

# CYTO-GENETIC AND TAXONOMIC INVESTIGATIONS ON MELANIUM VIOLETS

BY J. CLAUSEN

GENETICS LABORATORY OF THE ROYAL VETERINARY AND AGRICULTURAL  
COLLEGE, COPENHAGEN

---

## I. INTRODUCTION.

THE *Melanium* section of the genus *Viola* offers exceptional opportunities for a comparative study of hybrids between species with different numbers of chromosomes as well as for experimental taxonomic studies on the relationship and the origin of species. These opportunities are due to the facts that a unique series of numbers of chromosomes ranging from  $n = 7$  to  $n = \text{abt. } 30$  occurs in this section and that each species is capable for crossing with a number of other species, irrespective of their chromosome numbers, giving at least partially fertile hybrids. This makes possible a cyto-genetic analysis of the differences characterising the individual species of an entire section, or rather sub-genus, thus interlinked by fertile specific hybrids. The chief intention for the present study is, therefore, to clear up how the species of one natural sub-genus are constructed, cyto-genetically speaking, or at least to throw new light upon this problem.

The genetical part of the analysis, thus defined, was initiated with KRISTOFFERSON's investigations on hybrids between *Viola arvensis* and *tricolor* (KRISTOFFERSON 1914, 1916, 1923); in the last named paper a few notes on hybrids of *Viola Munbyana* with *tricolor* and *arvensis* are added. The present author contributed with a series of papers on the taxonomic and cyto-genetic analysis of *Viola tricolor* and *arvensis* (J. CLAUSEN 1921, 1922, 1926, 1927 a and 1930 a) and on cytology and taxonomy of a number of *Viola* species (1927 b, 1929, 1930 b and 1931 b).

Some of the experiments published here date back as far as to 1922 and 1924, but the main part of them have been under way only the last five years, from 1926. A series of species were selected for this comparative investigation. This selection was partly a natural one, because most alpine species and some of the subalpine ones do not succeed under the conditions of a lowland experiment field, and some species as for instance *lutea* and *Battandierii* suffer also unquestionably from inbreeding. In such cases propagation by selffertilisation was

impossible, and it was found necessary to take recourse to individuals produced by uncontrolled pollination in the open.

Fourteen species representing the haploid chromosome numbers 7, 10, 11, 13, 17, 18, 20, 24 and abt. 26—30 (oscillating) enter in the crossings. 38 different specific crossings were effected; of these the 23 were successful, inasmuch as they gave flowering  $F_1$ -plants. Probably repeated crossing using other varieties would in some cases have given a better result. Thus, for instance, *V. Battandierii* crossed with the *alba*-yellow variety of *tricolor* gave in two reciprocal directions 24 plus 14 seeds, but only one plant came to flowering in addition to a dwarfish and very cespitose one, which for three on each other following years did not flower at all. On the other hand, the same specific cross, but applying the *hortensis* variety (Line 519,  $n = 13$ ) as the *tricolor* parent, yielded a number of vigorous, abundantly flowering and comparatively fertile  $F_1$ -plants. A similar difference was seen among the hybrids of *V. lutea* with *tricolor*: using the var. *hortensis* of *tricolor* as one parent, a vigorous hybrid was obtained, while the hybrid of *V. lutea* with *tricolor alba* was very weak and suffered from »black leg», just as the inbred *V. lutea* itself.

Only some of the crossings were reciprocal. The annual species were often a little late for the perennial ones, so that these last ones had to be used as male parents or, if both were sown the same year, as females alone. In one case, namely *V. arvensis*  $\times$  *rothomagensis*, the crossing was possible only in one direction; the cross with *arvensis* as the female parent gave many good and well germinating seeds, while the reciprocal cross with *rothomagensis* as mother in eight repeated instances invariably gave weak and shrunken seeds, which did not germinate at all. Both of these species have the haploid number of 17 chromosomes. The different success of the reciprocal crossings is therefore not connected with any difference as to the number of chromosomes in the endosperm of the reciprocal crosses, as THOMPSON (1930 a, b) showed for wheat crosses. Two plausible explanations are here suggested, namely either disturbed genic equilibrium caused by two sets of *rothomagensis*-chromosomes with one set of *arvensis*-chromosomes being brought together in the hybrid endosperm, or incompatibility between the  $F_1$  chromosomic complement and the plasm of *rothomagensis*. In the eight other cases, where reciprocal hybrids were obtained, no differences between the two reciprocals were observed. If such were present, they would be so slight as to be uncertain at all.

The technique was mainly the same as described in the 1926-paper:

emasculatation with a needle, pollination 4—6 days later and the capsules bagged in parchment bags some time before ripening in order to prevent the seeds from being spread. Sowing was effected in March in hot beds and the plants planted out in the field in May. Plants for self-fertilisation were removed to the insect proof green house, and at least all large flowered ones had to be artificially pollinated with a needle. Some of the very sterile hybrids were back-crossed. This gave more seeds. For fixation CARNOY's fluid was applied during the first years; 1927 NAWASHIN's chromic-acetic acid was tried for some of the fixations, and the last three years the combination of immersion for 5—10 minutes in CARNOY's fluid followed by abt. 24 hours in NAWASHIN's fixative proved very successful. The slides made before 1927 were stained mainly with HEIDENHAIN's iron-alum hæmatoxylin, the more recent ones with iodine-gentian violet.

The research work was done almost exclusively during leisure hours, and it had not been possible to get through without the kind and very accurate assistance yielded by my wife, Fru ANNA CLAUSEN, during the seasonal work on the experiment field. Artificial pollinations, back-crossings, fixations, baggings and harvesting were made almost exclusively by her, and she assisted me also in the enumeration of segregated types. Through the years the investigations were financially supported by funds from the Carlsberg Foundation, which liberated me from taking paid work during the time left from departmental work, enabling me to devote such time to research. Part of the cytological investigations were done during a research fellowship of the International Education Board from September 1927 to May 1928, spent at Division of Genetics, University of California, Berkeley, in my friend, Professor E. B. BABCOCK's laboratory. The remaining part of the work was done at the Genetics Department of the Royal Veterinary and Agricultural College, Copenhagen, in close and friendly connection with Professor Dr. ÖJVIND WINGE. Grateful acknowledgements are here extended to the institutions and single persons, who in one or another way, also by supplying of seeds of wild species, have supported these investigations.

The scope of this paper will be to extract from the records of the experiments such data, which might be supposed to contribute to the clearing up of problems of a more general biological interest, and to select some few crossings for a more detailed report; these may then be taken as paradigms for specific hybridisations in the *Melanium* section of *Viola*.

## II. SPECIFIC MATERIAL, WITH A SURVEY ON HYBRIDISATIONS.

The diagram, fig. 1, reports the species applied and the hybridisations made. The species are arranged after increasing chromosome number with one exception, namely those belonging to the collective species *V. Kitaibeliana*, which are inserted in one group after *arvensis*. Successful hybridisations (such that gave flowering  $F_1$ -plants) are indicated by a full line and a numeral between the two parental

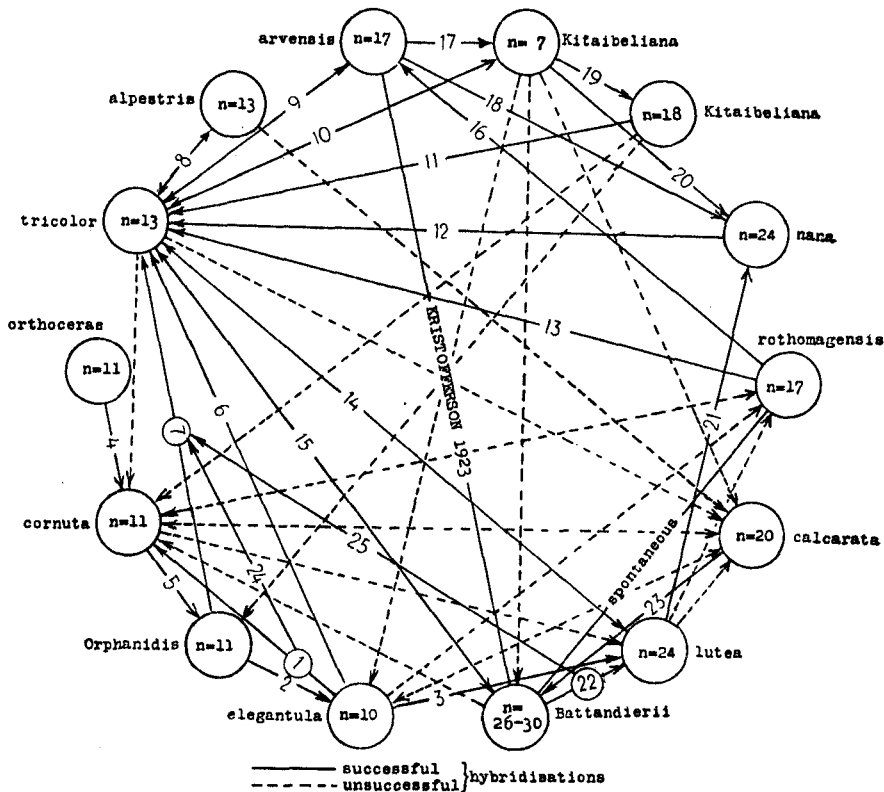


Fig. 1. Diagram of hybridisations in the *Melanium* section. Successful crossings are indicated by a full line between the parental species; the numerals on these lines refer to the cross no. in this paper; the arrows indicate the direction in which the pollen was carried. Stippled lines indicate unsuccessful hybridisations.

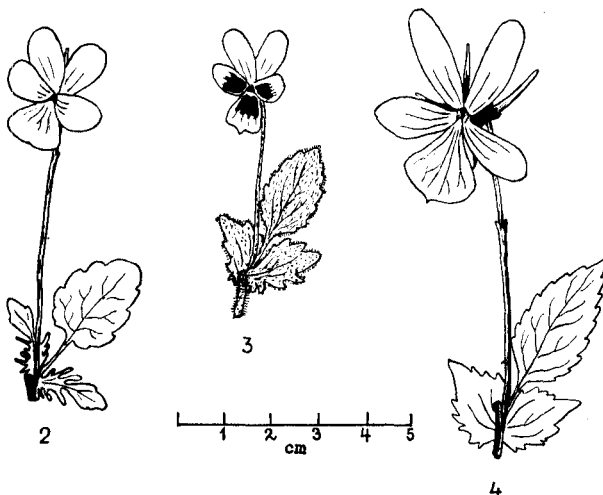
species. The numerals indicate the order in which the hybrids are described in this paper. An arrow indicates the direction in which the pollen was carried. Stippled lines similarly indicate unsuccessful hybridisations. In some cases no seeds were set, in other cases seeds were obtained, but these were not good (shrunk or empty). In most hybridisations, also of the unsuccessful ones, indeed, were good seeds with a plump and apparently good embryo formed, but they would not germinate. A number of them were treated in cold store for a week or two, the testa were caut-

iously removed from some few of them, but nothing would force them to germination. Tables 1 and 2 shortly relate about the hybridisations, the successful and the unsuccessful ones respectively.

In the following, the species applied in the investigations will be introduced to the reader. Many of them are not easily characterised in a brief description, also because they vary very much in nature. For description and figures of chromosomes see J. CLAUSEN 1927 b and 1929. For taxonomic questions compare W. BECKER 1905, 1923 and 1925 a.

*Viola elegantula* SCHOTT,  $n = 10$ .

Syn: *V. latiseptala* WETTST., *V. bosniaca* FORMANEK. Illustrations: WITTROCK 1897, Plate VII, figs. 82—88. Distribution: mountains in the northwestern part of Balcan Peninsula (1500—2000 mtrs.). A perennial species, but when cultivated in northern climate generally biennial. Characteristic by its long, straight and very narrow spur, which is thinner than that of *calcarata*. The flowers (fig. 2) are medium-sized, much smaller than *calcarata*'s and almost of the same size as *tricolor*'s. Herbage glabrate, light green, leaves ovate with cordate base, stipules lyrate-pinnatifid. Cultivated in rock gardens, the type applied in the experiments was the commonly cultivated red-flowering one (see the figures in WITTROCK 1897). No spot on the style.



*Viola Orphanidis* BOISS.,  
 $n = 11$ .

Syn: *V. proluxa* PANC. Habitat: Balcan Peninsula. Perennial, so-

Figs. 2—4. Flowers and leaves of 2: *elegantula*, 3: *Orphanidis* and 4: *cornuta*  $\times$  *orthoceras*  $F_1$  (Cross 4). All line drawings of flowers and leaves are reduced to abt.  $\frac{3}{5}$ ; the scale in centimetres applies to all of them.

meewhat prostrate, densely hirsute, stipules somewhat triangular in outline, dentate or a little lobed (fig. 3). Petals rather narrow, violet, often are the three lower ones dark *velutina* nearest to the eye. With or without spot in front of the style. Aberrant chromosomal types with  $2n = 21$  and 20 chromosomes were previously described (J. CLAUSEN 1930 b). Some of these are pollensterile. The type used for the crossings was the same as described in last named paper.

*Viola elegantula* and *Orphanidis* no doubt are nearly related and belong in group with a number of Balcan pansies as *V. Nicolai* PANT., *athois* W. BECKER., *dacica* BORB., *Dubyana* BURNAT and *declinata* WALDST. et KIT. Although typical populations of these species exist, the delimitations are in many cases very difficult. For a key to this group, see HAYEK 1917.

TABLE 1. *List of successful hybridisations. (I, II, III denote univalents, bivalents etc.)*

Cross No.	maternal species	paternal species	conjugation of chromosomes in $F_1$	fertility, seeds per plant, $F_1$	germination	carried to	including backcrosses to	$F_1$ cultivated year	remarks
1	<i>cornuta</i> n = 11	<i>elegantula</i> n = 10	ca. $10_{II} + 1_I$ (1-6 <sub>I</sub> ), III + IV occur	300-500, not poor	poor, 9 %	$F_2$	<i>elegantula</i>	1925	$F_3$ did not germinate
2	<i>elegantula</i> n = 10	<i>Orphanidis</i> n = 10, 11	multivalent association (III, IV, V, VI; I rare)	250-300, rather good	—	$F_1$	—	1930	spontaneous hybrid
3	<i>lutea</i> n = 24	<i>elegantula</i> n = 10	$14-15_{II}$ , 6-4, autosyn- desis, polysomic chains formed	50-100, poor	poor	$F_2$	—	1928	poor flowering of $F_1$
4	<i>cornuta</i> n = 11	<i>orthoceras</i> n = 11	$11_{II}$ , no elimination	rather good	poor	$F_2$	—	1927	—
5	<i>Orphanidis</i> n = 10, 11	<i>cornuta alba</i> n = 11	$8-11_{II}$ , 8-0 <sub>I</sub> , III and IV occur	200-400, rather good	poor, 10-20 %	$F_2$	—	1927	$F_3$ weak
6	<i>tricolor</i> <i>alba</i> -yellow n = 13	<i>elegantula</i> n = 10	$8-10_{II}$ , 7-3 <sub>I</sub> , polysomes (III-VI) rather common	abt. 30, very poor	—	$F_4$	<i>elegantula</i> , <i>tricolor</i>	1927	segregated cespitose, sterile dwarfs
7	<i>tricolor</i> <i>alba</i> -yellow n = 13	<i>Orphanidis</i> n = 11, 10	$0-11_{II}$ , 24-2 <sub>I</sub> , some III and IV; I split often	$30-130$ , poor	poor, 24 %	$F_4$	<i>tricolor</i>	1927	—
8	<i>tricolor</i> n = 13 <i>alpestris</i>	<i>alpestris</i> n = 13 <i>tricolor</i>	$13_{II}$ , rarely 2-4 <sub>I</sub> ; extranuclear nucleoli very common	1500-2000, very good	abt. 60 %	$F_2$	—	1926, 1928	three different reciprocal crosses

9	<i>tricolor</i> n = 13 <i>arvensis</i> <i>tricolor</i>	11-13 <sub>II</sub> , 8-4 <sub>I</sub>	500-2000, very good	good	F <sub>3</sub>	<i>tricolor</i> , <i>arvensis</i>	1921 and later	11 different reciprocal crosses
10	<i>tricolor alba</i> n = 13 <i>Kitaibeliana tricolor alba</i> n = 7	4-6 <sub>II</sub> , 12-8 <sub>I</sub> , occasional III	0-30, very poor	—	F <sub>1</sub>	<i>tricolor</i> , 10-30 seeds per plant	1930	—
11	<i>tricolor alba</i> n = 13	7-12 <sub>II</sub> , 17-7 <sub>I</sub> , non-re- duction observed, I split often	20-50, very poor	not good, 23 %	F <sub>2</sub>	<i>tricolor</i>	1927	—
12	<i>tricolor alba</i> n = 13	5-1 <sub>I</sub> , (=16-18 <sub>II</sub> ), III and IV occur; autosyn- dis	1000-2000, very fer- tile	—	F <sub>1</sub>	<i>tricolor</i>	1930	—
13	<i>tricolor</i> <i>alba</i> -yellow n = 13	ca. 1 <sub>IV</sub> , 2-3 <sub>III</sub> , 3-6 <sub>I</sub> (+ bivalents), splitting, elimination	12-37, very poor	poor, 15 %	F <sub>4</sub>	<i>tricolor</i>	1927	—
14	<i>tricolor</i> <i>hortensis</i> n = 13 <i>lutea</i>	8-11, mostly splitting; conjugation often in irregular chains	50-300, rather poor	poor, 10 %	F <sub>4</sub>	—	1925	<i>tricolor</i> , <i>alba</i> × <i>lutea</i> less viable
15	<i>tricolor</i> <i>hortensis</i> n = 13 <i>Battandierii</i> <i>tricolor</i>	few I; autosyn- dis; III + IV	ca. 100, rather good	not good, 25 %	F <sub>2</sub>	—	1927	<i>tricolor</i> <i>alba</i> -yellow × <i>lutea</i> less vi- able
16	<i>arvensis</i> n = 17	4-6 <sub>I</sub> , often splitting; 1-2 <sub>III</sub> , occasional IV; (+ II)	250-450, rather good	30 %	F <sub>2</sub>	—	1926- 1928	reciprocal unsuc- cessful

