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THE EXPERIMENTAL TAXONOMY OF *VIOLA LACTEA.* SM.

Abstract of the Ph. D. thesis presented by D. M. Moore, 1957.

1. *V. lactea* Sm. may be separated from *V. canina* L. on grounds of morphology, geography, ecology and cytology.

2. *V. canina* has a widespread,eurasiatic distribution; *V. lactea* is much more restricted,extending from S. W. Britain down the W. coast of Europe to C. Portugal.

4. The barrier primarily separating these species seems to be climatic; when they meet, as in Britain, the main isolating factors appear to be edaphic: exchangeable calcium is high in soils with *V. canina* and low in those with *V. lactea*

5. In Britain *V. lactea* is found in open communities on dry,acid heaths while *V. canina* occurs mainly on dunes or inland sand deposits and certain heaths. The allied species, *V. riviniana* Rchb., has wide edaphic and climatic tolerances and comes into contact with *V. lactea* and *V. canina* over much of their ranges.

6. Cytological studies show *V. lactea* to be a sub-hexaploid with 2n = 58, *V.canina* and *V. riviniana* both have 2n = 40.

V. lactea was crossed with *V. canina*, *V. riviniana* and *V. pumila* Chaix. and meiosis was studied in some hybrids to discover genomic homologies among the species. It was concluded that *V. lactea* is an allohexaploid derived from *V. canina* and another,as yet unknown, species. The possible identity of this unknown parent is discussed.

7. Field populations of V. lactea were sampled from most parts of its range in Britain and subjected to biometrical analyses for indications of hybridisation. Techniques were developed to deal with the three species encountered in field populations - V. lactea, V. canina and V. riviniana. Primary classification was by means of polygonal graphing after which the more detailed structure of the populations was determined by using pictorialised scatter diagrams.

The results show that there is a widespread introgression involving V. lactea and V. riviniana; a mechanism is discussed by which the sterility barrier between these species may break down. The possible implications of this introgression are also discussed.

THE EXPERIMENTAL TAXONOMY OF
VIOLA LACTEA. SM.

BY

DAVID MORESBY MOORE.

B.Sc. (DUNELM)

- being a thesis presented in candidature for the Degree of Doctor of Philosophy in the University of Durham, 1957.

CONTENTS

Page

Frontispiece

(Nat. size)

Plate I. Viola lactea Sm.

(1) Introduction.

Until the later years of the last century the relationships of a plant species were decided almost entirely by reference to its external morphology and geographical distribution - those characters which were most readily available in the herbarium. This kind of approach was, and still is, very useful for the treatment of large amounts of material obtained in the floras of newly explored countries. Since then, however, the basic knowledge of the floras of Europe and N. America is substantially complete, and more detailed investigations involving cytology, autecology, breeding and growth experiments, and statistics, as well as morphology and distribution, have been employed to give a more complete picture of the affinities and evolutionary position of species.

This 'experimental taxonomy' (Clausen, Keck and Hiesey 1940), which may be said to have been started by the Swedish botanist Turesson about 1920, is perhaps used to best advantage in the study of a single genus; a classic example of this is the work of Hutchinson, Silow and Stephens (1947) on *Gossypium*. By combining studies on the wild species in many parts of the world with detailed considerations of species and hybrids in the garden and laboratory they were able to learn a great deal about the evolution, origins and potentialities of the cultivated species. An extensive survey of this sort requires the large resources of time and facilities usually only afforded to plants of such economic , as cotton, potatoes, tobacco and the like, while many equally, or perhaps more, interesting groups have hardly been touched.

However, although the violets comprise a large genus, a start has been made on their study along experimental lines; an excellent basis for such work already exists, being provided b the world-wide studies and monographs of the violets carried out early this century by Becker. This work was closely followed by the field investigations of Brainerd (1921,-24) on N. American violets. The first applications of hybridization and cytological techniques to these plants were made by Clausen (1927-31) working both in Europe and N. America, and Gershoy(1928-34) who studied only North American violets. More recently Valentine (1949) employed a balanced and intensive approach for the study of two species - V. riviniana and V. reichenbachiana - in the sub-section Rostratae and discussed the status of these species in the terms of experimental taxonomy.

The work described in this thesis really developed from Valentine's studies and deals with another group of rostrate violets, the Arosulatae. In particular, it is centred round V. lactea and information is presented which, it is hoped, contributes a little to the understanding of the evolutionary position and requirements of this species. After defining its status and relations in terms of orthodox taxonomy, consideration will be given to other aspects revealed by crossing experiments in the greenhouse, cytological examination of the species and its hybrids, and autecological studies in order to supplement and amplify the data obtained from the herbarium sheet.

One of the most recent tools to be acquired by the experimental taxonomist is the application of biometrics to studies of wild populations, as described by Anderson (1949) and his pupils. By developing such techniques it has been possible to gain an insight into the present status of V. lactea and even very tentatively to look a little way into the future. Although as yet in its infancy such an approach, which is described in the later sections of this thesis, could well be applied to other groups of violets where there are as yet unresolved complexes of species and hybrids.

ACKNOWLEDGEMENTS

I should like to express my thanks to Professor D.H.Valentine who suggested that this group of violets may provide a suitable problem and who made available to me several stocks of artificial hybrids. I am further indebted to him for his constant invaluable advice and criticism throughout the course of this work and during the preparation of this thesis.

Thanks are due to Professor Dr. A. Fernandes of the Instituto Botanico Dr. Julio Henriques, Coimbra, for contributing so much to the success of a visit to Portugal for the purpose of studying violet populations.

Finally, I am indebted to the Department of Scientific and Industrial Research for providing a grant which enabled me to carry out this work and to the Research Fund of the Durham Colleges for generously contributing towards field expenses.

SECTION 2 .

GENERAL ACCOUNT OF VIOLA LACTEA.

(a) Taxonomy

  (i) Description

  (ii) Synonmy

  (iii) Relationships

(b) Distribution

(c) Literature Records

(2) GENERAL ACCOUNT OF VIOLA LACTEA.

(a) Taxonomy.

On the classification of Becker (1910) Viola lactea belongs to the Section Nomimium, Sub-section Rostratae, Group Arosulatae.

(i) Description. Perennial herb, 4-20 cm.; subglabrous; stems procumbent at base, then ascending, solitary or few; foliage and stems often purplish tinged. Leaves alternate, blade 1-3.5(-4)cm. lanceolate or ovate-lanceolate, subacute, rounded to cuneate at base, broadest at about $\frac{1}{3}$ distance from base to apex, shallowly crenate; stipules (except lower) large, middle ± lanceolate, c.$\frac{1}{2}$ length of petiole, upper ones equalling or longer than petiole, coarsely and irregularly dentate or fimbrio-serrate. Flowers solitary, erect, axillary on long peduncles which bear sparsely toothed bracts on their bend; sepals lanceolate; appendages rather large; corolla 10-20 mm., milky or pale mauve, spur greenish white; petals lanceolate, acute, three or more times long as broad: spur short, obtuse. Capsule glabrous, acuminate, longer than broad. Seeds oval, dark brown - black, c. I.5 x 0.8mm.

Not a very variable species, either in Britain or on the continent. Most typical populations have flower colour almost pure white, possibly the mauve tints are the result of influence from other species, notably V. riviniana. Rchb.

	V. LACTEA	V. CANINA
HABIT	Both species are perennial and arosulate.	
	Sub-glabrous Stems ascending.	Glabrous or sparingly pubescent. Stems decumbent to erect.
LEAVES	Lanceolate to ovate-lanceolate, subacute, shallowly crenate-serrate, cuneate at the base, broadest about ⅓ distance from base to apex. Foliage often purplish.	Ovate to ovate-lanceolate, obtuse or subacute, crenate or crenate-serrate, truncate or shallowly and widely cordate at base.
STIPULES	Large(except the lower), lanceolate to ovate-lanceolate, coarsely and irregularly fimbrio-serrate or dentate, equalling or somewhat longer than petiole.	Rather small, ± lanceolate, distantly serrate-dentate, few short and stout teeth, about ½ as long as the petiole.
COROLLA	Petals narrow (3-4 times long as broad), creamy to pale lilac	Petals obovate (I½-2 times long as broad), blue with little or no violet tint.
FLOWERING TIME	May - June	April - June.

Table I. Comparison of characters distinguishing *V. lactea* and *V. canina*

Plate 2.

Plate 2. Potted plants in greenhouse. (a) x$\frac{2}{3}$, (b) x I.

(a) V. lactea, Wickham Common, Fareham, Hants.

(b) V. canina ssp. canina. Cleeve Common, Gloucs.

(ii) *Synonymy.* *V. lactea* Sm. Engl.Bot.*7*,445,(1798).
V.lusitana Brot. Phytogr.lusit.*I*,ii,(1801)
V.lusitanica Brot. Fl.Lusit.*I*,306,(1804).
V.lancifolia Thore,Chlor.Land.,357,(1803); Reichb.,
Ic.Fl.Germ. et Helv.,iii,f.4506(1839); Gren. et Godr.
Fl.Fr.i,179(1847).
V. canina ssp. lactea. Syme Eng.Bot.,ii,22(1864).

(iii) *Relationships.* *V. lactea* is most closely allied to *V. canina* L. from which it was separated by J.E.Smith in Sowerby's 'English Botany' (1798). The author expressed doubt about their relative status even whilst so treating them since he comments:- "We are extremely doubtful of the permanence of this species, and now publish it as distinct from *V. canina* rather in conformity to the opinions of others, and in the hope of having the point cleared up by investigation and culture, than as willing to decide upon it ourselves". Later authors never really seemed certain whether to give it specific rank or to put it as a subspecies of *V. canina*. The principal characters of the two are set out for comparison in table I and these morphological data combined with that on geographical distribution and cytology given later strongly support the view that *V. lactea* is worthy of specific status.

Other members of the Arosulatae are listed below together with an outline of their geographical distribution.

V. canina L. - widely distributed throughout Europe and W.Asia.

V. stagnina Kit. - British Isles to W.Russia, northwards to S. Scandinavia and south to regions of Upper Rhone and Danube.

V. elatior Fries. - Turkestan and W. Siberia eastwards to N. France. Only Oland in Scandinavia, and reaches Serbia and Bulgaria.

V. pumila Chaix. - C. Europe to W. Siberia; to 58° N. in Sweden, Oland, south to E. France.

(b) Distribution.

Maps showing stations for V. lactea in Britain and in western Europe are given in figs. I and 2.

Apart from one or two inland habitats in Sussex, Bucks., and Gloucs. the distribution is clearly 'Atlantic' as defined by Kendrew (1927) when separating the major climatic regions of West and Central Europe. The general south-westerly trend obvious from both maps causes V. lactea to be included in Matthews' Oceanic West European Element. This species, like so many others in the group, is a member of the widespread "atlantic" heath communities of western Europe, to which region it is also endemic.

The eastern boundary of V. lactea seems to coincide fairly well with the 38° F January isotherm and is probably dependent on the winters being reasonably warm. Since (see sect. 5a) it inhabits dry, acid, rather sterile soils, and such a combination is most commonly realised in the south and west, it seems likely that the distribution of this species is ultimately determined by the interation of both climatic and soil factors.

Matthews' (1955) considers the general picture to indicate that V. lactea may have migrated 'along the fringes of the Atlantic seaboard, where a former wider area of distribution may have undergone disintegration'. This is an important clue when searching

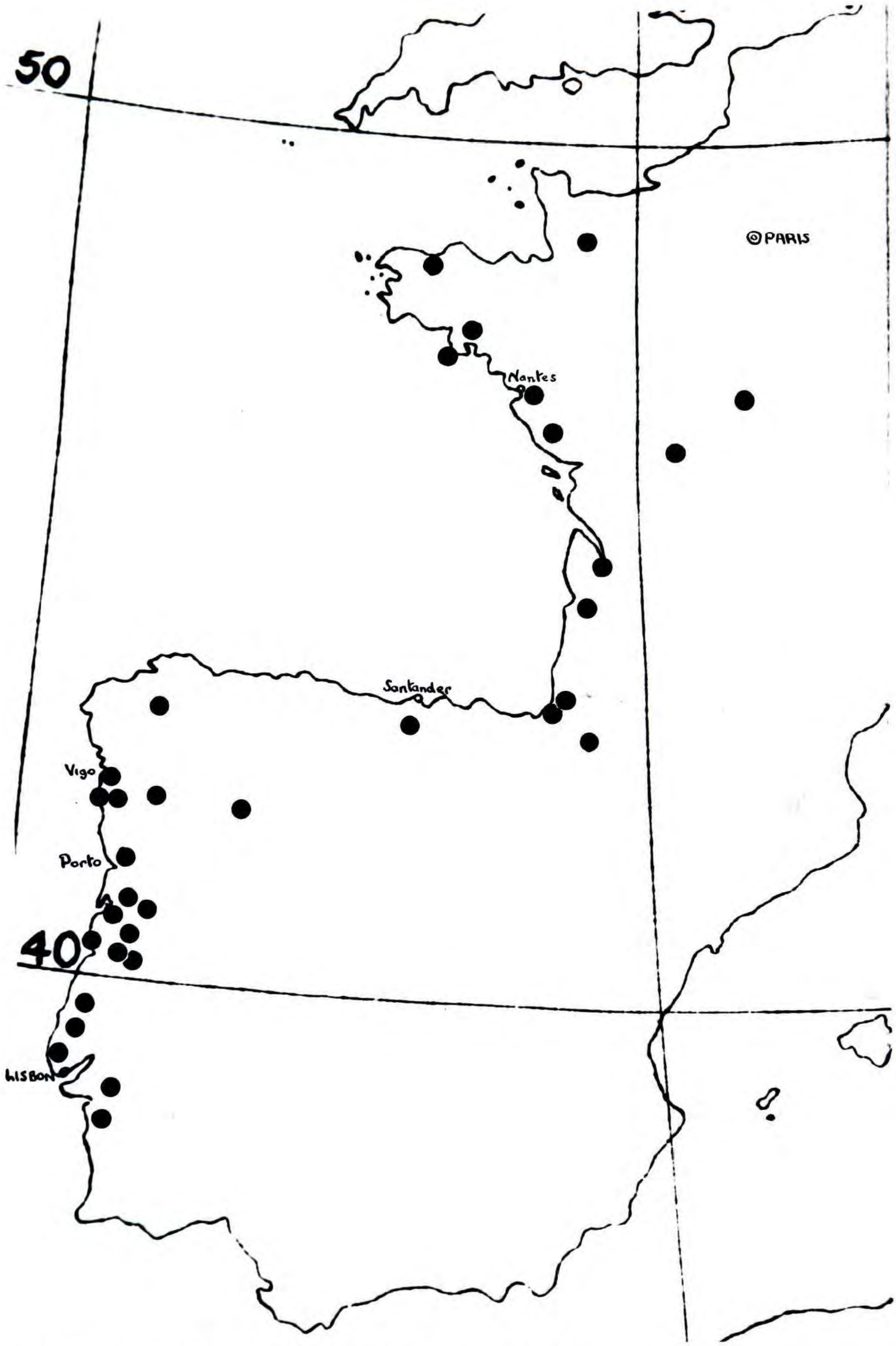
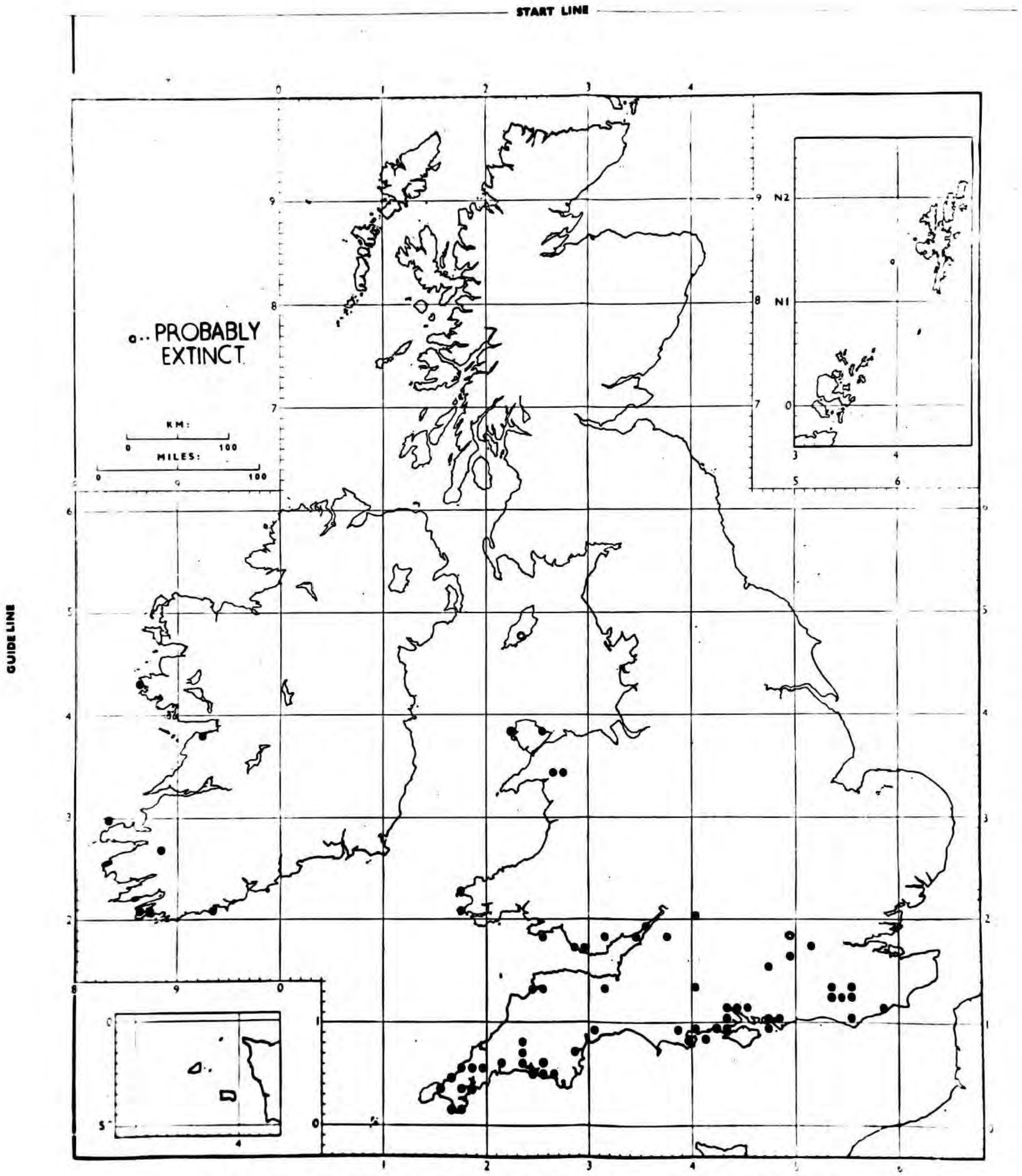


FIG. I. DISTRIBUTION OF V. LACTEA IN EUROPE.



BOTANICAL SOCIETY OF THE BRITISH ISLES DISTRIBUTION MAPS SCHEME

FIG. 2 DISTRIBUTION OF VIOLA LACTEA

IN BRITAIN.

for the origins of this species but will be considered in conjunction with other data in the final discussion (sect. 8)

(c) Literature records of hybrids involving V. lactea.

V. lactea x canina. In Violae Europaeae (p.138) Becker records that he himself has never seen this hybrid but cites several localities in France given by Fouillade (1902) who also reports it as sterile. Gregory (1912) describes the hybrid in detail from a specimen collected at Porth Towan, Cornwall, and considers it fairly widespread. Almost every local flora for counties where V. lactea is found reports the occurrence of V. lactea x canina.

V. lactea x riviniana. Becker (l.c.p.135) says this hybrid occurs in France and England and describes two forms :-

F. pseudocanina Fouillade - resembling V. lactea

f. pseudonemorum Fouillade - resembling V. riviniana.

Gregory (l.c.p.92) figures this hybrid and describes it as plentiful in Britain. She also reports that triple hybrids - V. lactea x canina x riviniana are not infrequently found in a number of localities eg. Chailey Common, Sussex. The influence of V. canina is inferred from the spur colour and shape of anther spur, neither of which has proved a reliable or constant character.

I have examined some herbarium specimens labelled by Gregory and others as V. lactea x canina and, although pressed violet hybrids are notoriously difficult to determine, several were undoubtedly V. lactea x riviniana. I was also able to visit

Porth Towan mentioned above and extensive searching in the field revealed that although *V. lactea* grew in conjunction, and hybridized freely, with *V. riviniana* there was no trace of *V. canina* with which it might cross. Local floras are often uncertain about dealing with this hybrid.

It is clear that there is no little uncertainty over hybrids involving *V. lactea*. This is very largely due to the impossibility of dealing at all effectively with violets in the herbarium so that if any idea is to be gained of the status of this species in field populations, and hence of its evolutionary relationships, a more intensive approach on living plants must be used. To this end the biometrical analyses of wild populations were devised and used to tackle the problems left unsolved by the methods of museum taxonomy. (See sects. 6 - 7).

SECTION 3.

EXPERIMENTS ON ARTIFICIAL HYBRIDIZATION

Introduction

(a) *V. canina x lactea*

(b) *V. lactea x riviniana*

(c) Other hybrids involving *V. lactea*

  (i) *V. lactea x pumila*
  (ii) *V. lactea x stagnina*

(d) Discussion

(3) EXPERIMENTS ON ARTIFICIAL HYBRIDIZATION.

During studies on the affinities of *V. lactea* with several taxonomically allied species a number of artificial crosses were made and they are described here. This account may be taken in conjunction with the cytological data on these hybrids given later (Sect.4) Pollinations were carried out on potted plants in a cool, insect-proof greenhouse; those flowers used as females were not emasculated and pollen was transferred on a sterile needle.

(a) *V.canina x lactea*

Since these two species are of undoubted close relationship it is not surprising to find that they can be crossed fairly readily. Table 2 shows that the direction of cross does not seem to affect either the ease of fertilization or the mean weight of seed produced but the hybrid seed seemed more viable with *V.canina* as the female parent.

Characters. The F1 hybrid is of vigorous and erect habit with much longer internodes than either of its parents,it is characteristically very floriferous and the corolla colour is intermediate between that of the parents,both in the greenhouse and in the few field samples seen. It also falls between *V.lactea* and *V.canina* in such morphological characters as leaf and petal shape and stipule form, with perhaps a tendency towards lacteoid leaves. This is clearly shown in Fig.3 by using some of the specific characters described for the biometric studies in Sect.6a.

TABLE 2

Female Parent	Male Parent	No. flowers pollinated / No. capsules formed	Mean No. seeds per capsule.	Seed wt. (mg)	% germination.
canina	lactea	3/2	37	1.30	91
lactea	canina	1/1	15	1.20	13.3
lactea	riviniana	13/13	26	1.44	6.7
riviniana	lactea	12/6	13.5	1.69	0
pumila	lactea	10/7	18.4	1.58	89.9
stagnina	lactea	4/3	16	0.97	0
lactea	stagnina	4/3	17	1.00	11.8 (both died)

NB. All fall within range of seed wts. of their parents.

TABLE 2 Summary of data showing fertility of artificial crosses involving V.lactea

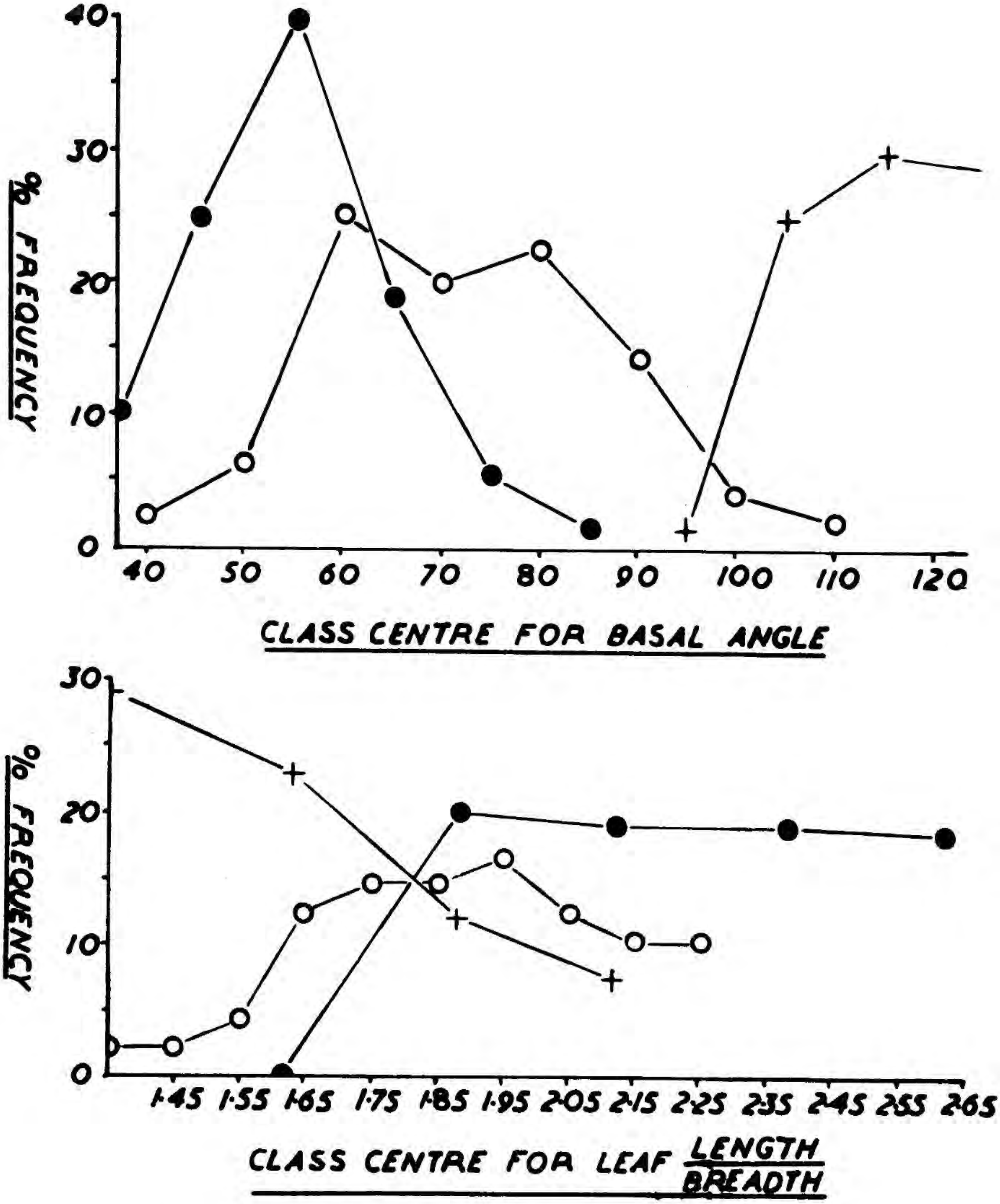


FIG. 3. Frequency polygons comparing leaf-shape in families of *V. lactea* (●), *V. canina* (+) and their artificial FI hybrid (○).

Fertility. Selfing of the hybrid automatically takes place in the cleistogamous flowers formed in summer and although many die and wither a number are successful (Table 3)

TABLE 3

No. of plants scored.	No. of cleistogamous flowers.	No. of capsules.	% flowers selfing successfully.
6	366	139	38

TABLE 3 Production of fruit from cleistogamous flowers of the artificial hybrid V. canina x lactea

The capsules produced vary widely but most are distorted to a greater or lesser extent (plate 3) the largest hybrid capsule shown contains 7 seeds. Many of the fruits scored in table 3 produced an empty white husk instead of viable seed but an estimate of seed production is given in table 4. and the proportion of 'ripe' seed germinating is quite high (91%) The same table also contains data on the seed production in cleistogamous flowers of the F2 generation, and the large increase in seed output per capsule is worthy of note.

TABLE 4

Generation.	No. of Plants.	No of Capsules	Total No. Good Seed.	% Good Seed.	Wt. of Good Seed Range and Mean.
F1	7	38	38	50	1.41 0.9 - 1.99
F2	10	90	629	76.7	1.56 0.97 - 2.04

TABLE 4 Production of seed from cleistogamous flowers in F1 and F2 generations of the hybrid V. canina x lactea

Plate 3.

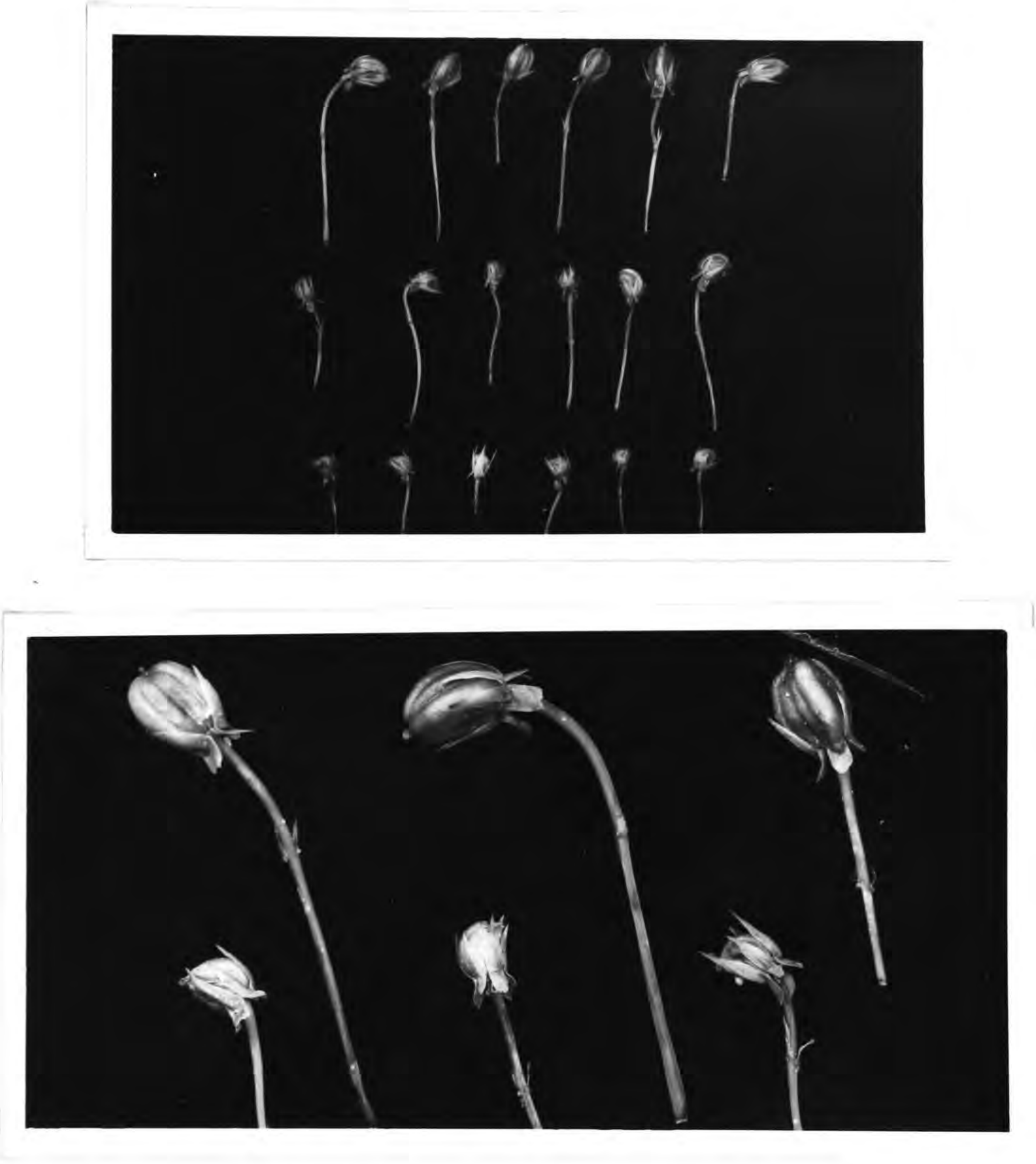


Plate 3. Cleistogamous fruits from plants of the interspecific hybrid V. canina x lactea growing in garden. Above x I, below x I½.

From the data of Table 4 it is possible to make an estimate of the productivity of the hybrid compared with that of the parents. Thus it is estimated that a plant of V. lactea would produce about 14 cleistogamous capsules with an average of 21 seeds per capsule; germination of these seeds was 96%. This gives an estimated productivity of 291 offspring per plant, compared with 21 in the hybrid, or about 14 times as much. V. canina averages 12 capsules per plant and 13 seeds per capsule with a germination of about 84% so that it has an estimated productivity of 130 offspring per plant or 6 times as much as the hybrid.

Backcross pollinations of the F1 hybrid by each of the parents have been successfully performed and a certain amount of viable seed procured (Table 5) In addition one lactea flower was fertilized by hybrid pollen and some good seed set.

