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Variation and evolution in the Genus *Viola*

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I. Introduction

In this paper, we shall discuss some of the evolutionary and taxonomic problems associated with the family *Violaceae* and in particular the genus *Viola*. This genus has long been studied by the botanists of Europe and North America; and though very much remains to be discovered, enough is known to provide a basis for an examination at all levels from the family to the species.

II. The Family

We shall begin with a brief consideration of the *Violaceae*, which appears to be a fairly natural family, and in which most of the genera are restricted to the southern hemisphere and particularly to S. America. This fact has been interpreted by CAMP (1947) to mean that the family originated in this area; and he has used it to support his general thesis that the *Angiospermae* originated in the southern hemisphere, a thesis which will not be further discussed here. It is, however, worth drawing attention to the relative sizes of the genera of the *Violaceae*, which are listed in Table 1. As regards both number of species and geographical area covered, *Viola* is clearly the first, and it is followed by *Rinorea*, a genus of small trees found in the tropics of both the old and the new worlds, and differing from *Viola* inter alia in having actinomorphic flowers. The remaining genera, apart from *Hybanthus*, are all small.

As was first pointed out by WILLIS (1922), and later in more detail by WILLIAMS (1944), this pattern of generic sizes in a family, i.e. few large genera and many small ones, is universal in all large groups of organisms, both animal and plant, which have been classified by systematists; and the question of its interpretation is an interesting one. In any particular group, we have first to ask ourselves on what characters the genera are based. In the angiosperms in general, and in the Violaceae in particular, the classification is based primarily on floral characters. Thus the *Violeae* differ from the *Rinoreae* in the symmetry of the flower; and *Hybanthus* differs from *Viola* in having a gibbous rather than a spurred anterior petal. Such floral characters may be interpreted as indicating patterns of adaptation to various types of insect visitors. Other character used in the classification, e.g. whether the fruit is a capsule or a berry, may be regarded as types of adaptation to seed dispersal. Both successful sexual reproduction by cross-pollination and efficient seed dispersal may well be regarded as vital to the survival of plant populations; and it may thus be argued that such reproductive characters are less liable to rapid evolutionary modification than vegetative characters which have, as it were, a wider margin of tolerance, and can safely vary within wide limits. In this way, the use of reproductive characters in constructing a natural classification can be justified. While this argument is probably soundly based, it admits of many exceptions. This has been well demonstrated in the wind-pollinated *Gramineae*, a family in which floral characters may be obscure, so that differences may be overlooked or misinterpreted. Here the use of a variety of vegetative characters, such as those of leaf anatomy, has led in recent years to the re-definition and re-orientation of a number of genera. A typical recent example is provided by the work of REEDER and ELLINGTON (1960), who have shown that the genus *Calamovilfa*, formerly placed in the *Festucoideae*, should be transferred to the *Eragrostoideae*.

We may however proceed on the assumption that the genera of the *Violaceae* are reasonably natural. How are we then to account for the great variation in generic size, and, for example, the very large number of species described in the genus *Viola*? We may put the statement of facts in another way by saying that large numbers of taxonomic species are known which show the characteristic zygomorphic *Viola* pattern. Other characters which these species have in common is habit (herbs or small shrubs) and life-form (predominantly chamaephytes and hemicryptophytes). It may also be added that sufficient is known about the genus to make it certain that most of the species are good species, efficiently isolated, and are not mere figments of the taxonomist's imagination. If we now compare *Viola* with its related genera in the rest of the family, it appears to show advancement and specialization in both its floral and vegetative characters; and this conclusion is supported by its geographical distribution. Like the family as a whole, it is widespread in the southern hemisphere; but unlike the other genera, it has succeeded in colonising large areas of the northern hemisphere, many of its species finding a home as vernal herbs in the field layer of deciduous forests. We may thus legitimately speak of the genus as successful; and we may interpret its characters as adaptions to temperate woodland conditions. This idea has already been put forward by CLAUSEN (1951).

Another general character which can be similarly interpreted is that of the breeding system. The violets, in so far as they are visited by insects, are to some extent regular out-breeders. Yet so far as is known, they are all self-compatible; and in large sections of the genus this self-compatibility has developed into a regular selfing mechanism, the cleistogamous flower (or cleistogene), by which selfed seed is regularly set. This combination of regular in-breeding with a certain amount of out-breeding may be regarded as a highly efficient breeding system; and the property of self-compatibility, which permits the establishment of new populations at a distance, by the agency of a single seed (BAKER, 1955) may likewise have been a significant factor in the establishment of the genus over a very wide area.

It is tempting to generalise from the example of *Viola*, and to argue that all large natural genera owe their success to a favourable combination of floral characters and breeding mechanism which, in the particular environments available to them, has allowed them to spread and to radiate. Other smaller genera could thus have remained small either because their overall adaptation was not so good or because they occupied an environment unfavourable to radiation. This interpretation of the pattern of generic sizes in terms of fitness and available environment is worth detailed consideration by taxonomists. The alternative view, put forward by WILLIS (1922), is that natural selection has nothing to do with the case, and that the size of a genus and the area which it covers are primarily governed by its age. There is, of course, no doubt that age is an important factor. The mere process of speciation, involving as it frequently must repeated migration and geographical isolation, takes time, so that a large genus must, of necessity, be fairly old. That *Viola* is an old genus is indicated by its wide range, and by the existence of endemic species in Hawaii, Australia, etc. But it does not follow that the small genera are necessarily young; some may be, but others will be small for a variety of reasons, which cannot be analysed in detail here.

III. The genus *Viola*

We come next to the question of variation within the genus. BECKER (1925) divided it provisionally into 14 sections, with an uneven size distribution pattern very similar to that of the genera within the family. Lack of knowledge prevents us from analysing the genus as a whole, and we shall have to restrict ouselves to those sections found in the north temperate regions. The relationship of these to the Andine violets, for example, has never been explored, and it represents one of the major outstanding problems in the genus.