Cross No.	maternal species	paternal species	conjugation of chromosomes in $F_1$	fertility, seeds per plant, $F_1$	germination	carried to	including backcrosses to	$F_1$ cultivated year	remarks
17	<i>Kitaibeliana</i> n = 7	<i>arvensis</i> n = 17	most common: $6_{II} + 12_{I_1}$ ; up to $2_{II} + 20_{I_1}$ ; many I split	500–600, rather good, but reduced	poor, 18 %	$F_2$	—	1926	—
18	<i>nana</i> n = 24	<i>arvensis</i> n = 17	$2-6_{II}$ , $37-29_{I_1}$ ; occasional one III	250–800, rather good	—	$F_1$	—	1930	—
19	<i>Kitaibeliana</i> n = 18 n = 7	<i>Kitaibeliana</i> n = 7 n = 18	abt. $6_{II} + 13_{I_1}$ ; sometimes one III	ca. 100, very poor	very poor, ca. 3 %	$F_2$	—	1929	—
20	<i>nana</i> n = 24	<i>Kitaibeliana</i> n = 7	$5-6_{II}$ , $21-19_{I_1}$ ; III + polysomes occur	250–300, not poor	—	$F_1$	—	1930	—
21	<i>nana</i> n = 24	<i>lutea</i> n = 24	$15-18_{I_1}$ ; polysomic conjugation	50–300, rather poor	—	$F_1$	—	1930	—
22	<i>lutea</i> n = 24	<i>Baltandierii</i> n = 26–30	—	50–100, not good	50 %	$F_2$	—	1923	—
23	<i>Baltandierii</i> n = 26–30	<i>calcarata</i> n = 20	—	16–60, poor	poor	$F_2$	—	1929	—
24	Cross 7, $F_1$ n = 13 × 11, 10	Cross 1, $F_1$ n = 11 × 10	—	33–325, variable	variable	$F_2$	—	1928	quadruple hybrid
25	Cross 7, $F_1$ n = 13 × 11, 10	Cross 1, $F_1$ n = 24 × 26–30	—	130–150	poor	$F_2$	—	1928	quadruple hybrid



TABLE 2. *List of unsuccessful hybridisations.*

crossings of	maternal species	paternal species	number of seeds obtained	notes on seeds
<i>elegantula</i>	<i>elegantula</i> , n = 10	<i>Kitaibeliana</i> , n = 7	8	apparently good
	<i>rothomagensis</i> , n = 17	<i>elegantula</i> , n = 10	8	
	<i>elegantula</i> , n = 10 <i>calcarata</i>	<i>calcarata</i> , n = 20 <i>elegantula</i>	2 0	empty
<i>Orphanidis</i>	<i>Orphanidis</i> , 2n = 21	<i>Kitaibeliana</i> , n = 18	10	
<i>cornuta</i>	<i>cornuta</i> violet, n = 11	<i>tricolor alba</i> -yellow, n = 13		empty seeds, apparently one good
	<i>cornuta alba</i> , n = 11	<i>Kitaibeliana</i> n = 18 (sparse pollen)	0	
	<i>cornuta alba</i> , n = 11 (two types)	<i>rothomagensis</i> , n = 17	14 30	{ at least four with embryo
	<i>rothomagensis</i>	<i>cornuta alba</i>	2	
	<i>calcarata</i> , n = 20	<i>cornuta alba</i> , n = 11	0	not good
	<i>cornuta alba</i>	<i>calcarata</i>	0	
	<i>lutea calaminaria</i> n = abt. 24	<i>cornuta alba</i> , n = 11	0	
	<i>cornuta alba</i> , n = 11 (two types)	<i>Battandierii</i> , n = 26-30	10 10	
	<i>Battandierii</i> , n = 26-30	<i>cornuta alba</i> , n = 11	0	
	<i>Battandierii</i> , n = 26-30	<i>Kitaibeliana</i> , n = 7	7	
	<i>rothomagensis</i> , n = 17	<i>lutea</i> , n = 24	0	
<i>calcarata</i>	<i>calcarata</i> , n = 20	<i>tricolor alba</i> , n = 13	0	
		<i>tricolor alba</i> -yellow	0	
		<i>alpestris velutina</i> , n = 7	0	
	<i>calcarata</i> , n = 20 (two plants)	<i>Kitaibeliana</i> , n = 7	0 15	
	<i>calcarata</i> , n = 20	<i>lutea</i>	6	

*Viola cornuta* L.,  $n = 11$ .

Illustrations: WITTROCK 1897, plate VII, figs. 93—96. Distribution: High Pyrenees and mountainous Northern Spain and an isolated locality in Krain (Yugoslavia). Strictly perennial by subterraneous offshoots, very large-flowered with petals very narrow, especially the two upper ones. Spur very long. Petals light violet or pure white, flower fragrant. Two types were applied, one from HAAGE and SCHMIDT, Erfurt, (violet and white flowering plants) and another from H. CORREVON, Geneve (violet flowered); this last one is identical with the wild growing type. Easily cultivated.

*Viola orthoceras* LEDEB.,  $n = 11$ .

Transcaucasia 1500—2600 mtrs. Very similar to *cornuta*; mainly characterised from it by larger stipules and shorter petioles. The entire plant is larger than *cornuta*. Very difficult to maintain in culture.

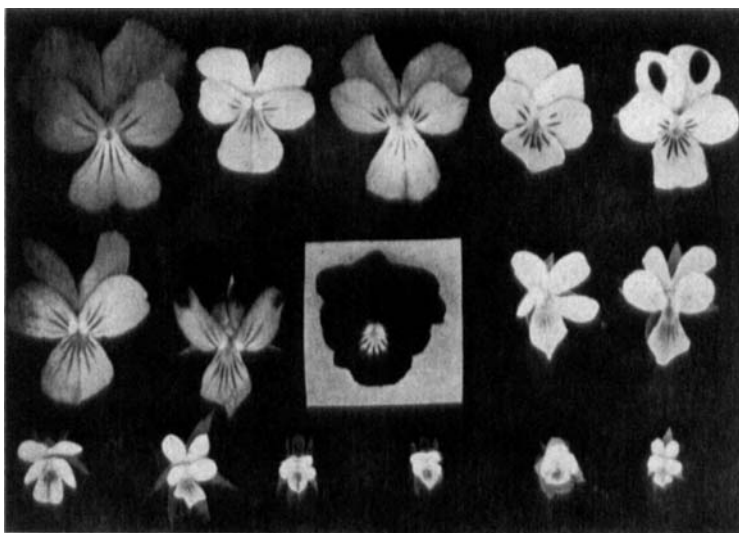


Fig. 5. Flowers of species of the group *Tricolores*; somewhat diminished. — From left, upper row: *Munbyana*, *lutea calaminaria*, *rothomagensis*, *alpestris macedonica* and *alpestris velutina*. — Middle row: *tricolor*-varieties, viz. *typica*, *maritima rosea* (velvety petals), *nigra*, *alba* and *alba-yellow*. — Lower row: *arvensis* Line C and Line 52, *Kitaibeltiana* ( $n = 7$ ,  $n = 8$ ,  $n = 18$ ) and *nana*.

Both of the species *V. cornuta* and *orthoceras* are exceptional among all other *Melanium* species by having downwards turned lateral petals and by having the centre of the flower (the eye) pure white. All the other species have at least the innermost part of the lower petal intense yellow. The stipules of *cornuta* and *orthoceras* are characteristic broad and triangular; a similar shape of stipules is found only in some species of the *Orphanidis* group, which may be more or less related with *cornuta-orthoceras*. Fig. 4 is from a line drawing of the  $F_1$  hybrid *cornuta*  $\times$  *orthoceras* and illustrates the morphological characters of leaves and flowers of this group. None of these two species have spot on the style and, notwithstanding their low number of chromosomes, they belong among the largest of the Pansies.

*V. tricolor* L.,  $n = 13$ .

For description of variation and types, see WITTROCK 1897 and J. CLAUSEN 1922 and 1926. The typical *V. tricolor* has rather large violet petals and palmate stipules (fig. 5). Most varieties are annuals but subspec. *maritima* is perennial. The varieties used for crossing are: *V. tricolor violacea*, Line 504 (violet flowers, the type of the species), *tricolor alba*, Line 320 (pure white flowers, without anthocyanin), *tricolor hortensis*, Line 519, velvet-violet flowered, an old cultivated garden type, (see J. CLAUSEN 1926, pp. 4—6 regarding these three varieties) and *tricolor alba-lutea* (J. CLAUSEN 1930 a, p. 351), an intense yellow flowering type free from anthocyanin; this type was extracted from the cross *tricolor lutea*  $\times$  *tricolor alba*. The middle row of fig. 5 shows flowers from a series of *tricolor* varieties.

*V. alpestris* (DC.) WITTR., W. BECKR.,  $n = 13$ .

Syn: *V. saxatilis* SCHMIDT (1794). Illustrations: WITTROCK 1897, Plate VI, figs. 77—79. *V. alpestris* is probably only a subalpine subspecies of *tricolor*. Growing on its natural habitats it is perennial; the flowers of most types are bright yellow, sometimes with a *velutina* blotch on the upper petals, those of other types are of a bleached violet colour. The two flowers most to the right in the upper row of fig. 5 belong to *alpestris*; the left one is of the Balcan type (*V. macedonica* BOISS., courteously sent by Professor KOSANIN, Beograd), the right one with *velutina* blotches is from Czechoslovakia (*V. polychroma* KERN.). It is very difficult to characterise *alpestris* from *tricolor*, the characters named may be found in varieties of both species; the shape of the upper leaves may be the most unfailing one; in *alpestris* they are broadly ovate (fig. 7) or even with a cordate base, while *tricolor*'s are lanceolate (fig. 6). The two types used for the crossings were both extracted from a collection of seeds received from the Botanical Gardens in Brno, Czechoslovakia, viz. the blotched variety named above and a yellow, non-blotched one. Both of them had dark spot on the style.



Figs. 6—8. Flowers and leaves of 6: *tricolor typica* Line 504, 7: *alpestris velutina* (var. *polychroma*) and 8: *arvensis*.

*V. arvensis* MURR.,  $n = 17$ .

See description of types and variation in WITTROCK 1897 (many coloured plates) and in J. CLAUSEN 1922 and 1926. Characterised from *tricolor* and *alpestris* by its small flowers (petals smaller than sepals, see the two left flowers in the lower row of fig. 5 and the line drawing, fig. 8), its yellowish white flower colour (not *alba*) and the absence of labellum under the stigma; the stipules are lyrate or pinnatifid.

By the structure of the flower the species is autogamous and it is also strictly annual.

*V. Kitaibeliana* ROEM. et SCHULT., sens. lat.

Syn: *V. nemausensis* JORD. — *V. Kitaibeliana* resembles *arvensis*, but it is in all respects smaller, and especially its flowers are smaller and more closed. The petals of *arvensis* have spread-out and flattened limbs, while those of *Kitaibeliana* form a small cup-shaped corolla (see the four last flowers in the lower row of fig. 5). Just as in *arvensis* the flowers are yellowish white without labellum.

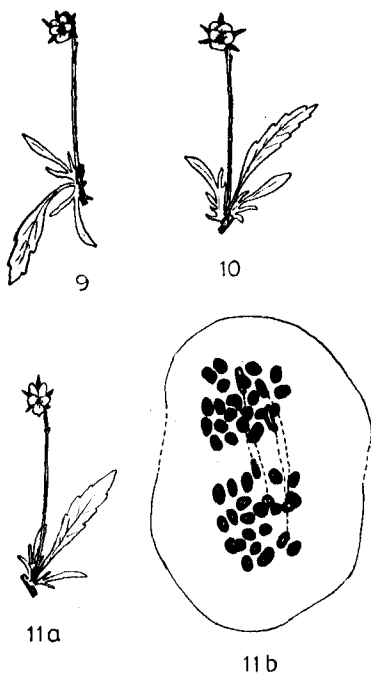
The species, although as to its content of types morphologically much more uniform than *tricolor*, comprises a number of chromosomal different types, namely with  $n = 7, 8, 12, 18$  and  $24$  and possibly still more numbers. The following were used for crossings:

$n = 7$ , (fig. 9) a type from Caucasia sent from the Tiflis Botanic Gardens. A tiny type with rather long internodes; no spot on style.

$n = 18$ , (fig. 10) also from Caucasia, courteously sent by Dr. G. WORONOFF. A stout and erect type, in its vegetative parts distinct from the preceding one but the flowers very similar; no spot.

*V. nana* DC.,  $n = 24$  (figs. 11 a and 11 b); the smallest of all the species and more tiny than *Kitaibeliana*  $n = 7$ . Its morphological characters are covered by the description of *Kitaibeliana*, except that the corolla is a little more open flowered. The seeds of this interesting type were collected on the island Jersey in the English Channel and kindly sent me by Dr. E. DRABBLE. Slight irregularities may take place during the progress of the meiosis, although it gives the impression of having a fairly constant chromosome number.

The group formed by the last mentioned species (*tricolor*, *alpestris*, *arvensis* and *Kitaibeliana* sens. lat.) contains the only annual and lowland species in the entire section; *alpestris*, indeed, forms a transition, being sub-



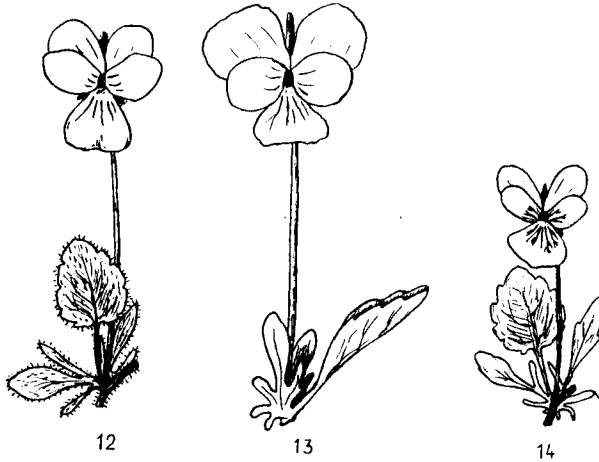
Figs. 9—11. Flowers and leaves of 9: *Kitaibeliana*  $n = 7$ , 10: *Kitaibeliana*  $n = 18$ , 11 a: *nana* and 11 b: heterotypic anaphase of *nana*,  $2n = 24 + 24$ ; for clearness sake are the two nuclear plates removed a little from each others ( $\times$  ca. 1800).

perennial and subalpine. They have the widest distribution of all the pansies; *tricolor* and *arvensis* occupy Northern and Middle Europe and push forward into Western Asia; *arvensis* goes more southern than *tricolor*, which in the subalpine areas is supplanted by *alpestris*. *V. Kitaibeliana* in its distribution is more southern than the other ones, mainly Mediterranean to West-Asiatic; the most western type of it is *V. nana*, whose area is the surroundings of the Channel. The small-flowered species of this group, as *arvensis* and *Kitaibeliana*, are the only *Melanium* species without labellum under the stigma or at least with a very small one and are there-

fore also the only autogamous ones. All large-flowered species have this labellum, which prevents spontaneous selfpollination.

*V. rothomagensis* DESF.,  $n = 17$ .

Syn: *V. hispida* LAM. Illustrations: WITTROCK 1897, plate XI, figs. 178—181. *V. rothomagensis* is a strictly local species from the calcareous tracts in northwestern France. The flowers (fig. 5, the middle flower in the upper row) are violet and remind about those of a large flowered *tricolor*, but *rothomagensis* is perennial, and the stems are more weak than *tricolor*'s. The upper leaves of *rothomagensis* are ovate with a cordate base (fig. 12), while the corresponding leaves of *tricolor* are lanceolate to linear-lanceolate. The most characteristic trait of *rothomagensis* is the straight, stiff and spreading hairs, with which the stems and the leaves and especially the margins of these are beset. They give the leaves and the stipules a ciliate appearance. The hairs of *V. Orphanidis* are more weak and also more dense than those of *rothomagensis*. With spot on style. The phylogenetic relationship of *rothomagensis* is not clear. Its position seems to be a little isolated, although it shows some relationship with *tricolor*, *arvensis* and *Battandierii*. The type used came from the Botanic Gardens of Rouen.



Figs. 12—14. Flowers and leaves of 12: *rothomagensis*, 13: *calcarata* and 14: *lutea*.