TABLE 5

Cross.	No. flowers pollinated.	No. capsules produced.	Total no. seeds produced.	Seed wt. (mg)	% germination.
V. lactea x Hybrid	3	1	1	1.04	100.
Hybrid x V. Lactea	25	4	5	1.69 (1.31 - 1.98)	60.
Hybrid x V. canina	17	4	3	1.50 (1.49 - 1.51)	66.66

TABLE 5. Backcrosses of the artificial interspecific hybrid.

Characters of subsequent generations From the seed produced in cleistogamous selfings of these hybrids F2 and, subsequently F3, generations have been raised and kept under observation in boxes. Salient morphological characters of leaf and petal shape were measured for all the individuals of one or two families in each generation and are summarized in the frequency polygons illustrated in figs 3 and 4.

The F2 family produced a much wider range than the F1 in both leaf characters taken. The polygon using basal angle gave a mode suggesting a partial segregation of lactea characters and another, larger, peak covering a range of values from those of the F1 to the canina parent. The leaf $\frac{\text{length}}{\text{breadth}}$ shows a gradation from the lactea region to a definite mode very near that of V. canina. This tendency is also continued in similar polygons produced for petal shape in the F2.

The F3 family gave a frequency polygon for leaf basal angle showing two maxima which suggests some segregation out of plants bearing characters close to one or other of the parents. Leaf $\frac{\text{length}}{\text{breadth}}$ shows a general tendency towards V. canina as in the F2.

These results may perhaps be partially due to multigenic control of the characters involved so that segregation out of parental combinations would, to some extent, be masked as was found by Hutchinson (1935) when working on the inheritance of leaf shape in Gossypium. However, more important is the general trend in the F2 and later

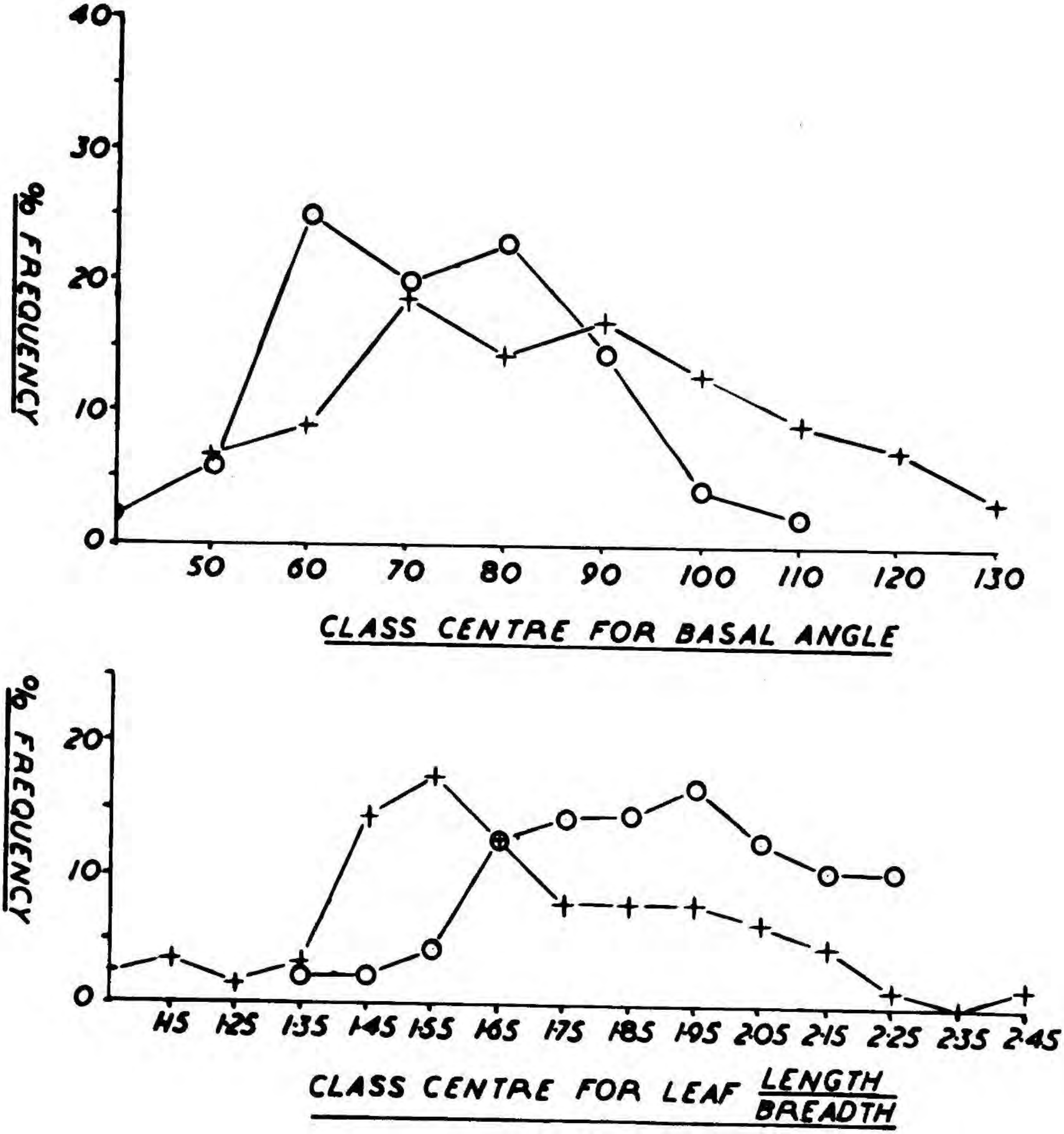


FIG. 4. Frequency polygons comparing leaf-shape in FI (O) and F2 (+) families of the artificial hybrid V. canina x lactea.

generations for plants more to resemble *V.canina* and this can undoubtedly be linked with the cytological observations recorded later (sect.4) that the F2 plants had chromosome numbers more akin to *V.canina* (2n = 40) than either *V.lactea*or the F1 hybrid. A few observationson the cultivation boxes suggested that those plants most strongly characteristic of *V.lactea* were of miserable stature, did not flower, and soon died.

We have seen that since the hybrid is about $\frac{1}{6} - \frac{1}{20}$ as fertile as the parents the genetic obstacle to gene flow between these two species is not so very great. Not only do selfings occur fairly readily to give F2 and later generations but cross pollinations can take place, to a more limited extent, between the hybrid and the parent species. It would thus appear that the factors isolating *V.lactea* and *V.canina* must be sought elsewhere, among their physical and physiological requirements.

(b) *V.lactea x riviniana*

As noted later the habitats of these two species often overlap in localities where *V.lactea*is found and the hybrid between them is found in most of these areas. The hybrid forms large floriferous clumps which may extend by means of soboles (See plate 4) to cover areas of several square yards. This vegetative propagation seems to be a constant characteristic of this hybrid in the field and may have important bearing on possible gene flow between the

Plate 4.

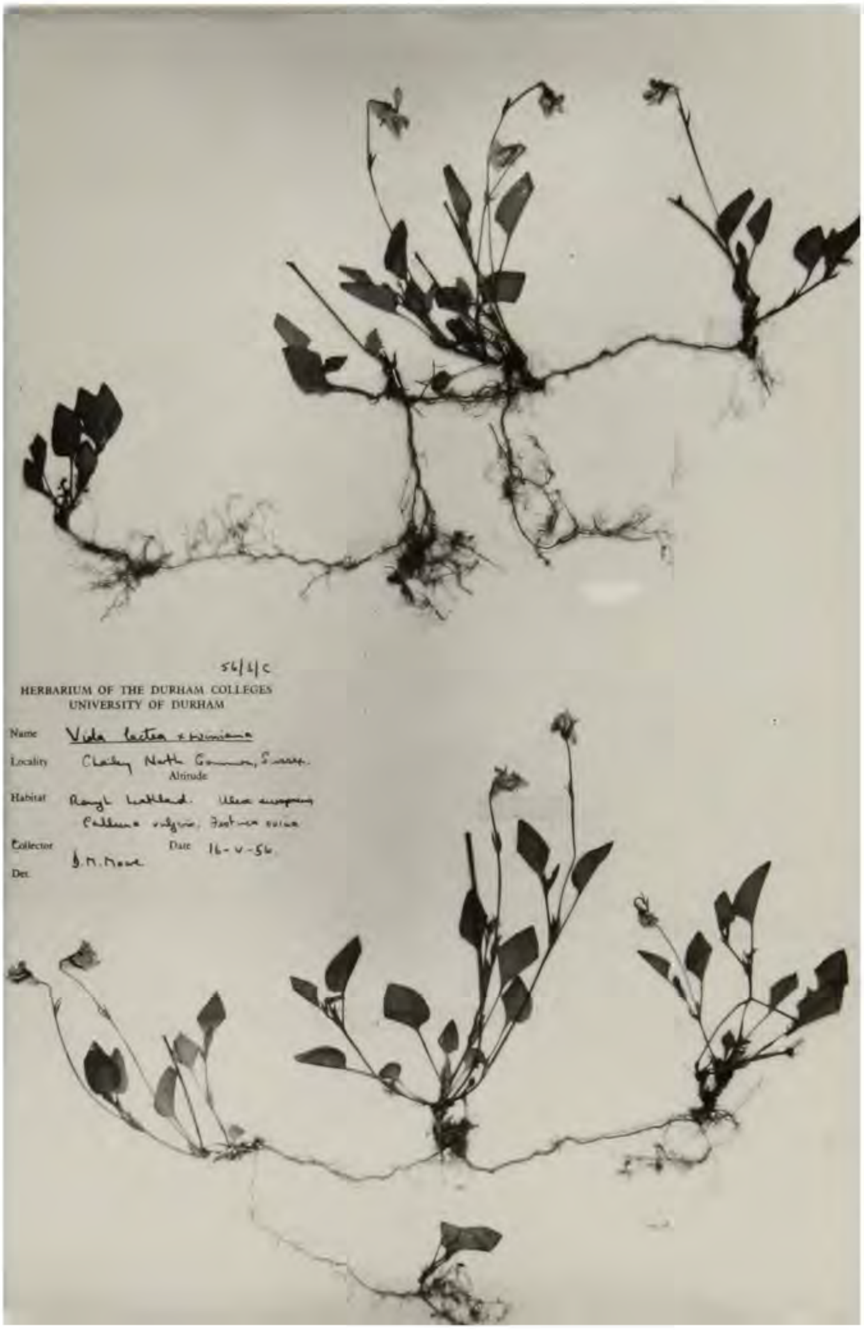


Plate 4. Herbarium specimens of wild hybrid between V. lactea and V. riviniana showing vegetative proliferation by soboles. Chailey Common, Sussex.

two species. Unlike the preceding hybrid it is highly sterile and often all the flowers, both open and cleistogamous, fail even to start fruit formation. Artificial crossing has proved fairly easy to effect though the pollinations always seemed most successful with *V.riviniana* as the male parent. From the data in table 2 it will be noted that seed from *riviniana x lactea* failed in all cases to germinate while in the reciprocal cross up to 13% germination was noted.

This hybrid produces little viable pollen, tests with aceto-carmine detect only about 18% stainable grains. A number of backcross pollinations were attempted on plants of the hybrid collected in the wild; 24 flowers were pollinated from *V.lactea* and 8 from *V.riviniana*; none was successful. Some of these same wild hybrids were kept in the greenhouse for observation over two years; each produced many cleistogamous flowers of which only one yielded a small distorted fruit with a single viable seed. (1.63mg)

These data when considered with that given in the cytological studies of the hybrid (Sect.4c11) provides information relevant to the examination of introgression in wild populations discussed later (Sec.7c) and its implications will be considered then.

(c) *Other hybrids involving V.lactea*

V.lactea x pumila The cross was made between *V.lactea* and its fellow arosulate species *V.pumila* Chaix. which is fairly widespread in Europe, during attempts to discover their genomic affinities with each other. Hybridization was only attempted using *V.lactea* as the as the male parent and 80% of the flowers pollinated set fruit (Table 2)

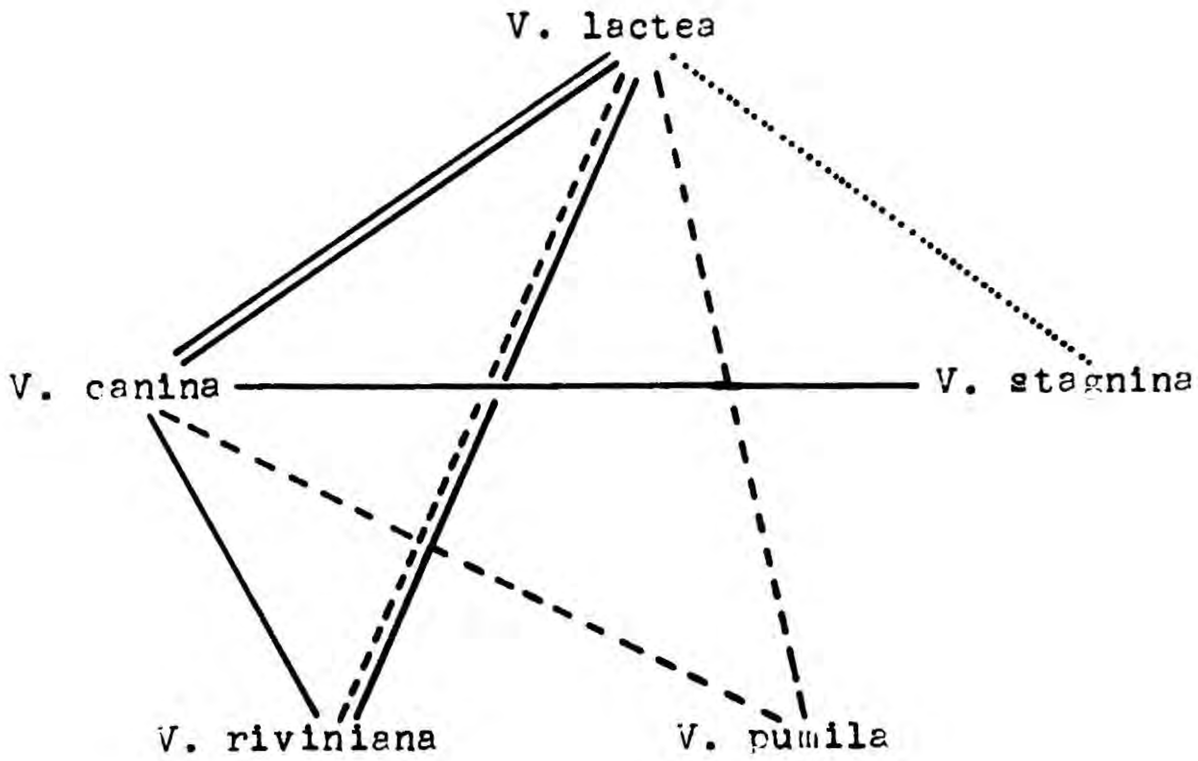
V.lacteax stagnina. The third British species belonging to the Arosulatae is V.stagnina Kit., a local violet of damp fenland in S.E, England and Ireland and attempts have been made to cross it with **V.lactea** (Valentine unpub). The results are given in table 2 and show that with V.lactea as female parent 1/4 pollinations were successful but the seeds germinated to give sickly seedlings which soon died. The reciprocal cross gave a similar capsule set but no seed germinated.

The hybrid V.stagnina x canina has been successfully produced by Valentine (unpub) and has proved exceedingly sterile. It may be noted in this connection that the wild hybrid bewteen V.stagnina and V.canina ssp.montana is found at Woodwalton Fen.

(d) The data just presented need very little analysis here and are much better considered together with other information in later discussions.

Fig.5. briefly summarizes the essential data provided by these crossing experiments. Details of three hybrids not directly involving V.lactea are included in the polygon since they are relevant to an understanding of the general scheme. The most interesting observation at this stage is the relative ease with which V.riviniana forms hybrids with V.lactea and V.canina although belonging to a different taxonomic group - the Rosulantes. In addition to this

Fig. 5.



═══ FI produced; vigorous, fertile seed set.

═ ═ ═ FI produced; vigorous, highly infertile but fruit and seed very occasionally produced.

——— FI produced; **highly** sterile.

- - - - FI produced but no data on fertility as yet.

………… Seed set but adult FI not raised due to the mortality shown by the seedlings.

Fig. 5. Crossing polygon showing the results of the artificial hybridizations attempted.

it may be noted that the most difficult cross of those attempted was that between the two arosulate species V.lactea and V.stagnina . In both these examples those species between which there are high internal barriers also have high external barriers, and vice versa. Thus V.riviniana overlaps ecologically with both V.canina and V.lactea while V.lactea and V.stagnina inhabit very different habitats and have never been reported together in the field.

The results gained from these artificial crosses help in elucidating the relationships of V.lactea with the other species considered. This is perhaps the most important aspect of these experiments and will be discussed in conjunction with the cytological data in the next section.

SECTION 4

CYTOLOGY OF SPECIES AND HYBRIDS.

(a) Methods

(b) Cytology of the parent species

V. lactea

V. canina

V. riviniana

(c) Interspecific hybrids

(i) V. canina x lactea

(ii) V. lactea x riviniana

(iii) V. canina x riviniana

(d) Discussion

Cytology of species and hybrids.

(a) Methods.

Meiosis was studied in pollen mother cells, the anthers being squashed and stained by an iron aceto - carmine method based on that of Belling (1926). Buds were fixed for 24 hours or more in Bradley's Fixative - 1 acetic acid: 2 chloroform: 3 ethyl alcohol - to which were added a few drops of ferric chloride as a pre-mordant (Thomas 1940). To every 25 mls. of the aceto-carmine used 5 drops of ferric acetate were added thus obviating the addition of iron by means of needles. After passing through the acetic-alcohol series slides were made permanent by mounting in Euparal.

Mitosis was examined in root - tips which were pre-treated with para-dichlor benzene for 4 hours before fixing in Bradley's solution; the drug tends to arrest mitosis at metaphase thereby facilitating the study of this stage. Following a technique described by Proctor (1955) maceration and staining were combined by heating the roots in a little aceto- carmine in a test-tube over a boiling water bath for 3 - 5 minutes. The root-tips were cut off on a slide, teased in a drop of 45% acetic acid, tapped out and squashed. Slides were again made permanent by mounting in Euparal.

(b) Cytology of the Parent species.

V.lactea. Material of this species was available from a number of localities represent-ative of its whole range. In table 6 are given the chromosome numbers counted from plants collected in Britain, France and Portugal. There is little doubt thst *V.lactea* has a

Plate 5.

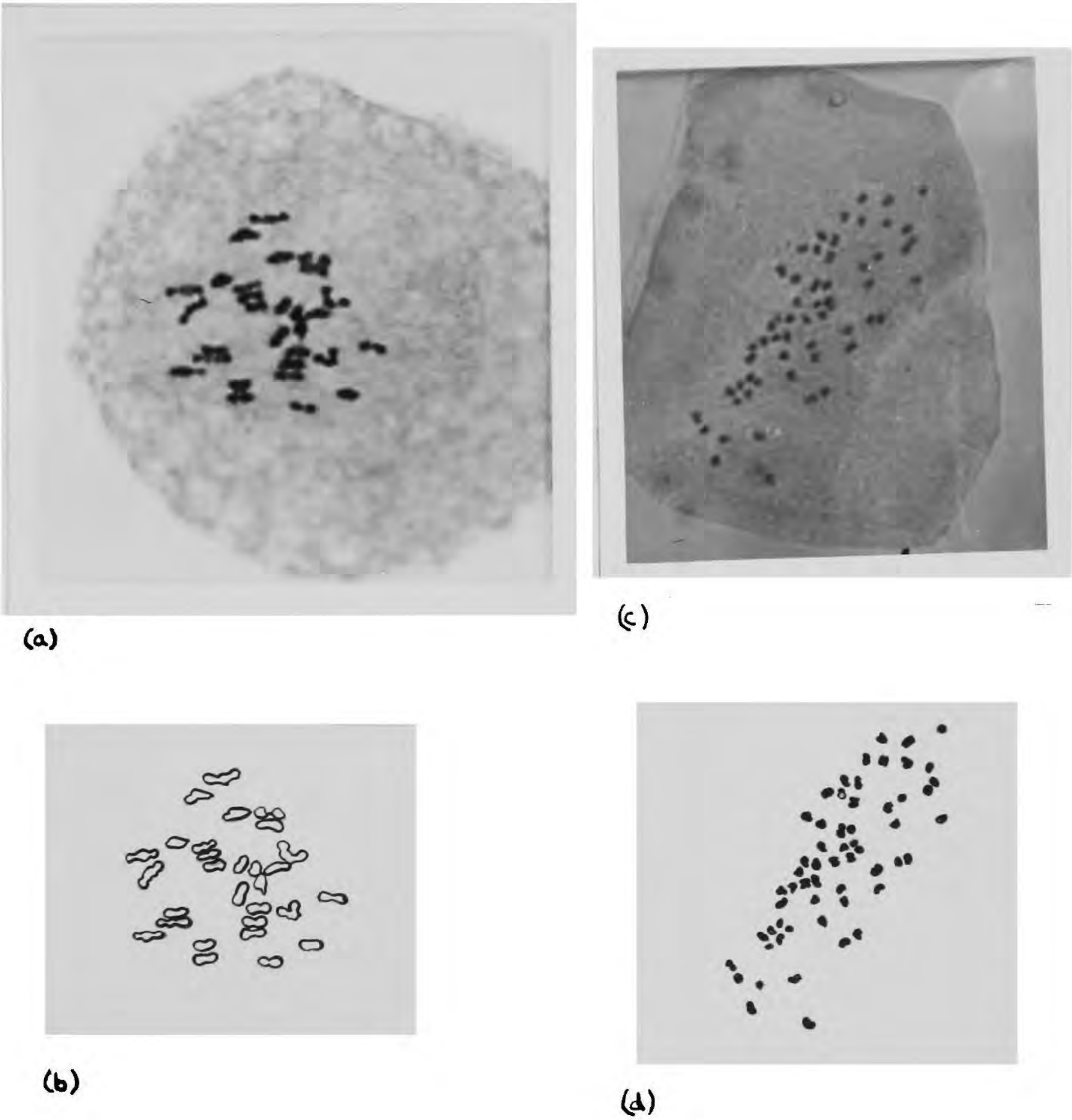


Plate 5. Chromosome number of V. lactea. (a) First meiotic metaphase - 29 bivalents - Lizard Downs. (b) Explanatory diagram for above. (c) Mitotic metaphase in root-tips - 58-chromosomes- Escheber, Basses Pyrenees, France. (d) Explanatory diagram for above. x 1250

Table 6.

Code	Locality	n.	2n.
29	Wickham Common, Fareham, Hants.	29	
VA9	Lizard Downs, Cornwall.	29	
EC1	Escheber, Basses Pyrenees, France.	29	58
LVI	Marinha Grande, nr. Leiria, prov: Beira Litoral, Portugal.	29	
57/C	Carrico, S. of Figueira da Foz, prov: Beira Litoral, Portugal.	29	

Table 6. List of chromosome numbers determined for V. lactea.

haploid number of 29. The basic number of the sub-section Rostratae, to which all species considered here belong, is x = 10 so that V. lactea can be regarded as a hexaploid which at some stage has lost two chromosomes. It is also interesting in being the only hexaploid so far discovered in the Section Rostratae.

Examination of the chromosome behaviour during meiosis showed normal pairing at metaphase in all cases (Plate 5), both first and second divisions being perfectly regular.

V. canina Early counts for this species were made by Clausen (1927) who recorded it as having 2n = 36 in Denmark. Later Bruun (1932) and Fothergill (1944) showed that it possessed the more likely number of 2n = 40. I have been able to examine British material of both ssp. canina and ssp. montana from a number of localities (Table 7),

Plate 6.

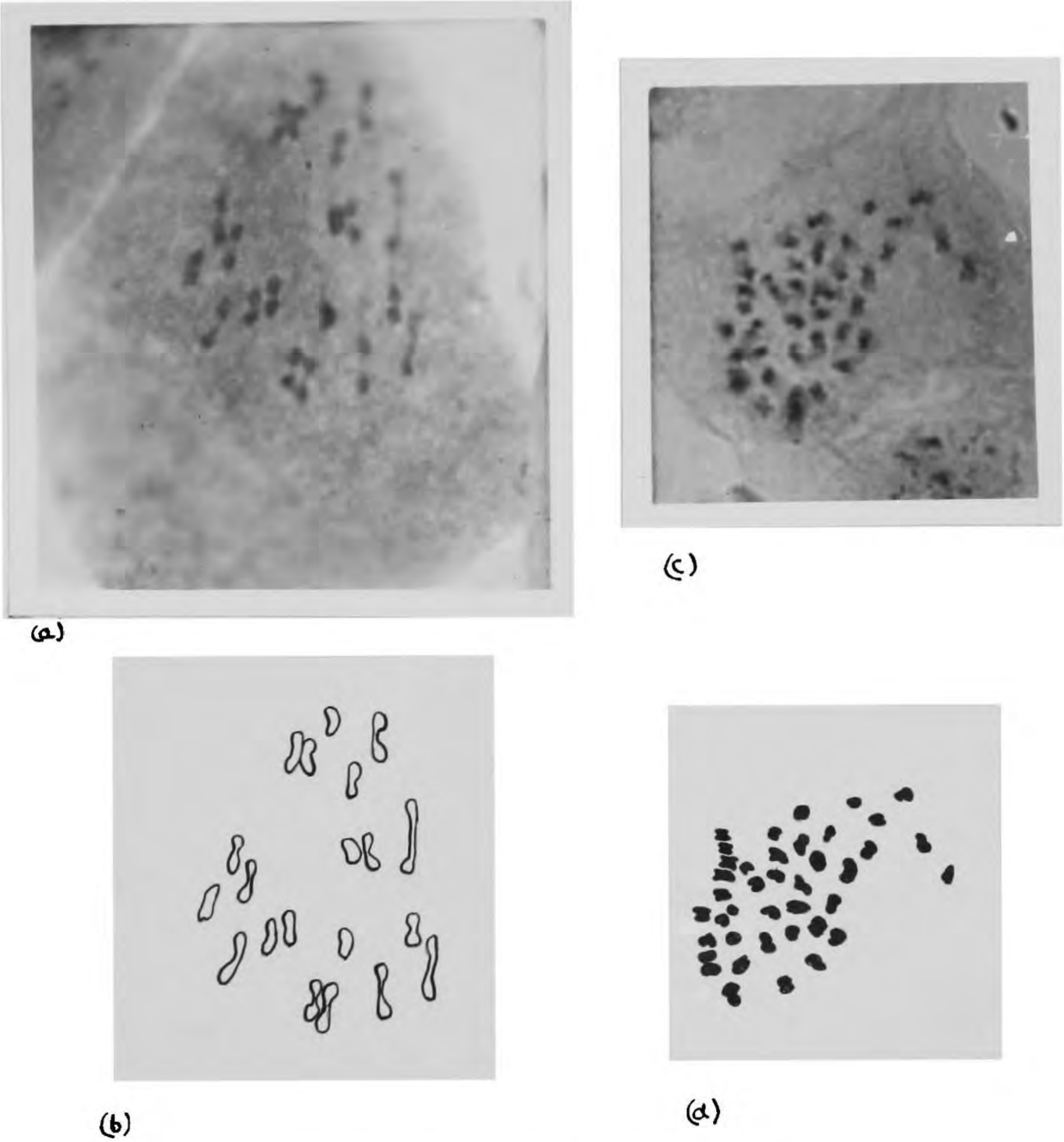


Plate 6. Chromosome number of V. canina ssp. canina. (a) First meiotic metaphase. 20 bivalents; Tuddenham, Suffolk. (b) Explanatory diagram for above. (c) Mitotic metaphase in root-tip. 40 chromosomes. (d) Explanatory diagram for above. x 1250

all counts confirm that V. canina is a tetraploid with 2n = 40. Meiosis is regular (Plate 6) and observations tend to agree with Fothergill (1944) who shows that this species has one chromosome pair smaller than the rest of the complement.

Table 7.

Code.	Locality.	n.	2n.
ssp. canina			
A	Piltdown Common, Sussex		40
U	Cleeve Common, Glos.		40
26n	Tuddenham Heath, Suffolk.	20	
150	Warkworth Dunes, Northumberland		40
ssp. montana			
10a	Woodwalton Fen, Hunts	20	

Table 7. List of chromosome numbers determined for V. canina

V. riviniana was reported by West (1930) as showing twenty bivalents at meiosis. This was confirmed by Valentine (1949) who summarized the chromosome numbers recorded for this species; he showed the number to be either 2n = 40 or 2n = 40 plus a variable number, usually 6 or 7, of small supernumary chromosomes. My observation on material of V. riviniana are in accord with the above.

(c) Interspecific hybrids.

(1) V. canina x lactea. The greater part of the material available for cytological study was of the hybrid derived from crosses using V. canina as female parent. However, in the few cases where pollen mother cells have been examined in the reciprocal hybrid meiosis seems to be identical with that described here.

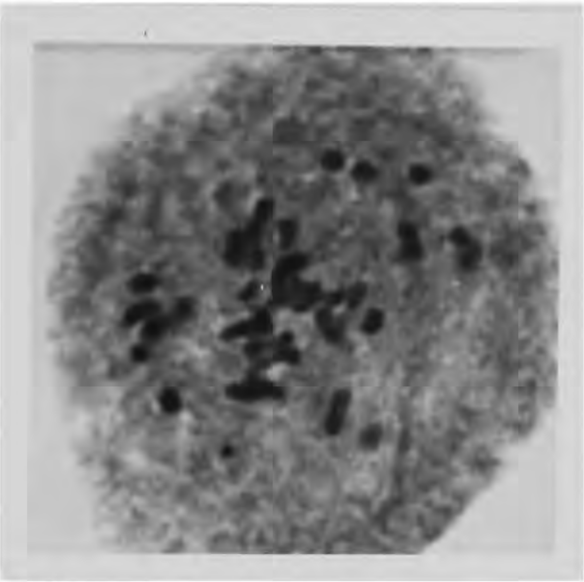
The results of examination of first meiotic metaphase are given in Table 8. Although rough counts could very often be made really reliable observations were possible only in the 35 cells listed. Clearly the most frequent arrangement is that giving 20 bivalents and 9 univalents (Plate 7). One of the cells almost certainly contained a trivalent while the other listed in Table 8 could possibly have been a bivalent in close juxtaposition to an univalent. Occasional failure of formation of one of the bivalents may be expected, thus giving the 19(2) and 11(1) arrangement, while the presence in five cells of 21(2) + 7(1) indicates that homogenetic pairing sometimes takes place

Table 8.

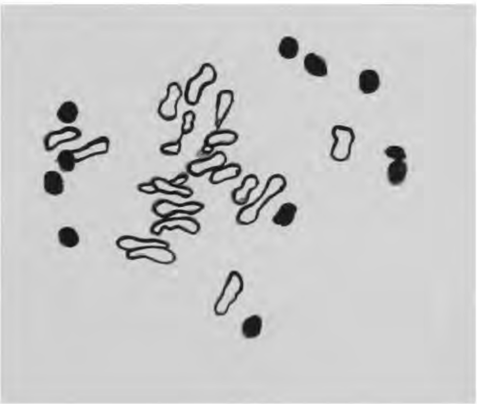
Type of pairing.	1(3) + 19(2) + 8(1).	21(2) + 7(1).	20(2) + 9(1).	19(2) + 11(1).
No. of cells.	2	5	25	3

Table 8 Pairing of chromosomes at first meiotic metaphase in the artificial F1 hybrid V. canina x lactea. 2n = 49.

Plate 7.



(a)



(b)

Plate 7. Meiosis in artificial FI hybrid *V. canina x lactea*. First metaphase showing I9(2) +II(I). Explanatory diagram in (b). x 1100

between lactea chromosomes. The general tendency shown at MI is for two of the lactea sets of chromosomes to pair with the canina chromosomes while one set is left as the unpaired univalets.

First anaphase begins with a regular disjunction of the bivalents which move normally towards the poles; a very common feature of their division is a tendency to retain a link as they move apart so that even quite late on it is possible to see clearly an attenuated process joining the two homologues of a pair.

