meiosis during frosty weather, the second sample during mild weather. This difference is reflected in the histograms of metaphase pairing obtained from each and shown in the top two histograms on p.79. In the first diagram there is a considerably reduced degree of pairing compared with the

second and the low result in the first is probably due to the action of frost on chromosome pairing during zygotene. In the second histogram most cells have the maximum number of bivalents possible. While no data are available from plants which have been grown entirely in frost-free conditions, it is thought likely that the second histogram represents essentially this state and that such failure of pairing as is shown is due to differences in the pairing abilities of the chromosomes and not to environmental factors. Since the other histograms were obtained from material collected during the continued mild weather subsequent to that from which the second V.conspersa x adunca (coastal) histogram was obtained, it may be concluded that they also have not been influenced by the weather. It should be added that there were no extremes of drought or heat during spring 1961 at Durham. In any case the histograms should be comparable, since with the exceptions of V.riviniana x mirabilis and V.riviniana x rupestris, all the material was collected during a period of uniformly mild weather during March and early April 1961. The material of the above two hybrids was collected in spring 1960.



## INTERPRETATION OF RESULTS

The results are discussed in four main groups as follows:-

- a) hybrids between diploid species.
- b) hybrids between V.adunca populations.
- c) hybrids between species with a genome in common.
- d) hybrids between species with no genome in common.

In Table 5, p.84, the meiosis results are given in order, starting with the highest pairing and ending with the lowest. It will be convenient to refer to this table from time to time.

### a) HYBRIDS BETWEEN DIPLOID SPECIES

Despite their low chromosome number, a few of these hybrids gave chromosome configurations which were among the most difficult to interpret of all the hybrids examined. The three most difficult were:-

V.reichenbachiana × rupestris

V.reichenbachiana × rostrata

V.reichenbachiana × labradorica

In these hybrids trivalents and irregular groups of chromosomes connected by thin strands were sometimes present, and at anaphase bridges were often seen. The other diploid hybrids showed only univalents and bivalents and were easily scored, (photographs p.151).

Since the three hybrids above gave the lowest meiotic pairing among the diploids, it seems reasonable to conclude that the pairs of species in each hybrid are the most widely <sup>divergent</sup> of those studied. In other words, in any scheme of relationships, V.reichenbachiana would have to be well separated from V.rupestris, V.rostrata and V.labradorica. In addition it is reasonable to expect the most widely diverged species to have accumulated the greatest number of translocations and inversions, assuming a common ancestry, and the irregular connections and bridges plus fragments noticed, are evidence of these. That the particular translocations and inversions are small is however shown by the fact that many cells still showed only bivalents and univalents and there was no consistent formation of a quadrivalent as noted in another hybrid, (V.rostrata x conspersa), which presumably in this latter case is evidence for a whole arm or large portion of a chromosome being translocated.

#### Relationships between the American species

The six American hybrids whose meiosis was investigated in detail were all of the same type and yielded histograms with a peak at 10, the maximum possible number of bivalents per cell, and the hybrid V.rostrata x conspersa although not investigated in such detail is of the same nature. From this it may be concluded that all the American species: V.striata, V.adunca, V.rostrata, and V.consperca, are closely related.

This is the same conclusion as Gershoy reached in 1934, since he was able to obtain F2 and later generations from similar hybrids although he did not examine meiosis.

Hybrid fertility in some of the hybrids at Durham was by no means as high as Gershoy seemed to have found when he stated that V.rostrata, V.conspersa, V.striata and V.adunca were a freely interbreeding group of species, although the fact that an extreme western form of V.adunca was used for the present experiments may explain the apparent difference in one or two cases.

The most fertile of these hybrids obtained at Durham was V.adunca (Mather) x rostrata and abundant seed was obtained which gave a germination of 33%, although there was a high seedling mortality later. With more difficulty an F2 and F3 generation were obtained from V.rostrata x conspersa. At the other end of the scale, V.adunca (Mather) x striata and V.adunca (Mather) x adunca (Burlington) have given no good seed during the course of two seasons, during which time the 18 vigorous plants of each hybrid produced an abundance of fine flowers. Thus in all these cases <sup>sterility barriers</sup> are present and even in the case of the most fertile hybrids seed production per capsule only reaches about a quarter that of the parents at the best of times.

Apart from revealing their close relation, the meiosis results do not allow of any finer divisions within the V.rostrata, V.conspersa, V.striata, V.adunca group. Neither does fertility seem to bear any relation to similarity of

morphology of the parents or their geographical position.

The freak cytological hybrid V. adunca (Mather) × labradorica has already been discussed but another stock of V. adunca, (coastal, California) gave a hybrid with V. labradorica which had a reasonable fertility and for this reason it is expected that its chromosomes will also mainly form ten bivalents at meiosis. V. labradorica may then be added to the four other American species as an additional member of this closely related group. The position of V. bellidifolia is more problematical since no hybrids have yet been obtained owing to the rotting off of the flower buds.

V. rostrata × conspersa was the first American hybrid to be examined and it was the only hybrid in which a ring quadrivalent was regularly seen at meiosis. For this <sup>reason</sup> it was at first thought that evolution in the rostrate violets had occurred in conjunction with a series of major translocations and inversions and that other hybrids would show related rings and inversion bridges and fragments. Such differences in chromosome structure between species have been found in several groups, eg. Clarkia, (Lewis, 1953) but in this latter genus hybrids with translocations show differences from V. rostrata × conspersa. Their fertility approaches that predicted from the mechanics of chromosome disjunction so that a hybrid with one or more translocation quadrivalents may have a considerable fertility, whereas the same meiotic metaphase picture in the violet hybrid is associated with a very much greater reduction in fertility which cannot be due

solely to irregularities brought about by the ring. In fact it is now known that the quadrivalent is only responsible for a small part of the reduction in fertility since the other American hybrids have a more regular appearing meiotic metaphase and yet some are of even lower fertility than V.rostrata x consperaa. It is obvious that the ability of the chromosomes to form bivalents at first metaphase in pollen-mother-cells is no guide to the fertility of the hybrid.

The problem of why these hybrids have not got a much greater fertility has not really been solved. Only a few observations have been made on later stages of meiosis and these have not revealed any great irregularities. Inversion bridges and fragments have been seen at first anaphase but are not frequent and in only a very few cases have second division bridges been seen, (photograph p.152). In short, the observed chromosome irregularities seem insufficient to account for the bad pollen and low seed production and yet a genetic cause of sterility would appear to be ruled out because colchicine restores fertility. Possibly the answer is that the chromosomes of the species differ by a large number of very small structural changes. One of the conclusions drawn from the Clarkia work referred to earlier is that some species which occur in a few restricted areas in California are of relatively recent origin. This recent (postglacial) origin could account for the high fertility of hybrids apart from the disturbances caused by translocations



and inversions, while for Viola it has been postulated earlier from the distribution of V.rostrata that they may be of ancient origin and it would not be surprising in this case if the chromosomes did not differ by a number of small changes. If the violet species are very old the problem is really to explain why the chromosomes still show such a high degree of pairing ability. This same problem has been found by other workers in other genera; the classic example being Primula kewensis (Newton and Bellew 1929). Stebbins discusses Primula and gives a list of similar hybrids, (Stebbins, 1950).

#### American - European connections

One of the prime aims of this investigation was the elucidation of the relationships between the European and American rostrate violets. The outstanding result obtained in this connection is the close affinity demonstrated between V.adunca from Mather, California and V.rupestris from Widdy Bank Fell, Durham. This is shown in the histogram of their hybrid on p.79.

The only other hybrid between V.rupestris and an American species whose meiosis could be studied was V.rupestris x labradorica and in this a peak at 8 bivalents per cell was obtained, p.80. In view of the morphological similarities between V.adunca, V.rupestris and V.labradorica this latter result is lower than expected and needs repeating, but even so the result does indicate a close affinity between V.rupestris and V.labradorica

V.rupestris and V.adunca had first been grouped together by Becker, and Clausen (1929), also pointed this out when he noted that the morphology and particularly the pubescence of V.adunca much more resembled V.rupestris than it did V.canina with which it had previously been classed. Clausen classified V.adunca as V.rupestris subsp. adunca and this could certainly be defended in view of the fact that the hybrid V.rupestris x adunca shows 97% of the possible bivalent formation at metaphase of meiosis. Despite this V.adunca merits status as a species distinct from V.rupestris since their hybrid is sterile and their geographical ranges different.

As pointed out earlier a much more remote relationship is shown by V.reichenbachiana on the one hand and V.rostrata and V.labradorica on the other. To the latter two species may now be added V.adunca and V.striata since they belong to the same closely related group.

Much the same considerations apply between V.reichenbachiana and V.rupestris, which show 70% bivalent formation in their hybrid. The similarity of the behaviour of their hybrid to the ones between V.reichenbachiana and the two American species is not surprising in view of the connection between V.rupestris and the American species.

The relationship of the diploid European Arosulatae, V.stagnina, has not been studied beyond the production of the hybrid V.stagnina x striata, which is of intermediate

morphology, vigorous and completely sterile. Colchicine treatment resulted in good seed production.

It has not been possible to make any diploid hybrids involving V.mirabilis.

#### b) HYBRIDS BETWEEN V.ADUNCA POPULATIONS

The results obtained from the work on V.adunca do not lead to any definite conclusions. Had there been available only the first year's results, (the uniform eastern flower type, the interfertile western populations and the two sterile east-west hybrids), there need have been no hesitation in concluding that V.adunca is an aggregate group composed of two species, an eastern and a western. As in many other cases the more facts are known the less easy it is to fit them into a simple hypothesis and the later (incomplete) results do not fully support the above simple view. Clausen (1929) was the first person to point out that the eastern and western plants looked different; he thought that the western plants more closely resembled V.rupestris but did not state where his plants came from, which is unfortunate in view of the extreme variation found in the west.

Any explanation of the origin of the observed populations of V.adunca must take into account the following facts:-

- 1.The floral uniformity of the eastern plants.
- 2.The floral variability of the western plants.
- 3.The sterility of some hybrids but not others.

How such a situation could have arisen it is not easy to say. One suggestion is that V.adunca looks as if it were a series of hybrid swarm derivatives, each of which has become adapted to its local conditions. If this is so, the question arises of, hybrids between what? It is difficult to invoke V.labradorica, V.conspersa, V.rostrata, V.striata or V.bellidifolia since these are of such high geographical and morphological integrity and on their side show no signs of introgression from the V.adunca direction. In any case the region of greatest variability of V.adunca, the far west, is just that region where it is the only rostrate violet present, except for V.bellidifolia in a restricted area at high altitude in the Rocky Mountains. Of course this need not always have been the case and the pre-glacial distribution of the species was no doubt very different.