*V. calcarata* L.,  $n = 20$

Illustration: WITTROCK 1897, plate VII,

figs. 97—98. *V. calcarata* is a decidedly alpine and perennial species. *Calcarata* itself in strict sense is not much variable, but taken together with its near relatives *V. Zoysii* WULF., *Bertoloni* SALIS., *nebrodensis* PRESL. and the widespread *heterophylla* BERTOL. it forms a very variable group of near related and not easily defined species. Characteristic for *calcarata typica* are the very short internodes, the broad upper petals and the long, straight spur; the colour of the flower is violet in the type, but yellow in the var. *flava* and in *V. Zoysii*. The type used for the crossings was received from the Botanic Gardens of Gothenburg as *V. Bertolonii* DE SALIS. It is a rather typical *calcarata* (fig. 13), except that the spur is a little shorter and the petals not just as broad as in the *calcarata* proper, which is very difficult to cultivate.

*V. lutea* HUDS.,  $n = 24$ .

This species may be related to the *tricolor-arvensis* group, but is perennial. It is subalpine and has a distribution from Scotland and Ireland through the Middle-European mountainous districts to Austria and Hungary. The upper leaves are short ovate, and it has rather large yellow flowers, in some of the types saturated yellow flowers. The type used in the experiments belongs to the western subspecies, *elegans*

KIRSCHL.; it has long and thin, underground stolons but was received from Glasgow Botanic Gardens under the name of *V. tricolor*. The flower colour of it is bleached yellow, and it segregated types with violet petals (var. *amoena*) and some with the two upper petals velvety (*velutina* 1). The flower of this type (fig. 14) is not larger than that of *tricolor*. Characteristic are the dark, very furcate striæ on the three lower petals. It has a dark spot in front of style. It suffers from inbreeding, and was therefore difficult to maintain in cultivation, except by spontaneous pollination between different individuals in the Copenhagen Botanic Gardens. Fig. 5 (upper row, second from left) shows a flower of the var. *calaminaria* (LEJ.) also belonging to subsp. *elegans*.

The cultivated *V. lutea* (see figure in WITTROCK 1897, plate VI, figs. 80—81) probably originated from the eastern type, subspec. *sudetica* WILLD., which is more erect, more deep yellow and more large flowered. From crosses between the cultivated *V. lutea* and old cultivated types of *V. tricolor* took our garden pansies *V. Wittrockiana* GAMS (= *V. tricolor maxima* hort.) their origin.

*V. Battandierii* W. BECKR., the garden type with oscillating chromosome number,  $n = \text{ca. } 26-30$ .

Syn: *V. Pseudo-Munbyana* W. BECKR. The type used of this species is completely identical with the *V. Munbyana* described and shown in WITTROCK 1897, plate XI, figs. 173—177, and it was received from Muséum d'Histoire Naturelle in Paris under the name of *V. gracilis* SIBTH. et SM. This type is commonly grown in Botanical Gardens under different names, and it was apparently also used by KRISTOFFERSON (1923) for his crossings with *V. arvensis* and *tricolor*.

There has been some confusion as to the identity of this type. It is not identical with the North African (Algerian) species *V. Munbyana* BOISS. et REUT., which has much smaller stipules and is considerably smaller in stature than the Botanic Gardens type, being one of the largest pansies at all. The true *Munbyana* is by transitions connected with *V. heterophylla* BERTOL. (subsp. *ovatifolia* W. BECKR.), *V. gracilis* SIBTH. et SM. and *V. nebrodensis* PRESL. [var. *grandiflora* (GUSS.) CARUEL], all belonging in the group of *V. calcarata*.

The question now remains, if the type from the Botanical Gardens is the replica of any other wild growing type. BECKER first (1905) supposed this and identified it with a type collected in Algeria by REVERCHON 1896 (No. 192) under the name of *V. Munbyana*, by BECKER named *Battandierii*. Later (1925 b) BECKER thought the Botanic Gardens type to have no known wild growing representatives, and in order to distinguish it from the North African one he named the garden type *V. Pseudo-Munbyana*. The present author thinks this is to overemphasize the differences and has already (1927 b) drawn attention to the fact that types collected in cedar forest at Teniât-el-Had in Algeria by C. M. POULSEN (1870) and by A. LETORNEUX (1888) very much resemble the types from the Botanic Gardens, also as to size and the characteristic shape of the stipules. BECKER himself drew this type to *Battandierii*. The writer, therefore, identifies the Botanic Gardens type as a *varietas hortensis* of the North African *Viola Battandierii* W. BECKR.

The type in question is strictly perennial, very vigorous and rich flowering. The leaves are large, ovate, and the stipules palmately lobed with a large foliaceous end-lobe and a number of lanceolate side lobes (fig. 15 a). The peduncles are very long and very easily detached from the stem by a kind of joint. The flowers are large,

violet and with a short spur; the flower in the upper left corner of fig. 5 is of *Battandierii* and shows the characteristic triangular shape of the lower petal.

In the Copenhagen Botanic Gardens it constantly crosses with *rothomagensis*. It suffers from inbreeding and has to be maintained by open pollination. During the winter 1928—29 the original culture at the experimental field at Lyngby completely disappeared. This was so more to regret, because the old chromosomal count meantime had proved unsatisfactory, due to primitive fixation with CARNOY's fixative and staining with DELAFIELD's hæmatoxylin. Two or three homotypic metaphases

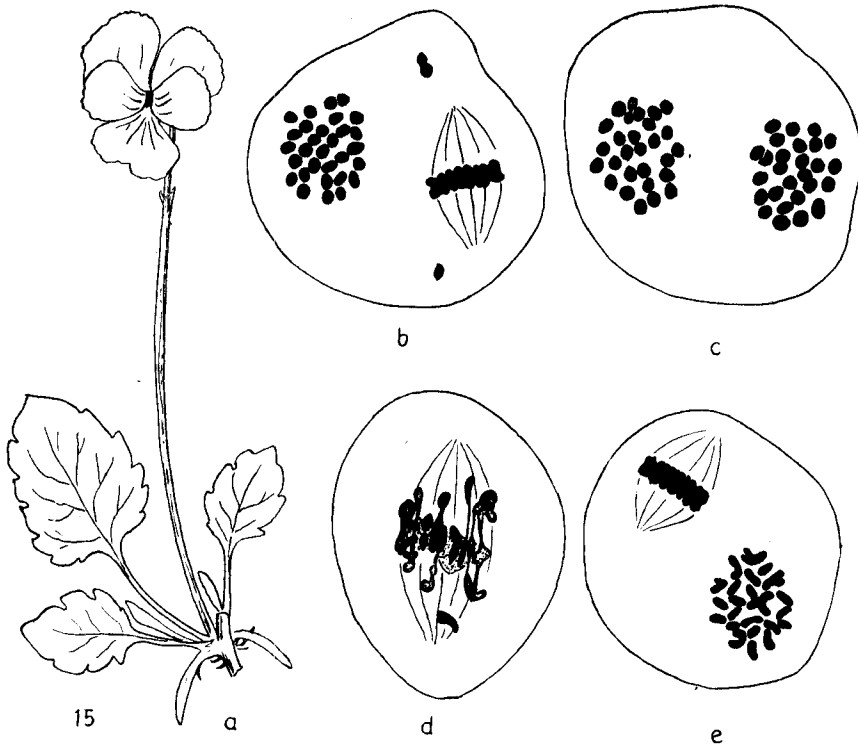


Fig. 15. *V. Battandierii*. a: flower, leaf and stipules; b—c: homotypic metaphases of pollen mother cells,  $n = 30$ , abt. 26 and abt. 27, in b two and in c one chromosome (divided, left) outside the nuclear plates; d—e: selfed individual (see text), d: heterotypic metaphase and e: homotypic metaphase,  $n = \text{ca. } 24-26$  chromosomes of a long shape. (b—e  $\times 1800$ .)

showed 30 chromosomes (fig. 15 b), but others not more than 26, 27 or 28, (fig. 15 c), and these last ones were thought to represent cut pollen mother cells with incomplete nuclei. In fact they were complete. In heterotypic metaphases of these old fixations the chromosomes appear conglomerated as if polysomes occur, but they are impossible to follow. From a selfpollination only one plant was obtained; it was very weak. Fig. 15 d shows the heterotypic metaphase from this plant with very long and irregularly disposed chromosomes, fig. 15 e a homotypic metaphase with 24—26 rather long, bent chromosomes. — The hybrids suggest that the gametes of the original *Battandierii* type generally carried not far from 30 chromosomes. It is thus the

*Melanium* species with the largest chromosome number known, but represents a cytologically irregular type similar to *Viola canina* (J. CLAUSEN 1931 b) although the type as judged from illustrations and herbarium specimens maintains itself morphologically.

### III. MORPHOLOGICAL NOTES ON $F_1$ . DESCRIPTION OF GENIC COOPERATION.

Instead of giving a detailed description on each  $F_1$  it is thought better here to treat the individual characters collectively for a number of hybrids. This may prove useful for botanists, who observe supposed hybrids in nature. A description on the action of genes will also be given, which is necessary primarily for the complicated system of genes effecting colouring of flowers.

#### DURATION OF LIFE.

The *perennial* type acts as dominant over the *annual* one. This character was involved in the Crosses 1, 13, 14, 15 and 16 (see the diagram, fig. 1 or table 1). The perennial character apparently is a very complex one, and several genes may be responsible for it, because the species used in the experiments are perennial to a very different degree, representing a true gradation regarding this character.

*V. cornuta* and *Battandierii* are strictly perennial, the same is *lutea* on its natural habitats, but under the conditions, which the experiment field offers and probably also due to inbreeding, it is weak. On the experiment field *rothomagensis* lives 2—3 years, *elegantula*, *Orphanidis* and *alpestris* behave as biennials, although they are told to be true perennials on their natural habitats.

The  $F_1$  hybrids here mentioned are not more perennial than their perennial parent: *tricolor*  $\times$  *rothomagensis*, *arvensis*  $\times$  *rothomagensis* and *tricolor*  $\times$  *lutea* live two years at least. *Cornuta*  $\times$  *elegantula* is still very vigorous after six years and the same is *tricolor*  $\times$  *Battandierii*, although the *tricolor* type applied is strictly annual.

Hybrids between two perennials are also perennial: Cross 22, *lutea*  $\times$  *Battandierii*, is still very strong and rich flowering after eight years lifetime, although its parents died (*Battandierii* probably due to winter conditions); Cross 4, *cornuta*  $\times$  *orthoceras*, is also vigorous after four years, although it was impossible to keep *orthoceras* in culture.

$F_1$  of Crosses 6, *tricolor*  $\times$  *elegantula*, and 7, *tricolor*  $\times$  *Orphanidis*, were partly biennial just as their male parents.

In  $F_2$  of the crosses named several types were segregated, of which a number had the general appearance of perennials and others the



character of annuals, but the plan of the experiments has not hitherto allowed a maintaining of the  $F_2$  populations through a number of years in order to test the length of life of the segregated types. Annuals crossed with annuals invariably have given plants of annual type, in  $F_1$  as well as in  $F_2$ .

Considering the evolutionary points of view, it should then seem more probable that the annual types originated from the perennial ones than *vice versa*, if they are of monophyletic origin at all. Annual violets are indeed the exceptions. Among the largely 500 *Viola* species recognised are no more than about five annuals, of which the four belong in the *Melanium* section in close relationship with *Viola tricolor*. They are: *V. tricolor*, *arvensis*, the *Kitaibeliana* group including *V. occulta* LEHM., *parvula* TIN. and related types, and finally *V. Rafinesquii* MUHL. from the Eastern United States of America. The fifth annual *Viola* species, viz. the East Asiatic *V. diffusa* GING., has the same number of chromosomes,  $n=13$ , as *V. tricolor* (MIYAJI 1929), a number not found elsewhere among the violets. BECKER (1925) places *diffusa* in the *Nomimium* section, but morphologically it is a *Melanium* violet. MIYAJI (1929) makes suggestion as to the character of its leaves and stipules being similar to those of the *Melanium* section, and its structure of style (J. CLAUSEN 1929, fig. 44) sooner places it in the *Melanium* than in the *Nomimium* section. This brings all the annual species into one section. It would be of interest to try a crossing between *diffusa* and *tricolor*.

#### SIZE OF FLOWERS.

The character *small flower* (i. e. petals shorter than the sepals) is prevalent over the *large flowering* type (petals larger than sepals). The Crosses 9, 10, 11, 12, 16 and 21 all have a large and a small flowering parent. All  $F_1$ 's were small intermediates as to size (see Table 3).

$F_1$  of Cross 16 showed some variation in flower size with a mean of abt. 7.5 mm. for the two upper petals; the other crosses had fairly constant flower size. For the three first named hybrids the pure white *alba* type of *tricolor* was applied. The two upper petals of its flowers are not 13, but only 10 mm., indeed, but this is due to the fact only that it contains none of the basal genes *A* for anthocyanin flower colours; as soon as one gene *A* is added, the flowers attain full size. As all  $F_1$ 's carry *A* (from their small flowered parent) the size value attributed to the *tricolor* parent must be that of the anthocyanous coloured type.

TABLE 3. *Length in millimeters of upper petals in  $F_1$  hybrids and in their parents.*

small flowered parent			$F_1$			large flowered parent		
	n=	mm.	Cross no.	actual length in mm.	calculated mean length in mm.	mm.	n=	
<i>Kitaibeliana</i> ...	7	3,0	10	6	8,0	13	13	<i>tricolor</i>
	18	3,5	11	5	8,25			
<i>nana</i> .....	24	2,5	12	6,5	7,75	13	24	<i>lutea</i>
			21	6,5	7,75			
<i>arvensis</i> .....	17	5,0	9	7	9,0	13	13	<i>tricolor</i>
			16	7,5	9,0			
						13	17	<i>rothomagensis</i>

There may be several genes affecting the flower size, some increasing it, others decreasing it, but the most superior one in its effect seems to be an inhibiting gene, *F*, present in the small flowered species (J. CLAUSEN 1926). Large flowering plants are then *ff* and may in addition have some minor genes, positively increasing the flower size, while real small flowered species have no such ones. Due to the interaction of these genes,  $F_1$  is not real small flowered but small intermediate. The hexaploid *V. Kitaibeliana*  $n=18$  may have at least two inhibiting  $F_1$ -genes as suggested from the very small flowers of  $F_1$  and from the segregation in  $F_2$ . Diploid *Kitaibeliana*  $n=7$  seems to have a less inhibiting effect than hexaploid *Kitaibeliana* upon the flower size of  $F_1$ , probably because it has no more than one principal inhibitor.

Otherwise the chromosome number itself seems to be of no effect in stamping the type; which species shall be the predominant one seems to be due to the effect of their genes only, not to their amount of chromosomes. Small size of flowers is prevalent as well in Cross 10, where it was introduced by the parent with the smallest number of chromosomes as in Crosses 9, 11 and 12, where the parents with the largest chromosome number introduced small flower size. KRISTOFFERSON (1923) measured length of petals in  $F_1$  and  $F_2$  of the Cross *tricolor*  $\times$  *arvensis* and found similar relations.

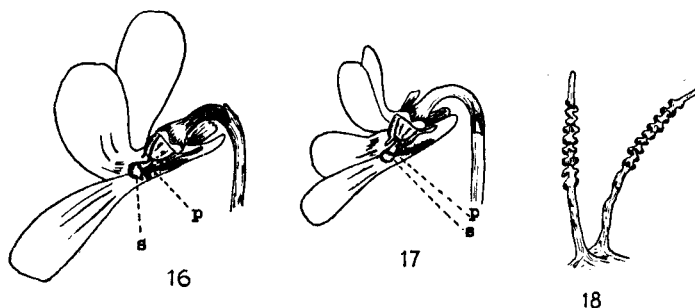
#### SIZE OF LABELLUM.

All large flowered species of the *Melanium* section have a minute *labellum* on the front side of the style under the stigma, while

the few species with small flowers, previously mentioned, have no such one.

This easily overlooked character is a significant one in the biology of the flower, as it practically determines if the species is capable of spontaneous selffertilisation or not. When present, it prevents the pollen from falling into the stigma.

Although presence or absence of labellum seems to be the principal factor in determining if the species shall be a hercogamous or an auto-gamous one, this outcome is influenced also by other mechanisms, which likewise seem to be correlated to labellum and flower size. The pollen, which fall from the anthers, are retained by the *pollen magazine* (figs. 16—17) a variably shaped groove on the proximal part of the spur



Figs. 16—18. Flowers cut through in order to show the interrelation between stigma (*s*) and pollen magazine (*p*); 16 is from *tricolor* (with labellum under the stigma and closed pollen magazine); 17 is from *arvensis* (no labellum, open magazine); 18: hairs from the pollen magazine (enlarged).

bearing petal. The walls of this groove are formed by peculiar hairs (fig. 18) irregularly fitted with wart-like processes on their surface, serving to retain the pollen — either for later removal by insects hunting the nectar secreted by the glandular projections from two of the anthers — or for a gradual dropping them into the unsheltered and somewhat backwards turned stigma of the small flowered species. Presence or absence of labellum, shape and relative length of the pollen magazine and direction of stigma seem to be the principal agencies determining the mode of pollination.

There seems to exist a mechanism keeping together all these characters in two blocs, namely (1) large flowers, stigma directed somewhat forwards sheltered by a labellum and placed outside a pollen magazine, which is closed in front (fig. 16) and (2) small flowers, with stigma directed downwards and somewhat backwards, its opening

pressed down in the pollen magazine, which forms a funnel-shaped channel open in front (fig. 17). It is for these species a lucky case that small, inconspicuous flowers coincide with a structure, which facilitates selfpollination. By the cross *arvensis*  $\times$  *tricolor* (J. CLAUSEN 1926, pp. 82—85) it was shown that the mechanism, which keeps »small flowers» and »no labellum» together is a genetical linkage, apparently because the inhibitors for flower size and for labellum are located in the same chromosome at some distance from each others.