In Europe and N. America, the sections *Nomimium*, *Melanium*, *Dischidium*, *Chamaemelanium*, *Xylinosium* and *Delphiniopsis* occur. The two latter are represented by a few species of very small shrubs in the Balkans and the Mediterranean region, and must be regarded as old relic groups. *Delphinopsis* with its linear leaves and very long-spurred flowers, is clearly highly specialised and has probably always been a small group. The only well-known species of the small section *Dischidium* is the circumpolar *V. biflora* L.; as CLAUSEN (1929) has pointed out, there is a case for including it in the section *Chamaemelanium*. Some of the distinguishing characters of these sections are shown in Table 2. *Nomimium* is much the biggest and most widely distributed; *Melanium* is mainly European and *Chamaemelanium* mainly North American. Some guidance as to the relationship of the three sections is provided by chromosome counts and hybridization experiments. Thus, all the species of *Chamaemelanium* which have been investigated have a basic chromosome number of 6; the basic numbers in the other sections are more variable. GERSHOY (1934), in an extensive series of hybridization experiments, was unable to obtain any viable intersectional hybrids (except from *Dischidium* × *Chamaemelanium*), whereas at least some intrasectional hybridization was always possible. Very extensive interspecific hybridization is one of the characteristic features of the genus, and we shall introduce at this point a short discussion on the evolutionary and taxonomic significance of such hybridization.

If we survey the genera of any well known flora, such as the European flora, we find a tremendous variability from genus to genus in the numbers of natural interspecific hybrids which are recorded. For example, in the genera *Vicia* and *Trifolium*, such hybrids are very rare, whereas in *Epilobium* and *Salix*, to take the other extreme, they are very common, and the reasons for this difference are by no means clear. As ANDERSON (1949) has pointed out, hybridization in natural populations is often associated with hybridization of the habitat; in other words, populations which under completely natural conditions are ecologically isolated may be brought together into new habitats created by man, and thus given opportunities for hybridization which they would not normally have. While recognising that this effect is of great importance (it will be referred to below), it cannot provide the whole explanation; for in some genera, e.g. *Trifolium*, the barriers to hybridization are internal and it is difficult or impossible to make artificial hybrids even between species which are taxonomically closely rélated (EVANS and DENWARD, 1955). Failure to hybridize in such cases may be due to failure of pollen to grow on the style, but it is probably more frequently due to post-fertilization breakdown of the endosperm and embryo (seed incompatibility).

On the other hand, it is possible in some groups to make very wide hybrids, such as intergeneric hybrids. These possibilities are only now beginning to be explored, and the results are likely to be of much taxonomic significance. ADAMS and ANDERSON (1958) have recently emphasised the frequency of intergeneric hybrids in the *Orchidaceae*, and STEBBINS (1956) has discussed their significance in the phylogeny and classification of the *Hordeae*. Two interesting examples, one of an artificial and one of a natural hybrid, have recently been reported in the *Rosaceae*. ELLIS (1958) has succesfully crossed species of *Potentilla* and *Fragaria*, and STEBBINS (1959) has reported observations made in North America on natural hybrids between species of the very distinct genera *Cowania* and *Purshia*. In the latter case the hybrids are partially fertile, and introgression in natural populations has been observed.

In these and similar cases, the taxonomist is led to consider some revision of existing generic limits. As has been pointed out, hybrids between members of different section of the genus *Viola* have not so far been obtained; and though negative evidence of this kind must be interpreted with caution, the result must certainly support the taxonomic divisions which have been made. It should however be pointed out that the barriers to hybridization here are of two kinds. Thus GERSHOY (1934) attempted to make crosses between *V. tricolor* L. (Section *Melanium*), and species of the sections *Nomimium* and *Chamaemelanium*, using *V. tricolor* as the seed parent; and he found that the failure of the crosses was due to the failure of the pollen to grow on the stigma. This in turn could be correlated with the fact that the optimum sucrose concentration for germination of *tricolor* pollen was widely different from that for the other two sections. On the other hand, in crosses between certain species of the sections *Chamaemelanium* and *Nominium*, Gershoy obtained seeds which contained embryos, but the seeds failed to germinate. This my perhaps indicate a relatively close relationship between the sections, and there is a possibility that further experiments might lead to the production of viable hybrids. Another interesting series of experiments by DODD and GERSHOY (1943) showed that it was possible to obtain successful grafts between members of all three sections. In some genera, such as *Gossypium*, HUTCHINSON, SILOW and STEPHENS (1947) have shown that success of grafting and of hybridization correspond fairly closely and give a useful assessment of relationship, and a similar conclusion was reached by EVANS and DENWARD (1955) for *Trifolium*. In *Viola*, the case appears to be rather different, in that grafts between Melanium and the other sections succeeded, whereas crosses always failed; but a good deal of further work on grafting is required to justify firm conclusions.

IV. The sections

We shall next examine hybridization within the section *Nomimium;* the extent of this is remarkable, and very large numbers of species can be linked, directly or indirectly, into a single hybridizing group or comparium, to use the term suggested by DANSER (1929). BECKER (1925) divided *Nomimium* into 17 sub-sections. The hybridizing relationship between five of the largest and most important of these, and their basic chromosome numbers are shown in Fig. 1. The *Rostratae* are a caulescent group, represented in Europe by such species as *V. riviniana* RCHB.; typical European representatives of the other groups are *V. odorata* L. (*Uncinatae*), *V. palustris* L. (*Stolonosae*) and *V. selkirkii* PURSH (*Adnatae*). The *Boreali-Americanae* are confined to N. America.