If the hybrid swarm origin is correct, the present situation could have arisen from contact between an 'original' V.adunca (the present eastern form?), and another species on the west coast of North America. This other species could either have disappeared, or be still present but not recognised, or just possibly might have been V.rupestris.

V.rupestris is not of course present in America but just reaches the Pacific coast of Asia (map.p.38), so there

is a definite possibility that it might have formerly extended across the Bering Straits. This is pure speculation and it will be difficult to arrive at any definite answers from further study of the nine samples in England. Having defined the nature of the problem, further work must be left to someone with facilities for extensive travel in both the United States and Canada.

It may be added that the type specimen of V. adunca Sm. came from somewhere on the west coast of North America.

c) HYBRIDS BETWEEN SPECIES WITH A GENOME IN COMMON

The European violets contrast with the American in having several widespread polyploid species, and many hybrids involving these polyploids show 10 bivalents (rarely 20) at meiotic metaphase. This group of hybrids included 3 triploids, 1 tetraploid and 3 pentaploids, and all except one showed a modal average of 10 bivalents per cell, (histograms p.81). The 11 bivalents per cell found in V. canina x stagnina appear to have been increased to that number by the presence of supernumerary chromosomes.

The presence of this pattern of pairing in hybrids involving polyploids is usually assumed to be evidence that the tetraploids have arisen, at some time in the more or less remote past, by hybridisation between distinct diploid species followed by spontaneous chromosome doubling in the hybrid. Hexaploids are postulated to arise by a simple extension of

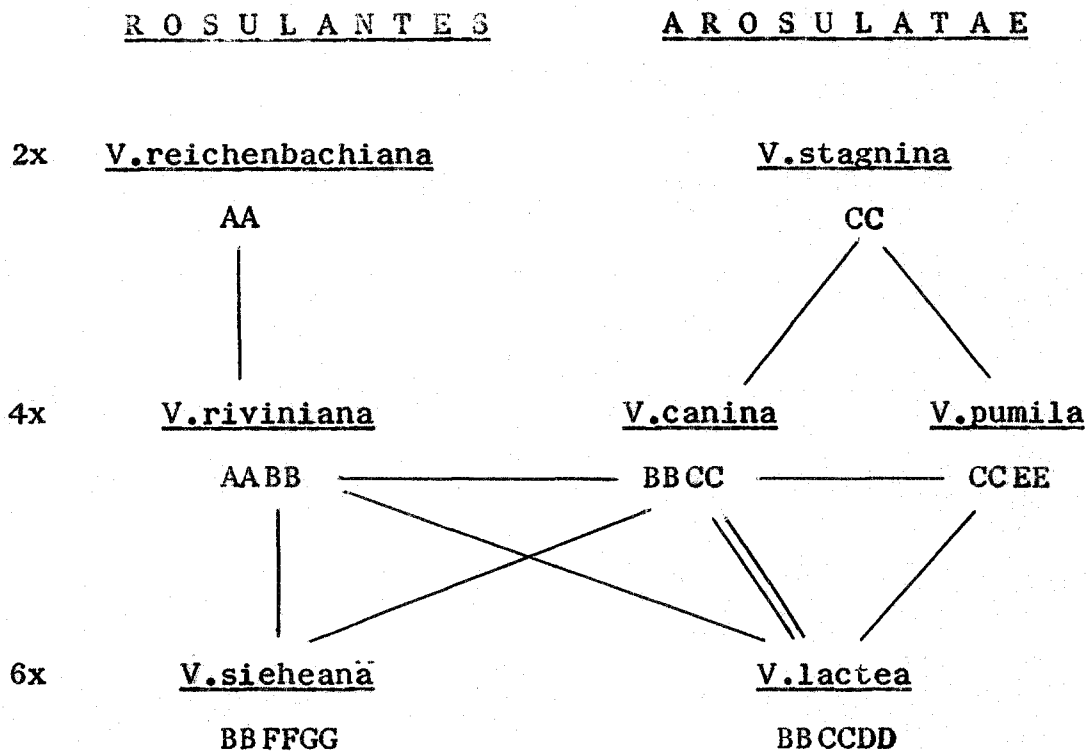
this by hybridisation between a diploid and an already formed tetraploid with, again, chromosome doubling.

The great majority of polyploid species found in the wild form bivalents but no multivalents at meiosis. One possible explanation of this which has frequently been adopted is to assume that in the hybrid between, say, the two diploids, there was no pairing of chromosomes at meiosis, but that on doubling of the chromosome number every chromosome had an exactly identical partner and consequently the tetraploid was bivalent forming. This is only one possible view and another explanation is put later; for the moment the idea above can be used to construct a scheme of relationships between a number of the European species by assuming each tetraploid and hexaploid to have been formed by the combination of the chromosome sets of two or three diploid species. In the diagram below each set is symbolised by a letter of the alphabet and a line connecting two species indicates that in the hybrid between the two an average of 10 bivalents has been found at meiosis.

The evidence of the connection between V.riviniana and V.canina is derived from Valentine (1958), and that between V.lactea and V.canina, and V.lactea and V.riviniana from the work of D.M.Moore in Moore and Harvey (1961).

PROVED GENOME CONNECTIONS

Fig.25



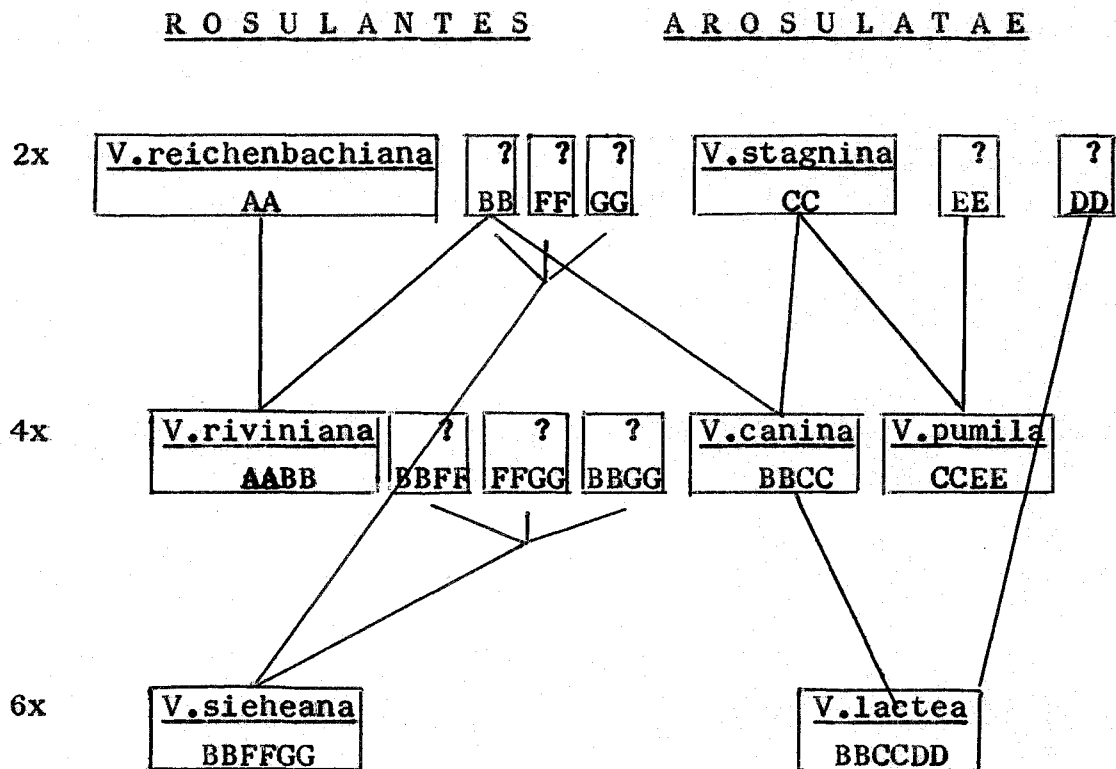
The above system is consistent even when extended to hybrids with no genome in common, eg. V.reichenbachiana x canina which has an average of only one bivalent per cell, histogram p.83.

Once sufficient hybrids have been examined to establish the identity of each genome, the meiotic behaviour of other hybrids within the group can be predicted. For instance, the hybrid V.sieheana x lactea has only so far been obtained as seed but examination of the above diagram shows that the two species have one genome (B) in common, and it is hence expected, with a good deal of confidence, that the hybrid will show about 10 bivalents and about 39 univalents at meiosis.

If, as pointed out above, the assumption is made that each genome has been derived from a separate and distinct ancestral diploid then the information in the previous diagram may be used to construct a theoretical scheme of the evolution of these violets:-

THEORETICAL SCHEME OF EVOLUTION

Fig.26



The lines in the diagram indicate which hybridisations have occurred to produce the various polyploids. In the case of V. sieheana so little is known of its ancestry that all that can be put is that it most likely arose by hybridisation between a diploid and a tetraploid to give its



present combination of genomes. Both these species are unknown at present. The most likely origin of V.lactea is indicated; there are two other ways in which its combination of genomes could have arisen, but the one indicated is much the most likely.

A number of points on the evolution of the rostrate violets emerge from the evolutionary diagram:

V.sieheana has not been derived from V.riviniana and has only one of its three genomes in common with it. Hence its designation as V.riviniana subspecies sieheana cannot be supported. As can be seen from the diagram it must have had a total of three diploid and one tetraploid ancestors, none of which have yet been found. The diploid ancestors include one with the B genome which is common to V.riviniana, V.canina, V.sieheana and V.lactea.

V.canina has arisen by hybridisation between a diploid with genome B in the Rosulantes and V.stagnina (C) in Arosulatae. It hence bridges the two sub-sections. Possibly the success of V.canina in colonising a wide variety of habitats (wet fen to dry grassland) over such a wide geographical range (Greenland, Europe and Asia probably to the Pacific coast), may be attributed to its combination of two widely differing genomes giving it a great plasticity and competitive ability.

To account for the origin of the five polyploid species investigated it is necessary to assume the existence of five

diploid and one tetraploid species whose identities are at present unknown; these are indicated in the diagram by question marks.