In crosses between species without labellum or with an extremely minute one and species with distinct labellum  $F_1$  has almost no labellum (compare also KRISTOFFERSON 1923, CLAUSEN 1926), and the principal inhibitor, *B*, for labellum is thus prevalent in its effect, just like the inhibitor for flower size. In some cases  $F_1$  is more or less hercogamous, in other cases autogamous (compare KRISTOFFERSON 1916) according to the factors, which in each special case influence the biology of the flower.

#### DIRECTION OF LATERAL PETALS.

The direction of the lateral petals is a valuable character in the classification of larger taxonomic units. All the violets of the *Nomium* section have downwards turned lateral petals and all *Melanium*

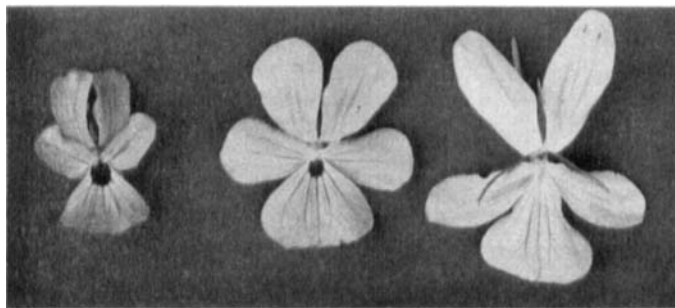


Fig. 19. From left: flowers of *elegantula*, *cornuta*  $\times$  *elegantula*  $F_1$  and *cornuta*. Portrait film without filter in order to show extension of bright yellow eye.

violets save the two exceptional species *V. cornuta* and *orthoceras* have upwards turned side petals. This character is involved in the Crosses 1 and 5.  $F_1$  is intermediate with »horisontal» lateral petals as fig. 19 (*cornuta*  $\times$  *elegantula*) shows. This behaviour in the cross was noted already by WITTROCK (1897, plate 7, figs. 89—90 and plate 9, figs. 125—126).

## INTENSE YELLOW EYE.

*V. cornuta* and *orthoceras* deviate also from all other *Melanium* violets by not having the intense yellow eye on the proximal part of the lower petal.  $F_1$  of the Cross 1 above had a yellow eye just as intense as in the *elegantula* parent, although its extent was smaller than in *elegantula*. Thus this character is dominant as to intensity but intermediate regarding extension. Fig. 19 is from a photograph on portrait film and shows very distinctly the difference of the yellow between the two parents and the  $F_1$ .

## HAIRINESS.

Two hairy species are included in the crossings, viz. *V. rothomagensis*, hispid by rather dense, straight and stiff, spreading hairs and *Orphanidis*, very densely hirsute by more soft hairs.

The *hispid rothomagensis* was used in the Crosses 13 and 16 with *tricolor* and *arvensis* (almost glabrous with few appressed hairs) as the other parent.  $F_1$  was somewhat intermediate but conspicuously hispid, although the cilia were shorter and more remote than in *rothomagensis*.

The *hirsute Orphanidis* was used in the Crosses 2, 5 and 7, with *elegantula*, *cornuta alba* and *tricolor* respectively. *V. elegantula* and *cornuta alba* are almost completely glabrous and  $F_1$  of Crosses 2 and 5 were minutely puberulent on young stems and had remote, short, appressed hairs on the leaves.  $F_1$  of Cross 7 with *tricolor* had rather few and remote hairs being not much more hairy than *tricolor* itself.  $F_2$  and later generations contained never a plant nearly as hirsute as *Orphanidis*; some of them were more or less puberulent, but never hirsute. This may be due to elimination of certain types, as  $F_1$  was very sterile.

Thus the *rothomagensis* hispid type of hairiness gives the impression of being dominant or at least prevalent, while the *Orphanidis* hirsute type seems to be recessive.

## FLOWER COLOUR.

*Anthocyanin.* The violet, red and more or less dark velvety flower colours depend upon the presence of at least one gene of the polymeric series (series of multiple genes)  $A_1$ ,  $A_2$  and  $A_3$  for anthocyanous colour in stems, leaves and flowers. They seem to be basal genes for flower colour also; they are dominant and give full effect in a single dose but may be present up to six times; the species with the largest chromosome number have also more  $A$ -genes.

If all *A*-genes are absent, the stem is pure green without anthocyanin and ordinarily the flower then is also pure white (*alba*). It may, nevertheless, be converted into *dilute mauve* by the action of a gene, *D*, which is hypostatic to all *A*-genes but dominant, when its action is not covered by *A*. This gene is present in *V. arvensis* (J. CLAUSEN 1926, p. 107) and in *alpestris*.

All plants recessive for *A* are smaller and have also smaller flowers than those with anthocyanin. It does not make any difference in this respect, whether they have *D* or not. *aaD*-plants may have a dilute mauve tinge on the stems, but this colour is different from the dark anthocyanous *A*-colour.

Genes of the *A*-series were analysed in the Crosses 1, 5, 6, 7, 8, 9, 11 and 13; 10 and 12 are not yet carried further than to *F*<sub>1</sub>.

*Velvety colours (velutina)*. The flower colour produced by the action of *A* alone without any other genes is really black velvety and extremely dark (fig. 5, middle row), but all wild species have a series of dominant and partial epistatic genes (*M*<sub>1</sub> to *M*<sub>5</sub>) which gradually modify this dark colour to violet; *M*<sub>1</sub> is the most superior and epistatic of these, able to change black to violet by almost one step (see J. CLAUSEN 1930 a, pp. 349—355). The flower colour contingent upon *A* is thus intensified by the absence of genes of the *M*-series. Dilute mauve *aaD* cannot become *velutina* or dark by absence of the *M*-genes.

*Reddish (rose) flower colour, rr*, is recessive. It demands the presence of one of the genes *A* for anthocyanous cell sap and it is changed to colours of the violet series by its dominant alternative, *R*, probably converging the reaction of the cell sap. The dilute colour contingent upon the presence of *D* exists also in both a dilute mauve (*aaDR*) and a dilute rose (*aaDrr*) edition. Reddish can be intensified to reddish *velutina* (velvet) by the absence of genes of the series *M*<sub>1</sub> to *M*<sub>5</sub>. Reddish or rose is often present in *V. elegantula* and was found in wild growing populations of *tricolor maritima*. It was analysed in Cross 6 and previously (J. CLAUSEN 1926) in Cross 9 (*tricolor maritima rosea* × *arvensis*) and three varietal crosses of *tricolor*. In all cases it was found to be recessive to violet (*AR*); *V. tricolor hortensis* and probably also *V. arvensis* were found to possess two polymeric *R*-genes.

*Intense yellow flower colour, LL*, is most intense on the spur-bearing petal (the lower one); it is namely epistatic to the violet colour contingent upon the action of *AMR*, and as this is most strong in the upper petals, *LL* cannot here suppress it to more than yellowish white; in spring time, when the plants are more anthocyanous, the upper petals

of *AMRLL*-flowers are often brownish purple. The *L*-gene occurs in some types of *V. tricolor* (J. CLAUSEN 1926), it is characteristic of most types of *alpestris*, it is present in *arvensis* but its effect is here covered by a bleaching gene (*W*), and this seems to be the case also with all *Kitaibeliana* types; the 18-chromosome type, at least, contains it. Finally, it is present in *Viola lutea*, but the type used in the crossing apparently also has a bleaching gene (called *Pal*) of a less bleaching effect than the *W*-gene.

It is obvious that a number of other genes govern the extension of yellow, for in some cases only the lower petal is intense yellow, the upper ones being more or less violet, while in other cases the *LL*-genes bleach the upper petals to yellowish white, and in still other types also they are intense yellow. A similar difference can be seen in the reaction of the heterozygotic type, *Ll*, which, if it is a pure *tricolor* type, begins as yellowish white, when the flower is just opened, but by and by the upper petals darken to pale violet, so that whitish and violet flowers may be present on the same plant, what is often noted on specimens occurring in nature; in this species the heterozygote can safely be distinguished from the violet and the yellow homozygotes. In Cross 8, *alpestris*  $\times$  *tricolor*, on the other hand, the *L*-gene from *alpestris* is not able to bleach the violet colour of *tricolor*, except when twice present; the heterozygote, *Ll*, must therefore be classified together with the homozygotic violet coloured *ll*-individuals. When violet colour is intensified by absence of the *M*-genes, *LL* has no visible effect on the upper petals. The gene *L* enters in the Crosses 7, 8, 9, 11, 14 and 22.

Yellowish white flower colour is due to the dominant or at least prevalent bleaching gene, *W*. Its bleaching of violet or rose of the *A*-series is not complete, except when together with *LL*. The gene *W* is present in the small flowered species *V. arvensis* and *Kitaibeliana* sens. lat. It may be present also in the large flowered *V. rothomagensis*, although the genetical basis of colouring of flowers in this species has not been fully cleared up yet.

$F_1$  of all crossings, of which one parent is yellowish white, are rather whitish flowered, although the older flower may have a tinge of pale violet. This has been found for the Crosses 9, 11, 12 and 16.  $F_1$  of Cross 10 (*Kitaibeliana*  $n=7$  as one parent) behaves in a different way, as it is rather violet of flower colour, although *Kitaibeliana* itself is whitish, at least during mid-summer. Probably this *Kitaibeliana* type has no *L*-gene.

*A superior gene for violet flower colour.* Apart from the two types

of genes giving more or less violet flowers, namely those of the A-series and the hypostatic dilute colour *D*, still a third type of violet flower colour may occur caused by a gene, *V*, which is epistatic over intense yellow, *LL*, (this again being epistatic over the basal violet flower colour, *AMR*, of the pansies). The play between these different genes for violet is very complicated. The gene *V* has been shown to exist in *V. Orphanidis* (Cross 7), and the violet flower colour of *V. rothomagensis* may depend upon this gene also (in cooperation with *W* and *L*), because the results of Cross 16 would otherwise be inexplicable. The *V*-gene is probably also present in an *alpestris* type received from Mount Bistria (Scardus) in Yugoslavia under the name of *V. elegantula*. It has 13 pairs of chromosomes and segregated an intense yellow type similar to the so-called *V. macedonica* BOISS. et HELDR. (= *V. alpestris*).

TABLE 4. *Some flower colours in Melanium violets and their gene symbols.*

$a_1 a_2 a_3$	$dd$ . . . . .	<i>alba</i>
	$D$ . . . . . $R$ . . . . .	dilute mauve
$a_1 a_2 a_3$	$D$ . . . . . $rr$ . . . . .	dilute rose
	$dd$ . . . . . $LLww$ . .	<i>alba</i> -yellow ( $aaLl = alba$ )
$A$ . . . . .	$D$ . . . . . $LLww$ . .	<i>alba</i> -yellow with dilute mauve upper petals
	$..m_1 m_2 m_3 m_4 m_5 R.llww$ . .	black velvet ( <i>velutina</i> 5)
$A$ . . . . .	$..m_1 m_2 m_3 M_4 . . R.llww$ . .	violet <i>velutina</i> 3 (= <i>tricolor hortensis</i> )
	$..m_1 m_2 m_3 M_4 . . LLwvv$ . .	yellow <i>velutina</i> 3
$A$ . . . . .	$..m_1 M_2 . . . . . R.llww$ . .	violet <i>velutina</i> 1
	$R.llww$ . .	violet (= <i>tricolor typica</i> )
$A$ . . . . .	$rrllww$ . .	rose
	$..Llwwvv$ . .	whitish to pale violet (sometimes violet)
$A$ . . . . .	$..LLwvvv$ . .	intense yellow (= <i>V. lutea</i> and <i>alpestris</i> )
	$..LlWvvv$ . .	whitish pale violet
$A$ . . . . .	$..LLWWvv$ . .	yellowish white (= <i>arvensis</i> )
	$.....V$ . .	violet (= <i>Orphanidis</i> , possibly also <i>rothomagensis</i> )

Table 4 gives a survey on the most significant types of flower colour and their formulæ.

#### DARK SPOT ON STYLE.

In Cross 9, *arvensis*  $\times$  *tricolor*, and in varietal crossings of *arvensis* and of *tricolor* (J. CLAUSEN 1926) it was found that the inheritance of the trivial character of a small triangular, dark spot in front of the style under the stigma was governed by an intricate cooperation of a number of genes, of which some were positive genes for spot and some



of them were inhibiting genes for certain of these, not for all of them. One gene for spot, at least, needed a complementary gene for its realisation, and the inhibiting genes were also complementary as to their effect. There was some evidence suggesting that the different genes for spot, reacting in different ways with the inhibiting and complementary genes, belonged to a multiple series of alleles. The interaction of inhibiting genes with positive genes for spot caused the character of spot sometimes to behave as a dominant, sometimes as a recessive. When the inhibitors are weak in their effect a safe classification of the types is impossible. *V. Orphanidis* has such weak inhibitors together with positive genes for spot. In all other specific hybrids the spot character behaved as contingent upon a single dominant gene; namely in the Crosses 8, 10 and 11 (spot gene from *tricolor*) and Crosses 13 and 16 (spot gene from *rothomagensis*).

#### IV. NOTES ON CYTOLOGY AND FERTILITY OF $F_1$ .

It is a matter of course that it will be impossible to enter into any exhaustive description of the cytological investigation on each of the many hybrids, how tempting it might be, because almost each one shows some interesting detail. But so much barren philosophy concerning the meiotic behaviour of assumed hybrids and species of assumed hybrid origin has been published during the later years that it might be worth while to have a short description on the behaviour of a number of hybrids, whose parents are known.

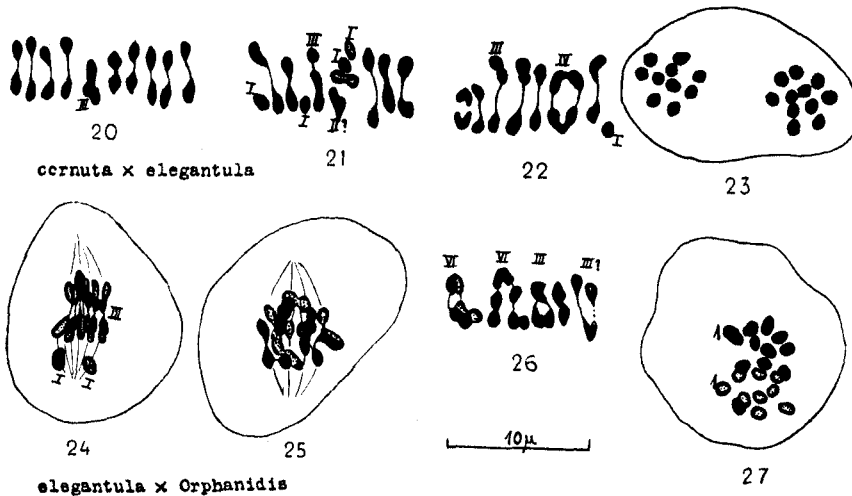
*Cross 1: V. cornuta, n = 11*  $\times$  *elegantula, n = 10*.

Heterotypic metaphases (figs. 20—22) show only few univalents (from one to six), but trisomes and tetrasomes are often present. One bivalent is often found lying »horizontal» (fig. 21). The parents show normal conjugation of 11 and 10 pairs without polysomes. Irrespective of multivalents and univalents the distribution of the chromosomes seems to proceed not too irregular, as homotypic metaphases (fig. 23) look rather normal apart from the fact that the number of chromosomes cannot be the same in both daughter nuclei. Of 16 homotypic metaphase plates there were 11 chromosomes in nine and 10 in the seven, and no elimination was observed.

*Cross 2: V. elegantula, n = 10*  $\times$  *Orphanidis, n = 11, 10*.

Some *Orphanidis* types throw gametes with only 10 chromosomes as previously demonstrated (J. CLAUSEN 1930 b). The plant investigated

was a spontaneous hybrid arisen after free (uncontrolled) pollination of *elegantula* in the field. Fig. 28 shows the flower of the hybrid



Figs. 20—27. Meiosis of Cross 1 and 2. When, in the following, nothing otherwise is stated, the figures show pollen mother cells (pmc.). Roman numerals in the figures indicate the number of chromosomes in the conjugated or single units. Abbreviations: het. = heterotypic, hom. = homotypic, met. = metaphase, ana. = anaphase. — The magnifications are all  $\times$  abt. 1800 (see scale referring to all chromosomal figures). — Figs. 20—23: Cross 1; 20—22: het. met., 22 not complete; 23: hom. met.,  $n = 10, 11$ . — Figs. 24—27: Cross 2; 24—26 show het. met. with mono- and polysomes and irregular chains of chromosomes (fig. 25), 24 is probably not complete; 27: het. ana.,  $n = 10$ , an unequal pair (marked 1) present.

between *elegantula* (left) and *Orphanidis* (right). The parental *Orphanidis* pollen carried only ten chromosomes.