Table 9

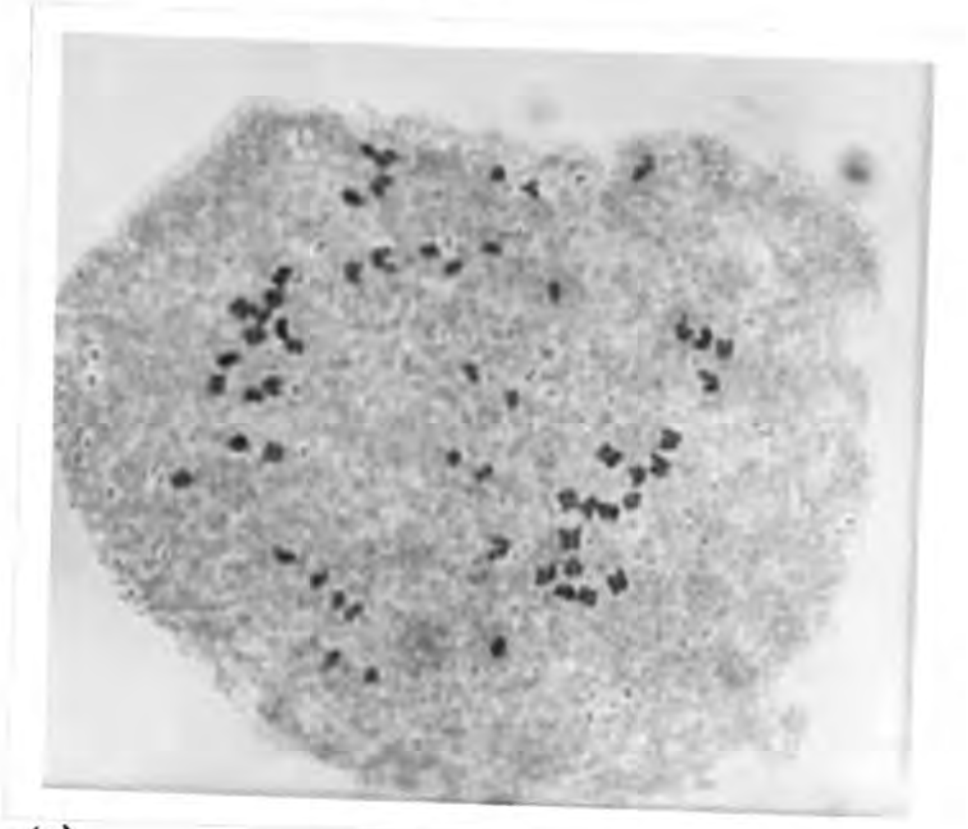
No. univalents dividing.	II	9	8	6	4	Total.
No. of cells.	2	12	2	1	2	19

Table 9. Numbers of univalents dividing at first meiotic anaphase in the artificial FI hybrid V. canina x lactea.

From Table 9 it is seen that usually nine, occasionally eleven, univalents are left in the equatorial region as the divided bivalents move to the poles. As anaphase progresses almost all the univalents are seen to divide longitudinally (Plate 8), those few cells in which some did not split were probably at an earlier stage.

In nearly all cases once a univalent has split the two daughter halves proceed to opposite poles and they usually join up

Plate 8.



(a)

(b)

Plate 8. Meiosis in artificial FI hybrid V. canina x lactea. First anaphase showing 20 chromosomes at each pole and 9 dividing bivalents along the equator. Explanatory diagram in (b). x 1250

with the daughter nuclei to form dyad cells of normal appearance. As a result, of 30 cells examined at first telophase, 24 showed a regular division, 3 lost one chromosome, 3 lost one dividing chromosome and one cell had 5 chromosomes not at the poles. These excluded laggards probably form one or more micro-nuclei.

At second metaphase most cells showed a perfect separation of the two plates with all chromosomes arranged regularly along the equator in side view. Some few had 2 - 3 laggards from the previous division lost between the plates and in one case a divided laggard still connected the two sets by a fine process. In all cases where no first division laggards were present the M2 plates showed about 29 chromosomes on either side, a small difference in numbers on the two plates does occur due to undivided univalents in first anaphase.

In nearly all cases the bivalents split regularly while the univalents, having once divided, seemed unable to do so again in the second division. They aree left along the equator as the chromosomes progress towards the poles and usually 9 are clearly visible, even quite late on. There is then fortuitous movement of the laggards to the poles which some of them reach before re-organization of the daughter nuclei

It is thus in the second division that the loss of unpaired lactea chromosomes is really seen and they can be observed at second telophase forming a number of micronuclei, singly or in multiples. Obviously, however, some of these are included in the large fertile nuclei forming the pollen grains and their number depends solely

Plate 9.

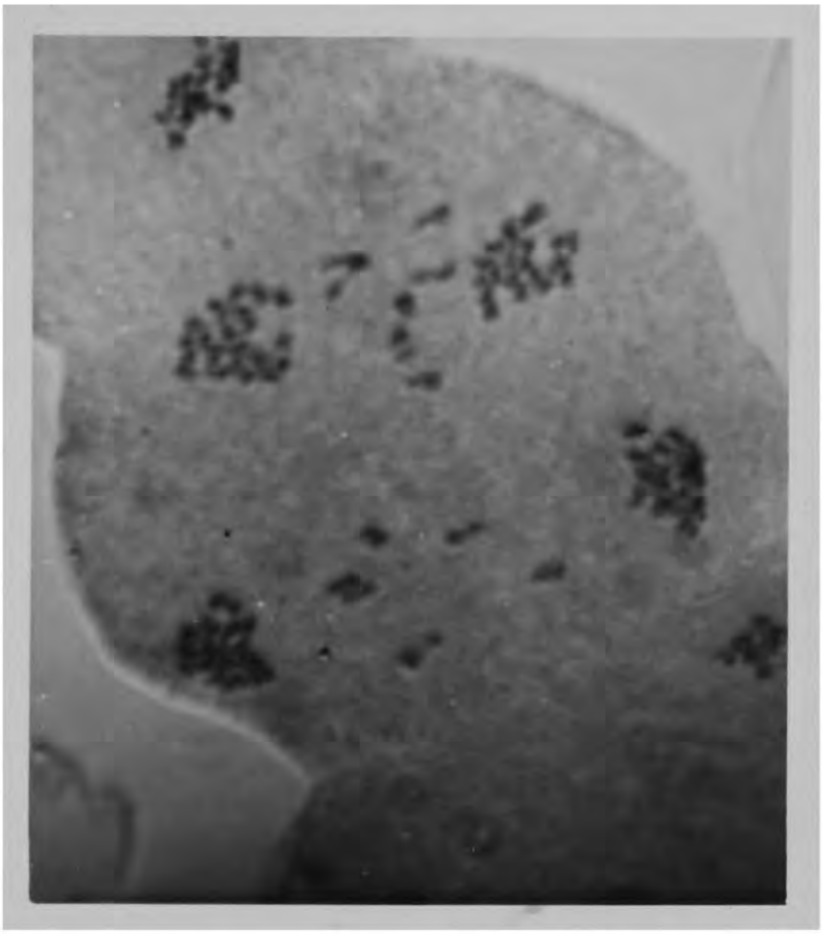


Plate 9. Meiosis in artificial FI hybrid *V. canina x lactea*. Late second anaphase showing laggards. x 1250

on their random movement towards the poles at second anaphase (Plate 9). Counting them at second telophase is uncertain but it has been possible to obtain a good idea of the numbers of chromosomes segregating in the gametes at meiosis by examining somatic metaphase in the root-tips of F2 plants produced by selfing in the cleistogamous flowers of this FI hybrid.

Tetrad formation showed that about 60 of the possible grains seem regular and these give 48 fertile pollen. A fair amount of tetrad irregularity is obviously demanded by the uncertain behaviour of the laggards in meiosis.

The behaviour of the chromosomes just described seems to be typical for hybrids of this type where homologous genomes from the two species pair to give bivalents which undergo normal meiotic division leaving the extra genome as unpaired chromosomes.
The subsequent behaviour of the univalents shown for this hybrid has earlier been described by, among others, Sax (1923) in *Triticum vulgare* (n = 21) x *T. durum* (n = 14) , Kihara and Nishiyama (1932) in pentaploid *Avena* hybrids , Kihara and Matsumura (1940) in pentaploid *Triticum* hybrids, Manton (1950 p.38) in triploid *Osmunda* and in 'Type A' cells of the triploid *V. riviniana x V. reichenbachiana* discussed by Valentine (1949). It may be noted here that Avers (1953) reports the univalents in *Aster* hybrids as undergoing two successive equational division and cites data which supports this from Clausen (1926) in certain *Viola* hybrids, Darlington (1930) in the triploid cherry *Prunus avium nana*

and Erlanson (1929) in the triploid hybrid Rosa blanda x carolina . There is no evidence of a second division of the univalents in the hybrid V. canina x lactea

In section 3 it was deduced from seed production and germination that the hybrid between V. lactea and V. canina was respectively about $\frac{1}{14}$ and $\frac{1}{6}$ as fertile as the parent species while the pentaploid Triticum and Avena hybrids mentioned were reported as being reasonably fertile. The probable reason for this is that there are, as has been shown, two pairs of homolgous genomes out of the five genomes involved. These were seen to form bivalents and to behave perfectly normally throughout the reduction divisions and there seems little reason to doubt that if they were considered alone mainly regular tetrads and good pollen grains would be produced. However the univalents, after fairly regular division at first anaphase, segregate fortuitously at second anaphase, some form micronuclei and are lost while others are incorporated into the major daughter nuclei. That the gametes produced are not rendered infertile by the addition of univalents is shown by the somatic numbers of F2 plants (Table 10) but they undoubtedly cause some sterility by interference with the genomic balance. On this basis it would be expected that

Table 10.

Somatic No.	c40	41	45	c47	50	Total
No. of cells.	11	1	1	2	1	16

Table 10. Chromosome numbers counted in the root-tip mitoses of F2 plants produced by selfing in cleistogamous flowers of the FI hybrid. V. canina x lactea

those gametes with least numbers of univalents would tend to be better balanced and more successful, this again is borne out by the data in Table 10. Most F2 plants produced by selfing show somatic numbers of c40 and probably further generations will reach a stable number with most of the univalents lost. F2 and F3 families show tendencies to revert to V. canina in morphological characters which indicates that the two canina genomes and their homologues in the lactea complement have the greatest influence; this supports the view that the univalents are mainly lost at, or shortly after, pollen formation in the FI hybrid.

It is interesting to compare these results with those given by Sears (1953) who noted a tendency in Triticum - Haynaldia hybrids for elimination of gametes with low numbers thus yielding an increased frequency of higher chromosome numbers in later generations. Watkins (1930) showed for pentaploid Triticum hybrids that in F2 and later generations there is a stabilization of the chromosome number at the hexaploid or the tetraploid level, plants with intermediate numbers being sterile. However examples can be cited which are much more compatible with the data presented for V. canina x lactea. In table II, taken from Sax (1923), the general tendency in wheat hybrids is for a gradual return to the tetraploid level in F2 and subsequent generations, though some plants do stabilize at the hexaploid number.

Table 11.

	Somatic chromosome number														
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
T.vulgare x T.polonicum(F2)	5				1		1	5	2						1
T.vulgare x T.durum (F3)	21		1	1	1	1	1	4	2	1	1				12

Table 11. Somatic chromosome numbers in F2 and F3 pentaploid Triticum hybrids. From Sax (1923)

Manton (1950 p.41) gives data for sporophytes derived from **autotriploid** Osmunda regalis in which the chromosome numbers are more akin to the lower valency :-

	c.2n	c.3n	c.4n
No of cells.	21	2	1

On this evidence it would be expected that in the event of V.canina and V.lactea coming into contact and hybridizing the more fertile, and presumably more successful, members of later generations would tend to resemble the first-named parent in gross morphology. **However,** lactea influence would naturally be also present as was seen in data for the F3 generation (Sect.3a), but more information is needed on the genetic control of physiology and other factors in these species before more exact details can be determined.

(ii) V.lactea x riminiana. Only one plant of the artificial hybrid was available, a cross made several years ago by Prof. Valentine. The female parent, V. lactea , came from the Lizard Downs and showed 29 bivalents at meiosis (See table 6) while the V. riviniana was one of the stocks described by Valentine (1949) as having $2n = 40$ plus 5 - 7 supernumary chromosomes.

This hybrid plant, possibly due to its age, produced buds very infrequently and only two anthers gave satisfactory squashes but the course of meiosis seems fairly clear from these. The chromosome pairing at first metaphase in five cells is given in table 12. The most usual arrangement is ten bivalents and twenty nine univalents with

Table 12.

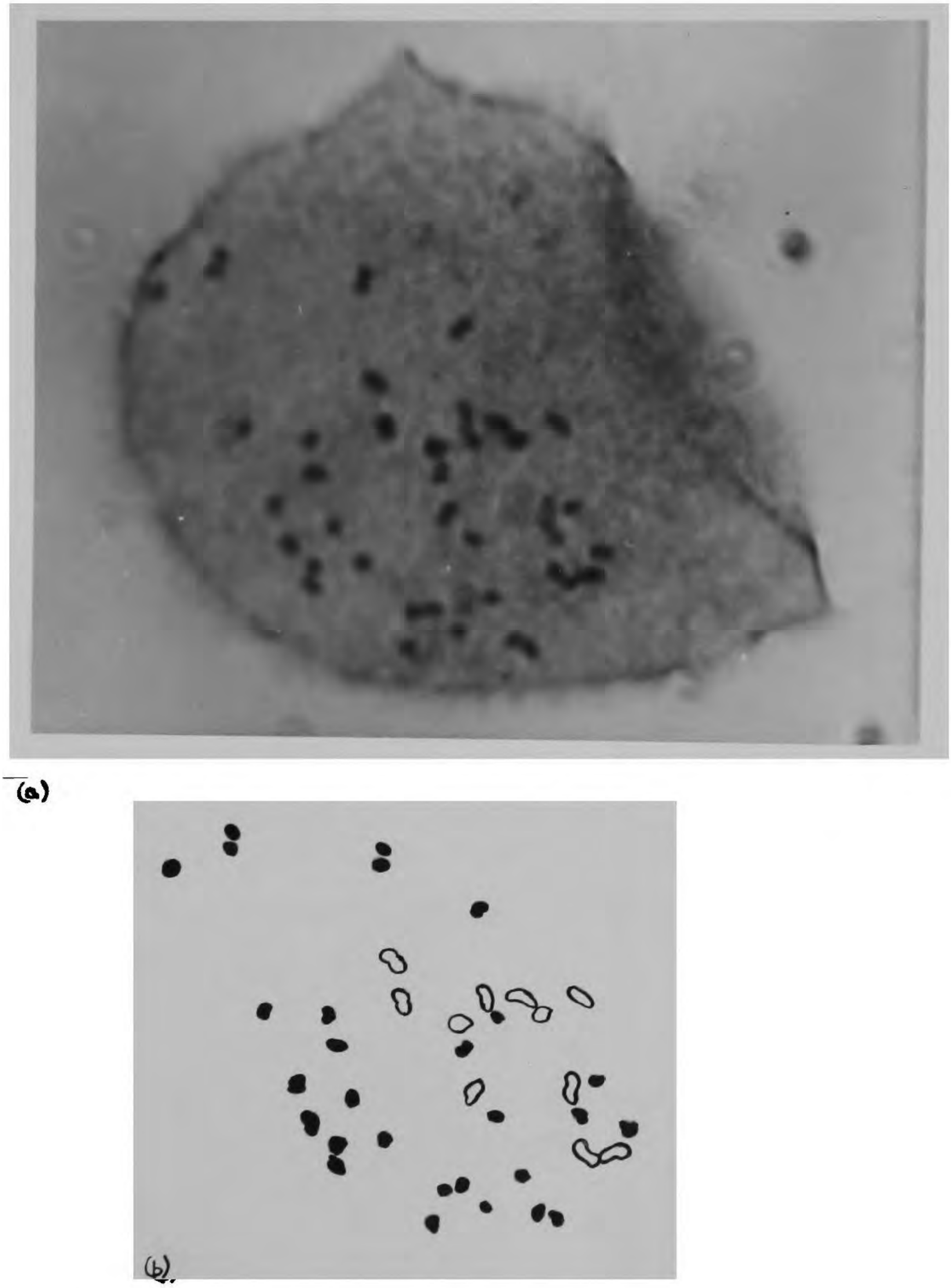
Type of pairings.	9(2) + 31(1).	10(2) + 29(1).	11(2) + 27(1).	12(2) + 25(1)
No. of cells	1	2	1	1

Table 12. Pairing of chromosomes at first meiotic metaphase in the artificial F_1 hybrid V. lactea **x** riviniana.

with extra, smaller, supernumaries and the others in the table can be based on this (Plate 10). Occasional failure of the formation of one of the bivalents is not unusual, thus giving 9(2) + 31(1), while one or two extra bivalents may reasonably be attributed to random pairing of homologous univalents.

Although anaphase has not been clearly seen in any

Plate IO.



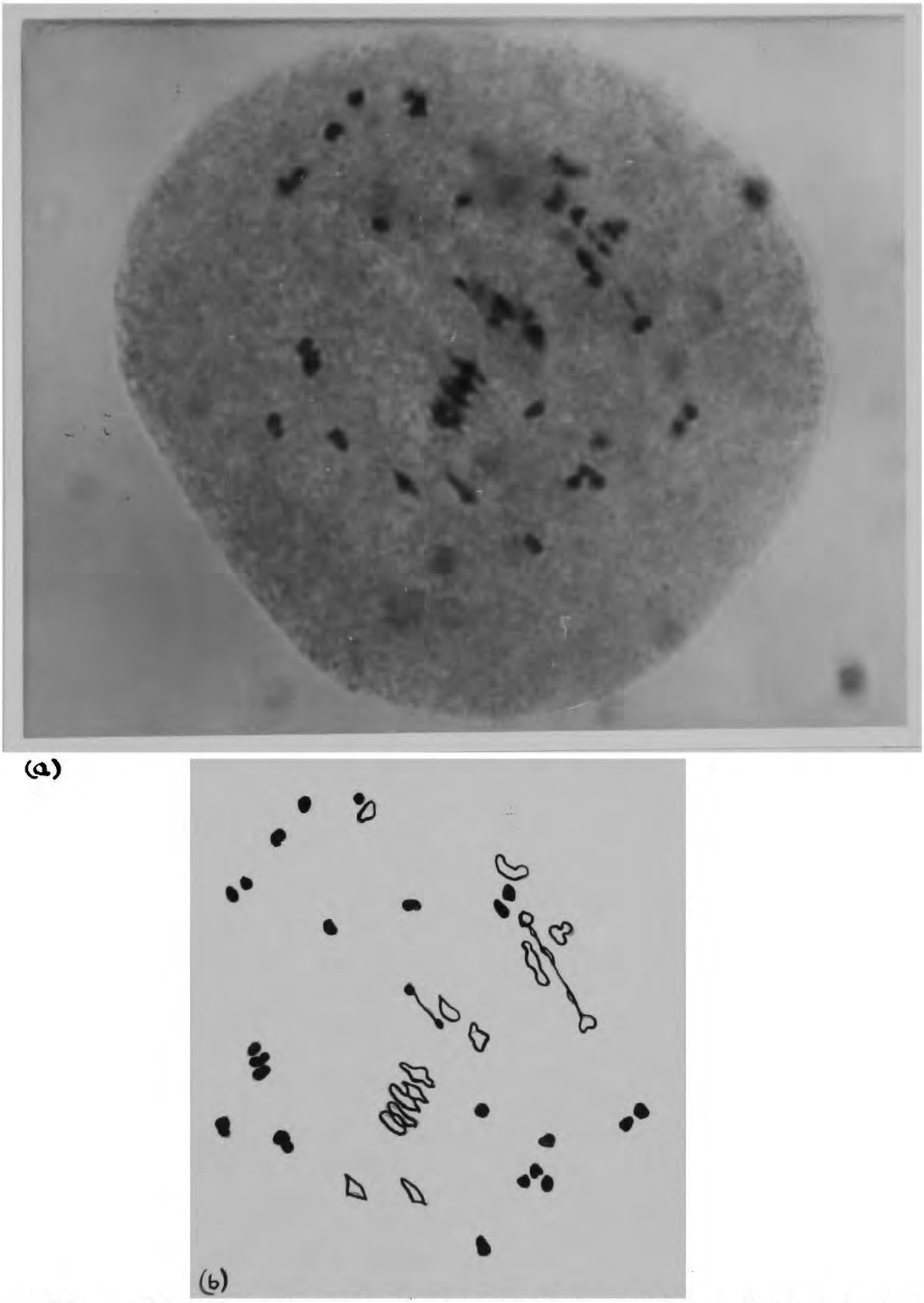
~~Plate IO.~~ First meiotic metaphase in artificial FIb hybrid V. lactea x riviniana. II(2) + 27(I) + I sn. Explanatory diagram in (b). x 1250.

preparations it seems likely that the bivalents will divide and separate normally. Whether the univalents will split and behave as described in the other two hybrids dealt with in this section cannot, of course, be settled, though it does seem likely. A consideration of telophase shows that in many cases all the univalents are incorporated into one or other of the two daughter nuclei; three cells showed lost univalents at this stage, one had a single laggard and one had two, while a third showed eleven lost chromosomes.

It has been possible to find several cells at late second anaphase and telophase. Hence there seems to be regular division of the bivalents but large numbers of univalents segregate irregularly and very many of them form separate micronuclei so that a very abnormal telophase is observed. This condition would be expected to give a high proportion of poor pollen in the anthers of hybrid plants, and several analyses based on staining with aceto-carmine showed that such plants had about 20% good pollen; germination tests have not been made.

The most likely interpretation of the data presented here is that in the hybrid one genome of *V. lactea* is homologous with one in *V. riviniana* and they pair to give about 10 bivalents; the other thirty chromosomes show little homology and for the most part remain as univalents. The irregular behaviour of all these unpaired chromosomes in later meiosis leads to very unbalanced gametes and quite high sterility of the hybrid. Some plants collected

Plate II.



<u>Plate II</u>. First meiotic metaphase in wild hybrid between <u>V. lactea</u> and <u>V. riviniana</u>. 14 (2) + 23 (I). Explanatory diagram given in (b). x 1250

in the field appeared on morphological grounds (see sect.7) to be hybrids involving V.lactea and V.riviniana, though not directly F1; meiotic pairing was observed in buds from two of these plants and the results are given in table 13. (See also Plate 11)

Table 13.

Type of pairing.	10(2) + 30(1)	11(2) + 31(1)	12(2) + 26 - 29(1)	13(2) + 24 - 27(1)
No. of cells	4	6	4	2

Table 13. Pairing of chromosomes at first meiotic metaphase in two wild hybrids between V.lactea and V.riviniana. The somatic numbers of these hybrids were 2n = 50 and 2n = 53. (The two values for numbers of univalents in the last columns are due to condensation of the table, not to uncertain counts).

If, as is suggested in section 7, hybrids between V.lactea and V.riviniana can occasionally produce offspring then the data presented indicate what cytological requirements may be involved. The plants considered here show a decided tendency to an increased number of bivalents over the artificial interspecific hybrid, and all have more univalents. This suggests that the few gametes which do prove fertile are those which get a large share of the univalents, one or two extra probably do not markedly upset the balance. This contrasts with the hybrid V.canina x lactea which loses chromosomes in the second and third generations. Finally, it may be noted that one of the wild hybrids whose meioses are considered here was the only plant to produce a fruit and seed. (sect.3b).

(iii) V.canina x riviniana . It has been found that both V.canina ssp.canina and V.canina ssp. montana exhibit similar cytological behaviour in their hybrid with V.riviniana. In the cross (code VRC) with ssp.canina the riviniana stock used possessed about 6 or 7 supernumary chromosomes (Valentine 1949). The stock crossed with ssp.montana did not have such supernumaries and the figures are therefore taken from this hybrid (code Q 13) to facilitate interpretation.

Results from the examination of first meiotic division are given in Table 14. Clearly, much the commonest arrangement is that of ten bivalents and twenty univalents (Plate 12a)

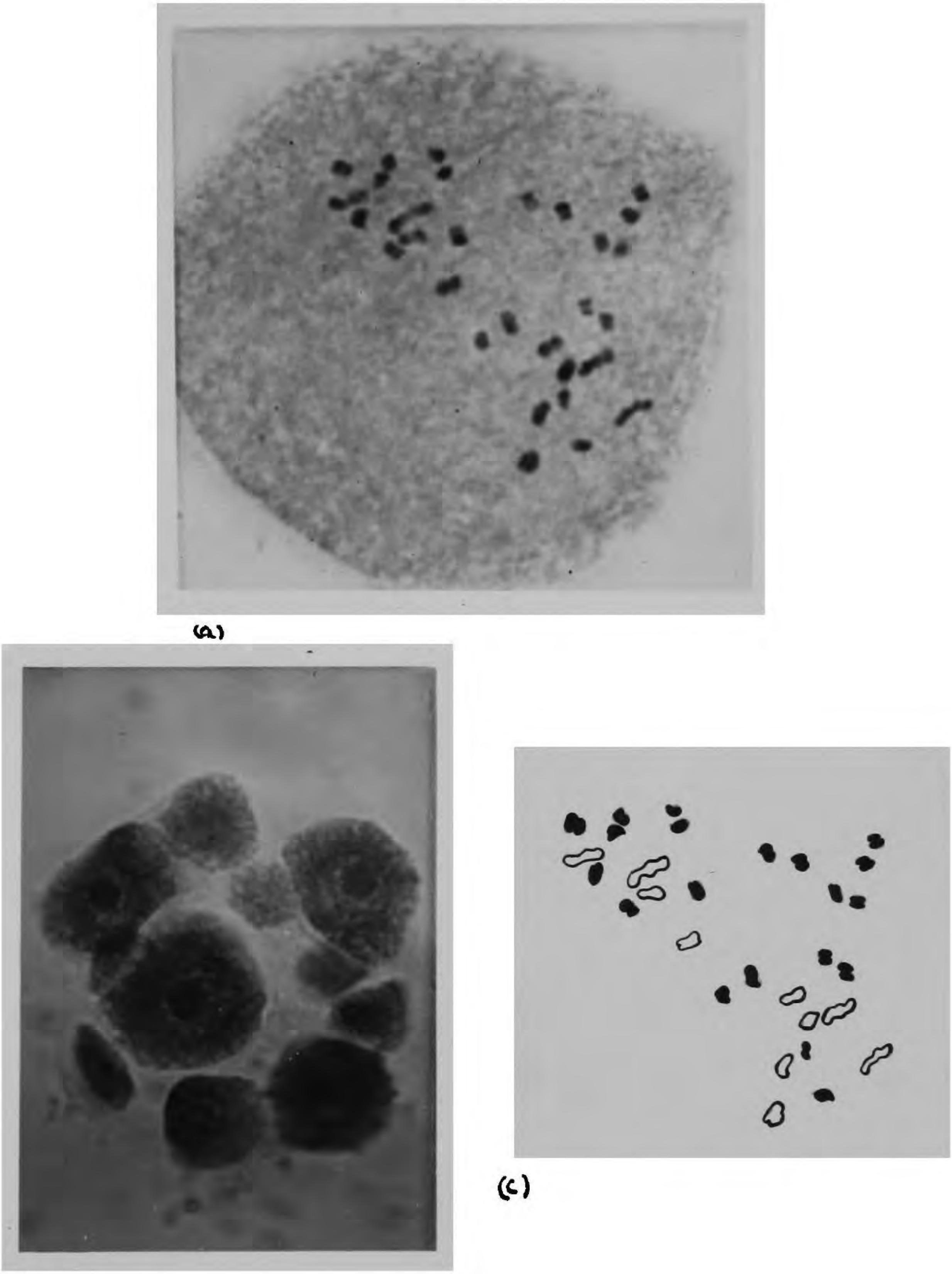
Table 14.

Type of pairings.	9(2) + 22(1).	10(2) + 20(1).	1(3) + 9(2) + 19(1).	Total.
No. of cells	1	11	1?	13

Table 14. Pairing of chromosomes at first meiotic metaphase in the artificial FI hybrid V.canina ssp.montana x riviniana. (2n = 40)

As in earlier hybrids, one cell is shown in which a bivalent has failed to form while in the other case a trivalent was doubtfully observed.

Plate I2.


(a)

(b)

(c)

Plate I2. Meiosis in artificial FI hybrid V. canina ssp. montana. 2n = 40.

(a) First metaphase showing I0(2) + 20(I). (b) Tetrads showing polyspory. (c) Explanatory diagram to (a).

x 1250.

If this is so then it is probably due to homogenetic pairing between chromosomes of one of the species. This pattern applies equally in the cross ssp. *canina x riviniana* (VRC) and table 15 shows that there are 10 pairs of homologous chromosomes and twenty asynaptic univalents

Table 15.

Type of pairing	10(2) + 22 - 24(1).	10(2) + 25 - 26(1).	11(2) + 20 - 22(1).	11(2) + 24(1).	Total.
No. of cells.	12	6	2	1	21

Table 15. Pairing of chromosomes at first meiotic metaphase in the artificial hybrid *V. canina* ssp. *canina x riviniana*. (Probably 2n = 40 + 2 - 6 supernumaries)

augmented by up to six supernumary chromosomes from the *riviniana* parent. Here also there is often an extra bivalent due to either homo- or heterogenetic pairing of the univalents. The most likely explanatio of this arrangement is that ten *riviniana* chromosomes are pairing with ten of the *canina* chromosomes, the remaining ten from each complement forming the univalents. The chance that the bivalents are formed by homologous pairing within one or other of the species is very remote. At anaphase the bivalents divide regularly and send ten chromosomes to each pole while, later, the univalents split longitudinally and follow them; identical behaviour with that observed in the hybrid *V. canina x lactea*.

The second meiotic division resembles that in *V. canina x lactea* with the bivalents dividing and going to each pole while the univalents move in a random fashion, some reaching the daughter nuclei with which they are incorporated, others lagging to form micronuclei of various sizes. Observations on second anaphase showed that numbers of chromosomes at the poles ranged from 9 to 20. This chromosome behaviour leads to a great variety in tetrad form and all those examined showed marked polyspory. (Plate 12b). An estimate of goodness of pollen showed that of 666 grains examined 128, or 19%, were judged to be good. It is worth noting here that this hybrid is highly sterile and in the greenhouse has never been observed to set cleistogamous fruit, nor have back-crosses to either parent been successfully accomplished.

(d) Discussion.

Earlier (sect, 3) details were given of the success or otherwise of crosses between a number of taxonomically fairly closely allied violets belonging to the Sub-section Rostratae. The results were summarized in Fig. 5 and, although these in themselves give a clue to the relationship of the species concerned, where hybrids were produced it is possible to gain much more evidence by examining the behaviour of the chromosomes at meiosis. Precise phylogenetic evidence can be obtained by analysing chromosome pairing at meiosis in such crosses, notable examples of this being the discovery of allopolyploid origins for *Spartina Townsendii* by Huskins (1931) and for *Nasturtium uniseriatium* by Howard and Manton (1946).

The data on chromosome pairing in the hybrids

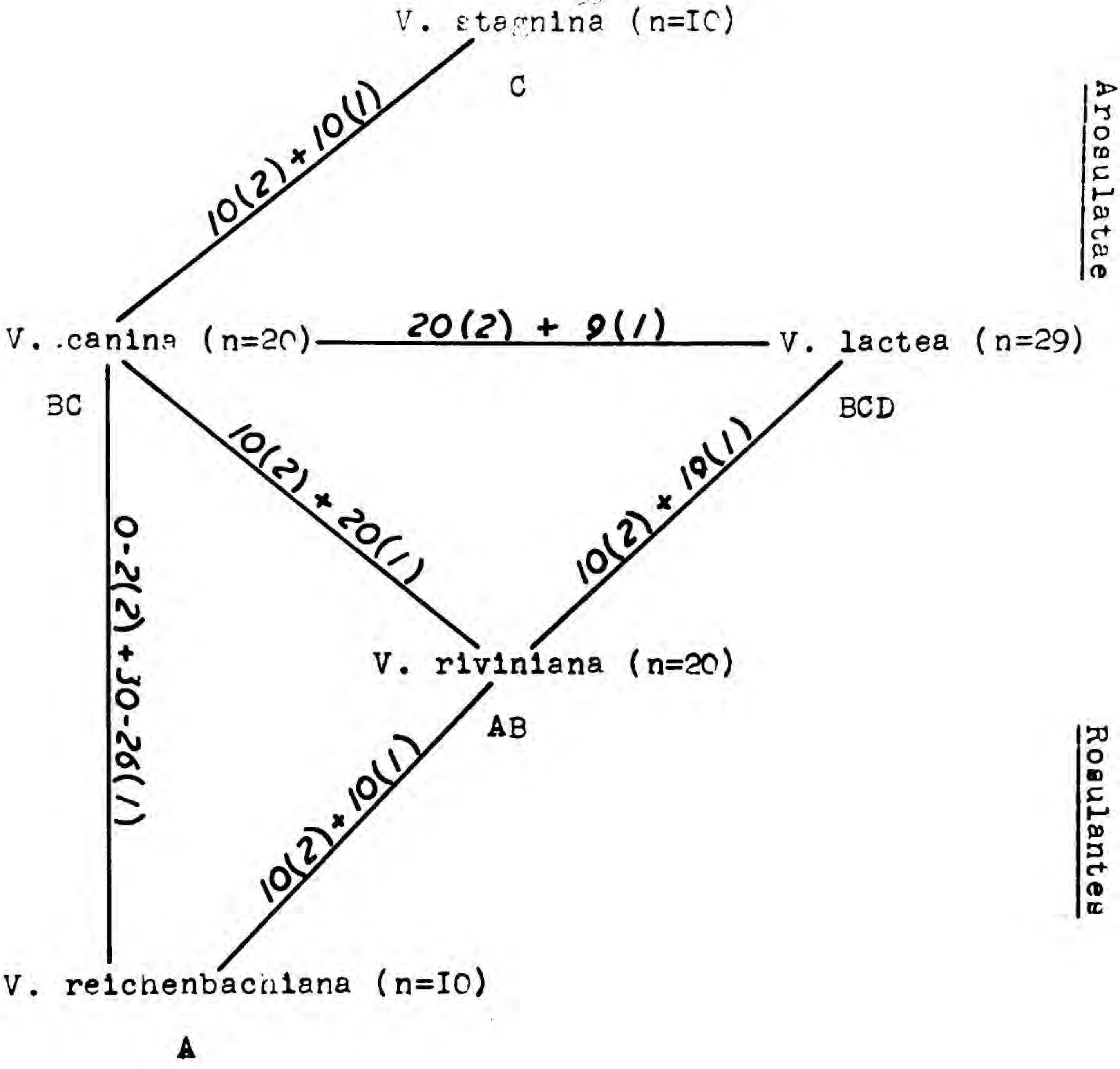


Fig. 6. Diagram showing chromosome pairing in the hybrids between species in the Sub-section Rostratae. The deduced genomic constitution of each species is also given.

described here has been summarized in Fig.6. Of the species considered, two were at the tetraploid and one at the hexaploid level, the two diploid species - V.reichenbachiana and V.stagnina - are included in the polygon since they help in its interpretation. The data on V.riviniana x reichenbachiana are taken from Valentine (1949), those for V.canina x stagnina are the result of preliminary observations on the hybrid (see sect.3d) which was made by Valentine (unpub) while those for V.canina x reichenbachiana are supplied by Valentine (unpub).