It has been objected that this scheme has a low ratio of known to unknown genomes but that some economy in assumed genomes could be made, presumably in response to Occam's razor, ('*Entia non sunt multiplicanda praeter necessitatem*'), by supposing V.sieheana to be an autoallohexaploid. Such a constitution has been put forward for several other hexaploids eg. Helianthus tuberosus, Phleum pratense and Solanum nigrum (Stebbins 1950) and must be considered for V.sieheana. Several points are however against such an origin; meiosis consists of uniform bivalent formation and regular separation so that evidence for partial polyploidy in the form of quadrivalents, is absent. Also V.sieheana is a widespread and successful species and this must presumably have come about by its having some advantage over its (unknown) ancestors. This advantage is more likely to have arisen from the genes in a third, distinct genome than from the mere duplication of a set already present in the tetraploid parent. Such a duplication is more likely to lead to unbalance of genes and decreased fertility than to increased vigour and spread. Against this latter point is the existence of the three hexaploids mentioned above but these are weedy or cultivated species depending on the cultivation of the land by man and not comparable with a species of woodland.

d) 3x,4x HYBRIDS BETWEEN SPECIES WITH NO GENOME IN COMMON

These hybrids, whose histograms of meiotic behaviour are shown on pp.82-83, show a fairly wide range of mean pairing: from a modal average of 4 bivalents per cell in V.canina x rupestris to 0 per cell in V.riviniana x pumila.

One of the reasons for making hybrids of V.riviniana with the various diploid species was to see if one of them was the species from which the 'B' genome had originated. From the results in this section it is obvious that 'B' is not to be found in; V.adunca, V.labradorica, V.mirabilis, V.rostrata or V.rupestris. And since all the American species studied seem to be closely related and there are no more European diploids it would <sup>appear</sup> that 'species B' is not one of the known species either in the New World or in Europe. It has become obvious that 'species B', if it still exists, will be found either in W.Asia or E.Europe. From the knowledge of it having taken part in the formation of so many natural polyploids it was at one time presumably reasonably widespread so there is every hope that it still exists somewhere, possibly passed over as an abnormal form of V.riviniana.

V.rupestris is seen to be not at all close to the other European species, in sharp contrast to its close affinities with those in America.

The average of 2 bivalents per cell found in V.sieheana x reichenbachiana shows that V.reichenbachiana was not one

of the parents of V.sieheana, and that the 10 bivalents per cell observed in V.sieheana x riviniana were hence derived from the B genome of V.riviniana and a similar one in V.sieheana. The A genome is absent from V.sieheana.

From V.sieheana x adunca it is also seen that V.adunca plays no part in the make-up of V.sieheana, which is not surprising in view of the geographical remoteness of the two. What is more valuable is the probable inference that V.rupestris also plays no part, since the close connection of V.adunca and V.rupestris has already been established. It was not possible to make the hybrid V.sieheana x rupestris and since the two species have an overlapping geographical distribution it was necessary to consider V.rupestris as a possible ancestor of V.sieheana. Hence the indirect demonstration that the latter hybrid most likely forms a low number of bivalents per pollen-mother-cell is most useful. In any case various features such as the boldly marked flowers and absence of pubescence rule out a V.rupestris ancestry.

V.sieheana seems to be of purely Asiatic origin and the most likely place to search for related species seems to be the western half of Asia and particularly Asia Minor. Of interest from this point of view are specimens labelled V.caspia (Rupr.) Freyn. in Herb. Brit. Mus (Nat. His.) which look sufficiently different from V.sieheana to be worth considering as a possible related species even though the name V.caspia is usually looked upon as a synonym of V.sieheana.

Some herbarium specimens from the mountains in north Persia, although very similar to V.sieheana, were seen to have a longer, narrower spur with a distinct upward curve. Since V.sieheana is probably the top polyploid member of a group of somewhat similar-looking species, many more living collections of dog violets are needed from the general area in which V.sieheana occurs.

#### DERIVATION OF THE GENOMIC RELATIONSHIPS OF THE POLYPLLOIDS

Table 5, p.84, summarising the results of the examination of meiosis in the hybrids, was used to derive the diagram of genomic relationships shown in fig.25, p.113., and some explanation of the steps by which this diagram was arrived at will now be given.

One limitation of working with rostrate violets is that the chromosome sets derived from the various diploids and other species are not distinguishable one from the other at meiosis. Hence it is impossible to examine meiosis in a hybrid and tell which of the parental chromosomes are forming bivalents and which univalents. Of course in some hybrids in other genera, eg. Raphanobrassica (Karpechenko 1928), the parental chromosomes are distinguishable by size and this enables the genomes pairing or not, to be distinguished visually but in the rostrate violets this can not be done. Thus in the hybrid V.riviniana x reichenbachiana,  $2n=30$ , there

is an average of 10 bivalents plus 10 univalents present at meiosis but it is not possible to tell the parental derivation of each bivalent or univalent. Actually this is a bad example to take as V.reichenbachiana has a pair of slightly larger chromosomes (Fothergill 1944), and one of these is sometimes distinguishable at meiosis in the hybrid, but in general in the rostrate violets the chromosomes cannot be assigned with certainty to a particular species by reason of their size or structure.

In a polyploid group the evidence of morphology, chromosome numbers and the behaviour in hybrids of the chromosomes at meiosis, can, when combined, give a good picture of the evolution of the species in the group. This approach has given notable results in a large number of plant genera, eg. Nicotiana (Clausen and Goodspeed 1925), many ferns and other groups (Manton 1950) and, of interest here, Viola (Valentine 1950).

In the case of the European polyploid violets there was a desire to derive the genomic relationships by a series of steps which would not involve any considerations of the floral or vegetative morphology of the species concerned. If such a scheme can be worked out it may then be compared with the taxonomic classification. In these rostrate violets sufficient knowledge will soon have accumulated to enable this to be done and the genomes may eventually be typified using only the knowledge of meiotic behaviour in the hybrids and such a scheme will hence be completely independent of

any theories of relationships based on morphological data.

If the triploid hybrid V.reichenbachiana × riviniana is considered it is seen that the 10 bivalents observed could have arisen by the pairing of the 10 chromosomes from V.reichenbachiana with an identical set of 10 from V.riviniana. In other words the two species might be related genomically as in the scheme, V.reichenbachiana = AA; V.riviniana =AABB, but this need not necessarily be the case since there are other ways in which 10 bivalents could have been formed. Suppose for instance that V.riviniana had had an entirely independent origin from V.reichenbachiana with genomes which could be designated PPQQ. Then in the hybrid, which would be of constitution APQ, let us suppose five chromosomes from V.reichenbachiana pair with five from P, and the other five with Q. This would again give a total of 10 bivalents. Hence the observation that a triploid hybrid forms n bivalents plus n univalents, unsupported by any other evidence or theory, does not itself prove any genomic relationship between the parents of the hybrid.

Somewhat similar arguments may be applied to the tetraploid hybrid V.riviniana × canina in which 10 bivalents have also been observed at meiosis; there are a number of possible ways in which the 10 bivalents could have arisen and to designate V.riviniana as AABB and V.canina as BBCC on that evidence alone is to consider only one possibility out of many.

When however the hybrid V.reichenbachiana x canina, with an average of only 1 bivalent per pollen-mother-cell, is considered, it can be said, purely from the cytological evidence, that these two species have no genome in common. This can form the starting point for a scheme of genomic relationships. V.reichenbachiana may be arbitrarily designated AA and V.canina BBCC, and the hybrids V.riviniana x reichenbachiana and V.riviniana x canina may now be reconsidered.

From the hybrid V.riviniana x reichenbachiana it is seen that V.reichenbachiana has 10 chromosomes in common with V.riviniana, and since V.reichenbachiana chromosomes are known not to pair with B or C genomes, V.riviniana and V.reichenbachiana have the A genome in common.

The next step is to consider the tetraploid hybrid V.riviniana x canina with 10 bivalents at meiosis. These bivalents cannot be A since V.canina does not possess A. The pairing set must therefore be the other set in V.riviniana, which may be put as either AABB OR AACC. Which of these two conventions is used does not matter if only the above three species are considered, but it does become important if V.stagnina is brought into the scheme and very unfortunately from the present point of view the hybrid V.riviniana x stagnina has not yet been examined. This means that the above hybrid cannot be compared with V.stagnina x canina (10 bivalents) and fig.25 is in fact still based partly on taxonomic considerations since V.stagnina and V.riviniana are



morphologically dissimilar and their hybrid will have to be assumed for the moment to show zero or very low pairing at meiosis. If this turns out to be the case then V.stagnina and V.canina share a genome but V.stagnina and V.riviniana do not and if, arbitrarily, V.stagnina is designated as CC then V.riviniana is AABB, and V.canina BBCC.

Similar reasoning shows that V.sieheana possesses the B genome but not A or C, and its other two genomes remain unidentified. Whether these are distinct from the genomes contained in V.pumila and V.lactea remains to be seen when the appropriate hybrids mature but here again considerations of morphology point to their being distinct.

However the above may turn out it should eventually be possible, when more evidence has accumulated, to work out fig.25 using only evidence from hybrid meiosis and not depending at all on morphological judgements as to a certain extent it does at present. Enough has been said to show that the essence of building up such a diagram is that first consideration must be to hybrids showing no (or very little) pairing and only then extending the scheme to hybrids showing  $n$  or  $2n$  bivalents.

### MEIOSIS RESULTS VIEWED AS A WHOLE

In Table 5, p.84, the meiosis results are arranged into three main groups; group (a), diploid hybrids; group (b), triploid and higher hybrids showing about 10 bivalents/cell; and group (c), triploid and higher hybrids showing less than 10 bivalents/cell.

Group (a) hybrids show chromosome associations ranging from an average of 10 bivalents per cell (100% of the possible) to 5 per cell (50%). Only a small number of the known diploid species were hybridised and in particular no species belonging to Arosulatae or Mirabiles were used; this is unfortunate from the point of view of investigating the connections between the diploid members of the three divisions of the Rostratae. However, within the Rosulantes, the hybrids involve species belonging to some of the more extreme morphological types with very different geographical distributions and it seems likely therefore that within this group, further hybrids that may be produced will not show a meiotic behaviour differing greatly from those already investigated. A particular feature is that none of the diploid hybrids show zero or very low average pairing, 5 being the lowest average recorded.

In group (b) the pattern of pairing can be neatly and consistently explained in terms of genomes, resulting in fig.25, p.113, as discussed previously.