CLAUSEN (1929, 1951) has suggested that the *Uncinatae* and the *Rostratae* be grouped together as the Section *Rostellatae*, and that the other sub-sections should form the Section *Plagiostigma;* this distinction, based on a difference in the shape of the stigma, has long been used by taxonomists. BECKER (1925) on the other hand, who was familiar with a much larger number of species of *Viola* than any other worker, preferred not to group the sub-sections in this way. While the evidence from basic chromosome number is in favour of placing together the *Rostratae* and *Uncinatae*, it is equally against uniting the *Boreali-Americanae* with the *Stolonosae* and *Adnatae*. Further, if we take the (admittedly incomplete) evidence from hybridization into account, it does not appear to favour Clausen's interpretation. It is worth while, at this point, discussing in a little more detail the specific case of the relationships of the *Uncinatae*.

The *Uncinatae*, a group which is absent from N. America, is unique in the genus in the form of its capsule and method of seed dispersal. In all other violets, the walls of the capsule are elastic, the capsule is erect or ascending at maturity, and the seeds are shot out as the elastic walls of the capsule valves contract. In the *Uncinatae*, on the other hand, the walls of the capsule are not elastic, the pedicels bearing the capsules (at least those of the cleistogamous flowers) are decumbent at maturity, and the seeds, with their conspicuous caruncle, lie loose in the open capsule or spill on to the ground, and may be collected and dispersed by ants. In some of the *Boreali-Americanae*, the cleistogamous fruits may also be decumbent, but just before maturity the capsules are erected and the seedsshot out in the normal way; but they are interesting in illustrating a possible intermediate stage in the transition from one method of seed dispersal to another.

A few hybrids between the *Uncinatae* and the *Rostratae* have been recórded in nature. BECKER (1910) cites, for example *V. riviniana* × *thomasiana* (Italy) and *V. alba* × *reichenbachiana* (France). I have attemped to make artificial hybrids between *V. hirta* (2n = 20) and *V. riviniana* (2n = 40); the crosses failed with *hirta* as seed parent, but gave a reasonable yield of seed with *riviniana* as seed parent. Most of the seeds, however, were empty or imperfect; the two heaviest (0.72, 0.94 mg.) were sown, but failed to germinate. The experiments are being repeated. DODD and GERSHOY (1943) were, however, successful. They used *V. riviniana* as one parent and *V. odorata* (2n = 20), which is closely allied to *V. hirta* and readily hybridises with it, as the other. The hybrids were vigorous and sterile; and Dodd and Gershoy were able, by colchicine treatment, to produce a fertile amphidiploid from the sterile F1 hybrids.

A number of conclusions can be drawn from these observations. First, the unique capsule and dispersal mechanism of the *Uncinatae* serves to distinguish the group from all other northern violets; its species can be hybridised with those of the *Rostratae* only with difficulty, and whether they hybridise with members of other sub-sections is as yet unknown. There is at least as good a case for giving the *Uncinatae* sectional status as there is for uniting them with the *Rostratae* to form the section *Rostellatae*; and it is clearly wisest for the present to retain the system of BECKER, in which all the sub-sections of *Nominium* have equal status, and to postpone re-grouping until further data are available.

The production of a fertile amphidiploid from the *V. odorata-riviniana* cross is a result of great interest, as it illustrates very well the way in which two related groups of species, which must have diverged a long time ago, are able to be re-united to produce a new type with new evolutionary potentialities. From this point of view, the persistence of interspecific crossability within the section *Nomimium* can be regarded as a character of considerable evolutionary importance.

V. The sub-sections

As has been pointed out by Clausen (1951), the patterns of evolution within the sections of *Viola* show a number of interesting differences. We shall attempt here to illustrate this by reference to two of the sub-sections of *Nomimium,* viz. the *Boreali-Americanae* and the *Rostratae.*

(a) *The Boreali-Americanae*

This group consists of some 28 to 30 species which are confined to North America, though there may possibly be one species in Mexico. Most of the species are furher confined to the eastern regions; only four or five occur west of the Appalachian mountains, and only one (*V. nephrophylla* Greene) reaches the western seaboard. The range of the group is from Newfoundland and Quebec in the north to New Mexico, Texas and Florida in the south.

The plants are all perennials, characteristically with fleshy rhizomes; lateral branches of these are easily detached, and the plants commonly form quite large clones. In spring, the flowers and leaves arise directly from the rhizome, on long peduncles; the flowers are violet, and the styles are swollen and somewhat flattened at the apex. Later in the year, more leaves are produced, and also numerous self-pollinated cleistogamous flowers. The group is divided into three sub-groups on the basis of the posture of these flowers, viz. whether they are prostrate (as in *V. palmata* L.) ascending, (as in *V. affinis* Le Conte), or erect (as in *V. cucullata* Ait.). A striking feature is the variation in shape of the mature leaves from species to species; the leaves, which are usually cordate, may be almost entire, or they may be three-lobed, or palmately or pedately divided.

What have long been recognized as natural interspecific hybrids are very common, and Brainerd (1924) who studied the group in detail, described and figured no less than 73 such hybrids. A little later, Gershoy (1932) succeeded in making 33 different artifical interspecific hybrids in the group, nearly all of which were vigorous and most of which were fertile; however, two or three of the hybrids (e.g. *V. affinis* × *cucullata*) showed reduced fertility. The chromosome numbers of all the species and hybrids investigated was found to be $2n = 54$. Relatively little work on the genetics of the hybrids has been done, though Brainerd (1924), especially in crosses between species with dissected and entire leaves (e.g. *V. fimbriatula* × *palmata*), was able to demonstrate very convincing segregation of leaf-shape in the F2 generation.