The basic haploid number of these species, as of most violets in the Sub-section Rostratae, is 10 and since all chromosome pairing in the hybrids can be derived from this number it is profitable to designate each genome of 10 chromosomes by a capital letter; those genomes which pair and are therefore most probably homologous being given the same letter. If we start by assigning 'A' to the genome of the most completely known diploid species, V.reichenbachiana, then it has this in common with V.riviniana which also has another genome, B, left as ten univalents in their interspecific hybrid. V.canina has one genome in common with V.riviniana and little with V.reichenbachiana so its complement must be BC. Both genomes of canina find homologues when crossed with V.lactea and the hybrid also shows an extra unpaired genome so that the hexaploid species can be BCD, this is also satisfactory when V.lactea x riviniana is considered. The genome shared by V.stagnina and V.canina is more likely to be C than the other, B, which is also present in the taxonomically more distant *V.riviniana*, but more work is needed to clarify this point.

Now that the relationships of the species can be represented in this way it becomes clearer why the hybrid *V.canina x lactea*, which has two pairs of homologous chromosomes, is much more fertile than either *V.lactea x riviniana* or *V.canina x riviniana* which both have only one genome in common. It does not explain the suspected difference in the fertility of the last two hybrids mentioned though probably *V.lactea x riviniana* is afforded slightly more latitude by the occasionally favourable segregation of its larger number of univalents; this was suggested in considering the chromosome numbers of wild hybrids between these species (p. 50).

From the general point of view of this thesis perhaps the most important fact is the relationship of *V.canina* and *V.lactea*. We have seen in section 2 that they are closely allied on orthodox taxonomic criteria and now the pairing of chromosomes in the interspecific hybrid confirms their close affinities. This type of chromosome behaviour and genomic structure indicates that *V.lactea* is an allopolyploid with *V.canina* as one of its progenitors. Thus, on this theory, sometime in the past *V.canina* crossed with a diploid violet species and, by doubling of the chromosome number in the hybrid, gave rise to *V.lactea*. Such an allopolyploid would, presumably, arise very occasionally and, being alone, would either have to fertilize itself or fail to become established.

In many species this involves a great change of the compatibility system for the new polyploid but in violets a mechanism is already present which allows the allopolyploid to reproduce. The cleistogamous flowers regularly formed by these violets provide a ready means of self-fertilization so that such an allopolyploid, if suitably genically balanced, can be perpetuated and fixed as a new evolutionary entity.

The problem now outstanding is to find the diploid species hypothesised as the other parent of V. lactea. Such a species would presumably have n = 10, the genome is designated **D** in Fig. 7, and the hybrid between it and V. lactea would be expected to give 9(2) + 21(1) at meiotic metaphase. The hypohexaploid number (n = 29) of V. lactea probably resulted from loss of two chromosomes in some period of instability during its establishment. Theoretically one would expect that by crossing this diploid species with V. canina and doubling the hybrid chromosome number by colchicine treatment it would be possible to synthesise V. lactea. This of course constitutes the ultimate proof of the theory put forward here and must await the more immediate task of discovering this putative diploid species, if it exists.

Since, in the early stages of such an investigation, the most useful clues as to the whereabouts of this species will come from an interpretation of historical, morphological and ecological data considerations of this aspect will be deferred until the final conclusions are presented in Section 8.

SECTION 5

ECOLOGICAL STUDIES

Introduction

- (a) Field notes on the ecology of the species
    - (i) Community types
    - (ii) Physical factors
    - (iii) Competition effects
- (b) Cultivation experiments
    - (i) Soil Boxes
    - (ii) Texture beds
- (c) Summary and conclusions.

(5) ECOLOGICAL STUDIES.

Introduction.

It has been shown earlier (sect 3a) that there is little genetic barrier between V. lactea and V. canina so one must look elsewhere for the factors separating these species. One obvious possibility is that they may differ in habitat requirements. In consequence, a number of localities for each species were visited and data on the habitats collected. All the areas in Britain for V. lactea were visited except those in Dorset, Hampshire and S.W. Ireland eg. Tourmore Estuary. Preliminary results are also available for comparison from localities for V. lactea in C. and N. Portugal visited in April 1957. (See Appendix B.)

In addition to the results gained by field observations a few elementary cultivation experiments were carried out to see if the physical factors governing the growth of each species could be ascertained.

(a) Field notes on the ecology of the species.

(i) Community Types. In table 16 are listed species closely associated with V. lactea in a number of localities. The most obvious general observation from the lists is that no species emerges as constantly dominant in those habitats it favours. Ten species occur in over half the localities but of these only Calluna vulgaris and Ulex gallii are dominant in at most three. The most constant species is Potentilla erecta which occurs, usually frequently, in all localities and is, suggestively, an indicator of light, acid soils. Only two other species, Viola riviniana and

Table 16.

1. Chailey North Common,,E. Sussex.
2. Nr. Yelverton, Roborough Down , S. Devon.
3. Nr. Buckland Monachorum, Roborough Down, S. Devon.
4. Kynance, Lizard Downs, Cornwall.
5. Nr. Bray's Cottage, Goonhilly Downs, Cornwall.
6. Cliffs between Portreäth and St. Agnes, Cornwall.
7. Hooper's Point, Dale, Pembs.
8. Gors Goch, Pentraeth, Anglesey.
9. 2m. south of S. Stack, Holy Isle, Anglesey.

	1	2	4	4	5	6	7	8	9
Viola lactea									
Achillea millefolium	-	-	-	-	c	-	-	x	-
Agrostis setacea	-	a	a-D	-	a.1D	-	-	-	-
Antennaria dioica	-	-	-	-	-	-	-	o	-
Anthoxanthemum odoratum	-	-	f	-	-	-	f	a.1D	O
Bellis perennis	-	-	o	-	-	-	o	x	-
Calluna vulgaris	o	a.1D	-	o-c	r	a	a-f	a.1D.	a-D
Carex flacca	-	-	-	c	-	-	c	f	-
C. panicea	-	-	-	-	x	-	-	-	-
C. pilulifera	-	1c	-	-	-	-	c	-	-
Centaurea nigra	c	-	-	-	-	-	o	x	-
Cerastium tetrandrum	-	-	-	-	-	-	-	o	-
Chrysanthemum leucanthemum	-	-	-	o-c	-	-	-	-	-
Cirsium vulgare	-	-	r	-	-	-	-	-	-
Dactylis glomerata	-	-	-	-	-	-	o	-	-
Daucus carota	-	-	-	-	-	-	x	-	-
Erica cinerea	-	x	-	-	6-c	-	-	f	-
E. tetralix	-	-	-	-	-	-	-	-	o
E. vagans	-	-	-	aD	alD	f	-	-	-
Festuca ovina	1D	a	-	f	-	alD	-	f	x
F. rubra	-	-	-	-	-	-	a-f	-	-
F. tenuifolia	-	1D	-	-	-	-	-	-	-
Filipendula vulgaris	-	-	-	o	c	-	-	-	-
Galium hercynicum	c	c	-	-	-	-	-	-	o
Genista anglica	-	-	-	1c	-	-	-	-	-
G. pilosa	-	-	-	r	-	-	-	-	-
Geranium sanguineum	-	-	-	o	-	-	-	-	-
Helianthemum chamaecystis	-	-	-	-	-	-	-	o	-
Hieracium pilosella	-	c	f	-	c	o	c	c-o	-
Holcus lanatus	-	-	-	-	-	-	o	-	-
Hypericum humifusum	-	o	-	-	o	-	-	o	-
Isolepis setacea	-	-	-	1c	-	-	-	-	-
Juncus conglomeratus	-	-	r	-	-	-	-	-	-
Leontodon autumnalis	-	-	-	-	-	-	-	f	-
Lolium temulentum	-	-	-	-	-	-	o	-	-
Lotus corniculatus	-	-	-	-	-	-	c	1c	-
Luzula campestris	o	o	-	-	-	-	-	o	-
Molinia caerulea	f	-	-	-	o	-	-	-	-
Orchis mascula	-	-	-	-	-	-	-	o	-
O. ericetorum	-	-	-	o	-	-	-	-	-
Pedicularis sylvatica	o	o	o	c	c	c	-	o	o
Plantago coronopus	-	x	-	-	-	-	-	-	-
P. lanceolata	-	o	o	-	-	o	o	r	r
P. maritima	-	-	-	-	-	-	x	-	-
P. media	-	-	o	-	-	-	o	-	-
Polygala serpyllifolia	c	c	-	-	c-f	-	c	a	-
P. vulgaris	-	-	-	r	-	-	-	-	-
Potentilla erecta	f	f	c-o	f	c-f	c	f	a	f
Poterium sanguisorba	-	-	-	-	-	-	-	r	-
Pteridium aquilinum	aD	-	-	-	-	-	-	1f.	-
Ranunculus bulbosus	-	-	-	-	-	-	o	o	-
R. repens	-	-	-	-	-	-	r	-	-
Rosa spinosissima	-	-	-	-	-	-	r	r	-
Rubus fruticosus agg.	-	-	-	-	-	-	r	-	x
Rumex acetosella	o	-	-	-	-	-	-	-	-
Salix repens	-	-	-	o	c-o	-	--	-	-
Saxifraga hypnoides	-	-	-	r	-	r	-	-	-
Schoenus nigricans	-	-	-	-	f-c	-	-	-	-
Scilla verna	-	-	-	o	o-c	c-f	-	1f	a
Spergularia rubra	-	-	o	-	-	-	-	-	-
Taraxacum offivinale	-	-	-	-	-	-	o	r	-
Teucrium scorodonia	-	-	-	-	-	o	-	-	-
Trifolium pratense	-	-	-	r	-	-	-	r	-
Ulex europaeus	f	-	c	-	f-1D	-	f	-	o
U. gallii	-	a	-	-	f-a	a.1D	a-f	alD	aD
Veronica chamaedrys	-	-	o	-	-	-	-	-	-
Viola canina	-	-	-	-	-	-	r	-	-
V. riviniana	o-c	o	c	r	c	o	o-c	o	r
Hypnum cupressiforme	-	-	-	-	-	f	f	-	-

Table 16. Species associated with V. lactea in representative localities.

Pedicularis sylvatica, seem fairly constant in these areas.

Besides the six dominants listed and the constant species just mentioned four species were found in 5 or more localities. Polygala serpyllifolia and Scilla verna, both typical of acid, heathy turfs and two ubiquitous plants having a wide pH tolerance but susceptible to strong competition - Plantago lanceolata and Hieracium pilosella.

The general picture emerging is of a heathy type of vegetation which has several more or less constant species but whose dominants vary according to the district. Since V. lactea almost invariably occurs where there has been destruction of the vegetation by burning, erosion &c., and subsequent recolonisation of the open ground, the dominant species is also dependent on the stage of colonisation attained by the micro-habitat. At Hooper's Point it was impossible to define any dominants, so broken and irregular was the community, this also tended to be the case in some other fairly recently burned localities, especially on Goonhilly Down.

The remaining species to be found in communities with V. lactea depended largely on the local conditions of the habitat and on the composition of the surrounding associations which provided many of the invading plants, hence the list is quite long. Diagrams of two representative metre quadrats are given in fig. 7 showing typical conditions favoured by V. lactea.

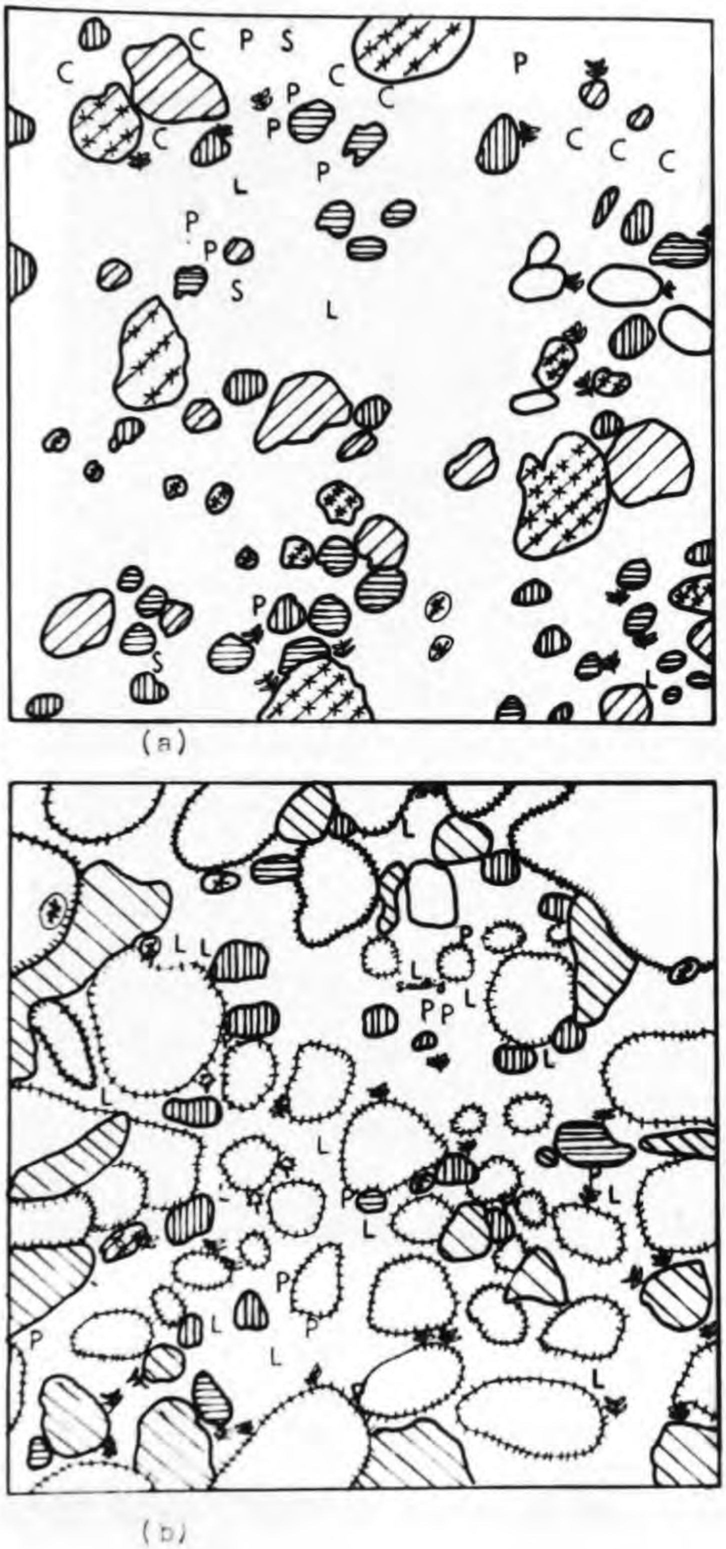


Fig. 7. Diagrams showing species composition of metre quadrats in representative habitats for Viola lactea at -(a) Gors Goch, Pentraeth, Anglesey and (b) Roborough Down, Devon.

Plate I3.

Plate I3. Typical habitat favoured by V. lactea. Open heath on cliffs at St. Agnes Point, Cornwall. One plant of V. lactea about centre. Other species visible include :- Erica cinerea, Ulex gallii, Scilla verna and Festuca ovina. Note evidence of burning viz. charred twigs in foreground.

Table 17.

1. Hooper's Point, Dale, Pembs. Cover 80%
2. Piltdown Common, Sussex. " 100%
3. Cleeve Common, Cheltenham, Gloucs. " 100%
4. Dune pasture, Silloth, Cumb. " 90%
5. Grey dunes, Pwllheli, " 90%
6. Grey dunes, Sandscale, Dalton in Furness, Lancs. " 40%

	1	2	3	4	5	6
Viola canina	O-lc	o-lf	lc	o-lc	o-lf	o
Achillea millefolium	-	c	o-c	c	-	-
Agrostis canina	o	-	-	-	-	f
Ammophila arenaria	-	-	-	-	f	a-D
Anthoxanthemum odoratum	a	a-D	a-D	-	-	-
Bellis perennis	o	-	-	-	-	-
Calluna vulgaris	aD	f	o-f	-	-	-
Carex arenaria	-	-	-	-	lf	o
C. echinata	lc	-	-	-	-	-
C. flacca	o	-	o	-	-	-
Chrysanthemum leucanthemum	-	o	-	-	-	-
Chamaenerion angustifolium	-	-	-	-	-	lf
Eryngia maritimum	-	-	-	-	r	-
Festuca ovina	-	-	lc	aD	-	-
F. rubra	lf	-	-	-	a-lD	-
Galium hercynicum	-	-	-	lc	-	-
G. verum	-	-	-	-	f	-
Geranium molle	-	-	-	-	-	o
Hieracium pilosella	o	-	-	-	-	o
Jasione montana	-	-	-	-	o	-
Leontodon autumnalis	-	-	-	lc	-	f
Lotus corniculatus	c	O-lc	-	c	-	c
Luzula campestris	-	a	f	-	-	c
Ononis repens	-	-	-	-	f-la	-
Plantago lanceolata	o	o	-	o	-	-
P. maritima	-	-	-	o	-	-
Poa pratensis	-	-	-	-	lc	-
Polygala serpyllifolia	lc	-	-	-	-	-
Potentilla erecta	f	c	f	-	-	-
Poterium sanguisorba	-	-	lc	-	-	-
Ranunculus acris	-	c	-	-	-	-
R. bulbosus	p	-	-	f	-	lc
Rosa spinosissima	-	-	-	r	-	-

(Table I7 contd.)

	1	2	3	4	5	6
Rubus fruticosus agg.	r	-	-	-	-	-
Rumes acetosa	-	o	-	-	-	-
Senecio jacobaea	-	-	-	-	-	c
S. vulgaris	-	-	-	-	-	c
Serratula tinctoria	r	-	-	-	-	-
Sonchus sp.	-	-	-	-	-	r
Taraxacum officinale	o	-	-	o	r	f
Thymus serpyllum	-	-	-	-	lf	lf
Tussilago farfara	-	-	-	-	-	c
Ulex europaeus	aD	-	o	r	-	-
Veronica chamaedrys	-	-	-	o-c	-	o
Vicia sepium	o	-	-	-	-	-
Viola lutea	-	-	-	-	-	f
V. riviniana	o	lc	o	-	-	lc

<u>Table 17</u>. Species associated with <u>V. canina ssp. canina</u> in representative localities.

In table 17 are listed the plants associated with *V. canina ssp. canina* in six representative localities. The dominants are rarely the same though if we split the communities into those found inland and those on coastal dunes then the two types of habitat can be outlined.

Those areas not on sand-dunes have *Calluna vulgaris* and *Anthoxanthemum odoratum* abundantly present with one or the other dominant. *Potentilla erecta* is frequent in all such places while *V. riviniana* is to be found. *Ulex europaeus* and *Carex flacca* seem to be normally present as is *Lotus corniculatus*, this is also common on dunes. No species is present in all the dune areas and, as would be expected, the associates of *V. canina* vary according to the species to be found on the dune pasture and 'grey dunes' of that locality.

Although not completely satisfactory these results do indicate the broad outlines of the communities occupied by *V. lactea* and *V. canina*. The former is a member of the more broken tussocky parts of a community which, although some of the more important species may vary locally, is generally typical of the acid heaths in the S. and W. of Britain.

V. canina, on the other hand, usually occurs inland in heath communities dominated by turf-forming species such as *Anthoxanthemum odoratum* and on dunes where obviously some effect of the physical environment is important, not the associated species.

(ii) *Physical Factors.* While studying field populations of these violets soil samples were collected from around the roots of the plants and packed in airtight tins. These were sent back to the laboratory, air-dried, and later tested for pH, exchangeable calcium and percentage loss on ignition; analyses for exchangeable potassium sodium and potassium failed to give satisfactory results. (For methods see Appendix C).

V. lactea inhabits a number of rock types, eg. old red sandstone, serpentine, slates, which are mainly Palaeozoic or Archaean and weather with difficulty to give a shallow, sterile soil supporting a poor, heathy type of vegetation. In one locality, Gors Goch on Anglesey, this species grew on a ridge of magnesian limestone but the soil in contact with this roots showed similar properties (table 18) to that from more typical areas, probably as a result of heavy leaching.

The physical conditions of the habitats favoured by *V. canina ssp. canina* seem, at least in Britain, to be a trifle easier to define. By far the most common substrate is sand, either as sea-dunes, principally on the west coast, or as inland deposits seen in the Breckland of W. Sussex and W. Norfolk, the cretaceous sands of N. Lincolnshire, and the glacial ridges of 'kames' of S.E. Scotland which are composed of sands and gravels. At Cleeve Common *V. canina* grows on a light sandy soil while at Piltdown the substrate was a fine yellow clay probably derived from the underlying Tunbridge Wells Sands. At Leyhill Common and Coombe Hill the clay resembling that just mentioned was probably a break-down product of the chalk. It seems to be associated with boulder clay deposits on Hoopers Point and has been reported as occurring on the same material in E.Yorks., though this is as yet unconfirmed and in much of that region it grows on blown sands over the Triassic

Some aspects of the ecological preferences of V.canina still remain unsolved. Why, for instance, it prefers the dunes on the west coast to those on the east coast, why it favours certain inland heaths and neglects others with apparently identical conditions; indeed the reasons for the widespread distribution and only local occurrence of this species remain largely hidden. These problems may have something to do with the fact that V.canina like so many other species, reaches Britain at the extreme limit of its westerly distribution. Here it is of remarkably constant form, being assigned to two recognizable subspecific categories, and is restricted to a few habitats, as it also is in N.Scandinavia. Eastwards into central Europe and possibly W.Asia it occupies a much wider range of habitats and forms a little understood complex of forms, many probably ecotypic, including the two recognized in the British Isles.

Analyses were carried out on samples of soil associated with either V.lactea or V.canina in several localities and the three

TABLE 18 .

Sample Code No.	pH.	% loss on ignition.	Exch. Ca. m.equiv/100 gms. air-dried soil.
V. canina			
A	5.00	7.73	3.64
A	4.50	6.63	-
U	5.05	-	15.40
L	5.62	14.17	12.88
Pwllheli	5.95	-	22.00
V	6.45	-	24.25
S	6.70	0.94	21.30
V. lactea.			
G	4.72	24.57	-
D	4.75	19.98	-
E	4.79	20.49	3.27
Z	4.88	20.11	2.45
Z	4.90	-	5.60
P	4.95	16.99	3.50
O	5.52	12.82	3.64
K	5.58	9.74	4.06
X	5.80	16.79	6.47
I	6.10	13.16	2.24
W	6.20	27.12	9.45
J	6.50	21.80	10.44

Table 18 . Analysis of soil samples from typical British localities for *V. canina* and *V. lactea* (Method as given in Appendix C)

properties considered can be readily seen and compared in table 18 . It is obvious that, although V.lactea tends to favour slightly lower pH values than V.canina, both species show a generally similar range.

The few analyses of percentage loss on ignition for V.canina suggest that this species shows lower values (range 0.94 - 7.73 [14.17]) than does V.lactea with a range of (9.74) 12.82 - 27.12% The two overlapping figures are placed in brackets and are very interesting since they were obtained from the only locality where these two species came into contact and formed hybrids.

The most interesting, and perhaps most significant, factor evaluated was the amount of exchangeable calcium present in the soil. Table 18 shows that, with one exception, the range of values for each species is quite distinct since V.canina has a range of (3.64) 12.88 - 24.25 me/100gm. soil and V.lactea one of 2.24 - 10.44 me/100gm. soil. The canina sample showing 3.64 me. came from a rather peculiar locality on Piltdown Common where it occupied a tiny area which seemed to be in danger of being overgrown by Calluna and Crataegus bushes. The geological formation was probably Tunbridge Wells sandstone which was also present at Chailey Common some 3 miles distant where it supported a heath containing V.lactea , this species was certainly not present at Piltdown. It may be noted that the value of 12.88 me% in the range forV.canina also came from the locality at Hooper's Point where it formed hybrids with V.lactea .

(111) Competition effects. In addition to the physical factors determining the habitats favoured by V.lactea and V.canina one other fairly constant feature distinguishing these two species is their relative ability to withstand competition and the biotic factors associated with it.

In all the localities visited V.lactea favoured fairly open conditions, frequently growing on areas subject to periodic and too frequent burning. In addition, many of its habitats - mainly on the Lizard and Roborough Downs - are scored by tracks (often of great age) across the downs and these, in common with burning, provide a focus for erosion of the plant cover so that here V.lactea is commonly found. Those populations which do grow among denser vegetation usually seem influenced by V.riviniana (see sct.7)

It is quite possible that the competition affects most strongly the seedling stage of V.lactea since field observations suggest that the young plant usually only succeeds in patches of open ground. Several times a mature plant was found to one side of a tussock with seedlings of varying stages on the bare soil and none in the vegetated area.

Although V.canina occurs on the open habitat afforded by the unfixed dunes it also flourishes in the closed sward of the dune pasture and in all inland localities visited by me seems to tolerate competition from associated species in the short turf which it favours. Like V.lactea, the habitat of V.canina appears much influenced by biotic factors. It depends to a large extent on the surrounding vegetation being of low growth since ssp.canina is adversely affected by shade and requires the open conditions of dunes or a sward which is kept fairly close cropped by sheep or rabbits.

(b) Cultivation Experiments.

To supplement the data given above on the physical factors involved in the field habitats a short series of experiments was designed to investigate certain of these factors by cultivating V.canina and V.lactea in soils of varying chemical and physical conditions.

(1) Soil Boxes.

Four samples of soil were prepared with their compositions so differing as to give varying pH and calcium content, both of which were at that time thought to influence strongly the choice of habitat of these species.

The composition of the four series is given below ;-

Basic medium - 7 parts loam
3 " peat
2 " sand.

Additional to basis -

T1. $\frac{3}{4}$ oz. chalk/bushel
$1\frac{1}{2}$ oz. hoof & horn/ bushel
$1\frac{1}{2}$ oz. superphosphate/ bushel
$\frac{3}{4}$ oz potash/ bushel

T2. As for T1. but without chalk

T3. Omit chalk and substitute Sodium phosphate for superphosphate

T4. Add extra chalk, 9x amount in T1.

pH of soil samples :-	T1.	T2.	T3.	T4.
	6.9	4.8	6.0	6.4

Several families of *V.canina* and *V.lactea* and their F1 hybrid were raised from the seedling stage and each one split up amoung the four soil types. These plants were grown in boxes for two years and observations made on the vigour and morphological characters to see whether any differences could be discerned, either between or within species, due to reactions of the soil types.

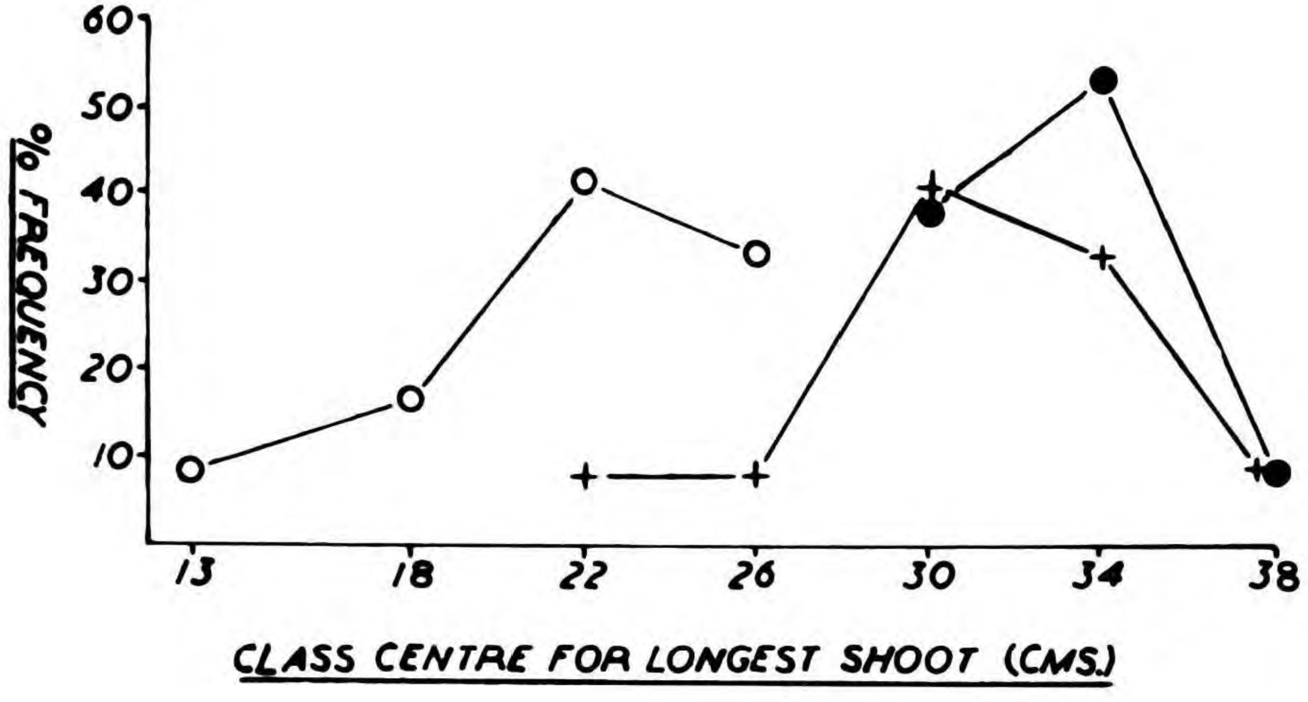
Individual vigour was estimated by measuring the number of, and length of the longest shoots for each plant; leaf length, breadth and basal angle (see sect. 6) were taken as giving a reliable morphological character which could be expected to vary with such environment changes. No positive results were obtained from these few experiments and it seems either that the conditions under which they were carried out proved unsatisfactory or that the physical factors chosen for investigation were not important to the growth of the two species. Both considerations are probably correct though, in view of the field data, the second probably exerted greater adverse influence on the experiments.

(ii) Texture Beds.

A very elementary trial was carried out using three newly formed texture beds to see if the growth of V.lactea and V.canina ssp.montana could be related to the physical structure of the soil. One bed was composed of raw clay, another of normal garden soil and a third of a mixture of equal parts of clay and soil. The characters mentioned in the preceding paragraphs were measured for each plant. V.lactea showed no preference for any bed and seemed equally suited to each, the general tendency being for the plants to be rather larger and more vigorous than field specimens. V.canina ssp.montana showed no morphological variation with the soils but the two characters used as indicators of general vigour showed that the clay seemed more favourable than the mixture which in its turn gave better growth than the garden soil. (Fig 8 .) This result accords with field observations that ssp.montana is found in Britain only in clayey soil at Woodwalton Fen while on the continent it shows marked preference for heavy clayey soils. Unfortunately no plants of V.canina ssp.canina were available for this portion of the experimental work.

c. Summary and conclusions.