While analogous results to those in groups (a) and (b)

could be quoted from research in other groups of plants, and the results were at first thought to be quite normal, as indeed taken separately they are, on closer study there were seen to be inconsistencies between (a) and (b). The question arose of why, in group (a) only a restricted range of pairing was encountered (from 5 - 10) since some of the hybrids between widely differing species might have been expected to show much lower, possibly zero, pairing averages, as in the hybrid between Raphanus and Brassica (Karpechenko 1927). Then in group (b) was the contrasting situation of none of the averages being over 10 (except for V.canina x stagnina with supernumerary chromosomes). Some of the group (b) hybrids had a considerable number of chromosomes which did not form bivalents, eg. V.riviniana x sieheana with 30 but in view of the results from group (a) these could have been expected to show a considerable degree of bivalent formation. Thus among 30 chromosomes a bivalent formation of at least 5 per cell would not be an unreasonable <sup>average</sup> to expect and hence V.riviniana x sieheana, assuming it to have two related genomes and three unrelated, might well show an average of say 15 bivalents per cell. In fact all group (b) show averages of exactly, or very close to 10; the results in other words are too good a fit. Either there has been a systematic error of scoring which has resulted in group (a) results being too high, or group (b) too low; or the behaviour of the non-pairing genomes in group (b) hybrids is

very much different from those of some of the apparently remotely related diploids used in group(a).

Yet another series of anomalies are found when the results from group (c) are considered because some of these can be predicted by combining those of groups (a) and (b). For instance from group (b) it is known that in the hybrid V.riviniana x reichenbachiana 10 bivalents are formed and this has been shown to be due to V.riviniana possessing a genome (A) in common with V.reichenbachiana. From group (a) it is also known that V.reichenbachiana x rupestris forms an average of 7 bivalents per cell, and it can therefore be predicted from these two results that the hybrid V.riviniana x rupestris should show at least 7 bivalents per cell as a result of chromosomes from the A genome of V.riviniana pairing with those from V.rupestris. A result in excess of 7 would not be surprising since a few more bivalents might form between some of the V.rupestris chromosomes and the B genome in V.riviniana. The actual result is a modal mean of 2 (arithmetic mean 2.9) and the difference of this from the expected 7 is too big to be confidently put down to observational error.

Similar discrepancies are found when similar comparisons are made using V.labradorica and V.rostrata in place of V.rupestris in the hybrids above:-

<u>group</u>	<u>bivalents/cell</u>
a) <u>V.labradorica</u> × <u>reichenbachiana</u> (A) .....	5
c) <u>V.labradorica</u> × <u>riviniana</u> (AB) .....	expected 5
	..... actual 1
a) <u>V.rostrata</u> × <u>reichenbachiana</u> (A) .....	5
c) <u>V.rostrata</u> × <u>riviniana</u> .....	expected 5
	..... actual 1

It is seen from the histogram of V.riviniana × reichenbachiana, p.81, that the 10 chromosomes from V.reichenbachiana still pair with 10 from V.riviniana, and it can be added that the bivalents so formed are of normal appearance and behaviour. The amount of failure of bivalent formation (8 cells with 9 bivalents, 2 with 8) represents only 0.9% of the possible assuming each cell potentially has 10 bivalents. Hence structural differentiation of the A genome since its incorporation into V.riviniana is insufficient to account for the differences noted above between the hybrids of V.reichenbachiana and V.riviniana.

#### A GENETIC HYPOTHESIS

One possible way of explaining the above anomalous results is by way of a mechanism of the genetic control of meiosis in polyploids such as was discussed by Darlington (1932) and for which recent evidence has been provided in the hexaploid bread wheats by Riley (1960).

Briefly, the idea is that in some allopolyploids there is a genetic system which acts at meiosis to prevent pairing between chromosomes derived from the two or three different sets contributed by the original parents of the polyploid. There are a number of possible advantages which such a system might give a plant possessing it. Multivalent formation would be prevented and with it the attendant possibility that the irregular separation of chromosomes might cause lowered fertility, (although this is not an inevitable result of multivalent formation). Another, more likely advantage, is that by preventing pairing and hence crossing-over between chromosomes from the two original parents of the tetraploid, the original chromosome sets are kept intact and this could possibly help to perpetuate the hybrid vigour of the original diploid cross. That the original chromosome sets have been kept separate by some mechanism is seen from the fact that several of the genome relationships in fig.25, p.113 can be drawn up consistently with several hybrids indicating the same results.

The genetic system is postulated to arise only after the formation of the polyploid. No similar system is suggested to restrict pairing in undoubled hybrids between diploid species. It is quite possible that many of the diploid rostrate violets may have evolved their distinguishing characteristics in isolation while at the same time retaining the ability of their chromosomes to pair in hybrids with other, distinct, diploid plants. This may represent the

situation found between V.rupestris and V.adunca, which are at present at least geographically isolated and whose hybrid forms bivalents almost completely. It is tempting to suggest that the ability of the chromosomes to pair in hybrids gets less the longer ago the two original stocks diverged. There is no evidence to support this suggestion but if it is true then the ancestors of V.rupestris and V.reichenbachiana diverged longer ago than those of V.rupestris and V.adunca.

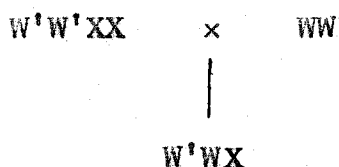
To discuss the genetic hypothesis for polyploids it will be convenient to draw up a theoretical scheme using symbols and then apply this to the results obtained with violets.

Diploid species may be symbolised thus:- WW, XX, YY, ZZ.

A wild tetraploid, formed from two of the above species may be symbolised:- W'W'XX.

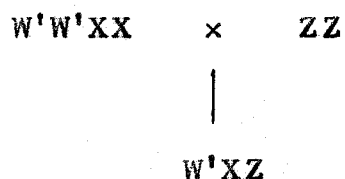
This tetraploid is postulated to possess a genetic system, denoted by ('), the action of which is to prevent chromosomes belonging to set W from forming bivalents with chromosomes from set X. The exact nature of the genetic system does not matter at this stage; it may be a single gene located on a particular chromosome of one genome, or many genes on one or both genomes. For the present it is represented as located on the W genome but this is not essential to the argument.

A triploid hybrid may be formed by backcrossing the tetraploid to one of its original ancestors:-



At meiosis in this triploid the only bivalents which can form are between W and W' since the genetic system prevents any association between W and X chromosomes. Univalents observed must belong to the X genome. Hybrids which show n bivalents and n univalents (V.riviniana x reichenbachiana and V.canina x stagnina) may be of this type.

The results from certain of the violet hybrids necessitate an extension to the postulated action of the genetic system. In V.reichenbachiana x rupestris an average of 5 bivalents per cell was observed but in V.riviniana x rupestris, where so far as can be deduced the same two sets are present together, the average is only 2. This was pointed out above as one of the anomalous results and can be given an explanation if the genetic system is additionally assumed to act in preventing, or at least lessening, pairing between the two sets in a tetraploid and a third, non-related set. Suppose the tetraploid is represented as before and the non-related diploid by ZZ; the two are hybridised to give a triploid:-



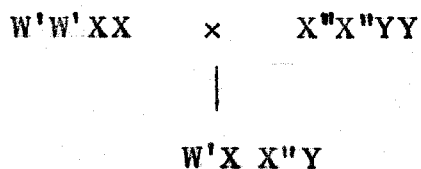


Then in addition to the genetic system preventing pairing between W and X, it must be additionally postulated to lower the ability of Z chromosomes to pair with W or X chromosomes. Thus pairing in the triploid W'XZ is lower than in the diploid hybrid WZ where there is no interference from any genetic system.

Thus there is a possible explanation for the higher result from V.reichenbachiana x rupestris (type WZ), than from V.riviniana x rupestris (type W'XZ), although it is difficult to see why there should be such an action since there has presumably been no selection for it. Any explanation for this extension of activity must await an investigation of the mechanism of operation of the genetic system. The same general effect also appears responsible for the low results obtained from V.labradorica x riviniana and V.rostrata x riviniana compared respectively with V.labradorica x reichenbachiana and V.rostrata x reichenbachiana.

Of more importance for the genetic hypothesis are the tetraploid hybrids, which may be of two types; those formed from parents with a genome in common, and those whose parents have no genome in common.

The first type can be formed by hybridisation as follows:-

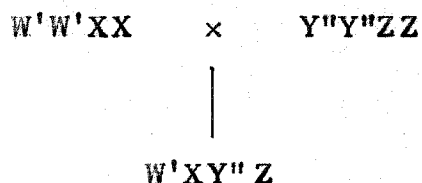


The (") indicates a genetic system which prevents pairing of Y and Z chromosomes but which is indicated by a different symbol from the system controlling W and X since it must have arisen independently.

In this type of tetraploid the chromosomes of the X genomes will form the n bivalents and the W and Y the 2n univalents. V.canina x riviniana and V.canina x pumila appear to be of this type.

By extending the action of the genetic systems as explained above, it may be supposed that the system preventing pairing of W and X chromosomes also prevents pairing of W with Y and that this is reinforced by the independent system derived from the X"X"YY parent. Thus, here is an explanation of why group (b) results p.84, have very sharp peaks at 10 bivalents per cell instead of being slightly in excess of this from pairing of some W with some Y chromosomes. The same argument applies to the pentaploid hybrids V.sieheana x riviniana, V.sieheana x canina and V.lactea x pumila, whose three non-pairing genomes also appear to be inhibited by the independent genetic systems derived from each parent. The strength of this effect is seen from the fact that the 30 chromosomes in the first two of the above hybrids only contribute about 2% of the total number of bivalents.

The second type of tetraploid hybrid is those where the two parents have no genome in common:-



The (') system prevents W from pairing with X and additionally, prevents W and X from pairing with Y or Z. The (") system prevents Y from pairing with Z with also an additional action of inhibiting Y or Z from pairing with W or X. The two independent systems may have a reinforcing influence one on the other.

This idea is given support by the group (c) hybrids, p.84, where those hybrids between a diploid (no genetic system), and a tetraploid or hexaploid (with a genetic system) have a single set of genes inhibiting pairing, whereas the hybrid between two tetraploids has a double set. It might therefore be expected that the latter hybrid would show a greater degree of pairing inhibition than the former group and this is supported by the hybrid between the two tetraploids, V.riviniana × pumila having a very much lower meiotic pairing average (0.3 bivalents per cell), than the rest.

Having given the main ideas for the genetic control of meiosis in violets it remains to use the hypothesis to predict some of the meiosis results for hybrids not yet examined.

It seems likely that further hybrids between diploid Rosulantes will have averages of about the same range, 5 to 10 bivalents per cell, as the hybrids already examined, and it is not expected that any will show zero average pairing. For instance the diploid species which has contributed genome B to several polyploids will probably show in its hybrid with V.reichenbachiana an average bivalent formation of something in the range 5 to 10 bivalents per cell.