Recently Russell has made a number of studies of wild populations in N. America, using mainly the character of the leaves. He has been able to show that in some cases (e.g. in *V. pedatifida* × *sororia*, Russell (1956)) hybridization is locally fairly common, but that there is little evidence of introgression. In other cases (e.g. *V. cucullata* × *septentrionalis*, Russell (1955)), introgression has proceeded to a considerable extent in both directions, so much so that in many localities, it is difficult to be sure what the original populations were like before hybridization had occurred. This difference in behaviour in the two examples may be related to the extent to which the habitats of the two species overlap and to the length of life and fertility of the hybrids, which in turn may be connected with the degree of genetic relationship between the species.

No detailed account of the isolating factors in the group has yet been published. Geographical isolation is certainly important, some species, such as

V. villosa WALT. being southern in range, and not overlapping with northern species such as *V. septentrionalis* GREENE. Ecological isolation is also important; the group occupies a wide range of habitats, from dry prairie to wet swamps; some occur only in specialised habitats, such as *V. egglestoni* BRAINERD, which is restricted to open calcareous soils in the south. However, two-thirds of the species are typically found in woodland of some kind or in habitats such as scrub and meadows which have been derived from woodland; and many show a considerable ovelap in geographical range. Most are spring-flowering plants, and have rather similar flowers, so that they are likely to have insect visitors in common; and as GERSHOY's experiments have shown, the internal barriers to crossing between many of the species are slight. The fact that hybridization is so extensive is probably mainly due to the breakdown of ecological isolation between the sympatric species. It has been suggested by RUSSELL and others that this is largely a result of man's activities in modifying natural communities and the natural ecological barriers. CAMP (1961) has also suggested that natural forces have been partly responsible for the breakdown of isolation. He has envisaged the development of the group as a series of species, differentiating allopatrically in geographical isolation, during pre-glacial times. During the Pleistocene glaciations these species were repeatedly driven southwards down the eastern regions of the United States, and repeatedly migrated northwards again during inter-glacial periods. This will have provided opportunities for the species to meet in their constricted southern area during glaciation, and will have allowed the species, modified by hybridisation and introgression, to spread northwards again to occupy new habitats. Thus the process of hybridization, and the blurring of species boundaries, may have begun long before the advent of man, though in recent times, it may have been accelerated by man's effect on the habitat, as indicated above. There is no doubt that at the present time, in many areas, it is difficult to match the natural populations with the taxonomists' descriptions. In a detailed study of a single area, CAMP (1961) has shown that the violet populations are in a state of flux, and are best intepreted as complex hybrid swarms.

The *Boreali-Americanae* provide an interesting example of a coenospecies, consisting of a large number of ecospecies or possibly even ecotypes, which is still actively evolving. The origin of the group is obscure, although it may have arisen from some of the *Adnatae* (x = 6), a group to which it shows some resemblances; whether it is monophyletic is uncertain, though this is the most likely hypothesis. Amongst genera which show both genetical and ecological analogies to the *Boreali-Americanae*, parts of the genera *Salix* and *Epilobium* may be mentioned. In *Salix*, wide hybridization and introgression occur and produce extensive hybrid populations, particularly in the Arctic, though the situation here is rather different in that both diploid and tetraploid chromosome levels are involved. The genus *Epilobium* in Europe, which has uniformly 2n = 36, and in which very extensive hybridization occurs, provides another parallel. Both genera are ripe for investigation, and detailed population studies, as well as experimental work, are much needed. As with the *Boreali-Americanae*, both repeated migrations, enforced by glaciation, and the modifying action of man on habitats, have probably been responsible for facilitating hybridization; and the weakness of internal barriers of isolation must have been an important factor in allowing the development of hybrid swarms.

(b) The *Rostratae*

The second sub-section of *Nomimium* to be described here is a little larger than the *Boreali-Americanae,* with about 35 species, and it provides an interesting contrast. To take the morphological characters first, it is caulescent, that is to say it produces leafy overground stems which bear the flowers; and its short, generally erect rootstocks are dry and not readily broken, so that vegetative reproduction, except for a few species which produce soboles, is not important. The style is characteristically beaked, or hook-shaped at the apex; and the capsules are erect or ascending, never prostrate. The range of habitats occupied is similar to that of the *Boreali-Americanae,* but the geographical distribution is much wider, extending all the way round the temperate regions of the northern hemisphere, and occasionally extending high into the mountains. Geographical isolation is important, and there are several groups of species which are confined respectively to Eastern North America, to Europe and to Eastern Asia.

Within these regions, and especially in Europe, where the group has been most intensively studied, ecological isolation may break down. Most of the species flower in the spring, floral differences are not great, and the species doubtless have insect visitors in common. Interspecific hybridization is in fact frequent, but unlike the *Boreali-Americanae,* most of the hybrids are highly infertile. This is in part due to the chromosomal structure of the group, which consists mainly of diploids and tetraploids (2n = 20, 40); and many of the hybrids are triploid (2n = 30); a good example is provided by the hybrid *V. stagnina* Kit. × *V. canina* L. But this is not the whole story; for some of the inter-diploid and inter-tetraploid hybrids are also practically sterile. This is the case with the hybrid between the diploid species *V. rupestris* Schmidt and *V. reichenbachiana* Jord., and also with the hybrid between the tetraploid species *V. rivianina* Rchb., and *V. canina* L. The reason for this, as will be shown, lies in the lack of homology between the genomes of the species concerned, as indicated by failure of pairing at meiosis; and this indicates that the genomes of these species are more highly differentiated from one another than those of the *Boreali-Americanae,* in which hybrids are generally fertile, and chromosome pairing (presumably) good. This fact, taken together with the wide geographical distribution of the group, gives us the impression that the group is a fairly old one, and that it has a longer history than the *Boreali-Americanae.*

It is convenient at this point to introduce Table 3, which shows all those species between which vigorous artificial hybrids have been made; the data are based partly on the work of Gershoy (1934) and Schmidt (1961) and are partly original. In addition, the Table indicates the level of polyploidy and also the geographical regions in which the species occur; no reference is made here to the numerous Asiatic and Japanese species, for which information about hybridization is lacking though six of these species are known to be diploid (Miyaji, 1929)]. 17 species are shown; and the number of artificial hybrids known at present is 44. 22 of these are within the European group, 8 within the American group, and 14 between European and N. American species. Many of the hybrids have made independently by Gershoy, Schmidt and Valentine.