A consideration of the distribution maps (sect 2b) showing the limited range of V.lactea compared to the wide-spread eurasiatic distribution of V.canina strongly supports the assumption that a climatic factor primarily separates these species, V.lactea



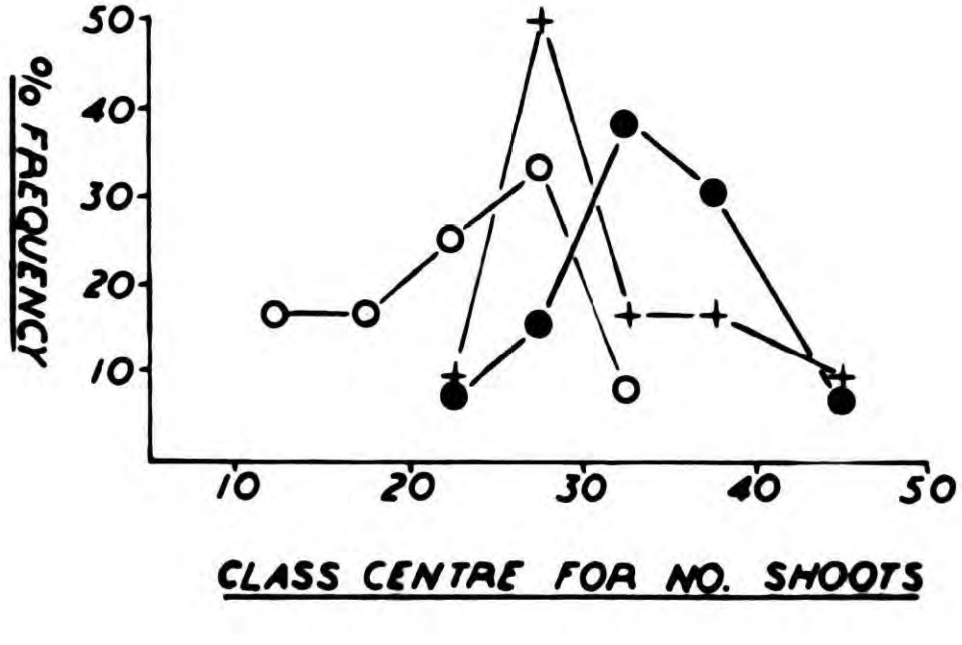


Fig.8. Frequency polygons comparing vigour (characterized by the two factors shown as the abscissae) of V. canina ssp. montana when grown in texture beds of clay (●), garden soil (○) and a 50 - 50 mixture of these.(+).

being much less tolerant of cold winters. Along their climatic boundary where they are likely to come together as, for instance, in S.W. Britain then edaphic factors have been shown to influence their choice of habitat.

V. lactea occurs on a variety of rock types having in common their support of a fairly well-defined heath community and with certain more or less constantly associated species. (see p.61) All the soils tend to have high values for ignition loss and have low amounts of exchangeable calcium. In addition, it is influenced by competition from other plants and depends a great deal on biotic factors such as burning, track erosion etc. preventing the attainment of climax communities.

V. canina can be considered as inhabiting two distinct types of habitat, open dunes and the closed communities of inland areas and the dune pasture. It's ability to survive in the former environment may well be due to it being able to withstand continual burial by drifting sand while *V. lactea* probably cannot do so. An examination of *canina* plants from open dunes normally shows that the primary stem is buried deep in the sand and it forks repeatedly to produce a number of prostrate flowering rosettes on the surface, these may fork when buried in their turn. The soils of such habitats also show a high exchangeable calcium status.

In the closed communites, especially inland, the only factor which could possibly keep *V. canina* apart from *V. lactea* is that it favours a higher percentage of exchangeable calcium.

Watt (1940) in his studies on the Breckland showed that *V. canina* would tolerate the highly calcareous conditions of his extreme 'Grassland A' but showed its highest constancy and frequency values in his grasslands C and D which support only the less exacting calcicoles.

The values of exchangeable calcium in the soil supporting *V. canina* suggest that it may usefully be compared with *V. lutea* which was studied in communities on the Derbyshire limestone by Balme (1953). She found it to be a moderately calcifuge species which is absent from highly calcareous soils, rare on podsols but is a characteristic indicator of transition conditions, invading rendzinas showing incipient surface leaching and is most successful in a Festuca-Agrostis turf on base-rich but not calcareous soils. It inhabits soils showing a range of exchangeable calcium 4.8-28.8 m.equiv. %. From the above ecological notes and soil analyses it seems likely that *V. canina ssp. canina*. shows similar ecological preferences to *V. lutea* favouring neither highly calcareous soils nor those with low base status which support *V. lactea*.

The ecological requirements of the rosulate species *V. riviniana* have been presented by Valentine (1949, 1950); it has wide climatic and edaphic tolerance and is able to come into wide contact with both *V. canina* and *V. lactea* over most or all of their ranges. In consequence opportunities for crossing with them are frequent and such data are of great importance when considering field populations, as will be seen later (sect. 7).

SECTION 6

BIOMETRICAL STUDIES.

Introduction

(a) Selection of characters

(b) Methods of analysis

- (1) Hybrid index
- (ii) Cyclic polygons
- (iii) Discriminant function
- (1V) Pictorialized scatter diagrams
- (V) Pollen fertility

(6) BIOMETRICAL STUDIES.

Introduction.

Earlier (sect. 1c.) reference to the literature suggested that in this country and on the continent V. lactea hybridized fairly frequently with both V. canina and V. riviniana. Since the experimental work described in sections 3 and 4 shows that V. lactea and V. canina cross fairly easily to produce a fertile hybrid it seems likely that introgression involving these two species might take place in the wild. However, ecological data (sect. 5) and field observations showed that they do not commonly occur together but that there is widespread contact in certain habitats between V. lactea and V. riviniana; these are known to cross quite readily to give a usually sterile hybrid (sect. 3).

It was previously noted (sect. 1c) that problems involving violet hybrids cannot be satisfactorily tackled using herbarium specimens alone since many important characters do not normally preserve well. This being the case, methods had to be devised suitable for studies on wild populations to discover whether introgression was taking place and, if so, between which species.

(a) Selection of characters.

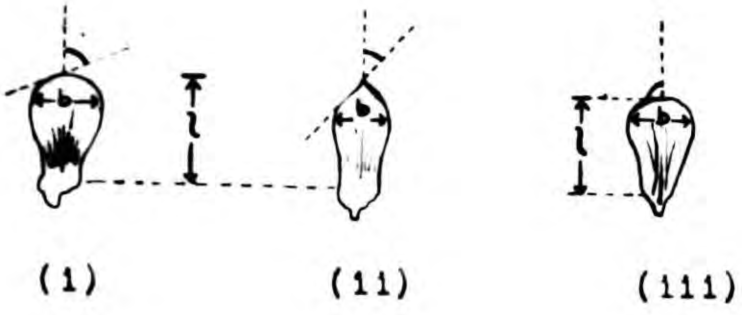
The first problem is to sort out the essential characters which distinguish the species and to express them in a form in which they can be either scored or measured. The relevant species obviously are V. lactea, V. canina and V. riviniana.

Investigations of herbarium and garden material, supplemented by field studies, showed the following eight characters to be most useful :-

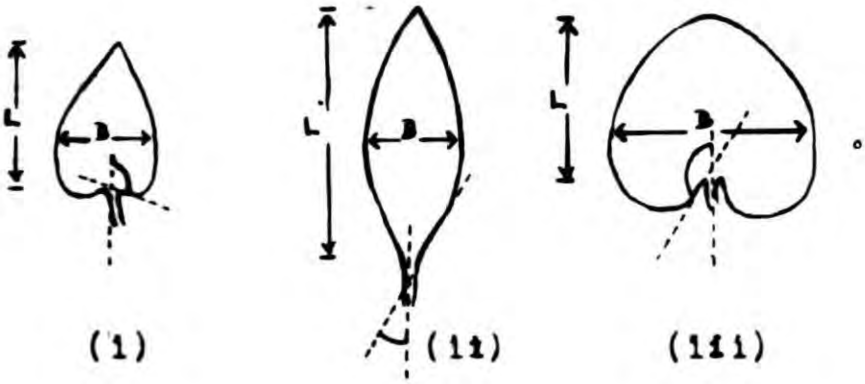
1. $\frac{\text{Greatest length}}{\text{Greatest breadth}}$ of leaf These characters (see Fig.9) denote
2. Basal angle of leaf. the leaf shape fairly well and are the only ones reliably found on most herbarium specimens. Russell (1952) used them in studies on hybridization in American violets. The leaf chosen for measurement was that which subtended in its axil the uppermost flower.
3. $\frac{\text{Greatest length}}{\text{Greatest breadth}}$ of lower (spur) petal. See Fig. 9. the shape of
4. Apical angle of lower(spur) petal. this petal is very useful but is a diagnostic character not normally available in pressed specimens.
5. Flower colour. Scored in the field by comparison with a numbered chart prepared to form an index for the range of colours involved.
6. Style length. Style shape was used by
7. Length of longest stigmatic papilla. workers on N. American violets, principally Clausen (1929) and Gershoy (1938), as a good specific character. I was able to characterize the organ biometrically by these measurements, easily obtained using a micrometer eye-piece.
8. Percentage pollen fertility. Measured by noting the degree of staining with aceto-carmine, using anthers dissected out from pickled flowers.

(b) Methods of analysis.

When studying groups, such as plant populations, including putative intermediates rarely does the consideration of a single character show at all fully the processes involved, no matter how intensive the methods used. Hence it is necessary to consider several of the more important characters separating the parents and combin-



Lower (spur) petal shape.



Leaf shape.

FIG. 9 . Diagrams showing measurements taken to obtain biometrical estimates of leaf and petal shape. Length, breadth, and angle are indicated as for typical (i) V. canina, (ii) V. lactea and (iii) V. riviniana.

ations of these are the most useful way of delimiting species and hybrids.

For instance, style length is one such single character which is distinct for each of the three species. It can be defined adequately (see above) and the results for several known populations are summarized in Fig. 10. Simple graphing is seen to separate the three species quite clearly and constantly and the hybrid V. lactea x riviniana falls into place rather as would be expected. In the case of V. canina x riviniana and V. canina x lactea the canina element seems to be completely dominated by the other parent from which no separation can be detected, and these are thus not plotted. But the spread shown by each element in the graph makes it impossible to characterize hybrid populations (which may include other than F1 hybrids) by this method and we must employ a number of characters.

However, it is difficult, even impossible, to consider several continuously varying factors at once and some methods of integrating the characters into a single expression are here considered.

(i) Hybrid Index. This method, devised by Anderson (1949), has been widely used, especially by American botanists, for the study of hybrid populations. The indices are formed by the simple addition of the measurements of several characters and a consideration of Table 19 shows why the method is of no use in this case. It is apparent that in most of the characters used V. canina is intermediate between the other two species and such an additive method could not resolve accurately the possible hybrids which might be involved.

Fig. IO.

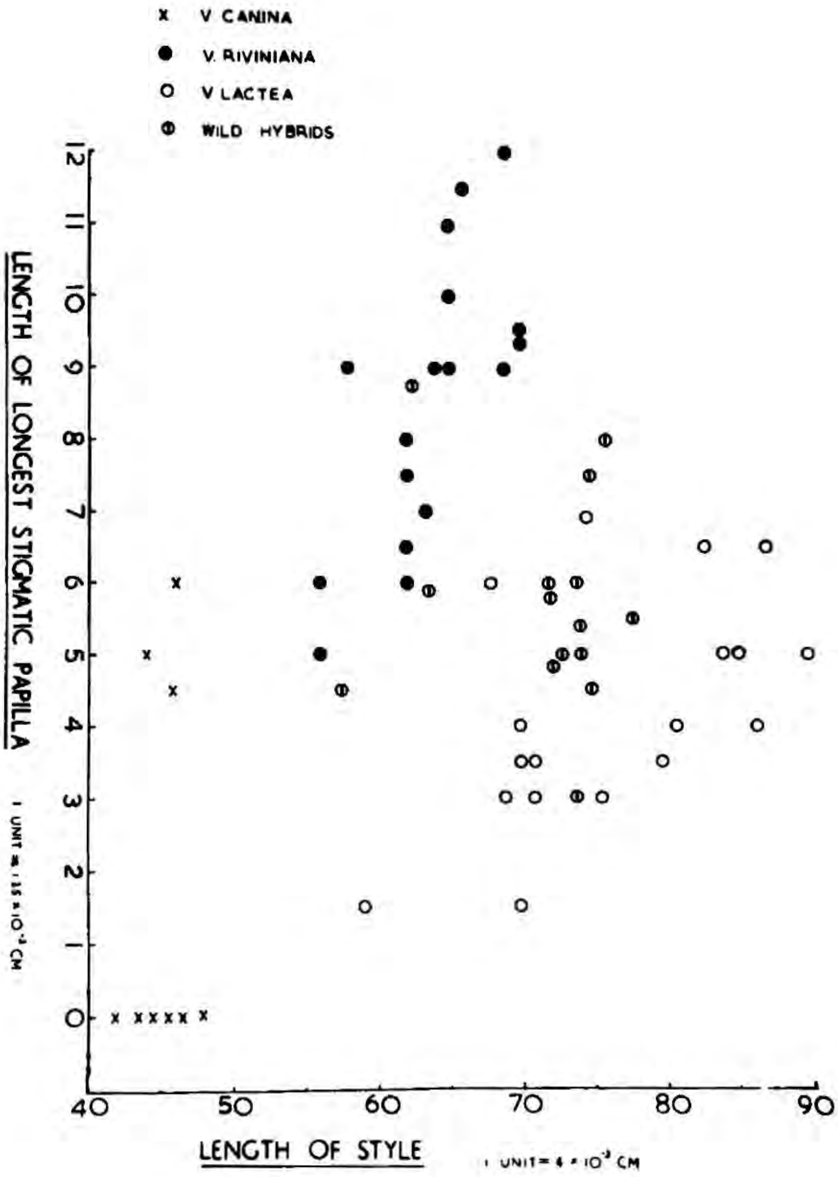


Fig. IO. Scatter diagram of style characters to separation of V. canina, V. lactea and V. riviniana. The wild hybrids scored closely resembled V. canina in vegetative characters, but it is evident here that they are intermediates between V. riviniana and V. lactea.

TABLE 19.

V. lacters	V. lactea	V. canina	V. riviniana
Leaf $\frac{\text{length}}{\text{breadth}}$	(1.4)1.8 - 2.8	(1.3)1.4 - 1.7(1.8)	0.85 - 1.3(1.4)
Leaf Basal Ang.	40 - 70(80)	90 - 120	107 - 155(165)
Petal $\frac{\text{length}}{\text{breadth}}$	(1.5)1.65 - 2.5	1.1 - 1.5(1.7)	1.14 - 1.53(1.6)
Petal Apical Ang.	48 - 74(80)	65 - 84	(78)80 - 90.

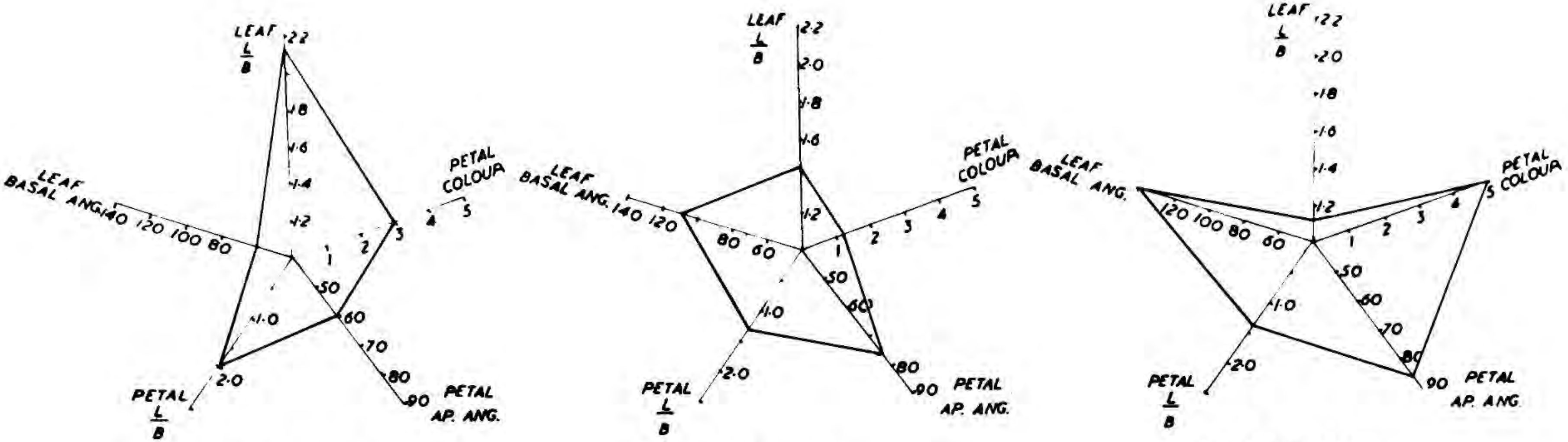
Table 19. Intra-specific range, in the three species concerned, of the characters based on direct linear measurements.

(ii) Cyclic Polygons. The use of these for simultaneously graphing a [illegible] number of variables has been evolved independently by several workers (Hutchinson 1936, Fassett 1941, Davidson 1947, Carson and Stalker 1947) engaged in the study of either inter-, or sub-, specific entities.

The construction of the polygonal graph can be readily seen from the numerous examples given. Five axes radiate from a point at an angle of 72 degrees to each other. For each sample the mean value is calculated for each of characters 1 - 5 listed above and they are then resolved along the axes to give five points which can be joined to form a polygonal outline. The standard error can be calculated for each variable and may be drawn in so as to form inner and outer polygons to that connecting the means. This has been omitted from the polygons illustrated since the reduced scale necessarily used for the figures makes distinction of most of the standard errors difficult. All standard errors are listed, on a separate sheet.

By this means it is possible to get a shape which is characteristic of the population and which speedily shows not only

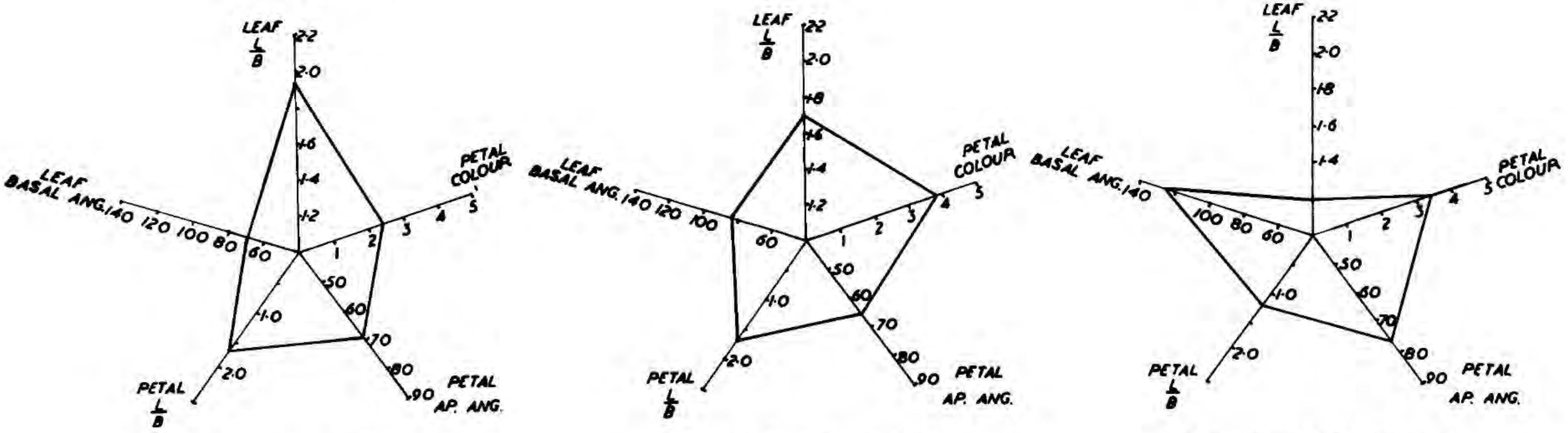
Fig. II. Cyclic polygons for samples from Viola populations of known species and hybrids.



V. LACTEA

V. CANINA

V. RIVINIANA

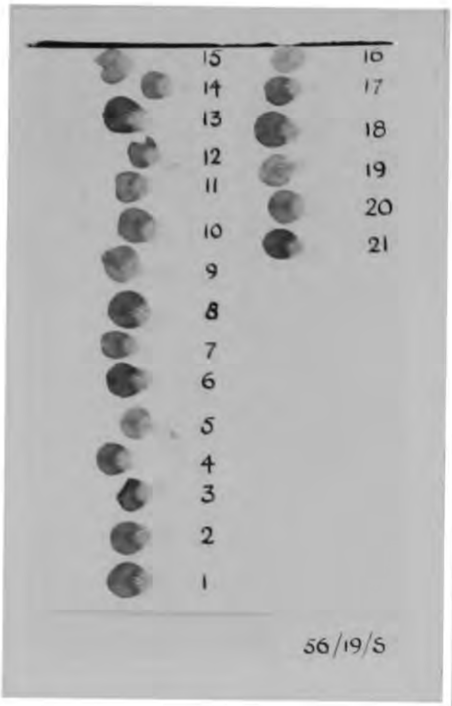


V. CANINA x LACTEA

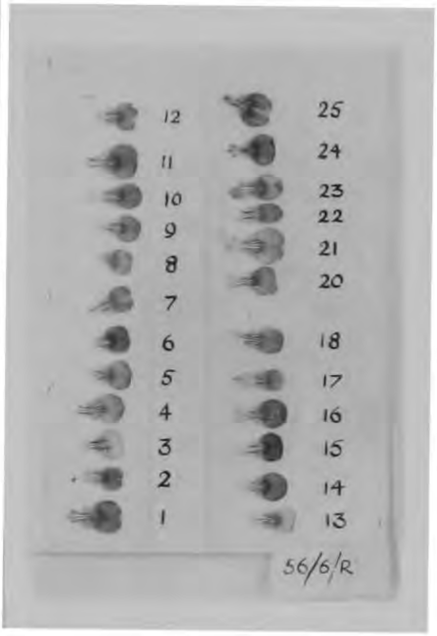
V. LACTEA x RIVINIANA

V. CANINA x RIVINIANA

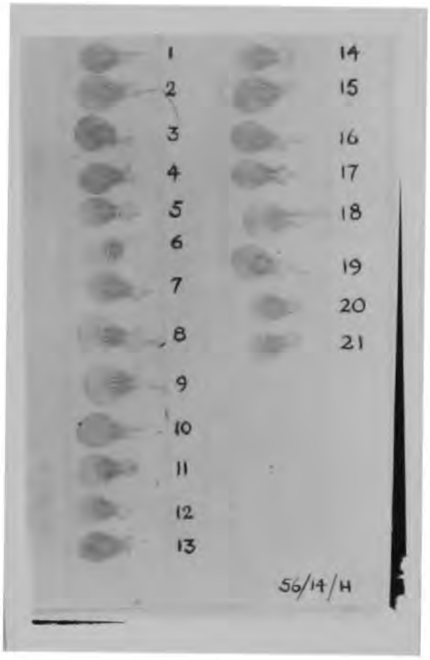
Plate I4.



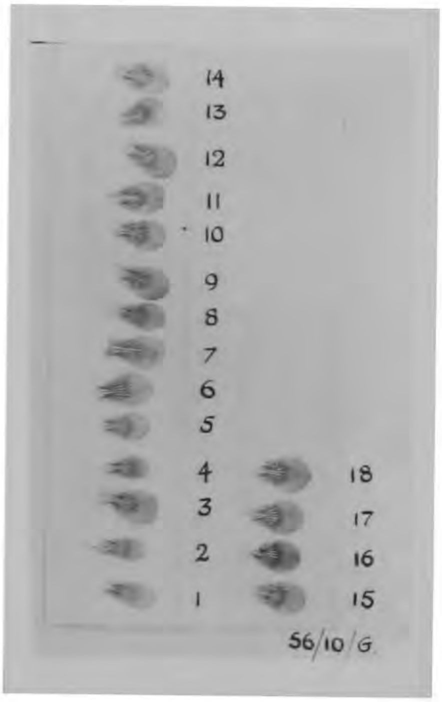
V. canina (S)



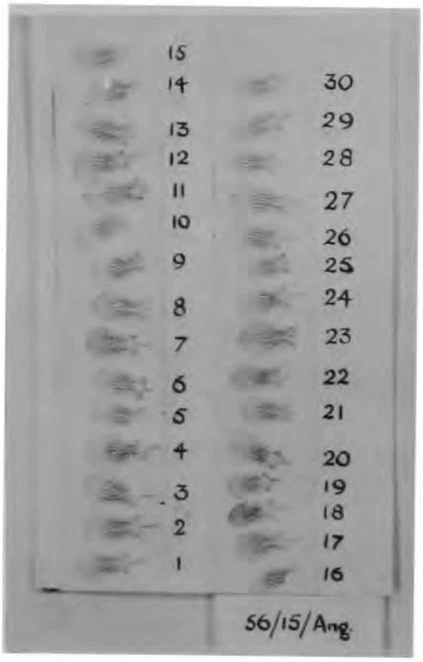
V. riviniana (F)



. canina x lactea (N)



V. lactea x riviniana (J)



V. lactea (O)

Plate I4. Samples of petals from typical populations of species and hybrids encountered in the field. Their origin can be traced from Table 21 using the code letter given in brackets.

× ½

which samples are the most similar and which the most different but also the particular characters which are most alike, and to a certain degree the amount of correlation between the characteristics. Some idea of the intra-sample variability can also be readily seen although it need not be considered so very important in this context.

The polygons given in Fig. 11 were drawn for samples of known species and hybrids from both field and garden populations; they show that a clearly defined and distinctive shape is produced for each entity. The series has proved constantly accurate when checked on several subsequent occasions so that it gives a set of standards by which to judge the later investigations.

(iii) Discriminant Function. An attempt was made to support the subjective method of comparing polygonal graphs by applying the statistical technique of multiple regression to the classification of population samples.

A method of multivariate analysis was suggested by R.A. Fisher(1936) to distinguish populations of *Iris setosa*, *I. versicolor* and *I. virginica*. By this method measurements on a certain number of characters in two or more groups can be combined into an expression termed the discriminant function (L) which affords the maximum discrimination between the two groups. Here the groups concerned are the population samples and the polygon shape can be replaced by a single function which takes into account the variability of each individual characteristic with regard to the others and which is, statistically, a perfectly valid and exactly definable term.

After much work it was found that although the samples could be classified on the basis of their discriminant functions significance tests showed that in fact, every population had a value for L significantly different from every other. It was thus deemed that such a statistical technique gave results which were too finely graded for the purpose required here and that the less objective cyclic polygons provided the only possible approach. The results obtained from multivariate analysis of several population samples are given in Appendix A, as well as details of the method.

Once a population had been roughly classified on polygon shape more detailed information regarding its composition can be obtained by using pictorialized scatter diagrams and pollen analyses as follows:-

(iv) Pictorialized Scatter Diagrams. These were described by Anderson (1949) and have been widely used, mainly in America, by those interested in natural hybridization and introgression.

The scatter diagrams used here have leaf $\frac{\text{length}}{\text{breadth}}$ plotted as the ordinate and leaf basal angle as the abscissa. Each point so defined was then used to show, pictorially, the two petal characters viz. $\frac{\text{length}}{\text{breadth}}$ and apical angle. Table 19 shows the range of these in the species here concerned and by using the symbols as illustrated in the keys the diagnostic leaf and petal characters embodied in each point can be assigned as nearer to one or other of the parental species, or as intermediate. This method is limited in that it only permits of a certain degree of exactness and suffers from the fault of subjectiveness applicable to all such visual methods. However, it proves to be the only method possible since, although the intimate details of structure may be obscured, the general trends seem to show up quite well in practice. It is to be stressed that the scatter diagrams are only reliable when the use of cyclic polygons has determined which two violet species are involved in a population. It is shown later that in most cases *V. lactea* and *V. riviniana* are concerned and the pictorial scatter diagrams have provided a satisfactory analytical approach. In the event of all three species being involved, as mentioned by Gregory (1912), it is doubtful whether a solution could be reached by any of the biometrical techniques at present available.

(v) *Pollen fertility*. In some cases it has been possible to supplement the information obtained from the above methods by testing the pollen fertility of plants within a sample. The method used was simply to dissect one or two anthers in aceto-carmine and examine under the low power of the microscope; all grains which were rounded and contained stainable contents were considered good while those which were either shrivelled or empty were taken as being infertile. For each plant the nu numbers of good and bad grains were counted for five random fields and the results presented as a frequency histogram of percentage good pollen. This suffices to give a broad outline of fertility trends among the individuals of a population. In the three species *V. lactea*, *V. canina* and *V. riviniana* this test shows that 'good' species have 85 - 100% fertile pollen so this may be taken as a basis on which to judge the histograms.

In many samples it was impossible to obtain any results since the anthers were dehisced and even in those described this was usually the case for a few individuals. Also, as is pointed out below (Sections 7 and 8), pickled flowers were packed ten to a tube so that these data cannot be correlated with individual plants used in scatter diagrams. Bearing this in mind care must be taken when comparing results and I have purposely not pressed matters too far. Suffice to note that striking support is given by the pollen data to the conclusions on biometrical grounds and although the data have little use alone they can supplement, at least partially, the principal analysis techniques used in these studies

The method finally evolved was to prepare a polygon for each sample taken and then to group like shapes together for comparison with the standard shapes shown in Fig. 11. This showed the general affinities of the populations and the details of structure could be obtained from analyses using pictorialized scatter diagrams and, in some cases, pollen analyses. The use of this procedure, and the results obtained, will be described next.

SECTION 7

FIELD POPULATIONS.

(7) Field Populations

(a) Notes on populations examined.

(i) List of localities. Details concerning the location of populations sampled are summarized in table 21 .

Table 21 .

Code	Locality.	Grid Ref.	Date Collected.
A	Piltdown Common, E. Sussex.	51445225.	15-5-56.
B	By Hermitage School, North Common,		16-5-56.
C	Chailey, E. Sussex.		16-5-56.
V	Leyhill Common, Chesham, Bucks.	4296-01-	5-6-55.
U	Cleeve Common, Cheltenham. E. Gloucs.	42004247	3-6-55.
D-F	Roborough Down, nr. Yeoland, S. Devon		18-5-56
G	Roborough Down, nr. Buckland Monachorum,		19-5-56.
H	S. Devon.		19-5-56.
W	Kynance, Lizard Downs, E. Cornwall	10689131	26-5-55.
X	Lower Predannack Downs, E. Cornwall	10675150	26-5-55
Y	Penhale, Goonhilly Downs, E. Cornwall.	10698193	27-5-55
I	Bray's Cottage, Goonhilly Downs, E. Cornwall	10727184	21-5-56
J	Arrowan Common, E. Cornwall.	10752177	21-5-56
Z	Cliffs nr. Porth Towan, E. Cornwall.	10677466	30-5-55
K-N	Hooper's Point, Dale, Pembs.		25-5-56
O	Gors Goch, nr. Pentraeth, Anglesey	23504816	27-5-56
P-R	2 miles S. of S. Stack, Holy Island, Anglesey.	23215804	28-5-56
S	Sandscale Dunes, Dalton-in-Furness, N. Lancs.	34186754	30-5-56
T	Dune pasture, Silloth, Cumberland.	3511-59-	2-6-56

(ii) Structure of populations. - V. lactea, as noted earlier (Sect.5), characteristically favours rather open habitats and in most localities such areas seemed of limited extent, being bounded by closed communities Even where a favourable habitat was more extensive as, for instance, the short heaths on the cliffs near Portreath which covered about 200-300 sq.m., local variations of topography and substrate caused discontinuities giving definable populations of V. lactea. Rarely did a population exceed 10-20 sq.m. and within this the spacing of individuals depended, naturally, on the disposition of open ground. Single plants were usually more than 6ins. apart, often much more; for example, 12 contiguous metre quadrats were laid in a typical habitat in Anglesey and they held respectively 4, 2, 2, 4, 0, 0, 9, 5, 6, 0, 2 and 8 lactea plants. Individual plants range from 4 to 20 cms. high with 1 - 8 flowering stems,

Although V. riviniana occurred fairly frequently intermingled with V. lactea it showed, in many cases, a tendency to be more plentiful and vigorous in patches of scrub and taller vegetation. This was most evident where the open habitat had been provided by fairly recent burning which V. riviniana seems less able to withstand than does V. lactea.