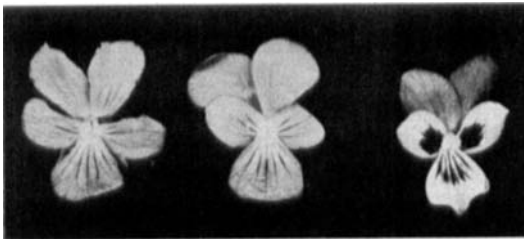


Fig. 28. From left: flowers of *elegantula*, *elegantula* × *Orphanidis*  $F_1$  (Cross 2) and of *Orphanidis*.

Characteristic for the heterotypic metaphase in the hybrid (figs. 24—26) is a *multivalent association* of chromosomes into open chains, but not in regular zig-zag (fig. 25); monosomes are rare, but on the other hand are hexa-, penta-, tetra-, tri- and ordinary disomes com-

mon. Fig. 26 shows the chromosomes of a nucleus in which only one bivalent was present. Rings of chromosomes are rare, and trisomes

form either a straight chain or a V as in fig. 24. The distribution of the chromosomes during the anaphase is surprisingly regular as regards their number at least. In most cases the daughter nuclei receive 10 chromosomes each (fig. 27). Very often a considerable difference between the members of one pair can be noted (see the two marked 1 in fig. 27).

*V. Orphanidis* shows occasionally trisomes and tetrasomes, at least the cytologically aberrant types do so (J. CLAUSEN 1930 b). But the polysomic arrangement observed in this hybrid far exceeds the sporadic irregularities in *Orphanidis*. One should think that many errors in the distribution of the chromosomes would occur by such a primitive and irregular mechanism of distribution, resulting in duplication and omission of single chromosomes ordinarily belonging to one of the two sets of ten.  $F_2$  has not been seen yet but the fertility of  $F_1$  was not bad, indeed, as abt. 250—300 seeds were obtained from each plant.

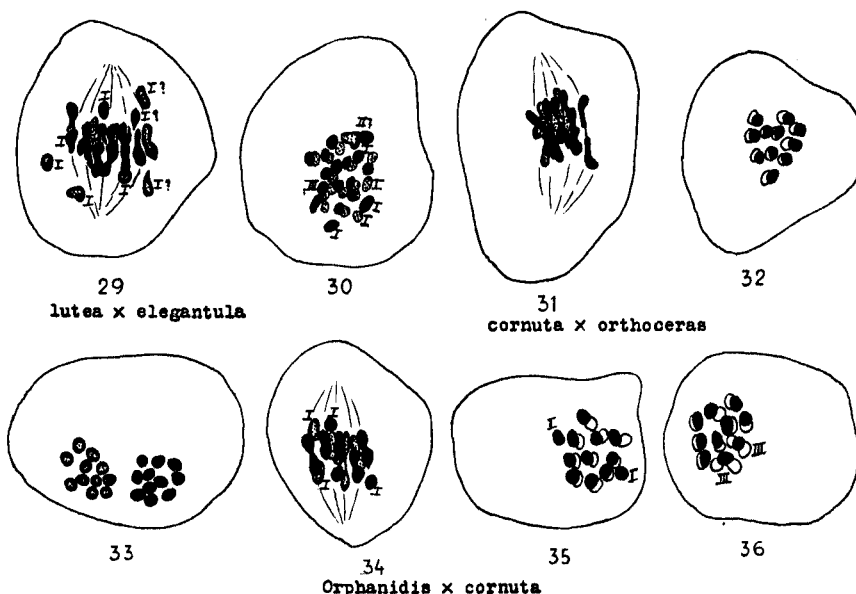
Cross 3: *V. lutea*,  $n = 24 \times$  *elegantula*,  $n = 10$ .

The pollen mother cells of this hybrid do not stain so well as in the preceding one, the chromosomes are small and the counting difficult. Characteristic for this hybrid is a certain amount of *autosyndesis*, apparently of the *lutea*-chromosomes. If all *elegantula*-chromosomes paired with *lutea*-chromosomes, fourteen would be left unpaired. No more than from four to six univalents are observed in most pollen mother cells, and heterotypic metaphases seen in polar view show not more than abt. 19 units; 13—15 bivalents may be counted in addition to 4—8 univalents (figs. 29—30). In some cells chains of chromosomes appear. The fertility of this hybrid was poor (50—100 seeds per plant), only very few of the seeds germinated and the plants did not thrive well at all. Genetic analysis, therefore, was impossible.

Cross 4: *V. cornuta*,  $n = 11 \times$  *orthoceras*,  $n = 11$ .

Characteristic for this hybrid is that the conjugation between the chromosomes seems just as complete as in the parent species. Eleven bivalents are observed during heterotypic metaphase and anaphase (fig. 31—33). Rarely an anaphase may show 12 chromosomes instead of 11; a single lagging chromosome may also be observed, but the main impression of the cytological behaviour is that of a pure species. The fertility is rather good, but the seeds germinate badly (12—20 %).  $F_2$  segregated for minor characters only, and the parents are morphologically also very alike. Fig. 4 shows the type of  $F_1$ , which is rather

luxurious. It is evident that the two species are very near related, also as to their chromosomal and genetical constitution.



Figs. 29—36. Meiosis in Crosses 3, 4 and 5. 29—30: Cross 3; het. met.; 29:  $13_{II} + 8_I$  (?); 30: abt.  $1_{III} + 13_{II} + 5_I$  (autosyndesis). — Figs. 31—33: Cross 4, het. met. in side- and polar view and hom. met.; regular,  $11_{II}$ . — Figs. 34—36: Cross 5, het. met.; 34: four univalents; 35:  $10_{II} + 2_I$ ; 36:  $8_{II} + 2_{III}$ .

Cross 5: *V. Orphanidis*,  $n = 11$ ,  $10 \times$  *cornuta*,  $n = 11$ .

The *Orphanidis* parent was V. 917—1, which had  $2n = 21$  chromosomes (J. CLAUSEN 1930 b). Consequently some of its gametes carried 11 and some 10 chromosomes. Of the five  $F_1$ -plants resulting from the crossing, two had  $2n = 21$  and three  $2n = 22$  chromosomes. There were no difference in morphological appearance or in the mode of meiosis of the two chromosomal types. The pollen mother cells gave the impression of being in good condition; the divisional pictures were clear, and the chromosomes stained well.

The conjugation in the plants with 22 chromosomes ranged from 8 to 11 bivalents with a corresponding number of univalents; some of the chromosomes were more or less clearly arranged into trisomes or tetrasomes (figs. 34—36). The plants with 21 chromosomes behaved in the same manner, but their largest number of bivalents was 10. The distribution of the chromosomes to the gametes was at random, as table 5 shows.

TABLE 5. *Distribution of chromosomes to the gametes during meiosis of Cross 5,  $n = 10 \times n = 11$  ( $2n = 21$ ).*

Number of chromosomes .....	8	9	10	11	12	total
number of nuclei counted .....	1	3	18	17	1	40

Elimination or splitting of one or a few univalent chromosomes occurred in some cells. The fertility was rather good but only 10—20 per cent of seeds germinated. The  $F_2$ -plants as well as the  $F_1$ 's were weak but a fairly good Mendelian segregation was obtained. No doubt *V. Orphanidis* must be considered one of the most near relatives of the taxonomical isolated *V. cornuta*, whether judged from the morphological appearance of the parents, or from the degree of chromosomal conjugation, the condition of the pollen mother cells and the fertility of  $F_1$ .

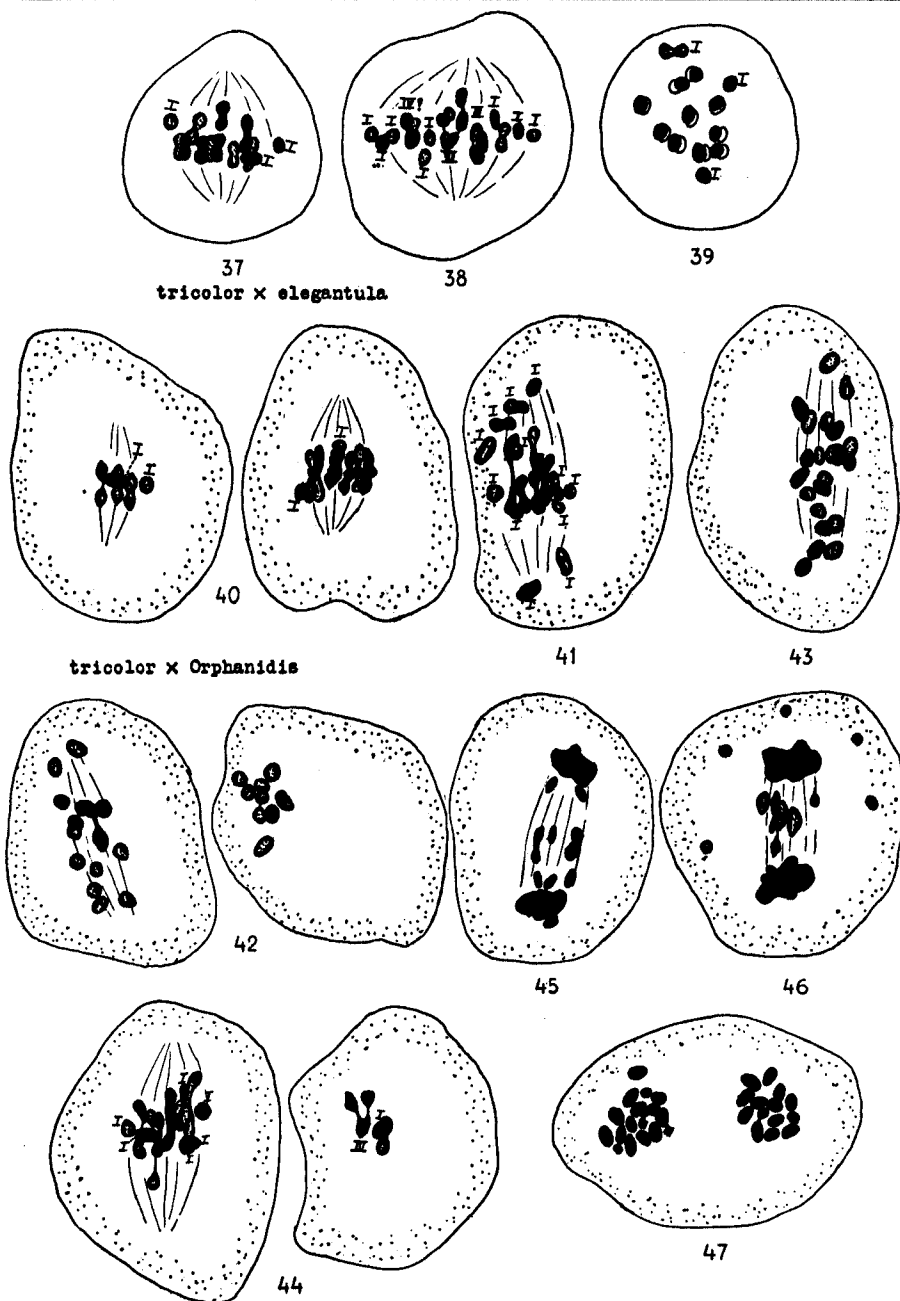
*Cross 6: V. tricolor,  $n = 13 \times elegantula, n = 10$ .*

The meiotic divisions in the pollen mother cells are good looking, and the cells in a good condition. From 8 to 10 bivalents are formed, and polysomes (II, IV, VI) occur considerably often (figs. 37—39). The univalents range from three to seven. Fertility very poor, abt. 30 seeds per plant, and germination even poorer.

*Cross 7: V. tricolor,  $n = 13 \times Orphanidis, n = 11, 10$ .*

The *Orphanidis* parent was 917—1 with  $2n = 21$  chromosomes, the same as in Cross 5. Consequently some  $F_1$ -plants had 23 and some 24 chromosomes according to whether the *Orphanidis* pollen carried 10 or 11 chromosomes. The conjugation of chromosomes was more *variable* in this cross than in the other ones, ranging in the same 24-chromosome plant from  $10_{II} + 4_I$  (fig. 40) to  $1_{II} + 22_I$  (fig. 42); probably also cells with 24 univalents occur. The cells with almost no conjugation were found together in one pollen sac, but even within the same pollen sac a great range of variation was observed. A great many observations were made on different plants of this cross.

The pollen mother cells appear well nourished and stain well, but the marginal zone of them contains numerous minute drops or grains, which stain with a colour similar to the nucleolus. It is a well known fact that the pollen mother cells of plants, which are in a less good condition, may contain nucleolar material outside the nucleus. In some cases a few larger nucleoli may be present in the plasm, in other cases more small nucleoli are scattered often most dense in the marginal zone (fig. 46); the many minute dots in the pollen mother cells of this



Figs. 37—47. Meiosis in Crosses 6 and 7. 37—39: Cross 6; 37 and 39:  $10_{II} + 3_I$ ; 38: abt.  $1_{VI} + 1_{IV} + 1_{III} + 1_{II} + 8_I$ . — 40—47: Cross 7; 40—44: het. met. with varying number of univalents, 42 and 43 (not complete) with almost no conjugation; 45—46: het. ana. with splitting univalents; 47: hom. met.,  $n =$  abt. 15 (+ one outside the group) and abt. 14. This cross has many minute grains or drops in the plasma of its pmc. and also extranuclear nucleoli (fig. 46).

hybrid, shown in the figures, may be of a similar nature. The writer observed dots just of a similar appearance in a non-hybrid *Phaseolus*. If stained with HEIDENHAIN's hæmatoxylin the larger extra-nuclear nucleoli are often mistaken for detached chromosomes, even by investigators, which should be regarded as trained cytologists. Although this condition may be most common in hybrids, it is also found scattered elsewhere in non-hybrid plants.

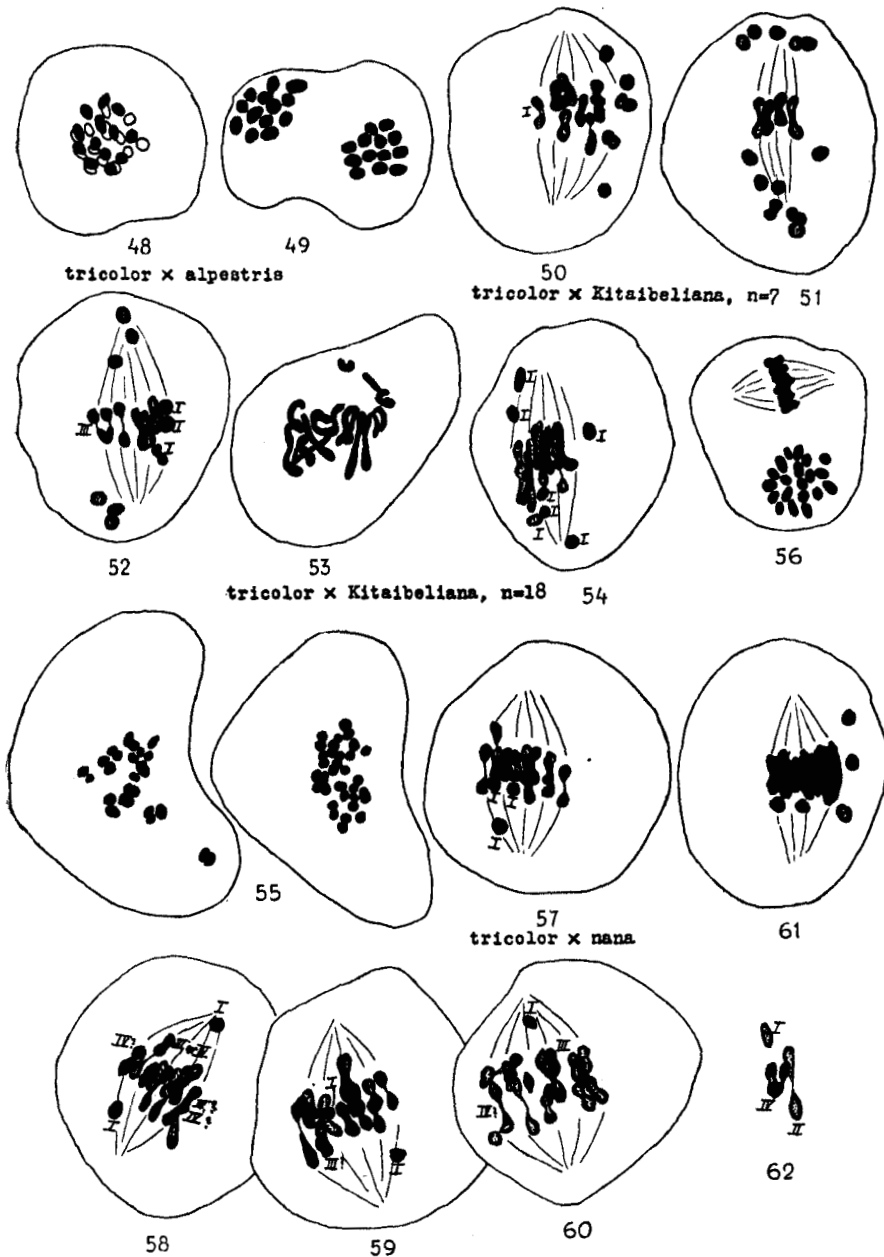
Some few trisomes and probably also some less typical tetrasomes (attached bivalents) are observed in some pollen mother cells of the hybrid *tricolor*  $\times$  *Orphanidis*, but polysomes are much more rare in hybrids of *Orphanidis* than in hybrids of *elegantula*. The figures 40—44 show heterotypic metaphases; in heterotypic anaphases (figs. 45—46) some of the univalents may be seen dividing, giving rise to homotypic nuclear plates with an increased number of chromosomes; fig. 47 shows such a pollen mother cell containing 15 chromosomes in one and 15 to 16 in the other nuclear plate together with one detached; some of the chromosomes here are very small (univalents recently divided). Elimination of chromosomes is not uncommon, and the formation of dwarf nuclei gives rise to pentads, hexads etc. Fertility and the germination rate of the seeds was poor.