The chromosome numbers of most of these species have been determined by several workers and references are given in Valentine (1958); but some are new or worthy of special comment. MOORE (1959) found that *V. lactea*, a species restricted to Western Europe, was a *sub-hexaploid*, with 2n = 58; this count was obtained from plants collected at several points in the range of the species. *V. sieheana* (2n = 60), is the only other hexaploid so far known in the group; the count was made by HARVEY (unpublished) working at Durham, and the plants came from the Pontic coast of Turkey and are thus not strictly European, though the species is known from European Russia, and may occur in the Balkan Peninsule. *V. jordani* (2n = 40) has recently been counted for the first time by SCHMIDT (1960) from material collected in southern France.

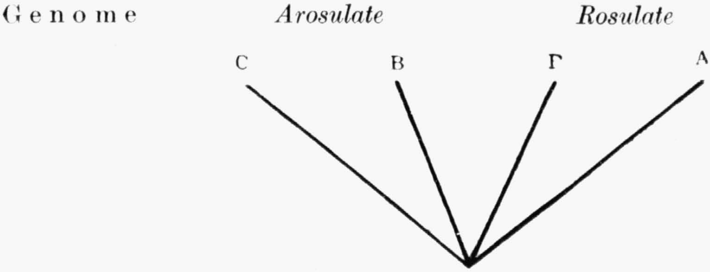
It may also be of interest to give some details about the success and failure of the hybrid crosses. Data for three groups of crosses, made mainly with British material, are given in Table 4; the polyploid species in this Table are all tetraploid except the sub-hexaploid *V. lactea*. The data will not be analysed in detail, but attention may be drawn to a few points of interest. First, the crosses between diploids were, on the whole, the least successful, in terms of yield of adult hybrids per pollination made. Secondly, as was pointed out above when discussing inter-sectional hybrids, there are several points at which a cross may fail. First fertilisation may apparently fail, probably because the pollen does not grow down the style; this effect was marked in the cross *reichenbachiana* ♀ × *rupestris* ♂. Secondly, fertilisation may occur, but the seeds may not develop to maturity. This is known as seed incompatibility, and it was well marked in the crosses *rupestris* ♀ × *reichenbachiana* ♀ and *reichenbachiana* ♀ × *mirabilis* ♂. Thirdly the seeds, though well-filled and apparently normal, may fail to germinate; and fourthly germination may occur but the seedlings may fail to thrive and die before reaching maturity, as for example in the crosses *stagnina* ♀ × *reichenbachiana* ♂ and *lactea* ♀ × *stagnina* ♂. A similar range of behaviour in interspecific hybridization has been recorded by GERSHOY (1934) in other sections of the genus *Viola* and by other observers in many genera. It may be added that all the hybrids recorded in Table 4 are sterile or highly infertile, with the exception of the *canina* — *lactea* hybrid.

Under these circumstances, the possibility of investigating the relationship of the species by genome analysis is clearly very interesting, and a beginning has been made. Several problems at once arise, of which two of the most obvious are first, the bearing of the cytological data on the existing taxonomy of the group, and secondly the relationship between the European and N. American species.

The classification of the *Rostratae* is based mainly on life-form and leaf-shape. The *Rosulatae* are chamaephytes, with a basal overwintering rosette of leaves, and the leaves are only slightly longer than broad; the *Arosulatae* are hemicryptophytes, in which overground leaves disappear during the winter; and the leaves are generally markedly longer than broad. (We shall not consider here the 2 species placed in the *Mirabiles*, which occupy a rather isolated position). Preliminary investigations by VALENTINE (1958) and more extensive investigations by MOORE and HARVEY have provided data for chromosome pairing in hybrids involving some European members of these two groups. The data are summarised in Fig. 2, which is taken from the paper by MOORE and HARVEY (1961). In this Figure it is assumed that homogenetic pairing generally takes place; letters (A, B, C, etc.) have been assigned to the 10 —

chromosome genomes to conform with the meiotic data. It should also be emphasised that the numbers of bivalents and univalents given in the Figure are averages, and that the occasional occurrence of polyvalents in some of the hybrids (notably those involving *V. pumila*) has been neglected.

If this scheme is correct, then it would appear that the *Arosulate* species *V. canina* and *V. lactea* have one genome in common (B) with the rosulate species *V. riviniana*. The diploid species carrying this genome has not yet been discovered, but according to HARVEY (unpublished data) it is not *V. rupestris*; and according to GERSHOY's data it is not one of the N. American species (this is being checked). A possible explanation is as follows. In the early history of the group, divergence occurred producing both rosulate and arosulate diploid forms. At the same time cytological differentiation occurred, producing genomes with characteristic pairing properties; but in some species, the habit diverged and not the genome, and these were the species with genome B, as shown in the diagram.



Subsequent hybridisation, followed by polyploidy, would then produce the arosulate BC species, and the rosulate AB species.

Extension of work on this problem to cover a wider range of species, especially those from Western Asia, is clearly needed. SCHMIDT (1961) has recently drawn attention to *V. jordani* HANRY, which ranges from S. France to Kashmir, and which was placed by BECKER (1925) in the *Arosulatae*, close to *V. elatior*. SCHMIDT has pointed out that in several characters this species, which is tetraploid, is intermediate between the rosulate *V. riviniana* and the arosulate *V. montana*, and that its hybrid with *V. montana* is sterile. It will be of great interest to determine the genome of *V. jordani*, and also to investigate it over the eastern part of its range, in which diploid relatives with the B genome may well occur.