Hybrid forms between these two species usually occur at points of contact between habitats typical of each, eg. on the edge of a scrub area surrounded by open sea heath. Such hybrid patches usually covered 1-3 sq.m., occasionally extending to 8 or 10 sq.m. The individuals were close together, extremely floriferous, and attained heights up to c.26 cms. with many stems.

In more open *lactea* areas smaller clumps of floriferous plants, even individuals, seemed of hybrid origin and these provided some of the most crucial and interesting samples since they seemed likely to be backcrosses, or some such intermediates, between the FI and the *lactea* parent.

V. canina tends to be local even in favourable habitats and the individuals so scattered that 30 plants may be spread over 9 or 10 sq.m. On dunes the plants of *V. canina* seem to be grouped more closely but in many cases this is due to a single rootstock forking when buried by the drifting sand to give a number of stems. Most individuals found fall in the height range of 2-10 cms.

In only one case was the hybrid found between *V. lactea* and *V. canina* and it formed floriferous clumps up to 30 cms. in diameter. The hybrids lay along the point of contact between a turfy, *canina* habitat and the more open, rocky, *lactea* areas.

(b) Method of sampling.

(i) Choice of population - Certain populations were needed to act as marker samples, or fixed points, in the biometric analyses undertaken. These were determined as typical of the parent species on field characters and taken from the most characteristic habitat types in several localities. Samples were also taken from hybrid clumps which seemed likely to be first generation crosses; such samples could not be random since the plants were probably all clonally related.

The remainder of the populations were chosen because of abnormal features which, if interpreted, might partially help to elucidate the general problem presented by V. lactea in the field. Cases, for instances, in which plants very like one species were found in a habitat typical of the other; populations containing a high proportion of morphologically aberrant individuals, or populations of one species which might be expected to show introgression due to close contact with another species and hybrids

(ii) Field techniques - From each suitable population a random sample of 30 plants was taken; in some cases the small size of the population did not permit of such a large gathering. As was seen earlier (Sect.6a) the data for analysis require that the whole plant be preserved for treatment on return from the field and the procedure adopted is described next.

While still fresh the spur colour was noted and that of the petals given an index number by comparison with a chart of standard colours. The flower was then removed and the rest of the plant labelled (pop. no./plant no.) and pressed. The diagnostically valuable lower petal was removed and stuck on to a postcard with cellotape; a method used by Heslop Harrison (1949) in his studies of field populations in the Dactylorchids whose flowers, like those of violets, deteriorate markedly on being dried. Each card could hold the petals for one sample and these were numbered to correspond with the pressed leaves. The remainder of each flower was preserved in a tube of alcohol so as to retain the pollen and stigma for later examination; it proved quite unmanageable to keep each flower separate so that they were preserved in bulk, ten to a tube.

By using this procedure it was possible to collect a reasonably representative number of population samples while travelling round several widely scattered areas and to keep the material in a form from which the necessary data could be extracted on return to the laboratory.

(c) Results.

By using the method described above of cyclic polygons and scatter diagrams it is possible to arrange the populations into six groups as follows :-

(I) V. lactea or very near. (Samples E, O, M, K, P, I, and B.)

(II) V. lactea x riviniana - FI. (Samples R, J, C and D)

(III) V. lactea x riviniana, showing evidence of approach to one or other parent. (Samples Y, Q, W, X and Z).

(IV) V. riviniana. (Samples F, H and G).

(V) V. lactea x canina - rare. (Sample N).

(VI) V. canina. (Samples A, L, S and T).

Following this order each of the above groups will now be considered in turn and the samples within them will be described individually using data obtained from polygons, scatter diagrams and pollen analyses, all of which are illustrated, and added to observations made in the field on habitat &c.

GROUP I.

12 Two samples, E and O, give polygons (Fig. 12) which agree exactly with the base shape for V. lactea and the scatter diagrams

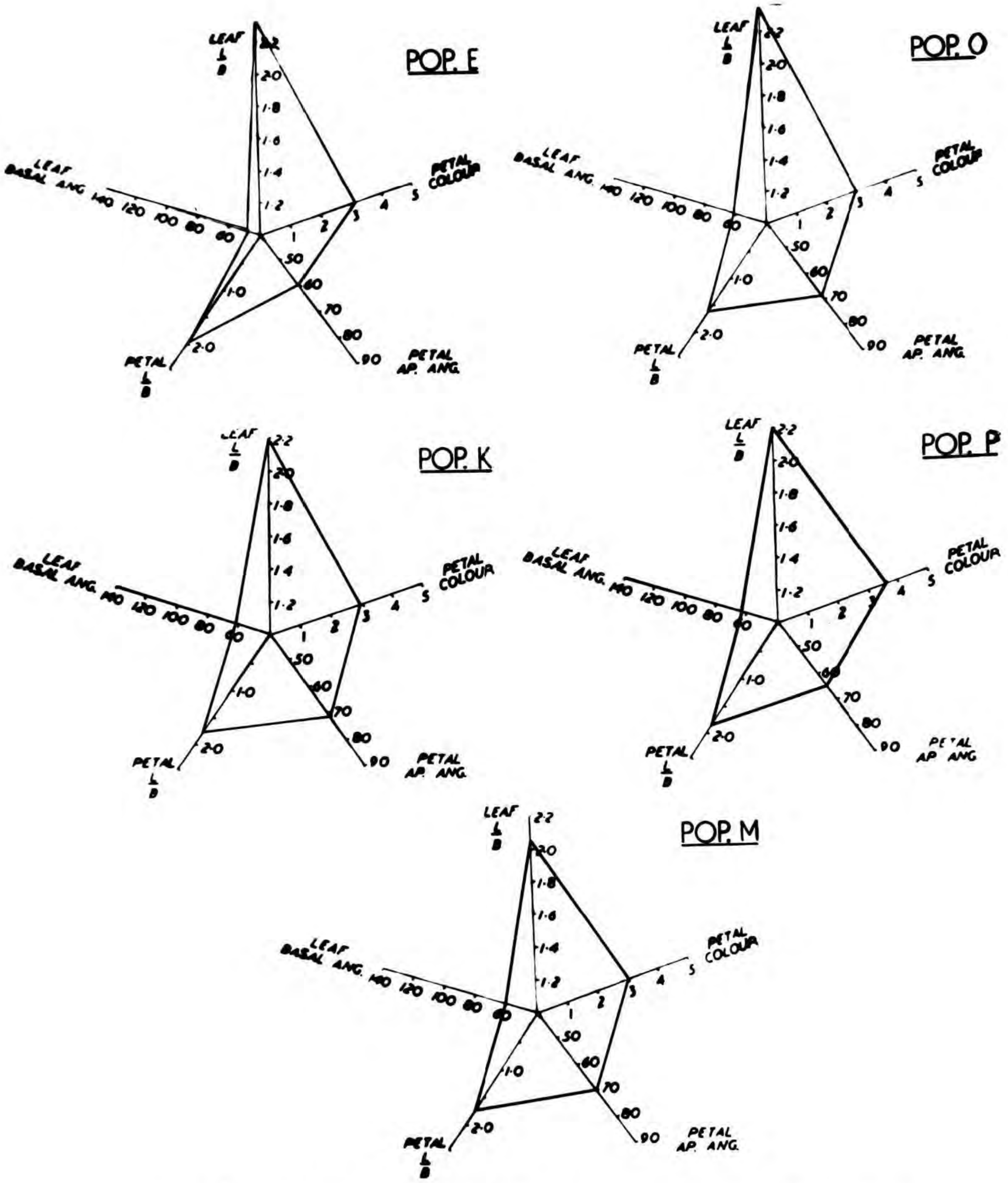


FIG.12 Cyclic polygons for five of the population samples included in Group I. Populations E and O are typical *V. lactea*.

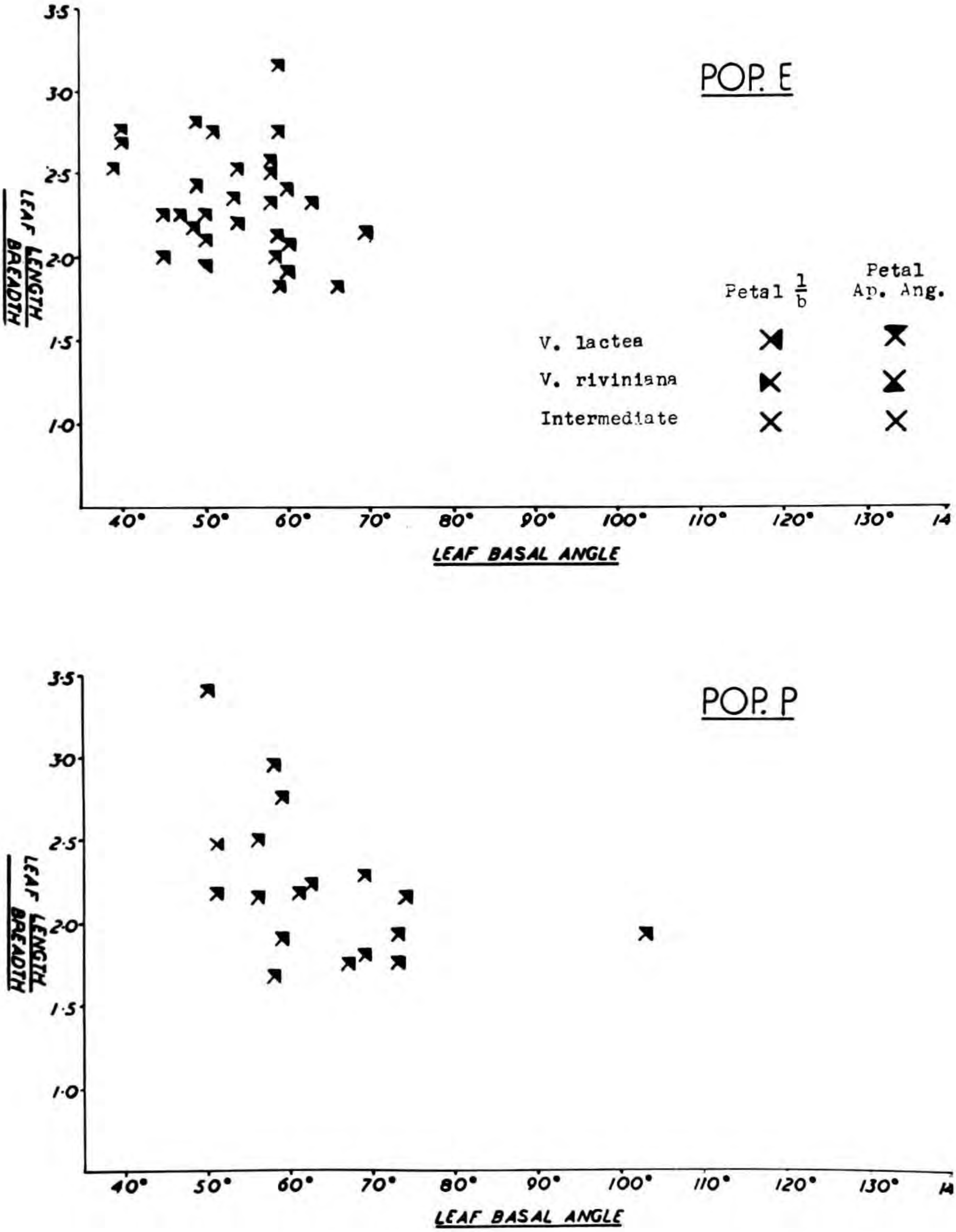


FIG.13 Pictorial scatter diagrams for population samples in Group I. E is 'good' *V. lactea* while P is influenced by *V. riviniana*.

(Figs. 13 &25) confirm that almost every individual plant is typical for this species.

K, P and M have polygons (Fig. 12) identical with, or very similar to, the forgoing but the scatters show them to differ slightly. M and K (Fig. 14) are almost entirely lactea, the former with 4 aberrant plants each showing one riviniana petal character, the latter having two plants intermediate in a single petal character and one plant with lactea leaf shape and riviniana petals. On field observations population P seemed nowhere to

Table 22.

Sample	Leaf $\frac{l}{b}$	Leaf Basal Angle	Petal $\frac{l}{b}$	Petal Ap. Angle
P	2.2I5 ± 0.1073	63.8 ± 2.944	I.86 ± 0.0522	65.0 ± I.405
E	2.334 ± 0.0587	53.8 ± I.405	I.98 ± 0.08I3	59.5 ± I.027
I	2.186 ± 0.0656	66.I ± 2.04I	I.62 ± 0.0247	76.7 ± I.272

Table 22. Comparison of means and standard errors in leaf and petal characters between sample of 'pure' lactea (E) and samples (P) and (I) showing introgression from riviniana.

be really pure lactea and every plant had a very slight tendency to vary towards V. riviniana. Although the polygon resembles V. lactea it shows a larger standard error in all characters (Table 22) and the scatter diagram (Fig. 13) demonstrates that in leaf shape some plants are like lactea but most are in the lowest limit of leaf ratio (I.5-2.0) which can be attributable to this species. In basal angle many plants also tend away from normal lactea.

Sample I (Fig. 15) is seen to give a less vertically

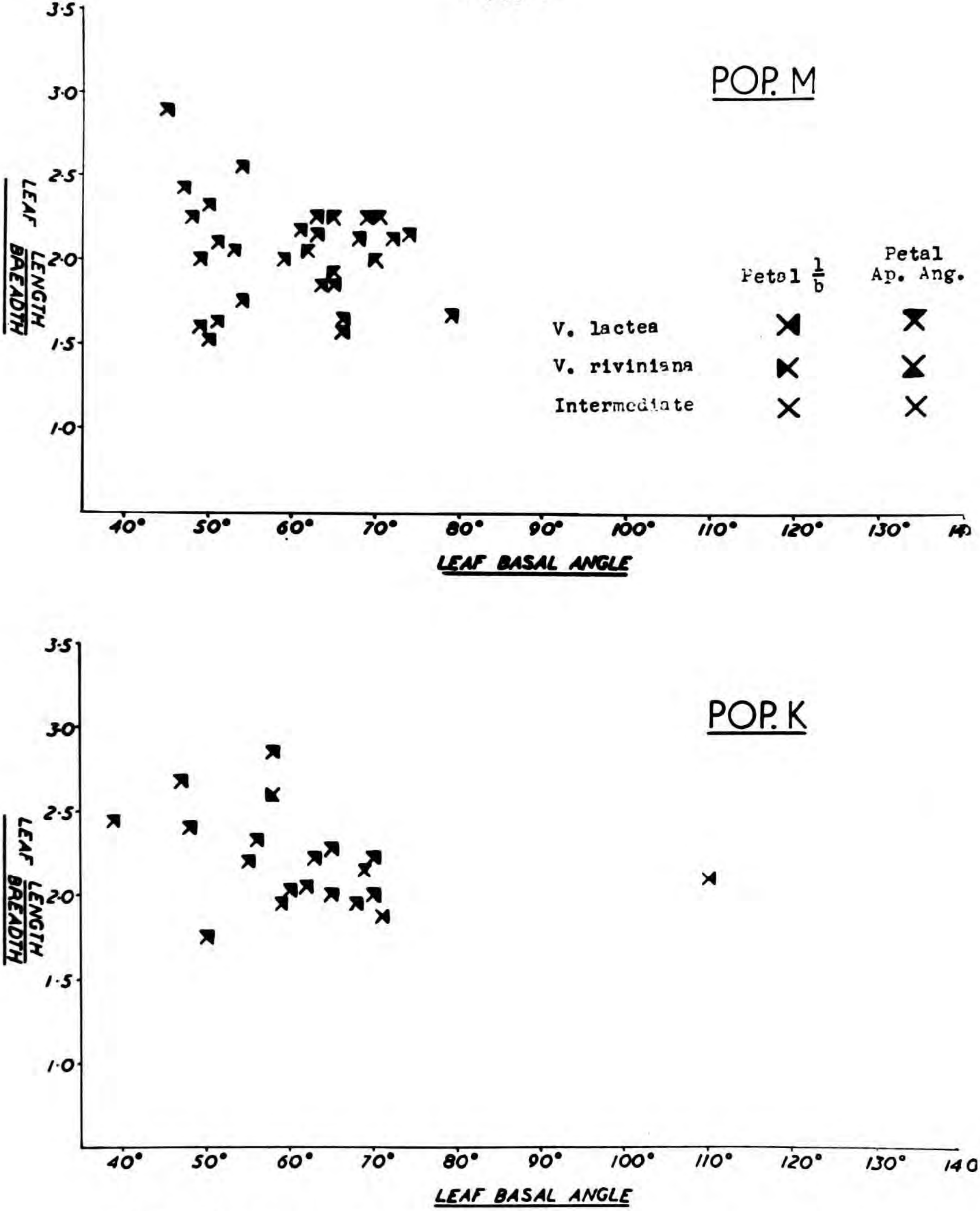


FIG.14. Pictorial scatter diagrams for population samples in Group I. Both have a few plants showing slight riviniana influence.

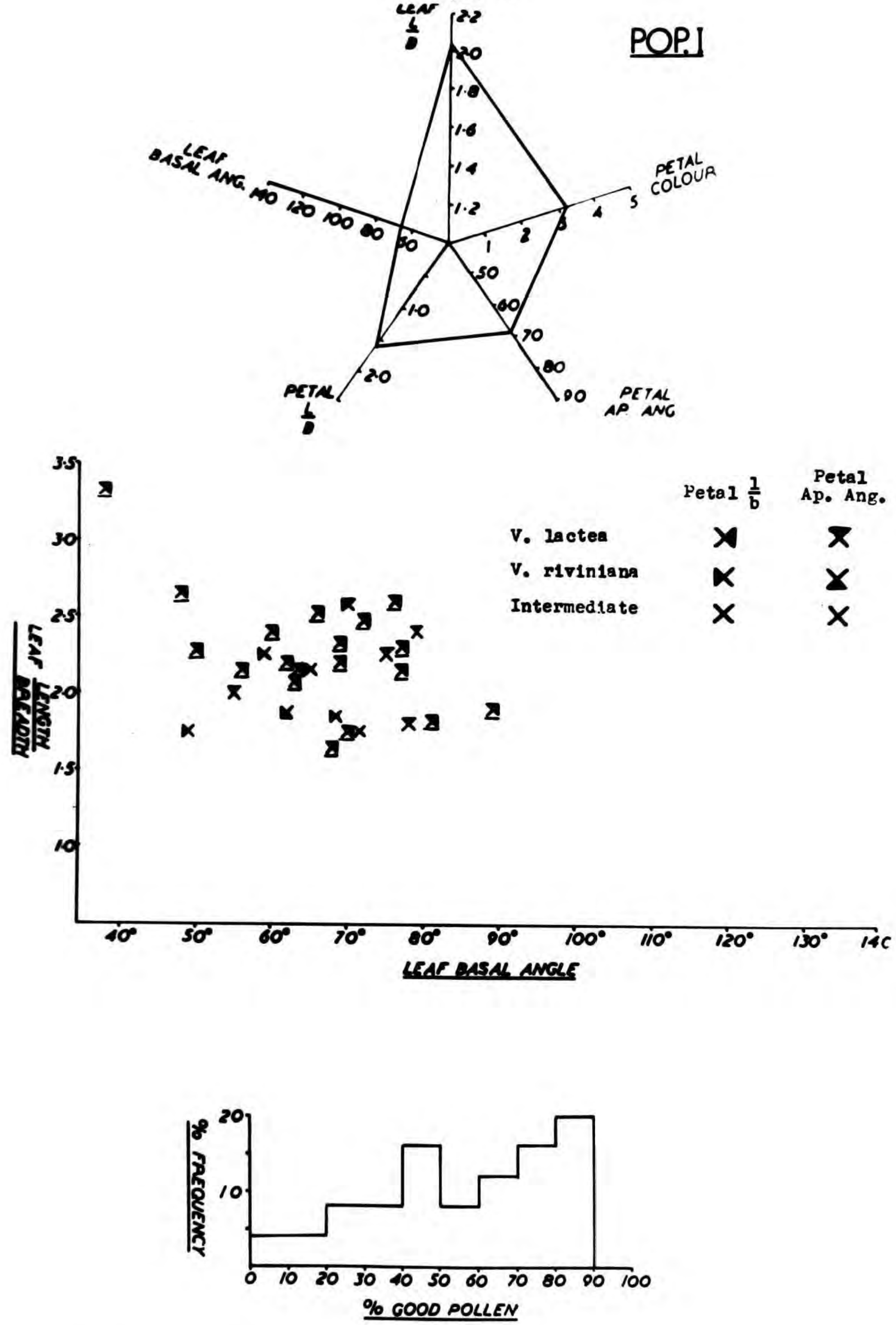


FIG. 15 Cyclic polygon, scatter diagram and pollen fertility histogram for sample I (Group I). Underlined plants are biometrically 'good' V. lactea.

elongated polygon shape than V. lactea from which it differs in leaf angle and petal ratio (Table 22) (note also high standard error). The scatter diagram (Fig.15) shows the lactea influence to be dominant but there is a tendency to an admixture of intermediate or riviniana characters in IO plants. This population was growing on Molinia tussocks in a damp 'pan' and the general impression in the field was that the plants seemed unusually large and luxuriant, some indeed showed vegetative proliferation which has always been found indicative of riviniana influence. Pollen fertility is seen (Fig.15) to vary greatly within the sample. Some of the plants (36%) have 70% good pollen, and thus do not differ significantly from V. lactea; but most have lower fertility, and some possess no good pollen. It is presumed that here is a population very like V. lactea in morphology but which shows certain riviniana characters such as tolerance of closed habitats and vegetative proliferation; this hybrid origin is reflected in the pollen sterility.

The final sample included in this group, B, shows a polygon (Fig.16) almost typical of lactea but with a high standard deviation in leaf characters and petal angle. The scatter (Fig. 16) shows only three plants to be completely lactea. In the field it was noticed that these plants were scattered in very floriferous small patches seemingly of hybrid origin, that they occurred near the rather more closely vegetated habitats favoured by V. riviniana and that there was evidence of vegetative propagation (**Plate 4**) Here, it seems, is a population which is one further step away from pure V. lactea in the direction of V. riviniana.

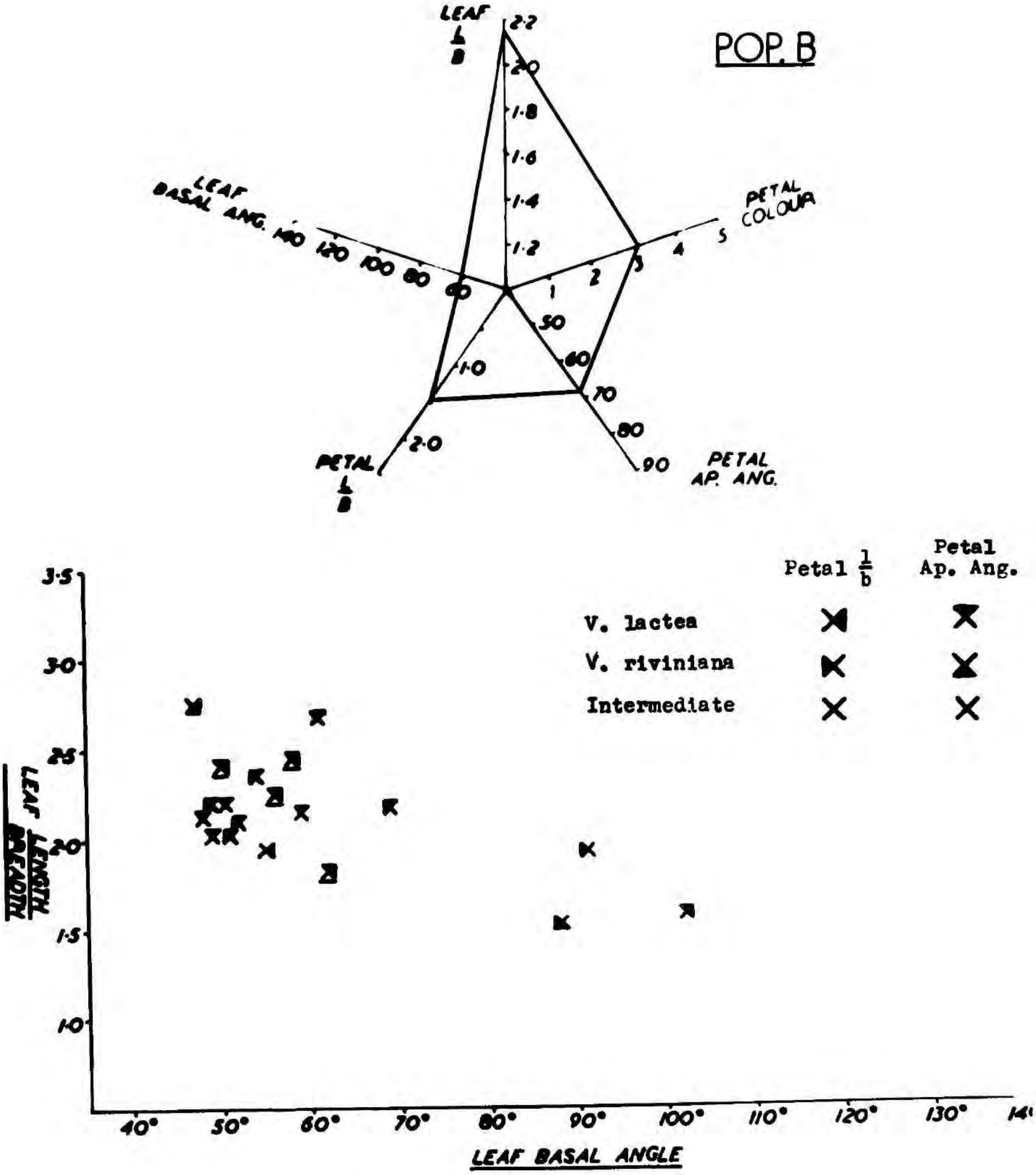


FIG.16 Cyclic polygon and scatter diagram for sample B (Group I). Underlined plants are biometrically 'good' V. lactea.

GROUP II.

The four samples included here all have polygons (Fig.17 & 18) resembling the standard for the F1 hybrid between *V. lactea* and *V. riviniana* given in fig.11 . At least one of them, R, is almost certainly an F1 hybrid since it occurred as three large floriferous clumps round the edge of a patch of scrub dominated by *Ulex europaeus* and *Rubus fruticosus agg.* which stood in an area of cliff heath. Obviously hybridization had been effected at the junction of typically *riviniana* and *lactea* habitats. In the scatter diagram (Fig.17 & Fig.18) it lies almost exactly intermediate between the two parents on leaf characters while the petals show mixed or intermediate affinities.

Sample J is seen to be very similar to the last in polygon shape but the swing towards *V. riviniana* shown in the scatter diagram (Fig.17) is a reflection of its habitat. This population grew on dampish ground among close, thick vegetation about I ft. high and which included *Juncus conglomeratus*, *Galium palustre*, *Ulex europaeus*, *Molinia caerulea* and *Vicia angustifolia*; in face of such competition *V. lactea* could not be expected to succeed.

The two polygons illustrated in Fig.18 , C. and D, are almost exactly similar to the above two. The first gives a scatter (Fig.18) showing it to be somewhere between the two parents in leaf characters, though nearer *lactea* than *riviniana*, and of mixed affinities in petal shape; no plant has all the characters of one of the parent species. Sample D is seen (Fig.18) to have a leaf $\frac{1}{b}$

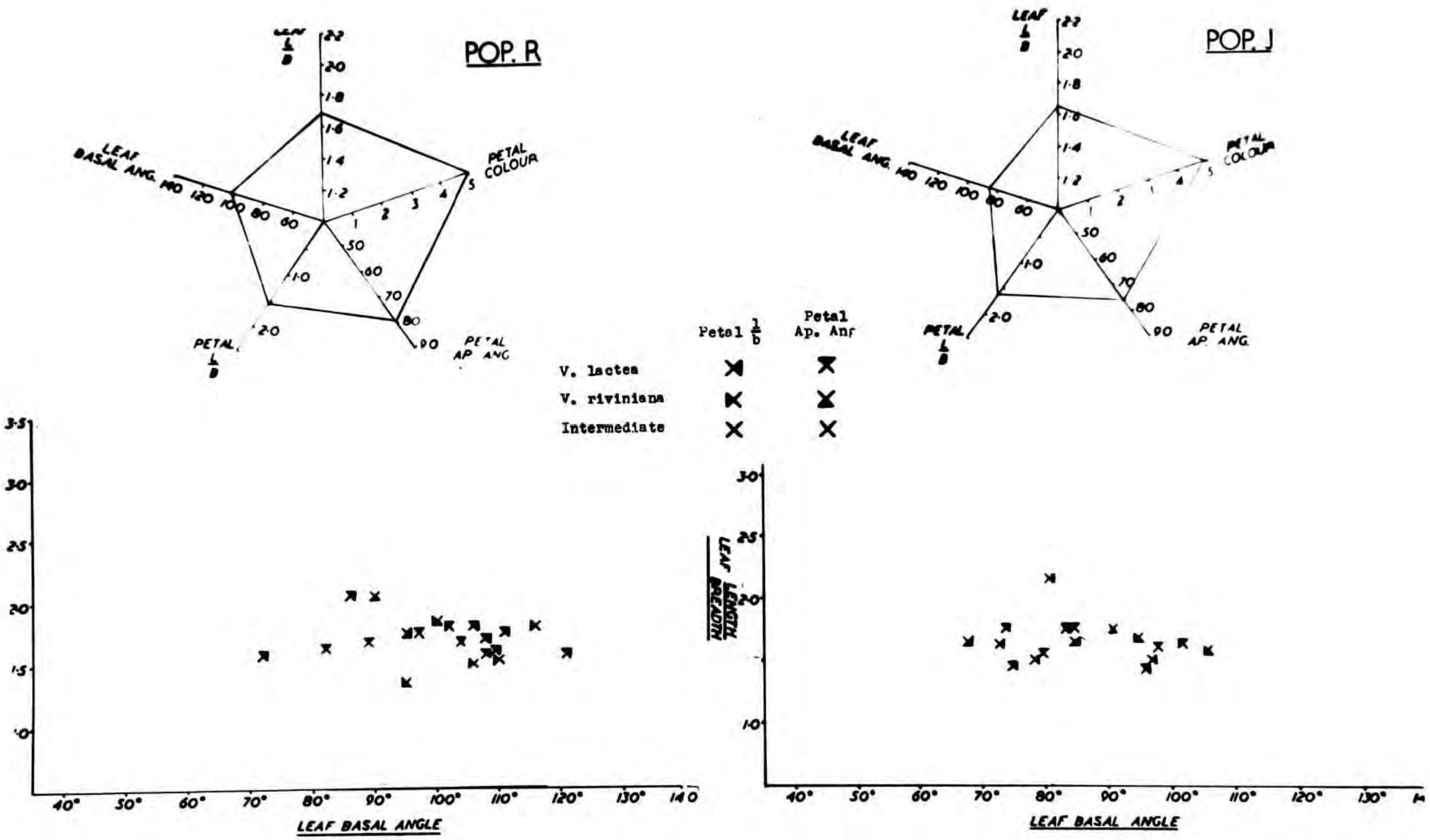


FIG.17. Cyclic polygons and scatter diagrams for population samples D(left) and C(right). Both are included with the FI hybrid V. lactea x riviniana in Group II.