Possibly low averages may be met with in diploid hybrids between the three groups Mirabiles, Rosulantes and Arosulatae. If this proves to be so it would provide cytological justification for the divisions. It is however proving difficult to make hybrids using V.mirabilis.

Very low average meiotic pairing can be predicted in hybrids where there is no common genome and where both parents contribute a genetic system controlling meiosis. This follows from the reinforcing effect of two combined systems discussed above. The essential condition here is that both parents must be at least tetraploid, and although the genomic constitutions of V.jordani and V.elatior are not yet known it is expected that the following hybrids will not show genomic pairing but will give very low results similar to V.riviniana × pumila:-

V.riviniana × jordani

V.riviniana × elatior

V.sieheana × pumila

V.sieheana × elatior

Further hybrids showing genomic pairing are expected to have sharp peaks in their histograms at 10 (or 20) bivalents per cell. No hybrid is expected with an average say, of 15.

The evidence for the genetic control of meiosis in the rostrate violets is much less sharply defined than that found by Riley in nullisomics of haploid wheat, where the presence or absence of a particular chromosome determined whether bivalents or univalents were formed. In the violets the evidence is entirely statistical and has only become apparent through the examination of fairly large numbers of cells. This provides some justification for the large amount of cytological observations done during the investigation. It has been found that individual cells may be selected to support almost any hypothesis and it is only when the average for a number of cells is taken that a sensible interpretation can be suggested.

### DIFFERENCES BETWEEN AMERICAN AND EUROPEAN VIOLETS

One of the striking discrepancies between the American and European rostrate violet floras is the low number of polyploid species in America, (1 out of 8 or 9) and the high number in Europe, (7 out of 11, including V.sieheana as a European violet). Is this distribution of polyploidy mere chance or can a more definite reason be suggested for it?

The first thing to point out about the American rostrate violets is that, possibly excepting V.adunca agg. they are not at all critical; that is to say, each species is readily identifiable from its vegetative and floral morphology. Some hybrids are reported as occurring wild but introgression has not been noted although this is possibly because of lack of study. In short, they are among the most readily distinguished of violets.

Despite these clear differences it has been shown that the chromosome sets of V.adunca, V.conspersa, V.rostrata, V.striata and very likely V.labradorica, are capable of forming 10 bivalents in their hybrids and that many of the hybrids are sufficiently fertile to give F<sub>2</sub> and further generations.

From the distribution of V.rostrata it has already been suggested that the separation of the two populations probably took place during the late Tertiary or at the latest during the early part of the ice age. From the similarity of

these widely separated populations we may deduce that the rate of evolution in V.rostrata has been low. If the striking morphological differences between V.rostrata and the other American species investigated have arisen at anything like the same rate, then it can be seen that the original series of evolutionary divergences which resulted in these species must have taken place long before the separation of the Japanese and N.American stocks; in other words the north American rostrate violets must have evolved into their present form well before the coming of the ice age. This, of course assumes a fairly steady rate of evolution in V.rostrata and the others, an assumption which is not necessarily true; it also assumes that the populations in Japan and America had a common origin and are not due to parallel evolution; the latter seems most unlikely since quite a large number of species belonging to other genera have similar distributions. Migration affords the only satisfactory explanation.

Thus it is postulated that the American species, 1), are of approximately equal age, 2), have evolved their characteristics in isolation from some common stock during the stable period of the Tertiary and, 3), were sufficiently distinct in their climatic, edaphic, pollination and other ecological characters by the beginning of the ice age to have remained separate and distinct throughout the climatic vicissitudes of that period.

The main mountain chains in N.America run in a general north-south direction and it may be supposed that with the advance of cold from the north, all the violets migrated south at roughly the same rate, keeping intact their barriers preventing introgression. It is as well to point out at this stage that several of the American rostrate violets may be regarded as primarily woodland plants and only secondarily as meadow plants. They are not plants which spread rapidly on newly disturbed areas, so the ice age would if anything, by reducing the amount of forest, restrict their habitat area. Some other groups of plants in N.America have been studied by other workers who have concluded that the ice age, by causing migrations and spread, has resulted in extensive introgression and/or polyploidy, so that some present-day groups are very complicated. This is the exact opposite of the history proposed for the rostrate violets. These other groups are composed of species which could take advantage of the disturbed and deforested conditions left after the retreat of the ice. Their populations probably greatly expanded into new areas and under these conditions it is usually supposed that introgression and polyploidy occur. A good example of such a group is the American blueberries, Vaccinium corymbosum agg., (Camp 1961).

In Europe, by contrast, the mountain chains run generally east-west and it seems likely that diploids in N.Europe would have been trapped at the onset of glaciation between ice or severe climatic conditions coming from the north and the ice



from the Alps etc. in southern Europe. This extermination hypothesis could explain the shortage of diploid violets in present-day Europe. The abundance of polyploids may be given a related explanation; those diploids which were not exterminated would have migrated either west via the Pyrenees and survived the ice age in Iberia, or east and survived somewhere in Asia or Asia Minor.

Such rapid east-west migrations, (rapid that is compared with movements during the Tertiary), would have brought into contact many ancient diploids which had previously been isolated for a very long time. These conditions would favour the production of hybrids and from these may have arisen the present-day polyploids.

Oddly enough the two areas into which the European diploids are most likely to have migrated with the onset of glaciation are just the two areas in which are found the only two known hexaploids; V.lactea in Spain and Portugal, and V.sieheana in Asia Minor. Thus a hypothesis is put forward which explains the present lack of diploids in Europe and their abundance in America. It appears to have been J.D.Hooker (1878) who first pointed out that the different alignment of the mountain chains in N.America and Europe could be used to explain the existence of many species in America which did not occur in Europe.

Ample grounds are available for speculation on the former distributions of violets. Some may have had much wider distributions than they have at present. For instance

V.rostrata might once have occurred in Europe; some of the other American violets might have occurred in Japan and since become extinct, or some of the Japanese diploids might have occurred in N.America. Then there is the Bête noire of the rostrate violets, V.howellii, distributed in N.W.America across what must have been an important migration route. Is its chromosome number really the  $2n=c.80$ , reported by Gershoy (1934)? If this is indeed so then it seems very likely that it will prove to have genomes of both Asiatic and American derivation.

#### VALIDITY OF BECKER'S CLASSIFICATION

As stated at the beginning of this thesis the rostrate violets are subdivided on the basis of life form and the validity of this ought to be examined in the light of the cytological evidence.

It is known that one genome, B, is common to species of both Rosulantes and Arosulatae (Valentine 1958). To this extent there may be some justification for regarding the whole group as one large polyploid aggregate containing a range of species, some extreme in character, some intermediate, the whole not being worthy of finer divisions. This mixing of Rosulantes and Arosulatae however only occurs at the polyploid level and the bringing together of different gene sets appears to be one of the advantages of polyploidy. V.canina certainly combines the chromosome sets of two greatly

differing diploids; the B genome from a diploid member of the Rosulantes and the C genome from V.stagnina in the Arosulatae. Perhaps the resulting genetic diversity has helped V.canina to achieve its very wide geographical distribution and great ecological range.

From its combination of characters it is suspected that V.jordani may similarly combine genomes from Rosulantes and Arosulatae. The broad leaves suggest Rosulantes influence while the overwintering buds and enormous stipules suggest the Arosulatae in which it is placed. Flower morphology, especially the slightly upturned spur, suggests that it may possibly have a genome (F or G) in common with V.sieheana and the very large stipules suggest that the Arosulatae parent is the same as that responsible for the very large stipules of V.elatior.

On the other hand if the Mirabiles-Rosulantes-Arosulatae classification is viewed with the diploids only in mind, then it works extremely well because they constitute the extreme types. Thus there may be some evolutionary significance behind the classification which later hybridisation has blurred. It may be that Becker's scheme has a good evolutionary basis at the diploid level but that later hybridisation has brought the divergent lines together again. Any judgement of the Mirabiles must wait until more hybrids, especially diploid hybrids, have been synthesised between V.mirabilis and members of Rosulantes and Arosulatae.

POSSIBLE CONNECTIONS BETWEEN MORPHOLOGY AND HABITAT

A consideration of leaf shapes in Rosulantes and Arosulatae was suggested as a result of reading D'Arcy Thompson's book 'On growth and form' (1942), and a series of papers by Melville (1951, 1953, 1960) on leaf shapes.

If the leaves on a single plant of V.riviniana are studied through a whole season it is seen that the early spring leaves tend to be broader than long, the later spring leaves may be almost circular and the last produced summer leaves are usually slightly longer than broad and have an acute apex. These changes may be ascribed to the greater relative extension of the leaf axis with the advancing season. and are illustrated in (a), (b) and (c) of fig.27, which are drawings of three leaves from the same plant of V.riviniana. Shape appears to be a function of the time of year at which the leaf is produced and seems to be independent of leaf size. Other species of Rosulantes behave similarly, as does V.canina where however the summer leaves become slightly more elongated. When other Arosulatae are considered, the same general sequence is observed, but the elongation takes place earlier and is much more extreme; the small rounded leaves which even V.elatior produces on first starting into growth in spring are soon lost through decay or other causes. The tendency to elongation which is present in Rosulantes is much more strongly developed in the 'pure' Arosulatae, V.stagnina V.elatior and V.pumila (V.canina, V.lactea and probably

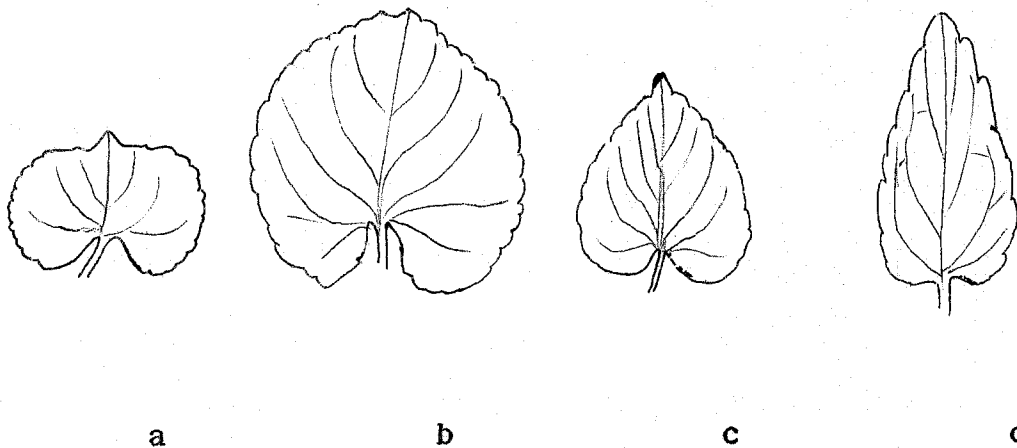


Fig.27, (a),(b),(c) V.riviniana, (d) V.stagnina

V.jordani are of hybrid origin between Rosulantes and Arosulatae). In fig.27 (c) is an upper leaf of V.stagnina which is seen to continue the trend in V.riviniana.