The second problem mentioned above, that of the relationship between the European and N. American violets, is at present under investigation; and it is too early to present any detailed results. GERSHOY (1932) found that *V. conspersa*, *V. rostrata* and *V. striata* all formed more or less fertile hybrids, indicating that their genomes were sufficiently similar to pair well at meiosis; and he also found 30 univalents in the hybrid *V. riviniana* × *striata*, which would indicate that the genome of these American species is neither A nor B. It is thus probably a distinct genome (F). A very interesting species, now under investigation, is *V. labradorica* SCHRANK, which is found in the sub-arctic regions of N.E. Canada and Greenland, thus forming a geographical link between the N. American species and the European. (*V. riviniana* occurs in Iceland but not in Greenland). Morphologically this species shows some resemblances to *V. conspersa* (rather than to *V. adunca* SM., with which it has been classified by FERNALD (1950)), but its exact relationships have not yet been worked out. The relationships of the *V. adunca* — *V. rupestris* complex, which covers the northern hemisphere, *V. adunca* in America and *V. rupestris* in Eurasia, are also being investigated.

It is of interest to compare the patterns of evolutionary divergence in the two sub-sections which have just been described. All the criteria, whether of morphology, cytology or crossability, indicate that the *Boreali-Americanae* form a natural group; and it seems likely that they are also monophyletic. We may imagine that hybridization has led, via allopolyploidy, to the formation of an ancestral population which, under the conditions in which it was established, was able to spread widely in North Amercia and form a large number of local populations of ecotypic or ecospecific status. During this process, barriers of crossability were only slightly developed and there was little cytological differentiation; and subsequent enviromental changes, which brought the species together, have allowed widespread hybridization and convergence. As already pointed out, this pattern of evolution is probably widespread; another good example is provided by the subgenus *Eugeum* of the genus *Geum*, which consists of some 25 species of which 15 have 2n = 42, and the remainder higher numbers in the polyploid series. The hexaploid species have a circumpolar distribution, but they are all capable of producing hybrids of at least moderate fertility and with good chromosome pairing; and GAJEWSKI (1957) considers the group to be monophyletic.

The *Rostratae* also appear to be a very natural group; but here the primary radiation has taken place at the diploid level. No less than 17 of the 25 species which have been counted are diploid. Although some of these diploids are crossable, many of the hybrids are sterile and some show little or no chromosome pairing. The 8 tetraploid or hexaploid species which are known have probably all been formed by allopolyploidy; but these polyploids, though again crossable, are themselves largely inter-sterile, as they rarely have more than one genome in common, and there is thus much failure of pairing. The possibilities of convergence by hybridization and introgression are thus much slighter than in the *Boreali-Americanae*, although some examples are known. Thus SCHÖFER (1954) has shown that in Germany the triploid hybrid *V. riviniana* × *reichenžachiana* (2n = 30) has limited fertility, and that *riviniana* populations have probably been affected by introgression from this hybrid. Again MOORE (1959) has shown that introgression appears to be occurring between *V. riviniana* (2n = 40) and *V. lactea* (2n = 58) in S. W. England and Portugal, in spite of the high infertility of the artificial hybrids. It is interesting to note here that although the hybrid between *V. canina* (2n = 40) and *V. lactea* (2n = 58) is moderately fertile, little or no hybridization occurs, because the species generally occur on soils of different base-status and are thus ecologically isolated.

With *V. canina* L. and *V. montana* L. the situation is different. While typical forms of these tetraploid species are both ecologically and morphologically quite distinct, numerous fertile intermediates occur. SCHMIDT (1960) has shown that while the species are often very efficiently isolated by differences in habitat, *montana* being mainly a woodland plant and *canina* a plant of more exposed habitats, artificial hybrids between them are completely fertile. There is thus a case for giving the taxa subspecific rather than specific rank; but further investigations over the whole of their range in needed.

The pattern of evolution found in the *Rostratae*, with a group of widely dispersed diploids on the basis of which localised polyploid groups have been formed, is of course widespread in flowering plants. It occurs again in the section *Chamaemelanium* of the genus *Viola*, investigated by CLAUSEN (1951). Another notable example, which spans the Atlantic Ocean, is to be found in the fern genus *Dryopteris*, recently described by S. WALKER (1960).