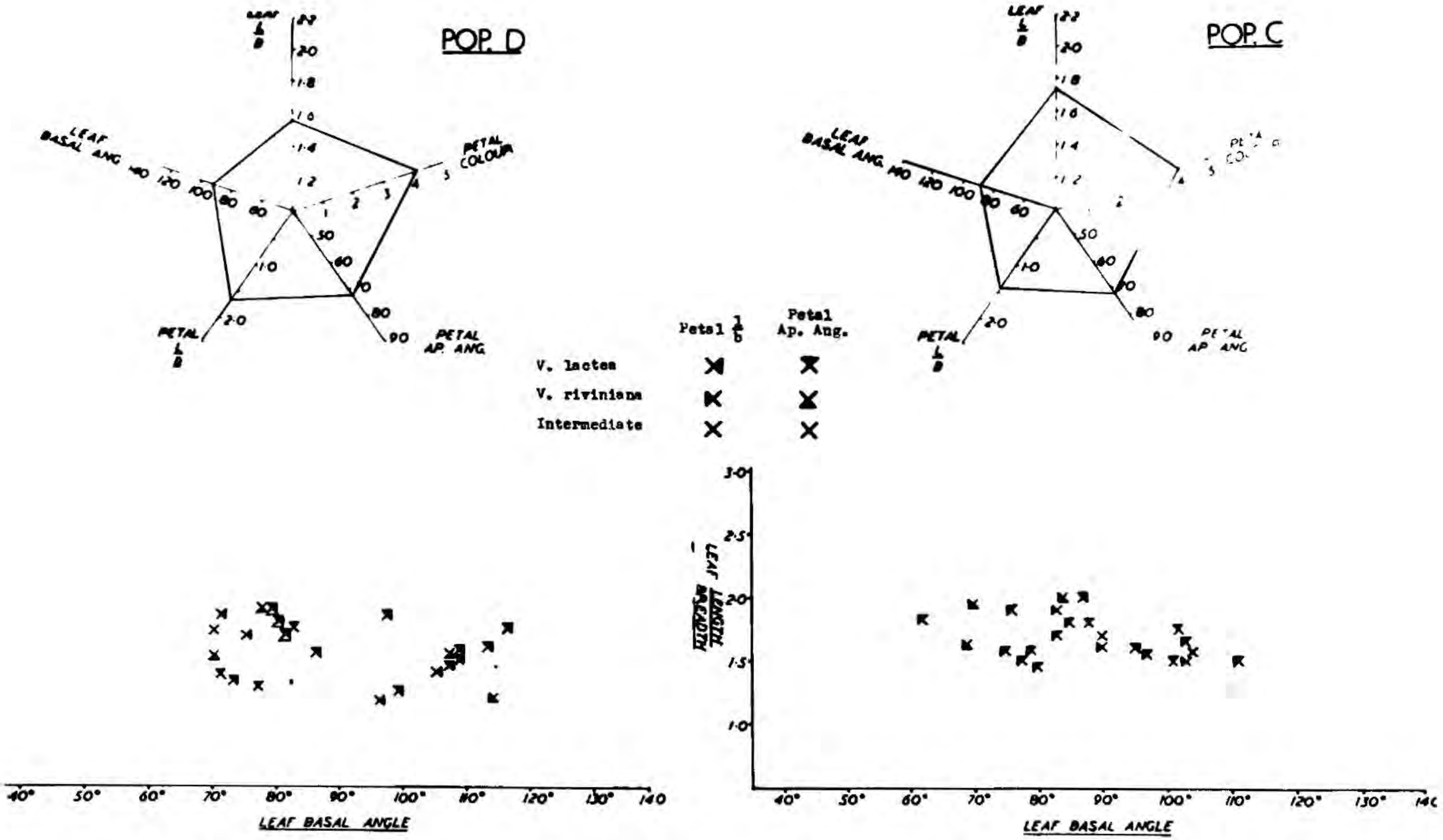


FIG.18 Cyclic polygons and scatter diagrams for population samples D(left) and C(right). Both are included with the FI hybrid *V. lactea x riviniana* in Group II.

less than in others of the group and differs from them in giving two distinct clusters of points. One of these is very close to lactea in leaf shape and three of the plants have affinities with that species in all characters. The other cluster just enters the range of leaf characters given by samples of V. riviniana while the petal shape shows mixed affinities. Field notes suggested the population to be hybrid and the sample was possible taken from one vegetatively spreading clone. However, the plants were not nearly so close together as usual and some typical V. riviniana grew nearby in the rather lactea habitat. This information, in conjunction with the scatter, suggests that it is not a direct F1 so much as a later segregate influenced by the habitat towards V. lactea; it is less likely to be a backcross (See Sect. 3b). Here, then, we see that the polygon shape suggests an aberration which is confirmed, and partially elucidated, by the scatter diagram - a process which is further shown in the next group.

GROUP III.

The polygons included here do not show very close affinities with any of the base shapes but they are all seen to be influenced to varying degrees by V. lactea and V. riviniana. This group contains those samples which are probably the most important for an understanding of the processes involved in the field populations. The samples considered here can be arranged in order of decreasing affinities with V. lactea.

Sample Y (Fig. 19) gives a polygon which lies just outside the range of shapes included in group I and it shows a slight trend

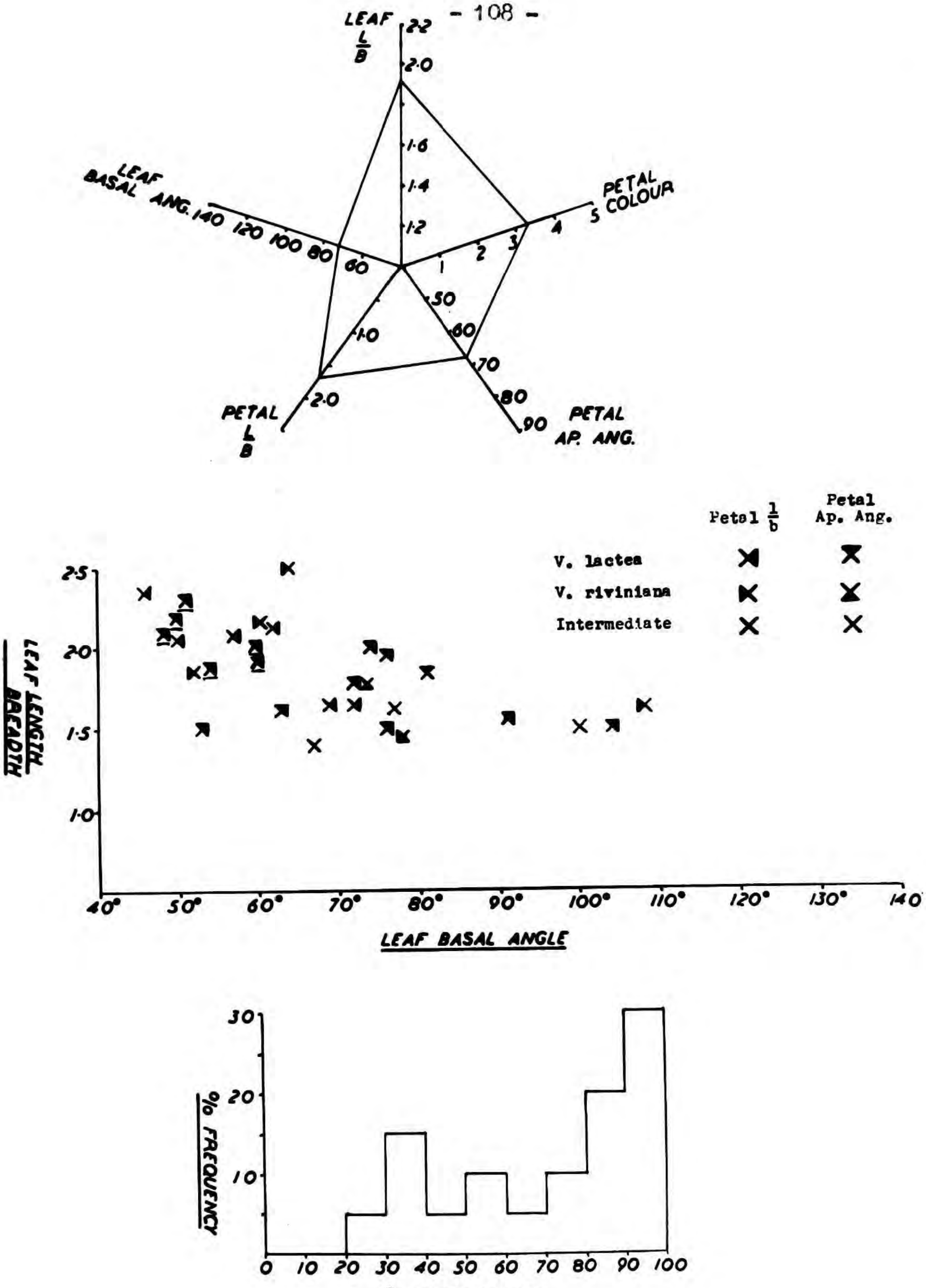


FIG.19 Cyclic polygon, scatter diagram and pollen fertility histogram for sample Y (Group III). Underlined plants are biometrically 'good' V. lactea.

away from this species in having a lower leaf $\frac{l}{b}$ and slightly rivinianoid petal colour. The scatter diagram shows that 11 plants (underlined) are lacteoid in all respects while the remainder have a mixture of characters and four individuals with the highest leaf angle tend towards the characteristics of lactea x riviniana hybrids described in group II.

Pollen analysis for this sample shows (Fig.19) a block of high fertility (80% good pollen) which probably coincides with those plants (35% of the sample) seen in the scatter diagram to have all the characters of lactea. The remaining plants have less good pollen and in the scatter diagram they can be seen to show varying combinations with riviniana characters, hence their low fertility.

Population Q occurred as clumps of floriferous plants in the more open sea heath, typically a lactea habitat, and seemed rather varied on field characters but with a decided tendency towards V. riviniana. Some plants had good capsules, on others they were smaller and misshapen, - long styles indicated their origin from chasmogamous flowers. The polygon (Fig. 20) bears out the above field observations by showing a conspicuous standard error in most characters, (Table 23) though their mean gives a lactea - like shape. Only two plants are shown by the scatter diagram to be outside the lactea range for leaf characters but many (not underlined) show varying degrees of riviniana influence in the petals. This general trend is perhaps more readily seen if each character is

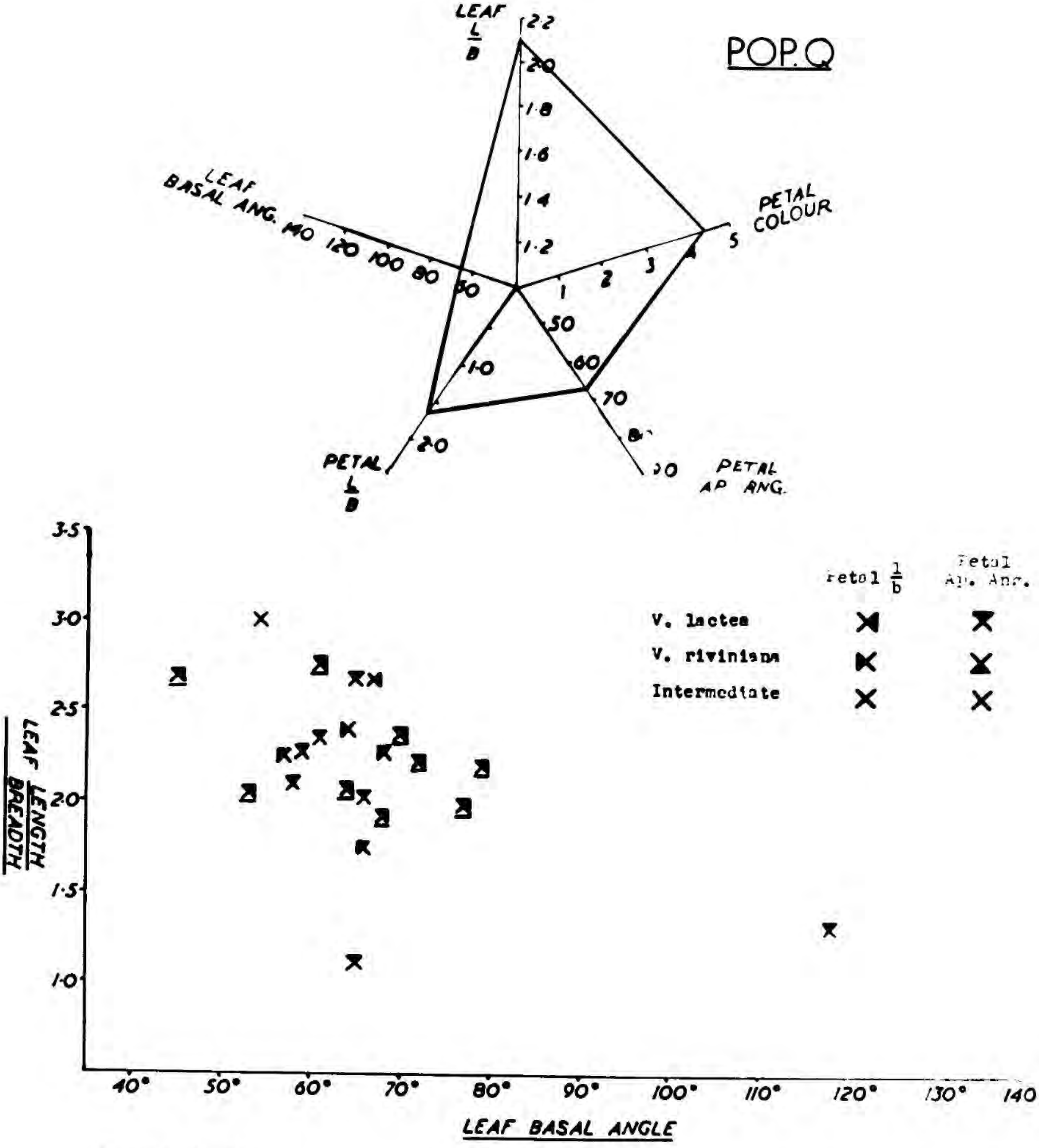


FIG.20 Cyclic polygon and scatter diagram for sample Q (group III). Underlined plants are biometrically 'good' V. lactea.

TABLE 23

Sample	Leaf $\frac{l}{b}$	Leaf Basal Angle	Petal $\frac{l}{b}$	Petal Ap. Angle.
E.	2.334 ± 0.0587	53.8 ± 1.405	1.99 ± 0.0381	59.5 ± 1.027
Q.	2.20 ± 0.0946	66.2 ± 2.928	1.63 ± 0.0265	67.1 ± 1.582

Table 23 Comparison of mean and standard errors of leaf and petal characters between sample of 'pure' *lactea* (E) and sample (Q) showing *riviniana* influence.

scored for its affinity with *V.lactea* (L), *V.riviniana* (R) or intermediate (O), and hence to produce some kind of hybrid index which shows the population to be closely akin to *V.lactea* but tending towards *V.riviniana* - viz. :-

LLLL - 9. LLLO - 7, LLOO - 1, LLOR - 3, LORR - 1.

All this tends to suggest that there is either a backcross to *lactea* or, more probably that they are lactea-like segregates from cleistogamous selfings of a *lactea x riviniana* hybrid or some derivative. (See sect.3b for experimental evidence)

Sample W is near the F1 hybrid *V.lactea x riviniana* in polygon shape (Fig 21) but deviates towards the former species. The population was growing in a habitat more suited to *V.lactea* and the scatter diagram shows $\frac{12}{28}$ of the plants to have strong affinities with this species (these are underlined) Three plants, indicated by r, have three of the four factors scored tending towards *V.riviniana* while the remainder have an admixture of characters tending in varying degrees towards *V.lactea*

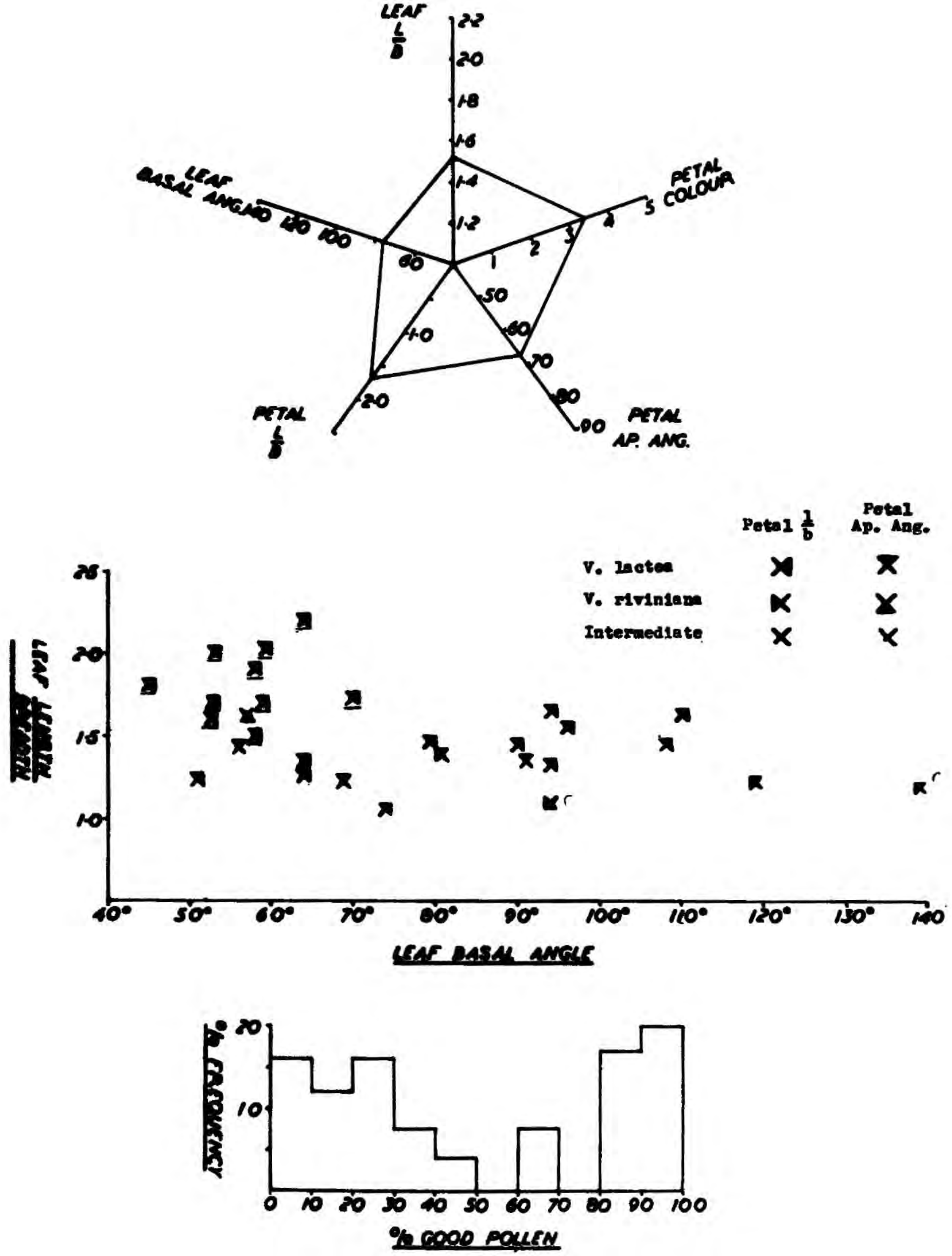


FIG. 31 Cyclic polygon, scatter diagram and pollen fertility histogram for sample W (Group III). Underlined plants are biometrically 'good' V. lactea.

The pollen fertility histogram (fig.21) shows that 36% of the individuals are equally fertile with the parent species; this compares with the scatter diagram where 12 plants, i.e.43% of the sample, show all the characters of V.lactea . 3 plants (10% sample) in the scatter diagram were shown to be very near riviniana and they possibly correspond with the 8% having 65 - 70% good pollen. If the rest of the plants were intermediate segregates they would show less than 40% good pollen, and this is the case.

Sample X follows on logically from the preceding one since it shows steadily more riviniana influence. The population grew in a clump of Empetrum and on field determination seemed influenced by V.riviniana. The polygon shape (Fig 22) agrees with that for V.lactea x riviniana in fig.11 but this sample was not included in group 11 because its comparison with the preceding population is most useful.

In the scatter diagram (Fig 22) most of the plants are intermediate in leaf characters between the parents and show petal characters with mixed affinities, much as in an F1 hybrid; four individuals (underlined) are identical with V.lactea. This is very strongly supported by pollen analyses which show (Fig. 22) 20% of the sample to have 80% good pollen, thus comparing very well with the four plants (19% of the sample) in the scatter diagram which resemble lactea on morphology. The remainder of the sample show low fertility and correspond to the high number of almost directly intermediate individuals figured in the scatter diagram.

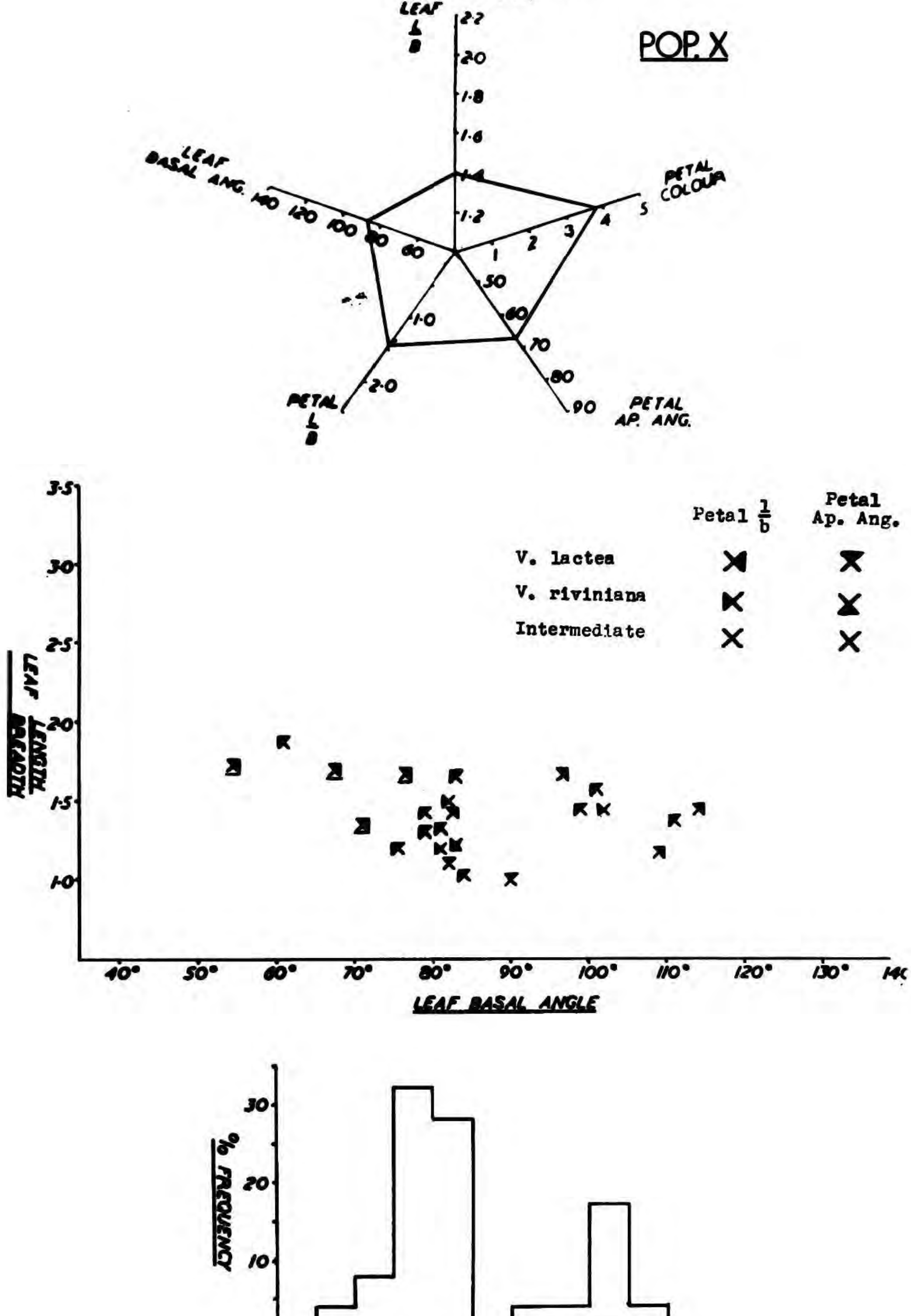


FIG.24. Cyclic polygon, scatter diagram and pollen fertility histogram for sample X (Group III). Underlined plants are biometrically 'good' V. lactea.

The trend towards V.lactea in population W and away from it in this one seem to be due to the different habitat factors involved. Thus the rather closed conditions of Z encourage the selection of riviniana characters in hybrid segregates. However, when applying the converse to selection of lactea characters in the more open habitats it must be remembered that V.riviniana was shown (Sect. 5) to tolerate such conditions. This tendency would be compensated for by the greater availability of V.lactea as a backcross parent in later generations but it must be borne in mind when examining such populations.

The polygon for sample Z (Fig 23) shows a marked trend away from the typical lactea x riviniana by reason of its large basal angle, lower leaf $\frac{l}{b}$ and smaller petal $\frac{l}{b}$. The population occupied a large hummock covered with a fairly dense stand of vegetation dominated by Calluna and Molinia caerulea so that in the event of segregation from cleistogamous seed of a hybrid, selection would be expected in the direction of V.riviniana

Although the general range of points on the two major axes of the scatter diagram for pop. Z (Fig.23) corresponds reasonably well to that shewn by lactea x riviniana samples in group, II, it tends further towards V.riviniana . In addition, no points show more than a single lactea character, and none have lactea leaves.

All the samples so far considered have been directed towards showing that introgression is taking place from V.riviniana to V.lactea.

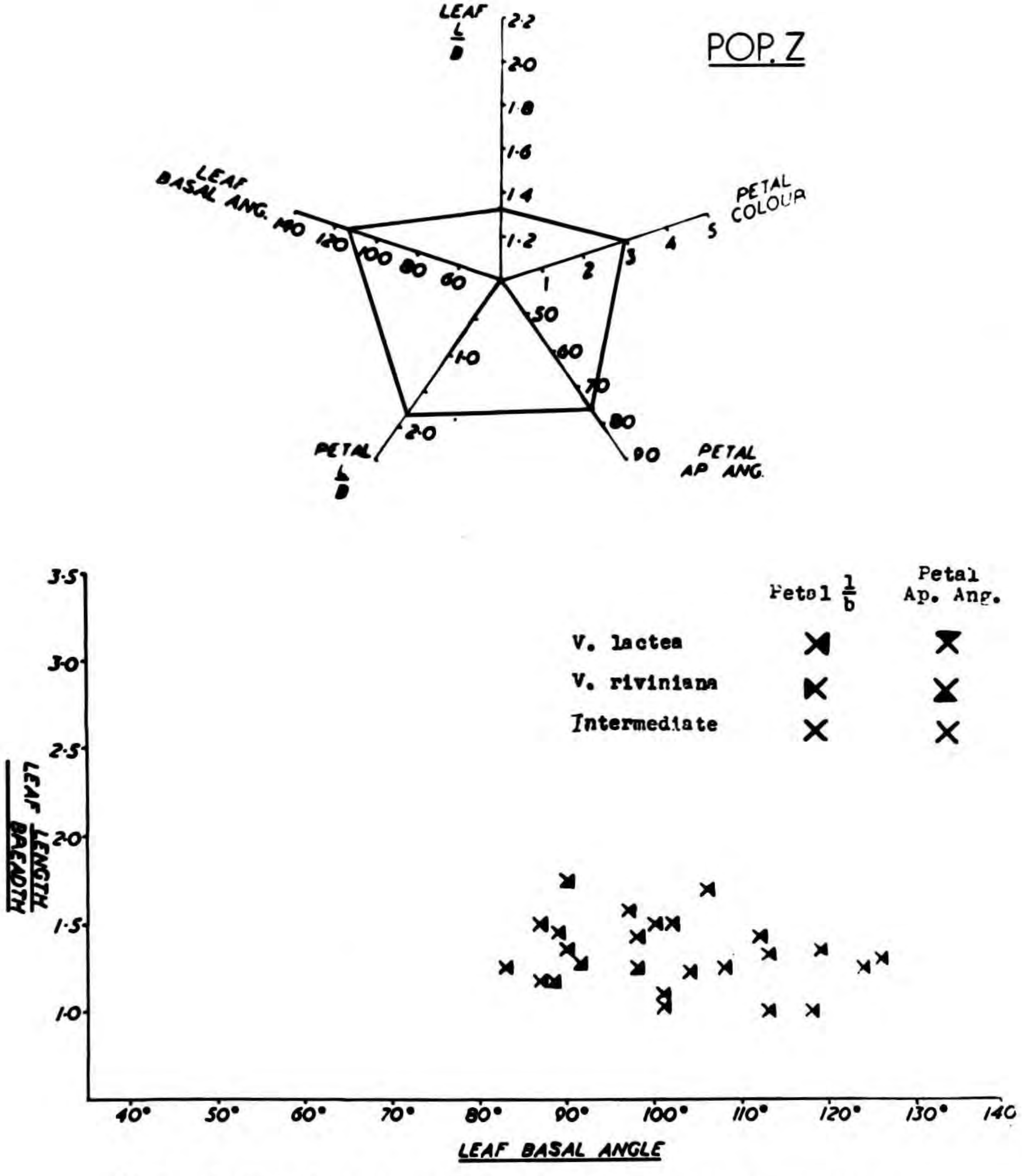


FIG.23 Cyclic polygon and scatter diagram for sample Z (Group III).

This population, and, to a less advanced extent, population X indicate that there is also a drift towards V. riviniana in a suitable habitat, and that lactea influence is being taken across.

GROUP IV.

Fig. 24 shows cyclic polygons drawn for three samples from populations of V. riviniana as it occurred in habitats near those occupied by V. lactea. Two agree closely with the basic shape for a known sample in Fig. 11 but a third, G, seems to be very lacteoid in petal characters. The scatter diagram, inset in Fig. 25 shows that most plants occur within the range of leaf shape for V. riviniana but all have some lactea influence in the petals. This population, like Z and X, tends to confirm that introgression does take place from V. lactea to V. riviniana under certain conditions.

Since V. riviniana plays an important part in wild hybrid populations a scatter diagram is given in Fig. 25 for sample F, and it is shown in relation to V. lactea and their interspecific hybrid.

GROUP V.

This group comprises a single sample taken from the only population (N, at Hooper's Point, Pembs.) found to contain the natural hybrid between V. lactea and V. canina. The sample gives a polygon (Fig. 24) agreeing with that shown in Fig. 11 for the artificial hybrid growing in the garden. From their close parallel it seems likely that the wild hybrid is near the F1, this also seems probable on field characters.

The habitat had been much disturbed during the construction of a war-time airfield thus probably accounting for the breakdown of

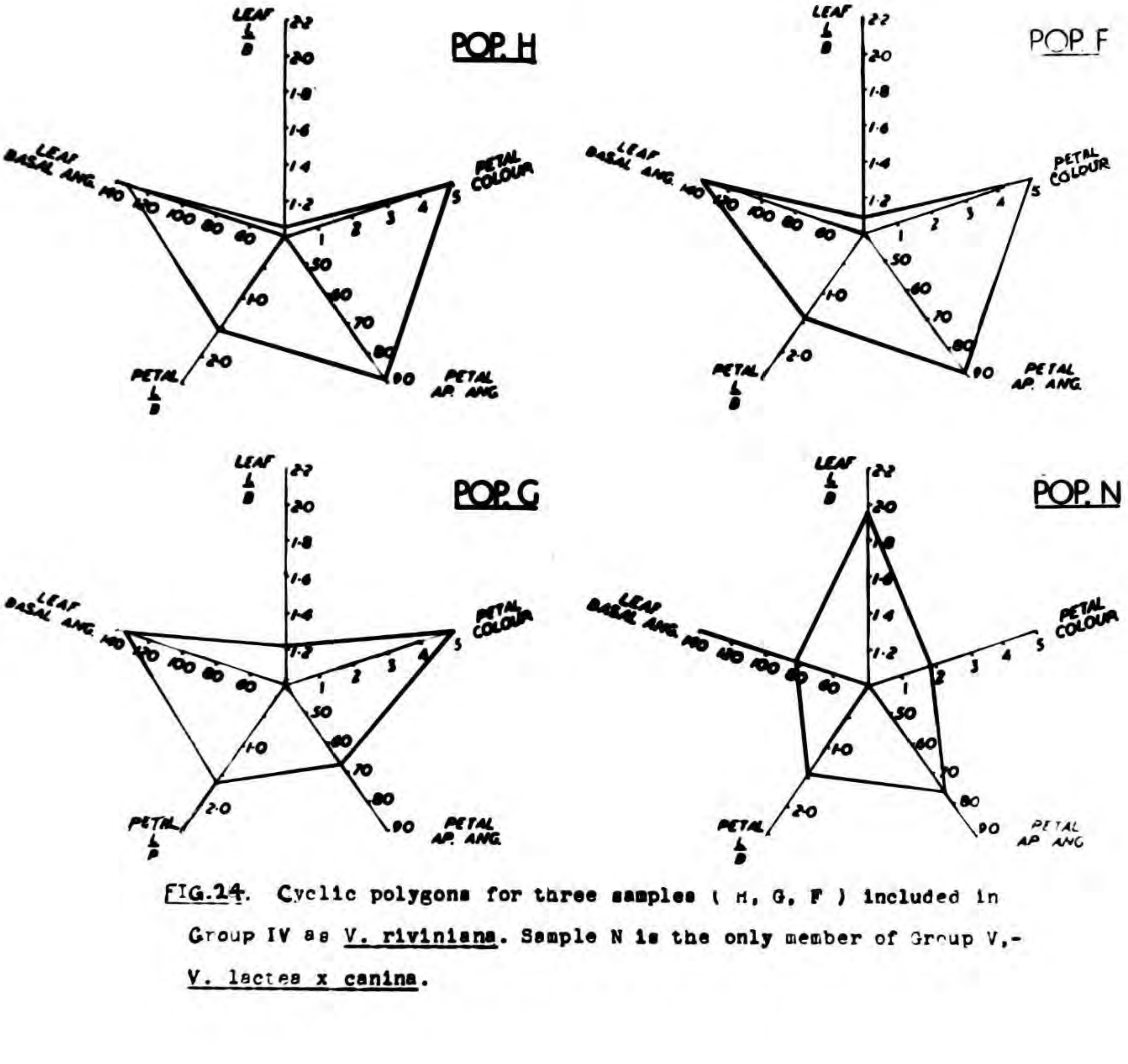


FIG.24. Cyclic polygons for three samples (H, G, F) included in Group IV as *V. riviniana*. Sample N is the only member of Group V,- *V. lactea x canina*.