*Cross 8: V. tricolor, n = 13*  $\times$  *alpestris, n = 13*.

There is a slight tendency to univalency of some few chromosomes in this hybrid, and figures showing univalent chromosomes (of which a number splitting) were previously published (J. CLAUSEN 1927 b, figs. 65—66). Such irregularities are, nevertheless, exceptions and occur in certain plants and crossings only; most hybrid plants show regular meiosis (fig. 48), in which only a loose conjugation or a not quite parallel placement of the thirteen bivalents reveals the hybrid nature of the plant in question. Heterotypic metaphases in side view may show univalents and anaphases lagging chromosomes, just as also dwarf nuclei occur occasionally. Extranuclear nucleoli were observed in the plasm of almost invariably all pollen mother cells. Apparently nearly all gametes receive 13 chromosomes (fig. 49). The fertility was extremely good, the  $F_1$ -plants gave from 1500—2000 seeds each. The conditions in this cross remind about those in Cross 4, and the two species or subspecies must be regarded as very closely related.

*Cross 9: V. tricolor, n = 13*  $\times$  *arvensis, n = 17*.

In heterotypic metaphases from 11 to 13 bivalents and correspondingly from 8 to 4 univalents are formed. No clear instances of



Figs. 48—62. Crosses 8—12. 48 het. met. and 49: hom. met. of Cross 8, regular,  $13_{II}$ . — 50—52: Cross 10, het. met. with  $6_{II}$ ,  $4_{II}$  and  $4_{II} + 1_{III}$ , respectively, in addition to univalents. — 53—56: Cross 11; 53: long, semi-prophasic chromosomes, no nuclear membrane; 54: het. met., at least  $7_I$ ; 55: het. ana., no reduction; 56: hom. met.,  $n=20$ . — 57—62: Cross 12, het. met., autosyndesis; 57 with three, 58 and 59 with two, 60 with probably only one and 61 with five univalents in addition to some tri- and tetrasomes (58—60); 62: tetra-, di- and monosome from the same cell.



trisomes or tetrasomes were found, but the arrangement of bivalent chromosomes in some heterotypic metaphases may indicate a slight secondary attraction between bivalents or between bivalents and univalents. The present writer is not in the possession of frankness enough to postulate that such slight and uncertain association of chromosomes be evidence enough of a true polysomic arrangement. DARLINGTON and MOFFETT (1930 b) appear to be inclined to see polysomic arrangement, not only in their own slides, but also in the figures of material, which they are not familiar with. But there is a great difference between the true polysomic arrangement, which is seen in some other *Viola* hybrids and also in cultivated races of *Pyrus* (DARLINGTON and MOFFETT 1930) and the slightly diverging arrangement of gemini in hybrids as *tricolor*  $\times$  *arvensis*. Some allowance for occasional placement of two gemini very close to one another must also be admitted.

Elimination of one or two chromosomes is very common in *tricolor*  $\times$  *arvensis* and the number of chromosomes has therefore a tendency to decrease during later generations. The fertility is very good and it gives from 500 to 2000 seeds per plant. This hybrid was previously dealt with in details (J. CLAUSEN 1926), but it is here mentioned for a comparison.

*Cross 10: V. tricolor, n = 13*  $\times$  *Kitaibeliana, n = 7*.

No more than six bivalent chromosomes were observed in heterotypic metaphase of this hybrid; in this case, which seems the most common, 8 univalents were present. But the combinations  $5_{II} + 10_I$  and  $4_{II} + 12_I$  were also often met with (figs. 50—52). A trivalent was observed in one cell (fig. 52). The pollen mother cells were not in a well nourished condition, but the chromosomes stained well and gave comparatively clear pictures. The hybrid was almost completely sterile, giving from none to 30 seeds per plant in spite of a good vegetative development and abundant flowering.

*Cross 11: V. tricolor, n = 13*  $\times$  *Kitaibeliana, n = 18*.

The pollen mother cells of this hybrid were poor-looking, and it was impossible to account for all chromosomes during the rather irregular meiosis. The nuclear membrane can dissolve before the nucleolus, and even before the chromosomes arrive to the typical diaphase with short chromosomes (see fig. 53). The number of bivalents in heterotypic metaphase could not be counted with certainty, but may be calculated from the number of univalents, which are much more easily counted. Seven univalents seemed to be the most common, what gives

12<sub>II</sub> (fig. 54), but 9<sub>I</sub> (11<sub>II</sub>) to 17<sub>I</sub> (7<sub>II</sub>) occurred and one pollen mother cell was found showing no reduction at all, the chromosomes dividing equational (fig. 55). On the whole, the chromosomes of this hybrid showed a great range of variation in their conjugation similarly as Cross 7. Elimination of chromosomes took place, just as also splitting of univalents, which may result in the formation of gametes with a higher number of chromosomes than any of the parents (see fig. 56 with 20 chromosomes).

The fertility of the hybrid was very poor, not more than 20—50 seeds per plant were obtained from hundreds of flowers, although these structurally were capable for selfpollination.

*Cross 12: V. tricolor, n = 13 × nana, n = 24.*

This hybrid is characterised by a certain amount of autosyndesis among the *nana*-chromosomes. Assuming that all *tricolor*-chromosomes conjugate with 13 *nana*-chromosomes, eleven *nana*-chromosomes are left without any mate, if no autosyndesis occurs. More than five univalents were never observed (fig. 61); the most common was three univalents, and even pollen mother cells with only one univalent were noted. If no trisomes or tetrasomes are formed this would give 16, 17 and 18 bivalents, respectively, the somatic chromosome number being 37. Trisomes or tetrasomes occur in almost all cells, but not more than one or two in each. Figs. 57—60 show heterotypic metaphases in side view, the three last ones are from a row in one and the same pollen mother cell; fig. 62 shows tetrasome, disome and monosome. The quality of the fixation did not allow the observation and counting of each conjugated unit, but the univalents were a rather safe indication of the autosyndesis, whether disomes or trisomes were formed. There is at least a striking difference between the behaviour of the *nana*-chromosomes in this hybrid and in Cross 18: *nana* × *arvensis* (figs. 86—89), in which only very few bivalents were formed.

Some elimination of chromosomes occurred and dwarf nuclei were formed, but the fertility of the hybrid was surprisingly good, as 1000—2000 seeds per plant were obtained.

*Cross 13: V. tricolor, n = 13 × rothomagensis, n = 17.*

As to chromosome number this cross is a homologue to Cross 9 (*tricolor* × *arvensis*), but the behaviour of chromosomes is different from this last one, because trisomes and tetrasomes together with a few monosomes are very common in the present hybrid (see figs. 63—64), not in the other one, and the fertility is also much less in

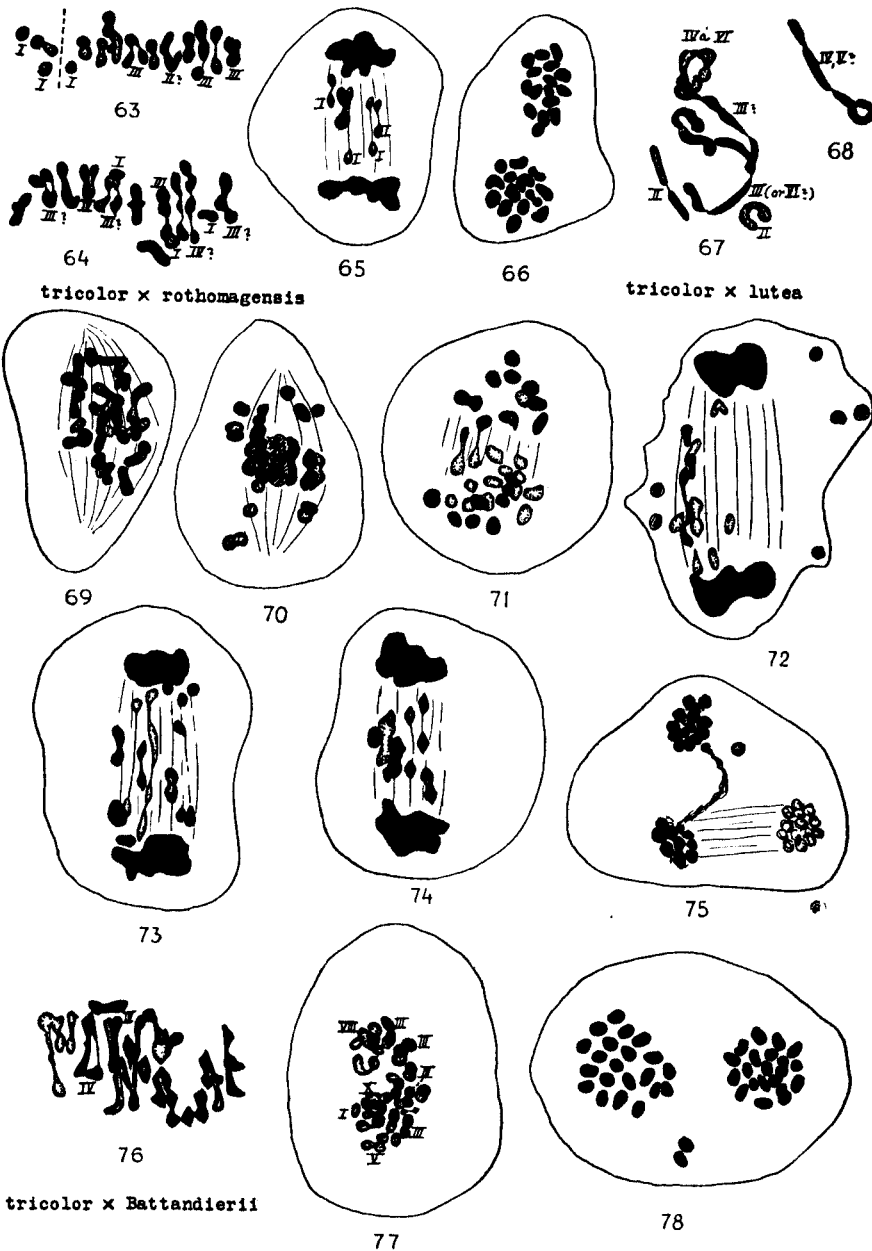
Cross 13 than in Cross 9; only 12—37 seeds per plant were obtained and but a small fraction of these germinated.

As in all *Viola* hybrids there were some variation regarding the amount of conjugated chromosomes in different cells (from two to six univalents were observed); some univalents divided in heterotypic anaphase (fig. 65). Most homotypic metaphases contain from 14 to 16 chromosomes (fig. 66); elimination of chromosomes and dwarf pollen were rather frequent.

*Cross 14: V. tricolor, n = 13*  $\times$  *lutea, n = 24*.

This cross is characterised by great irregularity in the progress of meiosis. The shortening of the individual chromosomes does not occur synchronously for all chromosomes of one and the same cell; some may be in the early diaphase (the so-called strepsinema) when others have shortened to the characteristic short shape of the late diaphase (fig. 67). As shown in this figure and in fig. 68 are some of the chromosomes connected to long, multivalent chains. The non-simultaneity and the chain formation often persists through heterotypic metaphase, but no regular zig-zag chains were observed, long and short chromosomes arrange themselves without any order in all directions between each others, sometimes connected to the most peculiar combinations; fig. 69 shows another type of heterotypic metaphase with short but irregularly arranged chromosomes. The fixation was done with NAWASHIN's fixative without previous application of CARNOY's fluid, and it did not allow the tracing of the individual bivalents through the heterotypic division. The univalents were most often belated enough for an observation during the heterotypic metaphase and anaphase; from 8 to 11 univalents were the most frequent (fig. 70), and there is a strong tendency for these to divide during anaphase as illustrated by the figures 72—74. The average chromosome number of the gametes increases by this process, fig. 71 shows 21—22 chromosomes at one of the poles of a heterotypic anaphase and in a previous paper (1927 b, fig. 72) a homotypic metaphase with abt. 23 chromosomes was reproduced. This is accordance with the fact that the cultivated pansies (*V. Wittrockiana* GAMS), which arose from this specific crossing in the years between 1830 and 1840 (WITTROCK 1896), have a chromosome number very near to that of the parent with the largest number, viz. abt. 24 (J. CLAUSEN 1927 b, figs. 73—77).

In some pollen mother cells were long chromosomes seen to persist to homotypic telophase connecting even the nuclear groups, which were



Figs. 63—78. Crosses 13—15. 63—66: Cross 13; 63: het. met. from two sections, abt.  $3_{III} + 9_{II} + 3_I$ ; 64: het. met., not complete, ca.  $2_{IV} + 4_{III} + 2_I$ ; 65: het. ana., univalents dividing; 66: hom. met.,  $n =$  ca. 16 and 14—15. — 67—75: Cross 14; 67: chromosomes from one cell in diaphase, unequally shortened, multivalent associations; 68: polysome from diaphase; 69—70: het. met., in 70 ca.  $11_I$ ; 71: het. ana.,

separated at heterotypic anaphase, that is, the connection has persisted through all the phases lying between them. Fig. 75 shows such a chromatin connection, which reminds very much about the abnormal divisions described by GOODSPEED and AVERY (1930, text figures 8—17) from X-rayed *Nicotiana Tabacum*.

In spite of all irregularities, the chromosomes are in homotypic telophase generally found arranged in four groups, pentads are rare. An enumeration through one slide gave 29 tetrads and three pentads. In the pentads the fifth cell was always small. The individual pollen in the tetrads were often slightly different as to size. The plasm contained plenty of nucleolar material. In spite of great efforts only 50—300 seeds were obtained per plant, and the germination was less than 10 per cent.

*Cross 15: V. tricolor, n = 13 × Battandierii, n = 26—30.*

As mentioned before, the chromosome number of the garden type of *V. Battandierii* is oscillating, and the gametes, therefore, do not all contain the same number of chromosomes. Anyhow, they contribute the largest number of chromosomes of any *Melanium* violet. The  $F_1$ -plants of Cross 15 had apparently not all exactly the same number of chromosomes. Contrary to expectation the univalents were few, less than six and usually not more than 3—4, not 13—17 as indicated by the difference in the chromosome number of the respective parents. This was due to a very extensive association of chromosomes into multivalent complexes (figs. 76—77) consisting of as many as up to ten chromosomes, as far as could be judged. This means a rather large degree of autosyndesis, but, in fact, even the non-hybrid *Battandierii* shows multivalent association of its chromosomes (fig. 15 d). The appearance of the heterotypic metaphase was very irregular and it was very difficult to interpret. Homotypic metaphases looked more ordinary, the nuclear plates contained most often 20 or 21 chromosomes (fig. 78) and the number oscillated between 23 and 17. Elimination of one to three chromosomes was very frequent, just as were pentads, hexads and heptads. The supernumerary pollen were generally minute

---

the upper chromosomal group not complete, in the lower 21—22 chromosomes; 72—74: het. ana.-telophase, univalents dividing, in 72 extranuclear nucleoli (hatched); 75: hom. telophase, a chromosomal connection has persisted from het. met. — 76—78: Cross 15: 76: het. met., multivalent association; 77: het. met. in polar view, abt.  $1_X + 1_{VIII} + 1_V + 4_{III} + 2_{II} + 1_I + ?$ ; 78: hom. met.,  $21 + \text{ca. } 20 + 2$  chromosomes.

dwarfs, obviously representing eliminated single chromosomes or groups of chromosomes. The fertility was rather good and germination not poor, abt. 30 %.

*Cross 16: arvensis,  $n = 17 \times rothomagensis, n = 17$ .*

These two species have one and the same chromosome number, but differ much as to their morphological characters. Meiosis of the hybrid was much more irregular than of the Crosses 4 and 8, in which the parent species also have the same number of chromosomes. From two to six univalents were noted (figs. 79—81), which means that one to three pairs of chromosomes failed to conjugate. In addition to the bivalents were one or two trivalents and occasionally a quadrivalent present. The cytological picture was not very clear and the chromosomes were often densely aggregated; this irregularity is probably connected with the fact that the nuclear membrane very often disappeared during the later prophase, before the diaphase. Univalents were seen dividing during heterotypic anaphase; detached single chromosomes, dwarf nuclei (rare), pentads and hexads were noted. Fertility was comparatively good, 250—450 seeds per plant were collected; the germination was no less than 30 per cent.