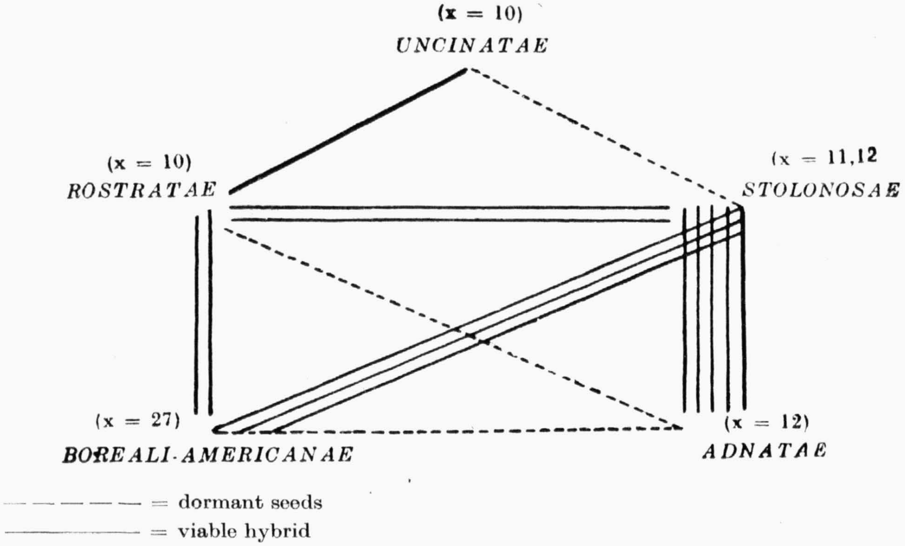
It is clear that the *Boreali-Americanae* and the *Rostratae* illustrate different genetical patterns of evolution and also that they represent different stages in the process of evolution via polyploidy. Looking into the future, it is conceivable that all the diploid species of the *Rostratae* might become extinct, leaving only some of the polyploids. One of these species, e.g. *V. riviniana*, which is already extremely variable (VALENTINE, 1956) might then become dominant, and produce a swarm of daughter species comparable to that found in the polyploid *Boreali-Americanae*. The difference in evolutionary pattern also helps to explain the taxonomical problems which arise in the two groups. The overlap in range and the inter-fertility of many of the species of the *Boreali-Americanae* make it very difficult to delimit hard and fast taxonomic boundaries. In the European *Rostratae* on the other hand, although the morphological differential characters are not as clear-cut as they might be, identification of hybrids by their sterility is generally easy and introgression rarely blurs the boundaries between the species. It may be added that identification has often been made more difficult in the past by failure to use floral characters, such as those concerned with the shape, colour and venation of the petals, which are often not usable in herbarium specimens, but which may be of considerable importance when fresh material is available.

VI. Conclusion

The work described in this paper, to which many authors have contributed over many years will, it is hoped, illustrate some of the ways in which the experimental taxonomist (or the biosystematist as he is sometimes called) selects and approaches his problems. His aims are twofold. First, he is interested in the pattern and process of evolution. He attempts to find out how populations are isolated from one another, under what circumstances this isolation may break down, and what the effects of such a breakdown may be. At the same time, he is concerned with the process of population differentiation, and with the factors, both internal and external, which are responsible for such differentiation. Secondly, he is concerned with the application of the knowledge gained in this way to the practical problems of taxonomy (e.g. to the revision of sections and genera) and also to the problems of phytogeography, such as the realtionship between the floras of Europe, Asia and N. America. As CAMP and GILLY (1943) and VALENTINE and LÖVE (1958) have pointed out, as an area is more intensively explored, and its flora becomes more perfectly known, the profit to be derived from classical taxonomical studies becomes less and less, and the desirability of the biosystematic approach becomes more obvious. It would be an exaggeration to state that our knowledge of the taxonomy of the European flora, for example, is complete; but the very fact that the Flora Europaea project has been successfully launched is sufficient to indicate that a great deal is known; and as the project proceeds, more and more problems for the biosystematist are revealed. This is well illustrated by the section *Melanium* of the genus *Viola*, of which little has been said in this paper. Thus, in some of the annual species of this section, e.g. *V. kitaibeliana* R. & S., CLAUSEN (1951) has shown that chromosome races exist, but little or nothing is known of the taxonomy and distribution of the races. Again in some of the perennial groups, such as the *V. cenisia* complex, there are many isolated populations in the mountains of southern Europe whose status is uncertain, and which need to be investigated by biosystematic methods. Studies of this kind will, in the future, throw much light on the evolutionary processes at work and will also help to place the taxonomy on a firm basis.

Figure 1

Artificial crosses and basic chromosome numbers in the section *Nomimium*
(From GERSHOY, 1934, 1943)



The number of lines indicates the number of different interspecific hybrids successfully reared to maturity. All the hybrids were sterile.

Figure 2

Pairing at meiosis in 8 interspecific hybrids in the *Rostratae* (from Moore & Harvey, 1961)

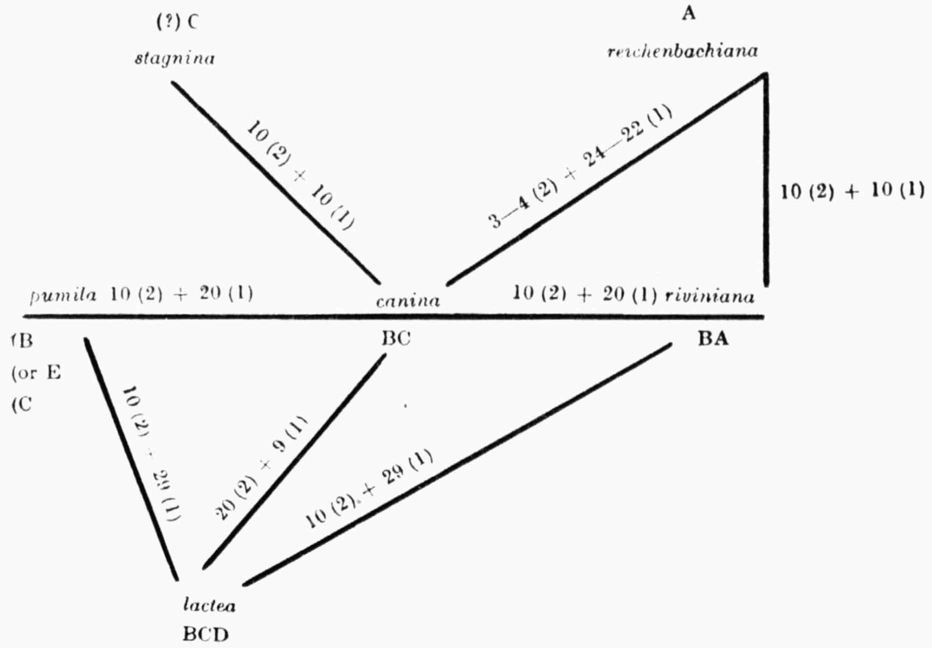


Table 1.