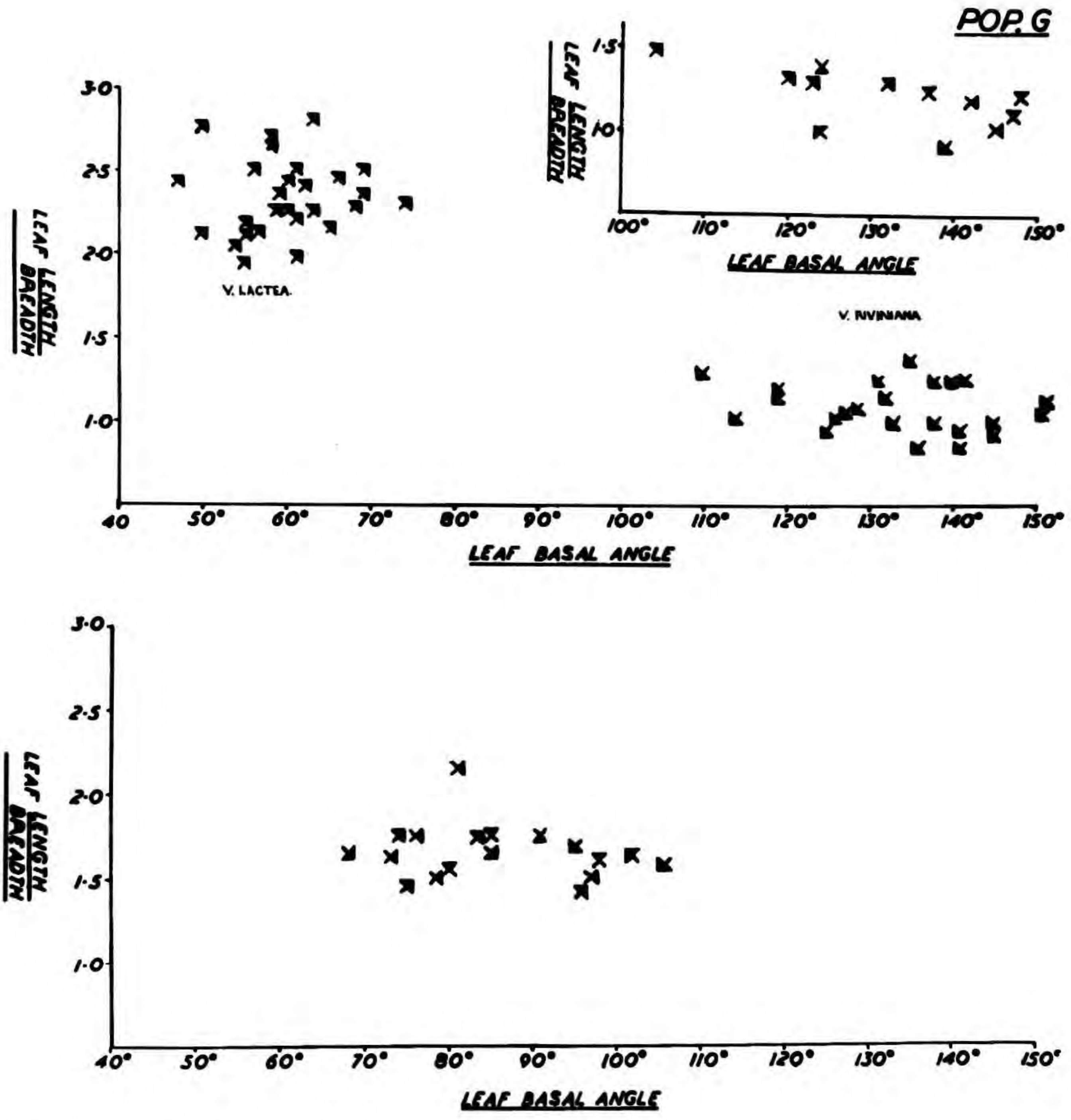


FIG. 15. Scatter diagrams showing the relationships of the main types of population considered. *V. lactea* (O) and *V. riviniana*(F) above, inset is the aberrant *riviniana* sample G. Below is shown the wild FI hybrid between *V. lactea* and *V. riviniana*.

ecological isolation (See sec. 5) between V. lactea growing over old red sandstone and V. canina seemingly restricted here to the close turfs on boulder clay drift deposits.

In view of the high fertility of this hybrid noted earlier (Sect 3a) it was thought that the canina population flanking the hybrid clumps may show some signs of introgression. Table 24 compares mean and standard errors of the leaf and petal characters measured in normal populations of V. canina with the population found at Hooper's Point. It shows that the two sets of means are much the same with canina at Hooper's Point showing, if anything, less affinity with lactea ; the standard errors are of the same order in both samples. It would seem of little use examining the lactea populations here since hybrids with V. riviniana have also been found and no conclusive results could be obtained. From the canina considered it seems that there is little introgression detectable between this species and V. lactea.

TABLE. 24

Sample.	Leaf $\frac{l}{b}$	Leaf Basal Angle	Petal $\frac{l}{b}$	Petal Ap. Angle.
L	1.49 ± .02678	107.9 ± 1.49	1.47 ± .02225	76.3 ± .7994
SS	1.56 ± .057	108.5 ± 2.34	1.42 ± .02132	80.7 ± .9046

Table 24 Comparison of means and standard errors of leaf and petal characters between 'pure' V. canina (S) and a sample of this species from Hooper's Point.

Possibly the disturbance of the habitat was too recent to allow extensive hybridization, but it is a most unexpexted result in view of experimental data. In this it may be compared with the results obtained by Avers (1953) who found in Aster that the species A. shortii and A. cordifolius were highly fertile in the greenhouse but was unable to detect any sign of introgression in mixed populations in the field.

It is to be stressed that field hybridization between V. lactea and V. canina may be more important than suggested here but it does look as though they are, in general, sufficiently separated by ecological preferences to prevent their crossing. If a few more localities could be found where these two species do overlap then biometrical techniques could be applied much as in the case of V. lactea and V. riviniana which have been shown to hybridize widely. However, most of the major British areas for V. lactea have been visited and V. canina is not associated with it anywhere else but the one place mentioned.

GROUP VI.

Here are grouped four samples (Fig. 26) from populations of V. canina in various localities. All give polygons seeming to be very similar whether collected from dunes or inland heaths, from North or South England, and all agree with the polygon in Fig. 11 drawn from a family of cultivated V. canina. It was thought that introgression from V. lactea to V. canina might be detectable in

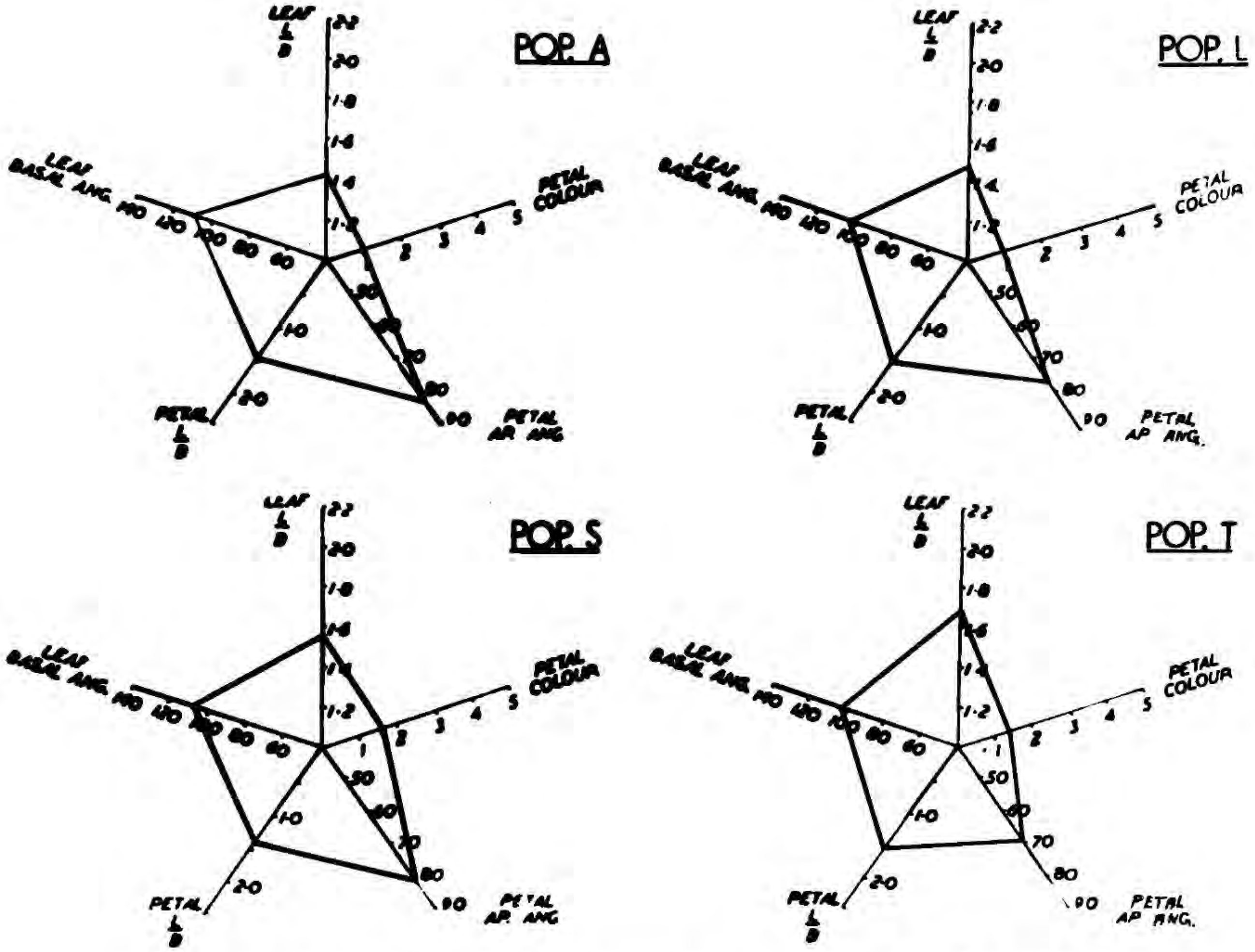


FIG.26 Cyclic polygons for those samples included as V. canina in Group VI.

population L from Hooper's Point where sample N - V.lactea x canina - was found, but no evidence of this could be obtained.

(d) Discussion

It has been shown that the methods developed above (sect. 6b) enable one to separate with confidence populations of the three species V.canina, V.lactea and V.riviniana, and their interspecific hybrids. On this basis it was possible to deal with all field populations encountered and a fairly constant and reliable picture emerged from the results.

Despite earlier reports (sect. Ic) that the hybrid V.lactea x canina occurred fairly frequently and the evidence of its high fertility (sects. 3a & 4c) only in one case have these two species been found growing together and hybridizing. The biometrical analyses showed all the remaining field hybrids involving V.lactea to be between this species and V.riviniana, with which it overlaps ecologically (sect. 5.)

In the field both parents and the F1 hybrid are easily distinguished while a number of populations, especially in lactea habitats, cannot be identified with certainty. The groupings possible on the analytical techniques used indicated that there were many stages of hybrid population between V.lactea and V.riviniana, these are clearly set out in groups I - IV in sect. 7c and the resultant pattern strongly suggested the occurrence of introgressive hybridization as defined by Anderson (1949) with the main trend being towards V.lactea .

The most obvious conclusion is that backcross pollination of the F_1 hybrid is being effected by one of the parent species and that in this way genes from V.riviniana are carried into V.lactea. But, as we have seen (sect.3b) artificial backcrosses have not so far been successfully achieved to either parent and the F_1 hybrid is highly sterile. At this stage the problem is very similar to that faced by Heiser (1951) when he reported biometrical evidence of introgression in natural populations of Helianthus Annuus and H.debilis var. cucumerifolius but found their artificial hybrid to be one of very low fertility.

However these violets are in rather a special position since in summer and early autumn the plants produce large numbers of cleistogamous flowers, each of which constitutes a chance to achieve self-fertilization. Observations in the greenhouse (sect.3b) indicated that the F_1 hybrid only very occasionally produces any viable seed by selfing but with this must be taken into consideration a number of facts. In almost every locality for V.lactea which I visited the conspicuous floriferous clumps of its hybrid with V.riviniana were to be found. These plants bore many more cleistogamous flowers than either parent, are able to propagate extensively, and seemingly indefinitely, by means of vegetative soboles and very probably possess the longevity normally associated with heterotic vigour in hybrids.

Once V.lactea and V.riviniana cross, a not infrequent occurrence from field observations, all the above-factors will tend to increase the chance of selfing so that a single hybrid may in fact produce much more seed than indicated by the few greenhouse plants. With the main sterility barrier of the F1 hybrid crossed it is easy to vizualize that subsequent plants will be more fertile so that cleistogamous selfings can occur more readily and backcross pollinations may be effected. Some evidence for this last comes from population Q which shows every biometrical indication of being intermediate between V.lactea and V.lactea x riviniana and several of the plants when collected were found to possess irregular capsules obviously produced by cross pollination since they had the long chasmogamous style still attached to the fruit.

Introgressive hybridization is usually associated with disturbed habitats since not only does this enable normally allopatric species to come into contact but it provides an open environment in which the hybrids can gain a foothold, as in the primary stages they might not be so well fitted to compete in parental communities. It was noted (sect.5a) that V.lactea characteristically favours open habitats so that any hybrids would be most likely to come into contact with this species. As a result much of the introgression is seen to be directed towards V.lactea , though the reverse process often occurs and V.riviniana shows lactea influence in populations G (p. 117) and Z(p. 115).

This work strongly suggests that the ecological overlap between V.lactea and V.riviniana noted earlier (sect.5) provides ample opportunities for introgressive hybridization between them and details of this process are revealed by the biometrical analyses employed. By means of greenhouse experiments and observations it has been possible to suggest a mechanism by which this could be effected and which would satisfactorily fit in with the biometrical data.

The hybrid *V.lactea x canina* has been shown to occur very rarely in the field since the parent species have, in general, distinct habitat preferences and only abnormal environmental conditions bring them together.

(8) GENERAL DISCUSSION AND CONCLUSIONS.

It has been seen that, after much early uncertainty, V. lactea was separated as a species distinct from V. canina. From genomic relationships determined by the cytological study of hybrids it seems probable probable that V. lactea is an allohexaploid derived from the tetraploid species V. canina and another, as yet unknown, diploid species.

V. lactea differs most strikingly from V. canina in having a cuneate leaf-base, large and coarsely dentate stipules and narrow, pale lilac petals which are 3-4 times longer than broad. From the data given in Section 4 on the F2 generation from the interspecific cross V. canina x lactea it seems that these distinguishing characters result from the action of genes carried in those extra chromosomes of V. lactea which form univalents at meiosis in the F1 hybrid and tend to be lost in later generations. This information enables a reasonable guess to be made as to the morphology of this unknown putative parent and, once found, positive proof could readily be furnished by crossing it with V. canina and V. lactea and examining meiosis in the hybrids. It is not yet yet known where, or indeed whether, such a species exists but sufficient data have been collected to make profitable and not altogether unfounded a certain amount of speculation on this subject.

V. canina is of a widespread eurasiatic distribution while V. lactea is much more restricted. While the barrier primarily separating the two species seems to be climate, along their boundary, as in Britain, the main isolating factors appear to be edaphic. Strong indications were given that V. lactea is very susceptible to competition from other plants and its present rather discontinuous distribution may merely represent the availability of suitable open habitats. The possibility that its range has once been greater and that it has been reduced by subsequent closing up of the plant communities could only be properly investigated by reference to the historical evidence of pollen analysis etc., but so far no worker has been able to assign anything less than generic rank to Viola pollen from fossil deposits. The open habitats favoured by V. lactea may suggest that it had the best chance of survival if it arose after the retreat of one of the Pleistocene ice-sheets when the open and disturbed conditions provided a suitable environment for the stabilization of a newly formed allopolyploid. This must again, however, remain pure conjecture.

The distribution of V. lactea resembles in many ways that of several Narcissus species studied by Fernandes (1951); there is not space enough here to discuss Fernandes' work in detail, but he concludes that many of these species have never extended much beyond the Iberian Peninsula and we may note N. triandrus var. Loiseleurii which reaches the Iles Glenans off the French coast at Finistère. There is thus a case for believing that V. lactea may have followed the same migratory routes as those postulated by Fernades and, in this case, would never have had a much wider range than at present.

If this is so, it seems likely that the other putative parent must be sought in southern Europe or further to the east. In that region, it may be noted, V. canina does not extend further westwards than the eastern regions of Portugal - the provinces of Beira Alta and Tras os Montes. The unknown species was possibly of fairly wide distribution at the time of its hybridization with V. canina and may be expected to have later become restricted to small areas where it still exists, or to have vanished altogether.

Such conjectures as these are based on the comparable example providied by the suggested origins of the hexaploid wheats. Percival (1921) has given evidence that they come from a cross between a tetraploid wheat and *Aegilops ovata* or *A. cylindrica*. In fact Popova (1923) actually found in Turkestan great numbers of hybrids of *Triticum vulgare* with *A. cylindrica* and *A. crassa*. More recently, McFadden and Sears (1946) have shown that the hexaploid *Triticum spelta* arose by doubling of the chromosome number in the hybrid between *Aegilops squarrosa* and the tetraploids *T. dicoccoides* or *T. dicoccum* and postulate that the ancestral hexaploid wheat arose, probably fairly recently, in south-eastern Europe or south-western Asia.

After searching the available information for a clue to the identity of the other possible parent only oneespecies, *V. pumila*, which belong to the Arosulatae, has the requisite morphological characters viz. large stipules, lanceolate leaves with cuneate bases and pale lilac flowers with narrow petals. However, indications are that this species is not a diploid, Gershoy(1934) reported its chromosome number as being 2n= 40, most probably for material from N. Europe as supplied by Clausen, and I have recently been able to confirm this number in root-tips of plants collected on Oland. *V. pumila* extends from Scandinavia to E. France and it is possible that races with 20 chromosomes exist in the southern localities but such a conjecture does not contribute materially to the present problem.

Even if, as seems likely from the data at present available, *V. pumila* is not the other putative parent of *V. lactea* it can be reasonably surmized from their morphological resemblances that they may have the missing diploid species as a common ancestor. If such is the case then they may possibly have originated to the N.E. of the Iberian Peninsula, *V. pumila* having been able to extend its range northwards while *V. lactea* spread to the south and westand has later become confined to open habitats in oceanic areas by a closing up of formerly suitable communities. Crossing experiments described in Section 3c have been successful in producing hybrids between *V. pumila* and both *V. canina* and *V. lactea*; it has not yet been possible, however, to study the cytology of these hybrids.

V..lactea,having arisen by allopolyploidy from *V. canina*, is kept apart from it by a combination of climatic and edaphic factors and ,, with very occasional exceptions, the isolation is complete. However, much confusion regarding the status of *V. lactea* has arisen because of the variety of forms which it appears to show in the field. Biometrical studies of wild populations including *V. lactea*have shown that it is undergoing introgression with *V. riviniana* which has a wide tolerance of the climatic and edaphic factors involved. Although their artificial interspecific hybrid is of low fertility, a mechanism by which the sterility barrier could be crossed was suggested in the discussion at the end of Section 7; data supporting this were provided by greenhouse observations and cytological studies on wild hybrid plants.

Assuming that introgression is taking place it is interesting to consider the effect which such an influx of genes from *V. riviniana* might have on *V. lactea*. In those populations postulated as showing the later stages of introgression it was seen that the plants tended to bear a very close resemblance to *V. lactea* in external characters while possessing additional properties, of which the most important are - ability to reproduce vegetatively by means of soboles and the capacity to withstand a good deal of competition from other plants. It has been pointed out that the open character of habitats occupied by *V. lactea* is due to biotic influences, principally to interference with the plant cover by man's activities. A change in the practice of heath burning, for example, would lead to a closing up of the plant communities so producing conditions which *V. lactea* would be unable to withstand. A graphic example of this tendency is presented in Portugal (see Appendix B)- where soil erosion is being checked by large-scale re-afforestation with *Pinus pinaster*; this policy is resulting in the closing up of habitats formerly favourable to *V. lactea* which is therefore being eliminated from many localities.

If, as was suggested, the distribution of *V. lactea* is partially dependent upon the availability of open habitats it seems likely that it has always been sensitive to a gradual closing up of the environment, a process which is not likely to decrease in the future. Following on from this idea it can now be seen how important the introgression with *V. riviniana*could be to the further survival of *V. lactea*. The additional characters would enable it to adapt itself to the new conditions and to compete on a more equal footing with the other species of the closed communities. It is not yet possible to describe in any detail the Portuguese populations but it may be said that *V. lactea* in the pinewoods of Portugal often resembles those British populations of this species which have been modified by introgression. It is clear that the future couse of variation and evolution in *V. lactea* is likely to be much influenced by the habitats available.

APPENDIX A.

Use of discriminant analysis in the classification of hybrid violet populations involving three species.

A procedure for the multivariate analysis of five characters per plant was applied to population samples for each of three species - *V. canina*, *V. lactea* and *V. riviniana*. The method adopted was taken from Hartree (1952) and Rao (1952) p. 317) and by this means linear expressions were obtained from which values for the discriminant function (L) could be obtained for any sample involving any one or two of the above species.

In table 20 are given values of L for those samples used as standard standard shapes and illustrated in Fig. 11. It is obvious that this function is markedly different for each of the three species, while the F1 hybrids have distinctive values much as would be expected. The significant difference for these factors have been calculated as

TABLE 20.

Species or hybrid	Discriminant Function (L)
V. lactea (Pembs.)	1330
V. lactea (Cornwall)	1464
V. lactea x canina	2021
V. canian	3301
V. lactea x riviniana	2733
V. riviniana	4253

Table 20. Values of the discriminant function (L) for known samples of species and hybrids.

suggested by Hartree and give positive results for all the values in Table 20, including the demonstration of a significant difference between the two samples for different samples of V. lactea.

It seems , on this trial, that the characters available for biometrical studies in these violets are not suitably balanced for the use of discriminant analysis but this type of approach may well be applicable when, for instance, more intensive considerations are needed of the individuals in a single population.

APPENDIX B.

Preliminary observations on V. lactea in C. and N. Portugal.

In April 1957 I was able to visit Portugal in order to study the status of V. lactea towards the limit of its range in Southern Europe. Coimbra was used a base and most of the known localities for V. lactea in the province of Beira Litoral were visited. Ecological observations were carried out and populations sampled using the biometrical techniques described in Section 6. It was possible to visit also North Portugal and populations weresttstudied in the neighbourhood of Porto and on the Serra de Gerez near the Spanish frontier.

In almost every case V. lactea favoured light soils overlying sandstones but in one area, at Leonte on the Serra de Gerez, the base rock was granite. Field analyses indicate that all these soils were acid and they are very probably of low base status.

The types of habitat were found to vary somewhat, but they could be divided into two main groups. In the higher localities, eg. Tovim (1100m.) on the Serra da Lousa, V. lactea occupied the open conditions afforded by eroding peat hags which bore a short heath reminiscebt of the habitats in S.W.Britain. The principal associated species being - Ulex nanus, Arrhenatherum elatius, Calluna vulgaris and Erica cinerea. At lower altitudes, at which are found most of the localities, the high summer temperatures and aridity prevent V. lactea occupying the more exposed and open parts of the heaths. Because of the rapid erosion of these lowland heaths they are now extensively planted with Pinus pinastre and Eucalyptus sp. (principally E. globulus) and V. lactea is found in the short, broken heathy tracts of the plantations.

Table 21.

1. Catraia (920m.), Serra de Lousa : Beiral Litoral.
2. Estacao do Lorrical, S. of Figueira da Foz : Beira Litoral.
3. Sao Gens, 3km. E. of Porto, : Douro.
4. Leonte, Serra do Gerez : Minho.

	1	2	3	4
Viola lactea	r	o-lc	o	o
Agrostis setacea Curt.	-	a	o-lc	-
Andryola integrifolia L.	-	-	x	-
Anemone trifolia L.	-	-	-	o
Anthemis repanda L.	-	-	x	-
Arrhenatherum elatius (L) Presl.	f-lD	-	a-lD	-
A. pallens Link.	-	-	-	o-c
Asphodelus lusitanicus P.Cout.	-	c-lf	-	f
Brachypodium silvaticum R.&Sch.	-	-	-	o-lc
Calluna vulgaris L.	f-lD	o	o-c	-
Carex asturica Boiss.	-	-	o	-
C. depressa Link.	o	o	-	-
Carlina racemosa L. or corymbosa	-	r	-	-
Cirsium tuberosum All.	-	o	-	-
Cistus crispus L.	-	-	x	-
C. villosus L.	-	-	x	-
Cuscuta epithymum Murr.	-	-	c	-
Daphne gnidium L.	-	o	-	-
Erica arborea L.	-	-	x	o-lc
E. ciliaris L.	lc	o	c	-
E. cinerea L.	f-a	o-lc	-	o
E. umbellata L.	-	-	o	-
Erythraea centaurium Pers.	-	r	-	-
Galium cruciata Scop.	-	-	-	x
Genista triacanthus Brot.	-	o	a-lD	-
Hypochaeris glabra L.	-	-	x	-
Leontodon tuberosus L.	-	-	o-c	-
Lithospermum diffusum Lag.	o	lc	c	o
Luzula campestris Gay.	-	-	-	a-lD
Montia lusitanica Samp.	-	-	-	r
Myrtus communis L.	-	o	-	-
Narcissus triandrus L.	-	-	-	lc
Pedicularis sylvatica L.	o-r	-	o	-
Pinguicula lusitanica L.	-	o-r	-	-
Pinus pinaster Sol.(seedling)	-	-	x	-
Pirus communis L.	-	o	-	-

(Table 21 contd.)

	1	2	3	4
Plantago lanceolata L.	-	r	-	-
P. major L.	-	-	-	o
Polygala vulgaris L.	o	-	o-c	o-c
Potentilla erecta Hampe.	o	-	-	-
Pulicaria odora (L.) Rchb.	-	r	-	-
Pteridium aquilinum L.	-	c	o	c
Ranunculus adscendens Brot. ?	-	-	-	o
Romulea bulbocodium (L.) Seb.& Mauro	-lc	-	-	-
Rubus sp.	-	-	o	o
Scilla hispanica Mill.	-	-	-	r-o
S. monophylla Link.	-	-	r	-
Scorzonera humilis L.	-	o	-	-
Sedum anglicum L.	-	-	-	r
Simethis planifolia Gr.& Godr.	-	o-lc	o-c	-
Stachys officinalis Trev.	-	o	-	-
Teesdalia lepidium D.C.	-	-	-	o
Ulex europaeus L.	-	-	c	-
U. micranthus Lge.	-	-	-	o
U. nanus Forst.	f-lD	-	-	-
Viola riviniana Rchb.	o	o	-	o-lc

Table 21. Species associated with V. lactea in several localities representative of its range in Portugal.

Most commonly it occurs in the damp gulleys where wind, water or biotic factors provide erosion surfaces where it is free from too much competition. Table 21 lists associated species from localities representative of its range in Portugal.

As is to be expected, such conditions favour *V. riviniana* and hybrids between this species and *V. lactea* were frequently encountered. In one pine plantation near Miranda do Corvo, about 5km. S.E. of Coimbra, V *V. lactea* occurred along the unstable rim of an erosion ravine while *V. riviniana* grew in the wet conditions at the bottom, about 15 ft. below; large patches of the floriferous hybrid between these species extended along the walls of the ravine about midway between the two parents.

About nineteen populations were sampled but the material has not ~~yet~~ been analysed. It is strongly suspected, however, that introgression involving *V. lactea* and *V. riviniana* will be demonstrated in many of the populations, especially in view of the success of *lactea*-like forms in the closed communities often found. It is certain that *V. lactea* no longer occurs in many former localities and this is probably due to the closing up of the ground flora resulting from the reafforestation policies and it will be most interesting to examine the structure of populations from areas threatened by the same fate. The results gained may well have a direct bearing on the speculations arising from the study of populations of *V. lactea* in Britian and give yet more clues as to the future status of this species.

APPENDIX C.

Analysis of soil samples; methods employed.

During the ecological studies on the habitats of V. canina and V. lactea samples of soil were collected from around the roots of these plants and packed in air-tight canisters. On return to the laboratory the soil was air-dried and analyses for pH reaction and exchangeable bases.

pH was measured by means of a wet electrode pH meter. About 20ccs. of the soil were shaken up with 80ccs. of distilled water and the suspension balanced against a known buffer. Two readings were taken at an interval of a minute and the results so obtained were accurate to the nearsest 0.1.

Exchangeable bases were fisrt extracted from the soil using William's method; the procedure being as follows :-

1. 25ccs. of air-dried soil were crushed so that it could pass through the meshes of a 2mm. sieve.
2. Add 150 ml. of N/2 Acetic acid to the soil.
3. Shake for 30 minutes.
4. Settle or decant through a filter funnel.
5. Add a further 150 ml. acetic acid to soil.
6. Shake for 20 minutes.
7. Transfer soil and liquid to funnel.
8. Pour N/2 acetic acid over soil in funnel to make volume up to 500mls. of extract.

This extract was then analysed for bases as follows :-

Calcium : Using 400ml. of the leachings, add 5ml. of concentrated ammonia and 10 gms. of ammonium chloride and boil the solution. When boiling add 1 - 2 gms. of ammonium oxalate and continue boiling for about one minute. Allow to stand overnight. The precipitated calcium oxalate is then filtered, washed well with hot water and dissolved in dilute sulphuric acid (25 mls. dilute acid to 100 mls. water). This solution is then titrated against $\frac{N}{10}$ potassium permangante.

1cc. $\frac{N}{10}$ potassium permanganate = 0.0028 gms. calcium oxide.

All results were expressed as milli-equivalents per 100 gms. of air-dry soil. eg. 0.400% Exch. Ca. is equivalent to 400 m.gms. of exch. Ca per 100 gms. In terms of m. equivalents this becomes $\frac{400}{20}$ or 20 milli-equivalents exch. Ca per 100 gms. soil.

Sodium and Potassium. Attempts were made to analyse the soil samples for these two metal bases but no positive results could be obtained. The method used was take about 10 mls. of the extract in acetic acid described above and analyse in a flame photometer; the instrument having been standardized against known solutionssof salts of these metals.

SUMMARY

1. *V. lactea* was given specific rank by Smith (1798) and may be separated from *V. canina* L. on grounds of morphology, ecology, geography and cytology.

2. The most important specific morphological characters are - leaf and stipule shape, flower colour, petal shape and style form.

3. *V. canina* has a widespread, eurasiatic distribution; *V. lactea* is much more restricted, extending from S.W.Britain down the west coast of Europe to C. Portugal.

4. The barrier primarily separating these species seems to be climate; when they meet, as in Britain, the main isolating factors appear to be edaphic. The most important of these is probably the base status of the soil; exchangeable calcium is high in soils with *V. canina* and low in those with *V. lactea*.

5. In Britain *V. lactea* is found in open communities on dry, acid heaths while *V. canina* occurs mainly on dunes or inland sand deposits and certain heaths. The allied species, *V. riviniana* Rchb., has wide edaphic and climatic tolerances and comes into contact with *V. lactea* and *V. canina* over much of their ranges.

6. Cytological studies show *V. lactea* to be a sub-hexaploid with $2n = 58$, *V. canina* and *V. riviniana* both have $2n = 40$.

V. lactea was crossed with *V. canina*, *V. riviniana* and *V. pumila* Chaix, and meiosis was studied in some hybrids to discover the genomic homologies among the species. From these it was concluded that V. lactea is an allohexaploid derived from V. canina and another, as yet unknown, species. The possible identity of this unknown parent is discussed.

7. Field populations of V. lactea were sampled from most parts of its range in Britain and subjected to biometrical analyses for indications of hybridization. Techniques were developed to deal with the three species found in the field populations. Primary classification was by means of polygonal graphing after which the more detailed structure of the populations were determined by using pictorialized scatter diagrams.

The results show that there is widespread introgression involving V. lactea and V. riviniana; a mechanism is discussed by which the sterility barrier between these species could be overcome. The possible implications of this introgression are also discussed.

8. Preliminary results are given from recent observations on populations of V. lactea in Portugal.

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