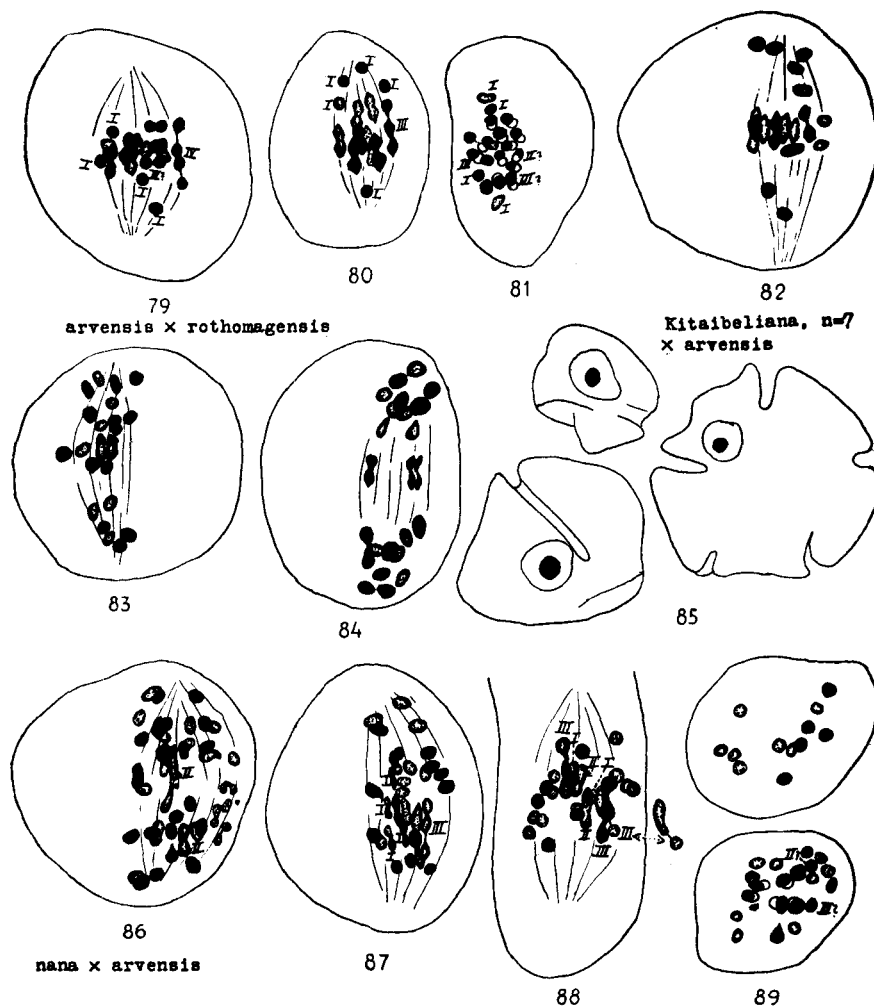
*Cross 17: V. Kitaibeliana,  $n = 7 \times arvensis, n = 17$ .*

*V. arvensis* has ten chromosomes more than the *Kitaibeliana* type applied for the present cross giving ten univalents as minimum (fig. 82), but twelve univalents (corresponding to only six bivalents) were seen in most of the pollen mother cells. Occasionally also cells with fewer bivalents occurred (fig. 83:  $2_{II} + 20_I$ ). Most univalents distributed themselves to the two poles, but detached chromosomes were noted. Some univalents divided during heterotypic anaphase (fig. 84) and, similarly, pentads and hexads were seen between more or less normal tetrads. The size of pollen was very variable (fig. 85), as it must be in a hybrid with so great difference between the parental numbers of chromosomes. The fertility of  $F_1$  was considerably reduced as compared with the parents, but not so poor indeed, as 500—600 seeds were obtained per plant. The germination rate, on the other hand, did not amount more than 17—19 per cent.

*Cross 18: V. nana,  $n = 24 \times arvensis, n = 17$ .*

This hybrid showed surprisingly few bivalents (more than six were never observed with certainty). The pollen mother cells were crowded with univalents (figs. 86—87). This is astonishing because Cross 12,

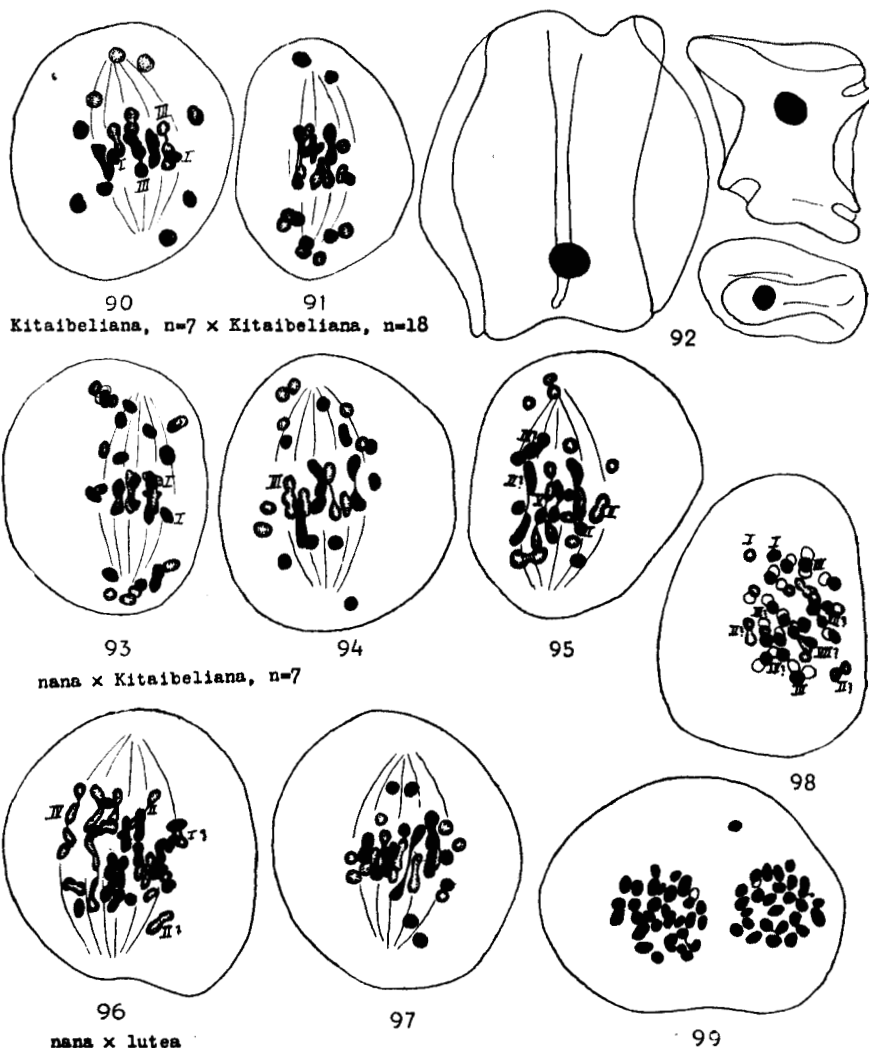
*tricolor*  $\times$  *nana*, showed surprisingly few univalents. If the *nana*-chromosomes are capable for autosyndesis in Cross 12, why do they not



Figs. 79—89. Crosses 16—18. 79—81: Cross 16, het. met.; 79—80 showing tetra-, tri- and monosomes; 81: abt. 12<sub>II</sub> + 2<sub>III</sub> + 4<sub>I</sub>. — 82—85: Cross 17; 82: het. met., ca. 10<sub>I</sub>; 83: het. met., ca. 2<sub>II</sub> + 20<sub>I</sub>; 84: het. ana., ca. 10 + 3 (dividing) + 11 chromosomes; 85: young pollen of different size (smaller magnification). — 86—89: Cross 18, all het. met., 88—89 from embryosac mother cells; 86: ca. 2<sub>I</sub> + 37<sub>I</sub>; 87: at least 21<sub>I</sub>, probably 26—27, some ones dividing, one trisome; 88: at least 18<sub>I</sub> and 3<sub>III</sub>; 89: polar view in two sections, at least 26<sub>I</sub>.

conjugate mutually, irrespective of any *arvensis*-chromosomes, in Cross 18? Embryosac mother cells of two plants were therefore inspected in

order to see, if the chromosomes showed better conjugation here than in the pollen mother cells, similar to what was observed in a pollen



Figs. 90—99: Crosses 19—21. 90—92: Cross 19; 90: het. met., ca.  $2_{III} + 4_{II} + 11_I$ ; 91: ca.  $6_{II} + 13_I$ ; 92: young pollen of different size, same enlargement. — 93—95: Cross 20, het. met.; 93:  $5_{II} + 22_I$ ; 94: ca.  $1_{III} + 5_{II} + 18_I$ ; 95: ca.  $1_V + 1_{III} + 6_{II} + 11_I$ . — 96—99: Cross 21; 96—98: het. met., in 96 one tetrasome and abt. 15 univalents, in 97 at least 15<sub>I</sub>; 98: (polar view) probably  $2_{VII} + 1_{IV} + 3_{III} + 11_{II} + 2_I$ ; 99: hom. met., in the nuclear plate to the right 26 chromosomes.

sterile type of *V. Orphanidis* (J. CLAUSEN 1930 b), but such was not the case (figs. 88—89).



Trisomes were rarely observed, and some univalents would split during heterotypic division. The fertility was rather good (250—800 seeds per plant) but considerably less than in Cross 12, *tricolor*  $\times$  *nana*, although *arvensis* and *nana* unquestionably are morphologically more near related than are *tricolor* and *nana*.

*Cross 19: V. Kitaibeliana*,  $n = 7 \times$  *Kitaibeliana*,  $n = 18$ .

Although these two parent types, morphologically considered, belong to one and the same species, the cytological irregularities are just as large as in species hybrids, and the sterility is more complete than in any other *Melanium* hybrid. There is a tendency to form six bivalents (leaving one of the seven chromosomes unconjugated) in addition to 13 univalents (figs. 90—91). One or two univalents may conjugate with bivalents giving trisomes. With so many univalents there is a possibility for a very uneven distribution of chromosomes during meiosis, and the pollen are actually very different in size (fig. 92).

*Cross 20: V. nana*,  $n = 24 \times$  *Kitaibeliana*,  $n = 7$ .

This is the widest cross within the collective species of *V. Kitaibeliana*, a cross of an octoploid type with a hyperdiploid one, the difference between the chromosome numbers of the parents being 17. There is some variation in the conjugation of chromosomes in the hybrid. All the seven chromosomes from one parent do not ever find a mate among the 24 chromosomes of the other as some pollen mother cells did not show more than five bivalents (fig. 93 with  $5_{II} + 22_I = 32$ , the *nana*-gamete contributing to this  $F_1$ -plant must have carried 25 chromosomes instead of 24!). Trivalents were met with (figs. 94 and 95) and even a pentasome was observed (fig. 95); in this last case some sort of autosyndesis must have taken place. The pollen mother cells were well developed and stained well. The fertility of  $F_1$  was not poor, 250—350 seeds were obtained per plant. Cross 20, as regards chromosome number and geographic habitat of parent types is a wider cross than Cross 19, but it shows less irregularity and less sterility.

*Cross 21: V. nana*,  $n = 24 \times$  *lutea*,  $n = 24$ .

These two species have the same chromosome number and their areas are not remote from each others, but they are morphologically very different. They cross easily, 72 crossed seeds were obtained, giving 50  $F_1$ -plants, which showed characters from both parents, although *nana* was prevalent regarding conspicuous characters as flower size and flower colour. The fertility was rather poor, from 50—300 seeds were

obtained from each of the richly branching plants. Pollen mother cells stained well, but the many chromosomes in no regular association troubled the disentanglement of the individual chromosomes and their associations. 15—18 univalents were observed in some cells (figs. 96—97) and irregular polysomic association occurred (fig. 96: a tetrasome, fig. 98: probably heptasomes, tetrasome and trisomes). Homotypic metaphases showed a little more or less than 24 chromosomes and occasionally detachment (fig. 99).

*Cross 22: lutea,  $n = 24 \times$  Battandierii,  $n = 26—30$  and*

*Cross 23: Battandierii  $\times$  calcarata,  $n = 20$ .*

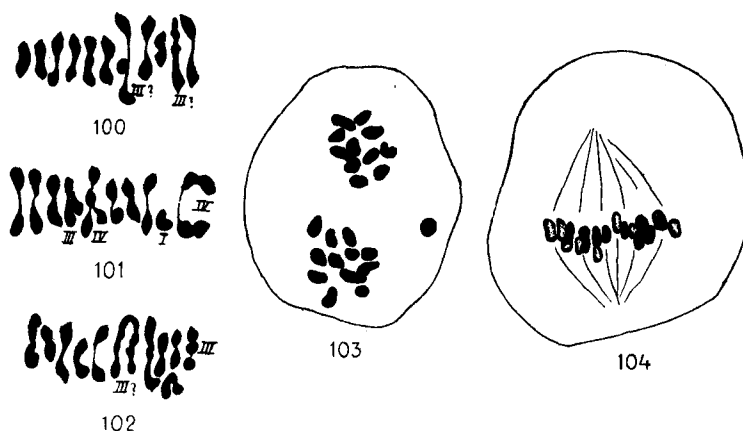
The two hybrids have not the primary cytological interest as the other ones mentioned due to the fact that the cultivated *Battandierii* type applied for the crossings had an oscillating number of chromosomes. *Cross 22* was fixed already in 1923 and owing to primitive technique then available (CARNOY fixation, DELAFIELD hæmatoxylin) it was impossible to get anything out of the heterotypic metaphase; the homotypic metaphases were fairly regular with about 24—26 chromosomes and occasional elimination of single chromosomes. The fertility was not good (50—100 seeds per plant) but the germination was fairly good.

*Cross 23* also showed elimination of univalents and a varying number of chromosomes in the homotypic metaphase (24, 28, etc.) so as pentads with dwarf pollen. The fertility was more poor in *Cross 23* than in *Cross 22*, but the present cross was, nevertheless, the only successful one with *V. calcarata* as a member. Only 18  $F_2$ -plants were obtained, showing some segregation as to shape of leaves and flowers etc., but all were violet flowered.

*Cross 24: (V. tricolor,  $n = 13 \times$  Orphanidis,  $n = 11, 10) \times (cornuta, n = 11 \times$  elegantula,  $n = 10)$ .*

As a matter of course, the two  $F_1$ -hybrids crossed together form gametes with a somewhat differing number of chromosomes, and the individual plants of the quadruple  $F_1$ , therefore, differ as to the number and the initial origin of their chromosomes so as to their morphological appearance and their fertility. It was surprising to see how regular the division was going on in hybrids with chromosomes from four species brought together. Figs. 100—102 illustrate heterotypic metaphase in *V. 1205—4* with a total number of chromosomes of abt. 24, almost all conjugating, either as ordinary disomes or as tri- and tetra-

somes. This plant was somewhat fertile, giving 123 seeds and 22  $F_2$ -plants from these. Figs. 103—104 illustrate another plant of Cross 24, the completely sterile V. 1205—2, also with a total chromosome number of 24 (somatic); fig. 104 shows two homotypic metaphases uniting into one very large group of chromosomes, which would give diploid pollen grains. The young pollen of this plant showed a tendency to disintegration, filling the pollen sac with a fibrillous mass similar to what was observed in pollensterile V. *Orphanidis* (J. CLAUSEN 1930 b).



Figs. 100—104. Cross 24 (quadruple hybrid). 100—102: V. 1205—4, het. met.,  $2n = 24$ ; 103—104: V. 1205—2 (sterile), hom. met.,  $2n = 24$ , in 104 are both nuclear plates united to one.

## V. TYPES OF SEGREGATION IN $F_2$ AND LATER GENERATIONS.

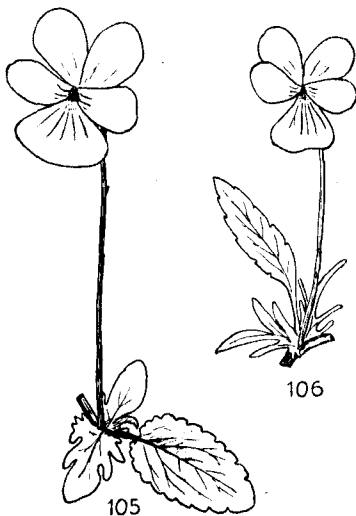
Cross 1: V. *cornuta* (Aa, violet)  $\times$  *elegantula* (AA, reddish).

A line drawing of  $F_1$  is reproduced in fig. 105; it shows the characteristic intermediate stipules and leaves (compare figs. 2 and 4). Fig. 19 shows the characters of the flower (direction of lateral petals and extension of yellow eye) of the parents and of  $F_1$ .

Segregation of the A-gene (anthocyanous:alba) in  $F_2$  and of the character yellow eye will be mentioned in chapter VI. For a number of other characters were segregations noted, but in these cases numerous gradations were observed covering the total range of variation with the characters of the two parents as the extremes, while classification was impossible. This holds true for the shape and diversion of the stipules, for direction, size and shape of petals, length of spur and partly also

for colour of flowers, except that true red flowered plants did not appear in  $F_2$ , but a large range of variation into more or less reddish types was noted (in addition to pure white). This may be connected with the fact that the *elegantula*-parent, V. 639—2, was not true red but of an intermediate colour and probably not constant for this colour,

as the much more red types of later accessions were. Already in  $F_1$  darker or lighter reddish types were observed, but all were more reddish than *cornuta*. Some plants of  $F_2$  were of a perennial type and others looked just more like annuals.



Figs. 105—106. Flower and leaves of *cornuta* × *elegantula*  $F_1$  (105) and of *tricolor* × *elegantula*  $F_1$  (106).

In a backcross of one  $F_1$ -plant with a pure red and constant *elegantula* of another accession it was tried to classify the characters of the individuals into two classes, according to whether they belonged to the  $F_1$ -type of the character in question or they were similar to *elegantula*.  $F_1$  was intermediate with respect to all the four characters tested. The enumeration is shown in table 6.