GENERA OF THE *VIOLACEAE* (from Melchior, 1925)

Subfamily I			
VIOLOIDEAE	Stamens introrse; fruit a capsule or berry		
Tribe I *Rinoreeae*	Flowers actinomorphic; lower petal not saccate or spurred		
Genera	*Rinorea*	Tropics, excluding Australia	260 species
	Allexis	Tropical W. Africa	3
	Gloeospermum	Tropical C. America	7
	Melicytus	New Zealand, Fiji	4
	Hymenanthera	New Zealand, E. Australia	5
	Isodendrion	Sandwich Is.	4
	Amphirrox	Tropical S. America	5
	Paypayrola	Tropical S. America	7
Tribe II *Violeae*	Flowers zygomorphic; lower petal saccate or spurred		
Genera	*Hybanthus*	Tropics and sub-tropics	75
	Agatea	New Caledonia, New Guinea	12
	Anchietea	Tropical S. America	8
	Corynostylis	Tropical C. & S. America	4
	Schweiggeria	Tropical C. & S. America	2
	Noisettia	Tropical S. America	1
	Viola	Cosmopolitan	c. 400
Subfamily II			
LEONIOIDEAE	Stamens opening terminally; fruit nut-like		
Genera	*Leonia*	Tropical S. America	3

Table 2

SECTIONS OF *VIOLA* IN THE NORTHERN HEMISPHERE (from Becker, 1925)

1. *Nomimium*	Herbs; stipules small; flowers blue; style beaked. With cleistogamous flowers.	N. Hemisphere. Many species.
2. *Dischidium*	Herbs; stipules small; flowers yellow; style two-lobed at apex. With cleistogamous flowers.	N. Hemisphere, S. E. Asia. 8 species.
3. *Chamaemelanium*	Herbs; stipules small; flowers yellow; style capitate. With cleistogamous flowers.	Asia and N. America. Many species.
4. *Melanium*	Herbs; stipules leaf-like; flowers blue or yellow; style capitate. Lacking cleistogamous flowers.	N. Hemisphere mainly Eurasia, Many species.
5. *Xylinosium*	Sub-shrubs; stipules small; flowers blue; style neither capitate nor beaked.	Mediterranean & the Cape. 4 species.
6. *Delphiniopsis*	Sub-shrubs; stipules small; flowers with very long spur; style neither capitate nor beaked.	S. Europe. 3 species.

Table 3

SPECIES OF THE SUB-SECTION *ROSTRATAE* KNOWN TO HYBRIDIZE DIRECTLY OR INDIRECTLY

	European			N. American
	Mirabiles	Rosulatae	Arosulatae	Rosulatae
Diploid (2n = 20)	*mirabilis* L.	*reichenbachiana* Jord. *rupestris* Schm.	*stagnina* Kit.	*striata* Ait. *conspersa* Rchb. *labradorica* Schrank *rostrata* Pursh *adunca* Sm.
Tetraploid (2n = 40)		*riviniana* Rchb.	*canina* L. *pumila* Chaix *elatior* Fr. *Jordani* Hanry	*howellii* Gray (also octoploid)
Hexaploid (2n = 60)		*sieheana* W. Bckr.	*lactea* Sm. (2n = 58)	

Table 4

Seed-set and percentage germination in interspecific crosses in *Rostratae*

	Cross (seed-parent first)	No. of pollinations	No. of capsules	Mean seeds per capsule	Mean percentage germination	Hybrid offspring
A. Inter-diploid	*reichenbachiana* × *rupestris*	30	14	7	2	Moderately vigorous
	reciprocal	8	5	6	0	—
	reichenbachiana × *mirabilis*	31	19	7	0	—
	reciprocal	4	3	11	0	—
	reichenbachiana × *striata*	5	2	3	0	—
	rupestris × *mirabilis*	2	1	25	0	—
	stagnina × *reichenbachiana*	9	5	22	5,5	Died in seedling stage
	stagnina × *rupestris*	1	1	18	33	Moder. vigorous
	stagnina × *striata*	6	5	30	13	Vigorous
B. Inter-polyploid	*canina* × *riviniana*	6	6	31	72	Vigorous
	reciprocal	4	4	24	0	—
	lactea × *canina*	1	1	15	13	Vigorous
	reciprocal	5	4	37	91	Vigorous
	lactea × *riviniana*	4	4	22	7	Moder. vigorous
	reciprocal	6	4	14	0	—
	pumila × *lactea*	10	7	18	90	Vigorous
C. Diploid-polyploid	*riviniana* × *reichenbachiana*	20	20	27	92	Vigorous
	reciprocal	9	9	17	84	Vigorous
	riviniana × *rupestris*	11	5	8	58	Vigorous
	reciprocal	4	2	4	0	—
	riviniana × *stagnina*	5	2	2	0	—
	reciprocal	1	1	28	11	Moder. vigorous
	riviniana × *striata*	2	2	10	94	Vigorous
	reciprocal	9	2	3	0	—
	riviniana × *mirabilis*	9	7	21	18	Vigorous
	rupestris × *lactea*	1	1	18	0	—
	reciprocal	3	1	19	11	Died in seedling stage
	stagnina × *lactea*	4	3	16	0	—
	reciprocal	3	1	17	12	Died in seedling stage
	canina × *reichenbachiana*	3	3	18	66	Vigorous
	canina × *rupestris*	1	1	21	0	—
	canina × *mirabilis*	2	1	13	0	—
	stagnina × *canina*	1	1	26	69	Moder. vigorous
	reichenbachiana × *lactea*	7	6	4	0	—

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Note added in proof

The interesting papers by WALTERS (1961), on the sizes of families and genera, and of BEUZENBERG (1961) on hybridization between two genera of the Violaceae (*Melicytus* and *Hymenanthera)* in New Zealand, were received too late for consideration in this paper, but they are included in the list of references.