Another point considered was the leaf mosaic which different species show when viewed from above. Rosulantes have widely spreading branches with leaves on fairly long petioles; Arosulatae have erect branches with the leaves clustered round the stem on short petioles. Thus, viewed from above, Rosulantes have a loose, spread-out mosaic, Arosulatae show a tight clustering. In V.elatior and V.jordani this concentration of the photosynthetic tissue close to the axis is further added to by the enlarged stipules, which in these two species must contribute an appreciable amount to the plant's photosynthetic output.

These two extreme types of leaf mosaics are perhaps adaptations which make the best use of the available light

in the habitats of the plants possessing them. V.stagnina and V.elatior are fen plants growing in fairly tall, dense vegetation and in such conditions the illumination is predominantly from above since the density of plants prevents any appreciable light coming from the side. Tall stems with a clustered leaf arrangement would seem to be a good arrangement for utilising a vertical light distribution in dense vegetation.

The other extreme in leaf mosaics is shown by V.riviniana, V.reichenbachiana and V.sieheana, all of which commonly grow in woods. In the herb layer of a wood the illumination is of a diffuse, dappled quality, filtering through the tree canopy from all directions and a well spaced out arrangement of leaves of an unspecialised shape (about as long as broad) will best trap the available light.

Other genera which have woodland and fen species show the same differences. Ranunculus lingua and Lysimachia vulgaris are fen plants with elongated leaves; Ranunculus ficaria and Lysimachia nemorum are woodland plants with spread-out leaves which are about as long as broad.

The overwintering form of the two groups may also be connected with habitat differences. Rosulantes have overwintering leaves which presumably are able to photosynthesise early in spring and so help the early flowering which is over by the time the leaf canopy becomes fully formed. In fens consisting of dense vegetation a cover of rotting vegetation and possible submergence would

prevent the persistence of overwintering leaves and in fact the Arosulatae have small overwintering buds and flower much later than Rosulantes. The main trend in Arosulatae appears to be adaptations to fen conditions.

#### A RECENT RECLASSIFICATION OF ROSTRATAE

Recently a fresh attempt at classifying the Rostratae has been made by a Japanese botanist, Tamotsu Hashimoto (1959), and is of interest since it cuts across Becker's 1925 classification. In it Mirabiles are kept separate as before but species in the categories Rosulantes and Arosulatae are merged and redivided according to the presence or absence of hairs and papillae on the petals and style giving two new subsections, Glabristylae and Capillostylae. The English summary to the Japanese text is given below without any change in its original wording:-

#### SUMMARY OF HASHIMOTO'S CLASSIFICATION

'The writer classified the group of long-spurred violet or wood violet and he put Arosulatae into the subsection Capillostylae.

Sect. Rostratae (section of long-spurred violet)

1).Subsect. Glabristylae (nom.prov.)

Style and petals, smooth (no papilla) and has longer spur than the next.

Viola rostrata, V.Kusanoana, V.grypoceras, V.obtusa,  
V.ovato-oblongata, V.Grayi, V.rhizomata, and V.Faurieana.

2).Subsect. Capillostylae (nom. prov.)

Hairy at apex of style and inside of the lateral petals,  
often short and twice-branched spur exists at the apex.

i) Ser.Sylvestres (nov. emend.)

Basal leaves rosulate. Leaf-blade cordate or reniform.

Viola sylvestris, V.Riviniana, V.rupestris, V.labradorica,  
V.sacchalinensis, V.conspersa etc.

ii) Ser.Caninae (Arosulatae)

Leaves not rosulate, blades oblong or lanceolate. Stem  
erect. Flower not so large.

Viola canina, V.acuminata, V.persicifolia, V.pumila,  
V.erecta etc.

3).Subsect. Mirabiles

Style smooth and oblique, dense ciliate at the inside of  
the lateral petals, stipules entire, plant delicate, pale  
green. Rhizome with hard many (or very complicate) lateral roots.

Viola mirabilis, V.Willkommii etc.'

-----

It is seen that Arosulatae are kept together in Series  
Caninae but that Rosulantes are about equally divided among  
the first two subsections.



From the few species seen in cultivation in England, this is a good attempt at reclassifying the Rostratae and in picking on the presence or absence of hairs on petal and style, what is probably an important aspect of their biology has been made the main feature of the key.

The presence or absence of hairs and the length of spur probably have a function in determining what insect or group of insects pollinates the flower and in the case of V.rostrata, with its glabrous style and petals and very long spur, it seems very likely that it is pollinated by some long-tongued lepidopteran insect. Thus the classification depends on a fundamental and fairly easily determined feature of their reproductive biology.

On the other hand in separating V.rostrata from V.conspersa the scheme does violence to what is known of the evolution of these violets since chromosome and breeding studies have shown that V.rostrata and V.conspersa appear to be closely related, but a representation of the evolution of a group is not an essential qualification for a taxonomic classification and its absence need not distract from its usefulness. The new classification reflects how two species, which are known from botanic garden studies to be interfertile and to give a moderately fertile hybrid from which F2 and further generations have been obtained, may nevertheless be kept separate in the wild by having different pollinating insects.

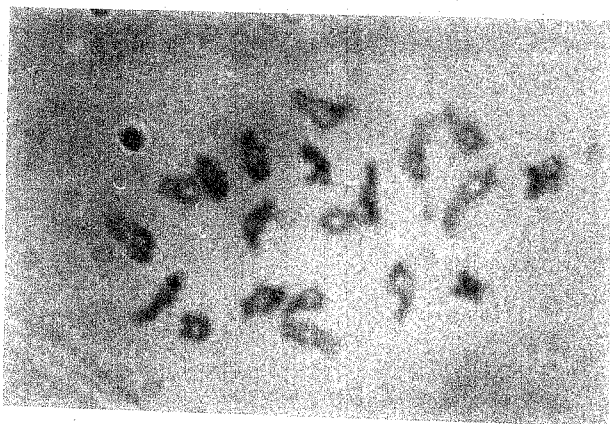
## P H O T O G R A P H S

Although the cytological evidence in this thesis is essentially statistical and given in the histograms on pp.79-83, a few photographs are added to show the range of chromosome behaviour encountered. The examples selected are not necessarily typical of the hybrids concerned since some show unusual but interesting features.

The photographs were taken using a Reichert microscope and camera attachment with a  $\times 100$  oil-immersion achromatic objective and either a  $\times 8$  or  $\times 12.5$  eyepiece, on  $2.5 \times 3.5$  " cut film. No standard degree of enlargement has been used in the prints following but most are between  $\times 2000$  and  $\times 4000$ . All preparations were mounted in 'Euparal' and all were stained with aceto-carmines except the root-tip of V.rupestris which was stained by Feulgen's technique.

Abbreviations used in the captions are:-

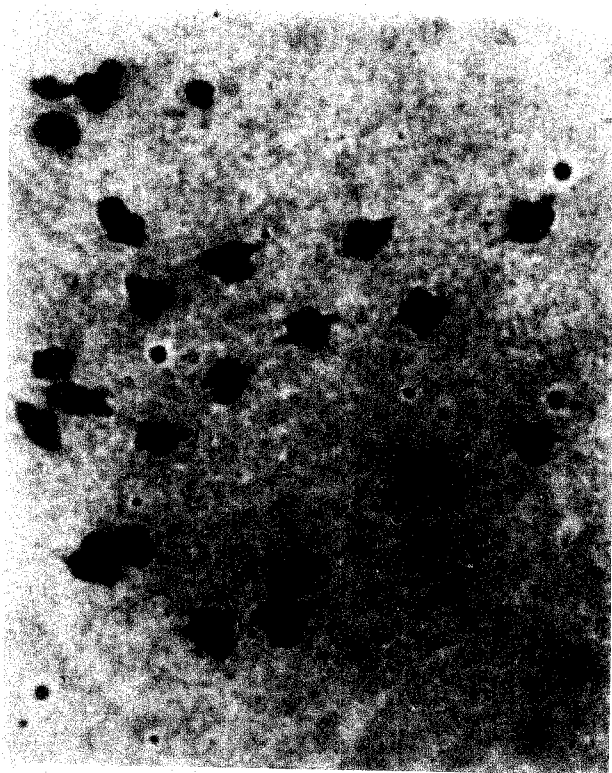
RT	root tip
M	metaphase
A	anaphase
T	telophase
PMC	pollen-mother-cell
I	univalents,      II bivalents
III	trivalents,      IV quadrivalent



1. *V. rupestris*, Long Fell, West 'ld.  
2n=20, R.T. early A, treated 8 hyd  
quinoline, Feulgen, 2 satellites.



2. *V. labradorica*, Mt. Albert, Gaspé,  
2n=20, M1, P.M.C. showing ring IV.



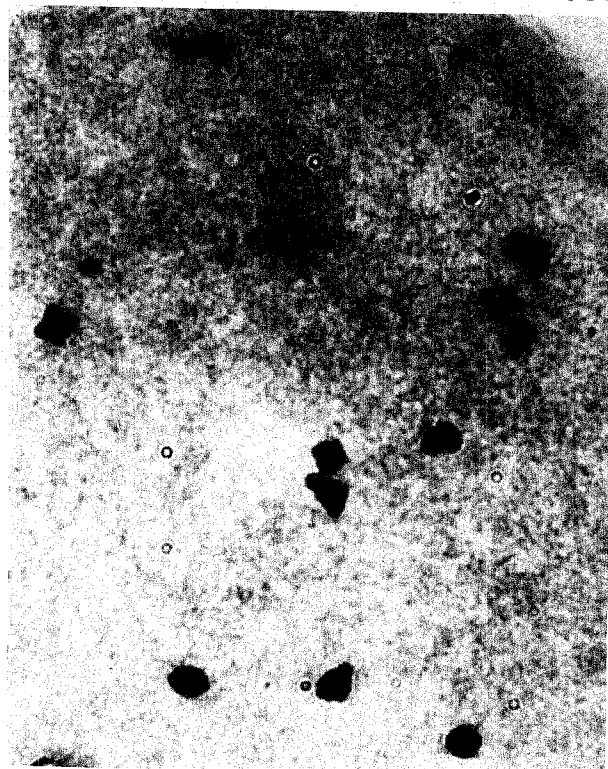
3. *V. riviniana*, England, 2n=46,  
21 II + 4 I, P.M.C. M1.



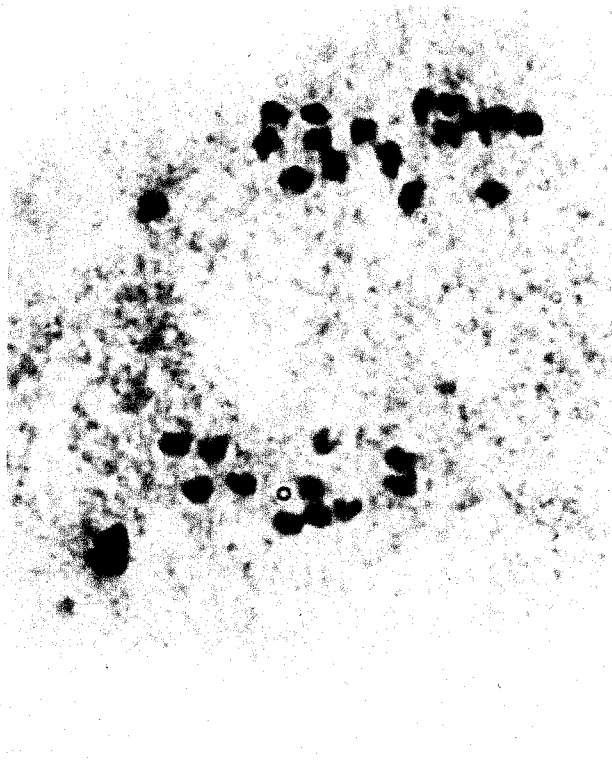
4. *V. sieheana*, Rizé, Turkey, 2n=60.  
P.M.C., A1 showing 2 groups of 30



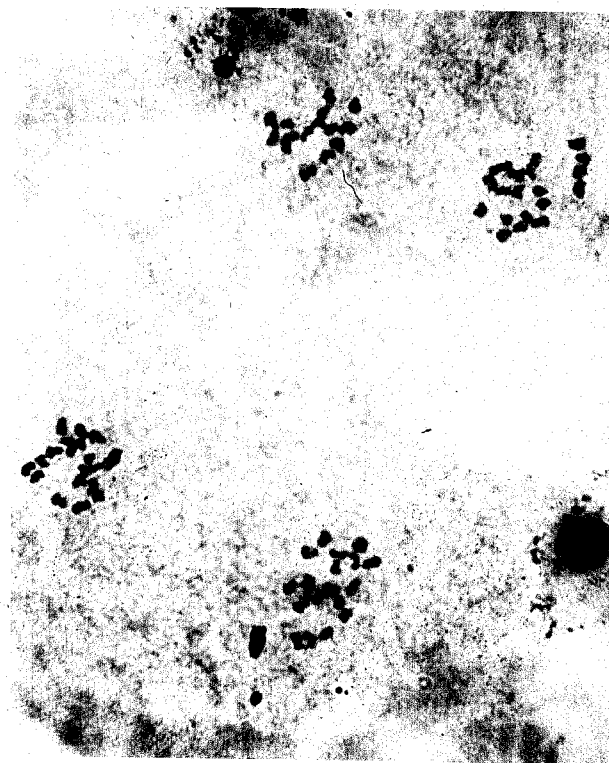
1. V. reichenbachiana, England,  
triploid plant,  $2n=30$ , P.M.C.  
M1, 10 III.



2. V. reichenbachiana, England,  
tetraploid plant,  $2n=40$ , P.M.C.  
prometaphase, 10 IV.



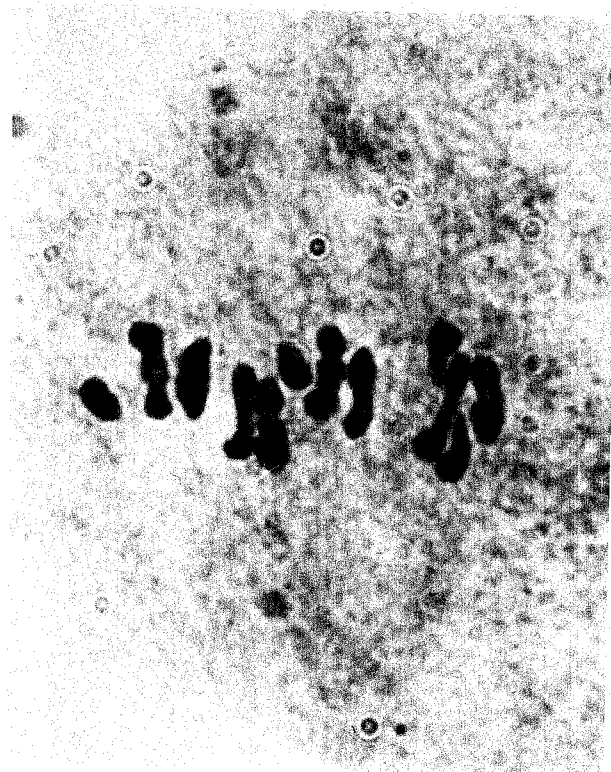
3. V. reichenbachiana, England,  
triploid plant,  $2n=30$ , P.M.C.  
unequal A1, 13:17.



4. V. reichenbachiana, England,  
tetraploid plant,  $2n=40$ , P.M.C.  
T2,4 groups of chromosomes.



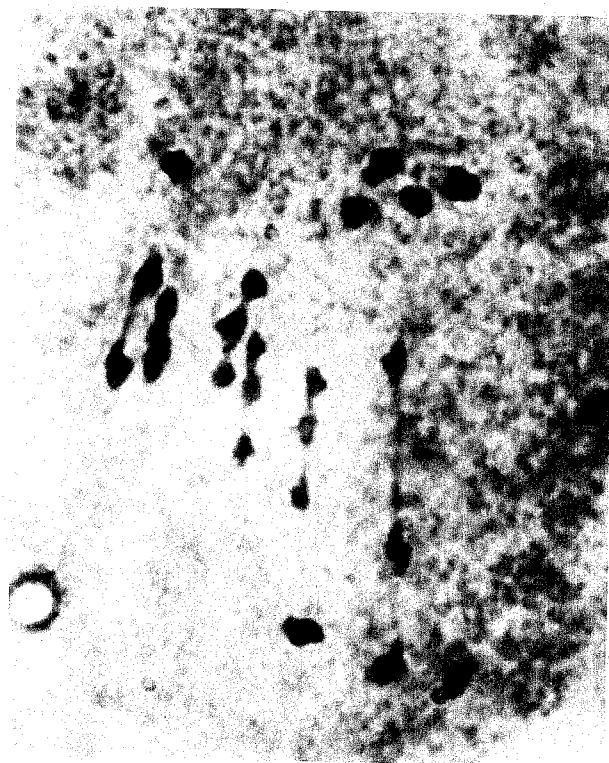
1. V. adunca × rupestris,  $2n=20$ ,  
P.M.C., M1, 10II.



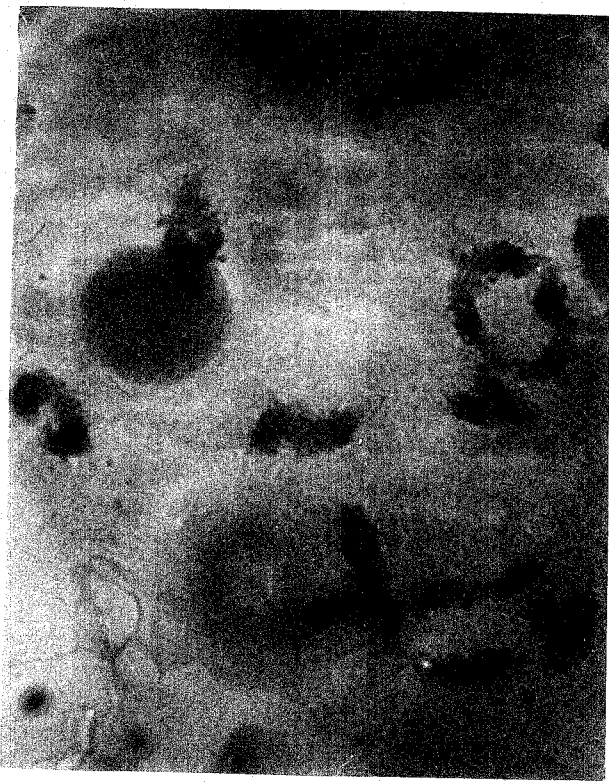
2. V. adunca × conspersa,  $2n=20$ ,  
P.M.C., M1, 9 II + 2 I.



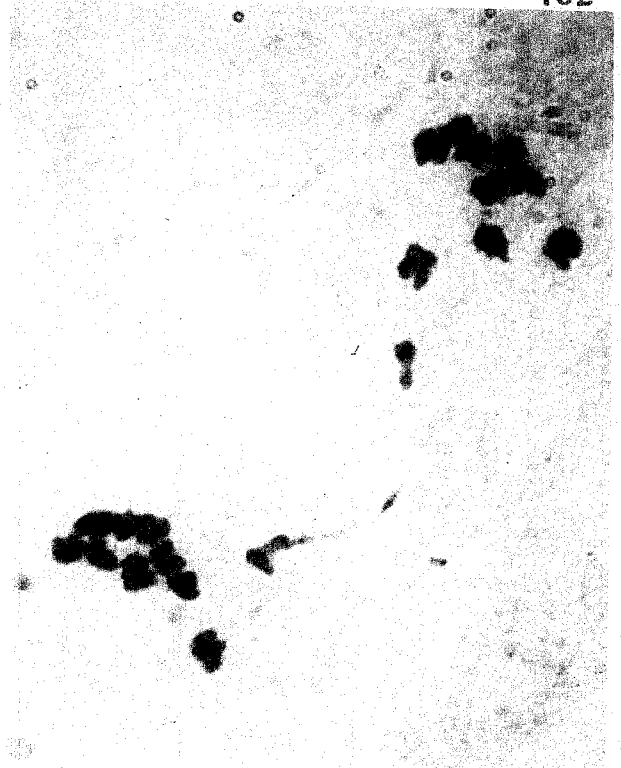
3. V. rupestris × labradorica  
P.M.C., A1, 8 II + 4 I.



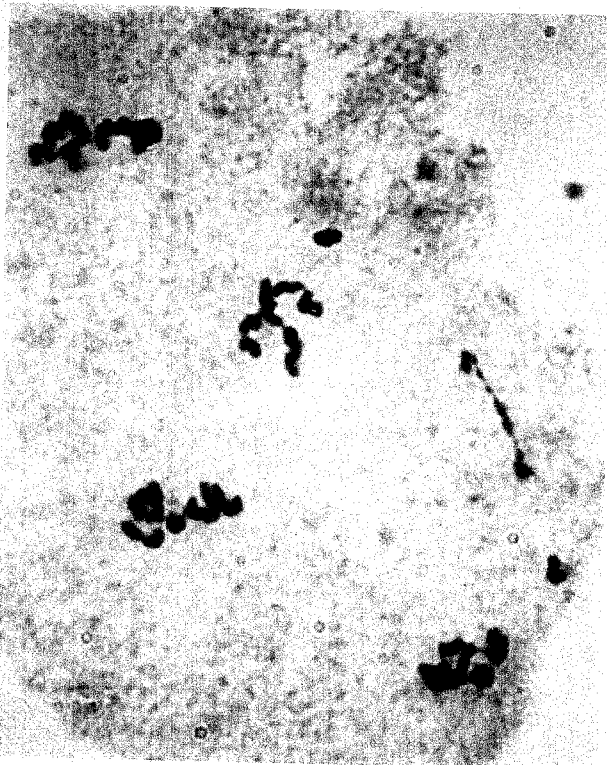
4. V. reichenbachiana × rupestris  
P.M.C. M1, 6 II + 8 I.



1. V. rostrata × conspersa,  $2n=20$ ,  
late P1, P.M.C. ring IV.

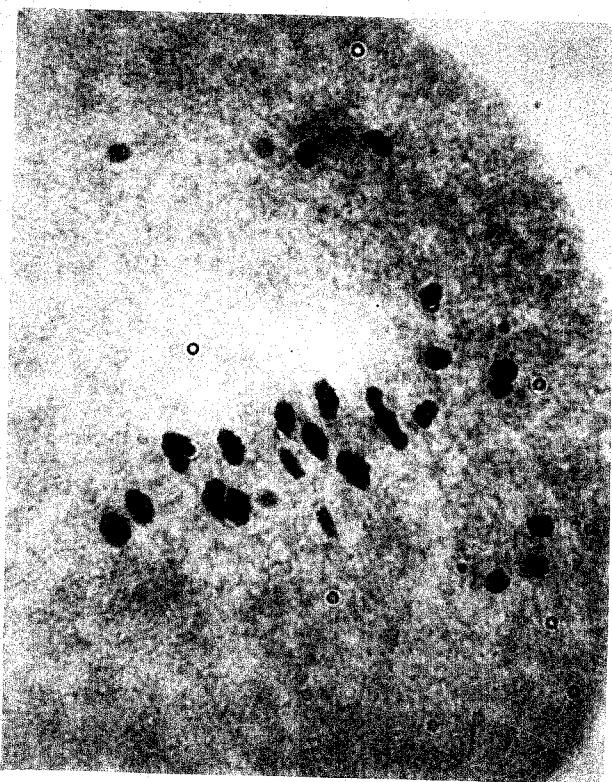


2. V. adunca × rostrata,  $2n=20$ ,  
P.M.C., A1, bridge + fragment.

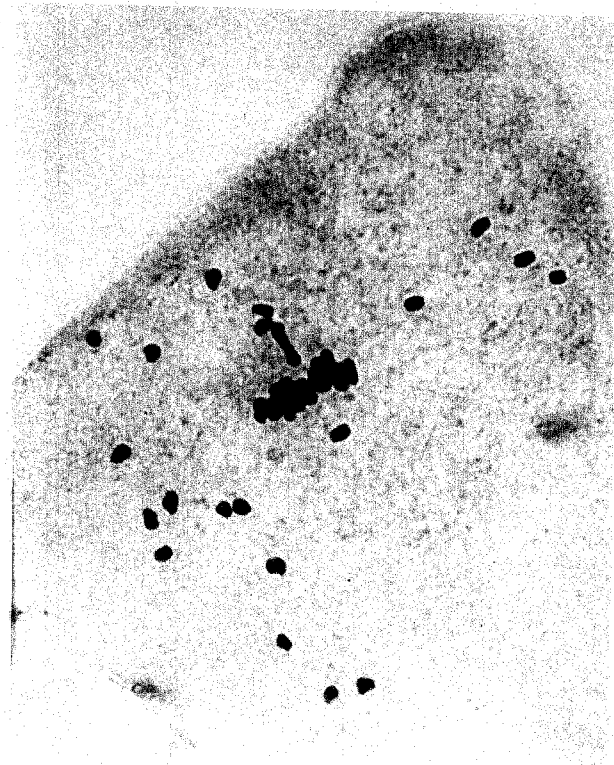


3. V. adunca (M) × adunca (B),  
 $2n=20$ , P.M.C., A2, showing bridge

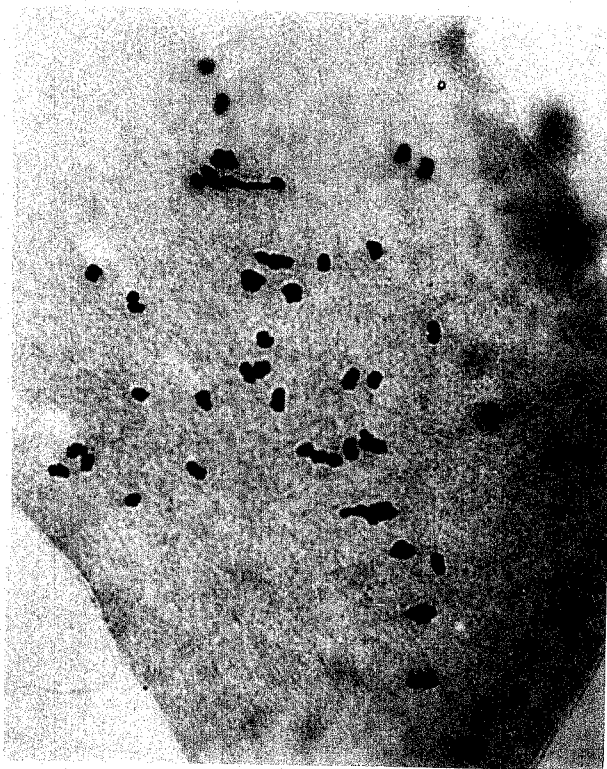
4. V. labradorica × reichenbachiana  
 $2n=20$ , PMC. M1, multivalents.



1. V.canina × stagnina,  $2n=34$ ,  
PMC. M1, 11 (or 12) II + 12 I.



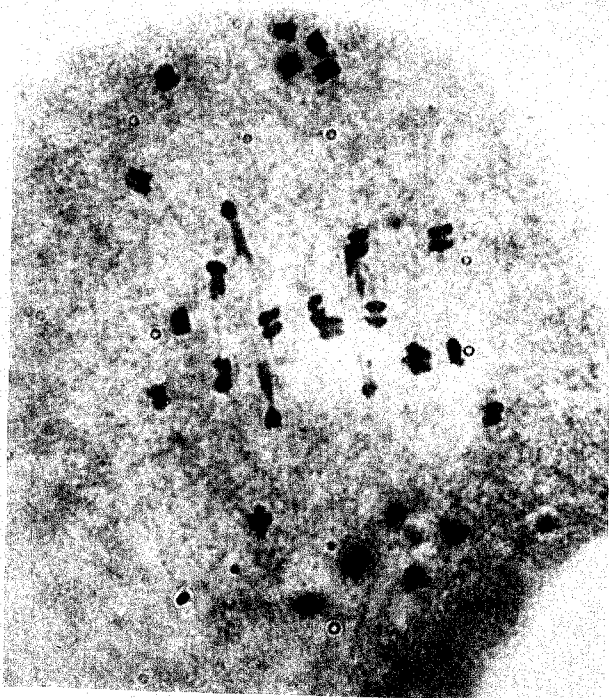
2. V.canina × pumila,  $2n=40$ ,  
PMC., M1, 10 II + 20 I.



3. V.riviniiana × sieheana,  $2n=53$ ,  
P.M.C. M1, 10 II + 33 I.



4. V.riviniiana × pumila,  $2n=42$ ,  
P.M.C. M1, 42 I.



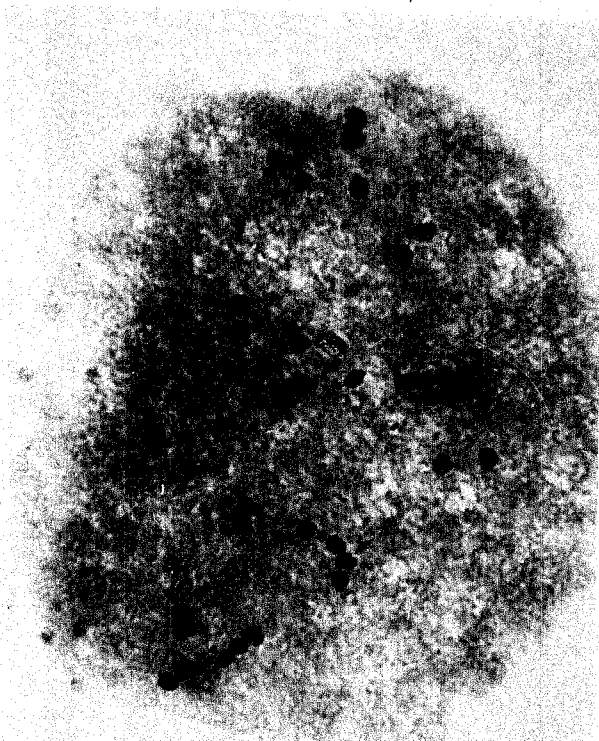
1. *V. canina* x *reichenbachiana*,  
 $2n=30$ , P.M.C. A1, 3II + 24I, the  
 I on equator starting to divide.



2. *V. adunca* x *labradorica*,  $2n=20$ ,  
 cell from anther,  $2n=c.293$ ,  
 ie. about 32-ploid.



3. *V. adunca* x *labradorica*,  $2n=20$ ,  
 P.M.C. M1, 19I + fragment.



4. *V. adunca* x *labradorica*,  $2n=20$ ,  
 P.M.C. late A1, 48 + 2 fragments.



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"What's the use of their having names?" the Gnat said.

"No use to them," said Alice; "but its' useful to the people that name them, I suppose. If not, why do things have names at all?"

"I can't say," the Gnat replied. "Further on, in the wood down there, they've got no names."

Carroll, L. (1871)

Through the Looking Glass.