The 88  $F_2$ -plants germinated from abt. 1000 seeds sown, and the 27 backcross-plants from abt. 140 seeds. Of 400 seeds collected from selfpollinations of four  $F_2$ -plants no one at all germinated. It is impossible to say what classes the non-viable combinations belonged to.

TABLE 6. Classification of the individuals in the backcross V. *elegantula* × (*elegantula* × *cornuta*).

Type	flower colour	flower shape	direction of lateral petals	shape of stipules
= <i>elegantula</i> .....	17 (red)	14	9 (upwards turned)	18
= $F_1$ .....	10 (reddish violet)	13	18 (horisontal)	9
total	27	27	27	27

No *alba*, all with yellow eye as expected.

This cross is of interest, because it shows that also characters distinguishing larger taxonomical units in this section segregate and

must depend upon the interaction of genes, apparently not very few. Cross 5, *V. Orphanidis*  $\times$  *cornuta alba*, showed a similar segregation as to shape and direction of petals so as to presence or absence and extension of yellow eye.

Cross 6: *V. tricolor alba-yellow*  $\times$  *elegantula, red*.  
( $a_1a_1a_2a_2RRLL$   $\times$   $A_1A_1 \dots rrl.$ )

The 30  $F_1$ -plants germinated from 40 seeds and varied a little (the *elegantula* parent did not belong to any pure line). The  $F_1$ -type of flower, leaf and stipules is shown on fig. 106 (compare the parental species, figs. 2 and 6, but the *tricolor* parent had smaller flowers due to the absence of all *A*-genes). The shape of leaves and stipules was greatly influenced by *V. tricolor*. Young flowers of  $F_1$  were whitish, changing to paler or darker violet (action of one gene for yellow, *L*, and the *R*-gene, both from *tricolor*). Some minor variation as to intensity of colour was noted. A number of the  $F_1$ -plants belonged to a sterile bushtype, which did not flower, neither the first, the second or the third year. Backcrossing of  $F_1$  to one of the two parents gave considerably more and considerably better seeds than the selfpollination (see table 7).

TABLE 7. Average number of seeds per capsule of *V. 1104*, Cross 6,  $F_1$ .

Plant no.	1	2	3	4	total of seeds	plants germinated
self pollination .....	1	0,8	1,4	2,6	50	6 (from 32 seeds)
$F_1 \times$ <i>tricolor alba-yellow</i> .....	3,3	3,9	—	—	181	64
$F_1 \times$ <i>elegantula</i> .....	—	—	10,8	5,6	181	96

The segregation as to the *A*-gene in  $F_2$  and backcrosses is mentioned in chapter VI. A considerable number of plants of both of the backcrosses never attained flowering; they belonged to a peculiar minute and tufted dwarfish type, many of them much more extreme than the corresponding types in  $F_1$ . The numbers of this type were the following:

	normal	sterile dwarfs	total
$F_1 \times$ <i>tricolor alba-yellow</i> .....	34	24	58
$F_1 \times$ <i>elegantula</i> .....	67	23	90

As to flower colour *elegantula* seems to possess some intensifiers and extension genes obscuring the segregation. By the action of

these, *Ll* may become rather bright yellow, not yellowish white changing to pale violet as usual. Table 8 gives the classification of the different categories of flower colour.

TABLE 8. Segregation for flower colour in Cross 6, *tricolor* × *elegantula*  $F_2$  and back crosses.

Antho- cyanin	flower colour	formula	$F_2$	$F_1 \times \textit{tricolor}$ <i>alba</i> -yellow <i>AaRrLl</i> × <i>aaRRLL</i>	$F_1 \times \textit{elegantula}$ <i>AaRrLl</i> × <i>AArrll</i>
with	violet.....	<i>ARll</i>	—	10 (violet + <i>pal- lida</i> )	24 dark violet
	rose .....	<i>Arrll</i>	1		32 dark rose
	<i>pallida</i> .....	<i>A.Ll</i>	3		11 yellowish
	yellow .....	<i>A.LL</i>	1		—
without	<i>alba</i> .....	<i>aa</i> . { <i>Ll</i> <i>ll</i>	—	9	—
	<i>alba</i> -yellow...	<i>aa.LL</i>	—	5	—
total			5	34	67

TABLE 9. Segregation of supposed *RrLl*-plants of Cross 6.

Flower colour	$F_2$	$F_3$		total	calcu- lated	ratio
	V. 1218	V. 1345	V. 1348			
<i>ARll</i> violet .....	—	7	28	35	29,8	3
<i>Arrll</i> red .....	1	1	6	8	9,0	1
<i>A.Ll pallida</i> .....	3	8	79	90	79,5	8
<i>A.LL</i> yellow.....	1	7	18	26	39,8	4
total	5	23	131	159	159,0	16

Four of the five  $F_2$ -plants were selfed for an  $F_3$ . Two of them segregated types similar to  $F_2$  (see V. 1345 and 1348, table 9). A pale rose plant gave two pale and three darker rose.

A fourth  $F_2$ -plant, yellowish flowered with upper petals rose, (classified as »*pallida*»), gave 20  $F_3$ -plants; 8 of them were yellow with upper petals rose and 12 were of an entirely new type with all petals yellow. Fig. 107 shows flowers of these types with their parent species. *tricolor alba*-yellow and *elegantula* in the upper row and two flowers of each type in the lower row, the intense yellow type to the right; this has larger flowers than the type with upper petals rose. The intense

yellow type was constant, two  $F_4$ -sowings of it gave 19 and 43 intense yellow, respectively. A plant with upper petals rose segregated 18

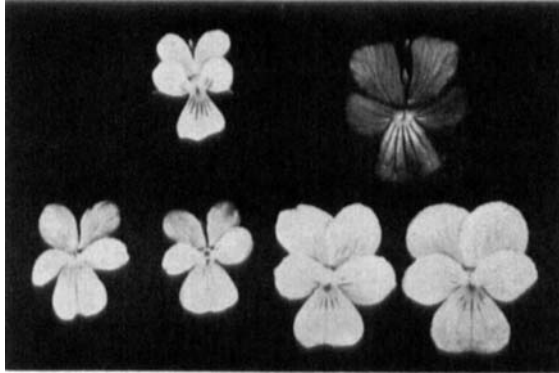
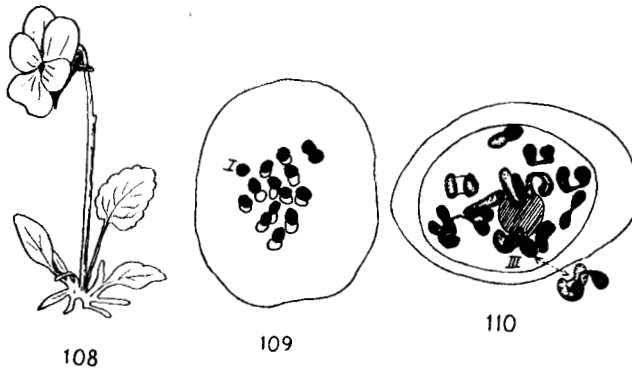


Fig. 107. Upper row: *tricolor alba*-yellow (left) and *elegantula* (right); lower row: flowers of  $F_4$ -types from this cross; left: yellow-rose, right: intense yellow on all petals (*V. phaeno-elegantula*).

yellow-rose : 11 intense yellow. The growth habit and entire vegetative appearance of this new type, which may be referred to as

*Viola phaeno-elegantula*,

(after its bright and very showy flowers) is that of a *Viola elegantula*, but the stem nodes are shorter; it is very caespitose and each plant forms



Figs. 108—110. *Viola phaeno-elegantula*. 108: Flower, leaf and stipules; 109:  $F_3$  of the original cross, het. metaphase,  $13_{II}$  (one horizontal) +  $1_I$ ; 110:  $F_4$ , diaphase,  $11_{II}$  +  $1_{III}$ .

a low, dense tuft with numerous, bright yellow flowers raised above the decumbent stems. Fig. 108 is reproduced from a line drawing of leaves and flower; the spur is thin and straight, but somewhat shorter

than of *elegantula*; the flowers are broader than of any of the parental species. From the *tricolor*-parent the *L*-gene for yellow flowers has been transferred, while *elegantula* probably has contributed the intensifying gene or genes. Although *V. phæno-elegantula* morphologically spoken has been built up mainly of characters from *elegantula*, it presents several new traits in its structure, in first line its flower colour, and it proves that genes from one species may be added to a bloc of genes from another and still form a viable combination. *V. phæno-elegantula* is namely very fertile and gives large capsules with many seeds. Although morphologically of a fairly constant type, it is not in chromosomal equilibrium as yet. Fig. 109 shows heterotypic metaphase of  $F_3$  of it; the total chromosome number of this plant was 27, most often conjugating  $13_{II} + 1_I$ , but one of the bivalents is usually placed horizontal outside the other bivalents together with the univalent one (compare  $F_1$  of Cross 1). Fig. 110 shows diaphase of an  $F_4$ -plant, selfed offspring of the  $F_3$ -plant represented in fig. 109. The total chromosome number is 25 and a number of diaphases show 11 bivalents plus one trisome, but occasionally may also three univalents occur. Dwarf pollen are frequent in tetrads of this plant, and the type may be supposed to stop at the chromosome number of  $12_{II}$ .

Cross 7: *V. tricolor alba-yellow*  $\times$  *Orphanidis*, violet.

$(a_1a_1a_2a_2LLvv \quad \times \quad A_1A_1 \dots llVv.)$

Although the *Orphanidis* type applied for crossing had one univalent chromosome, it was a constant type, never showing any conspicuous morphological segregation. Hundreds of plants from self-pollination were always violet flowered (J. CLAUSEN 1930 b), never yellow.  $F_1$  of the present cross showed a conspicuous and surprising segregation as one half of them were dark violet (not pale violet as expected from the presence of one *L*); another half had intense yellow lower petal with the upper petals of young flowers whitish or pale mauve, later on violet, while the lower petal remained yellow. The actual numbers of  $F_1$ -plants were 46 of the violet type as compared with 56 of the yellow one, being a total of 102 plants. As the *tricolor* parent was known to be homozygotic, this segregation suggested that the *Orphanidis* parent, V. 917—1, was heterozygotic for a gene, whose segregation was concealed in the pure *Orphanidis*, because it needed some activation of a gene from *tricolor*. But it seems more natural to admit two types of genes for violet colour: one of these, probably



identical with the *A*-genes, is the usual basal violet colour of all *Melanium* species. It is hypostatic, may be concealed by *LL* and *WW* and changed to red in the absence of *R* and to more or less dark *velutina* by the absence of genes of the *M*-series. The other gene for violet colour, *V*, is supposed to be dominant and epistatic over yellow, *LL*, but it needs the presence of one *A*. It is a condition in analogy with what WINGE (1920) assumed for the colour of horses, namely (1) a dominant gene for red, partly epistatic to black, and (2) a recessive red, the red basal colour of the horse, hypostatic to black.

If *Orphanidis* is *Voll*, it cannot segregate yellow because it has no *L*-gene, and the *voll*-plants segregated are violet due to the presence of *A*-genes. But if this *Orphanidis* is crossed with *tricolor alba*-yellow, *vvLL*, half of the plants get *VvLl* and are violet by the epistatic action of the *V*-gene; half of them get *vvLl* and are yellow, not pale violet as usual, due to some dominance of an intensified *L*-gene and the absence of the *V*-gene.

The  $F_1$ -plants had stipules intermediate between the two parents (compare fig. 111 with figs. 5 and 3), the leaves, flowers and direction of stems (erect) resembled more *tricolor*; the herbage was minutely puberulent of very short but dense hairs. It was extremely sterile, and only by a careful self-pollination and troublesome back crossing were seeds enough obtained for segregations. The fertility was considerably better in the back cross. Table 10 gives the numbers of seeds obtained per capsule and the germination rate.

In  $F_2$  (see the diagram, fig. 112) the violet  $F_1$ -plants segregated yellow ones and the yellow  $F_1$ -plants segregated violet ones. This sounds like a paradox but is nothing but what should be expected from the formulæ suggested. Likewise violet  $F_1$  backcrossed with *tricolor alba*-yellow segregated violet and yellow, while yellow  $F_1$  fertilised with the *tricolor* parent gave nothing but yellow plants.

$F_2$  corresponded to expectation as regards types except that some yellow  $F_2$ -plants coming from violet  $F_1$  should again segregate violet in  $F_3$ ; four plants gave constant yellow offspring, although  $\frac{2}{3}$  of them should be *vvLl* and segregate violet, *vull*. With the small number tried



Fig. 111. Flower, leaf and stipules of Cross 7, *tricolor*  $\times$  *Orphanidis*.

there is a chance for them all being *LL*, especially if differential viability or elimination of chromosomes plays in with. The segregation of the basal gene *A* for anthocyanous colour is mentioned in chapter VI.

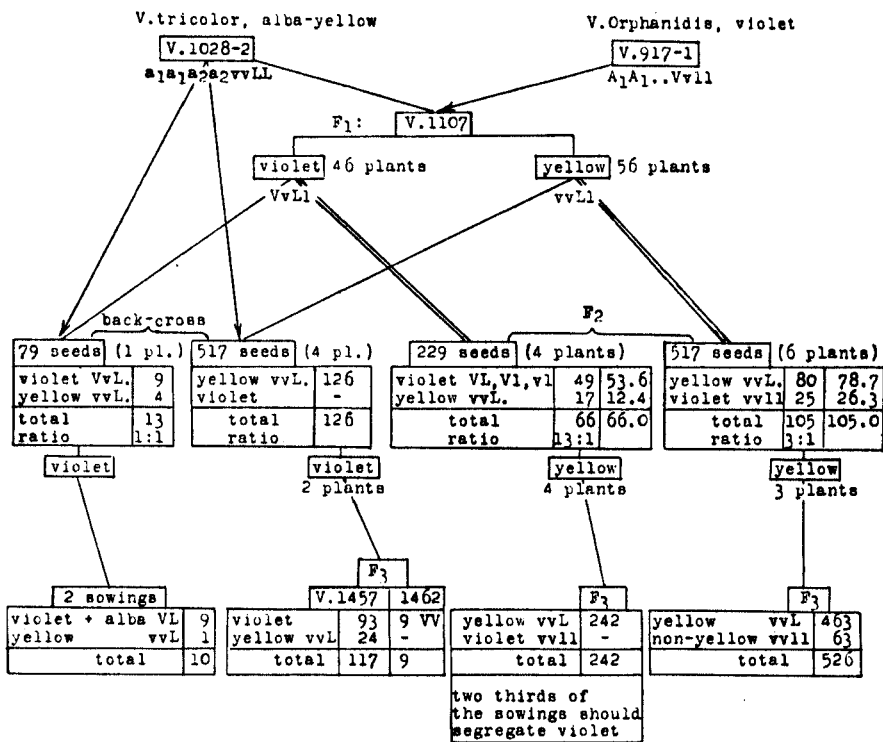


Fig. 112. Cross 7, diagram showing the segregation of flower colouring. (The ratio in *F*<sub>2</sub> from violet *F*<sub>1</sub> plants erroneously given as 13:1, read: 13:3.)

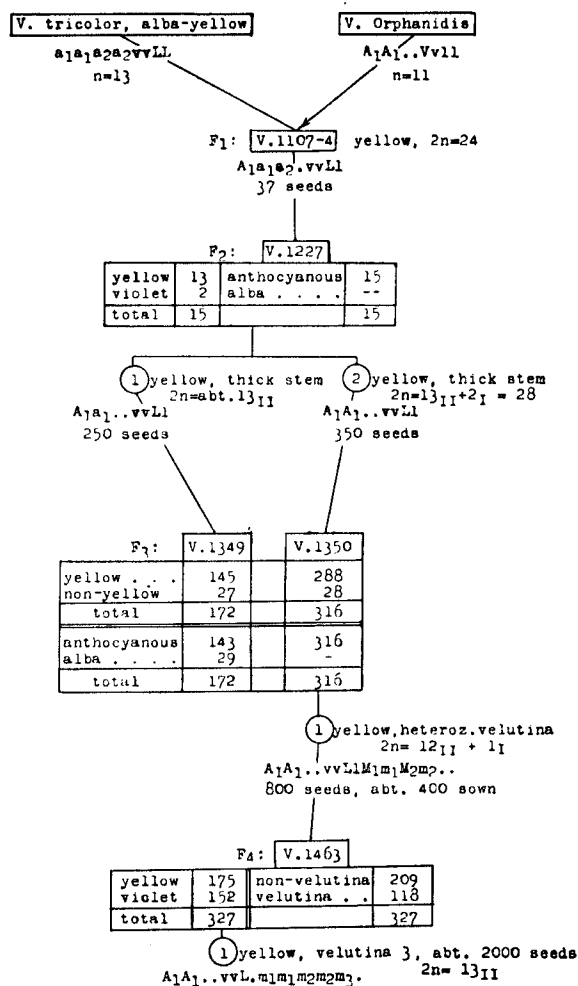
TABLE 10. Average number of seeds per capsule of *V. 1107*, Cross 7, *F*<sub>1</sub>.

Plant no.	1	2	3	4	5	8	seeds		plants germinated
							total	sown	
selfpollination .....	2,4	3,8	2,6	1,5	3,9	5,5	766 (11 plants)	746	178
<i>F</i> <sub>1</sub> × <i>tricolor alba</i> -yellow	4,4	8,1	6,9	7,7	6,0	3,3	641 (8 plants)	596	142

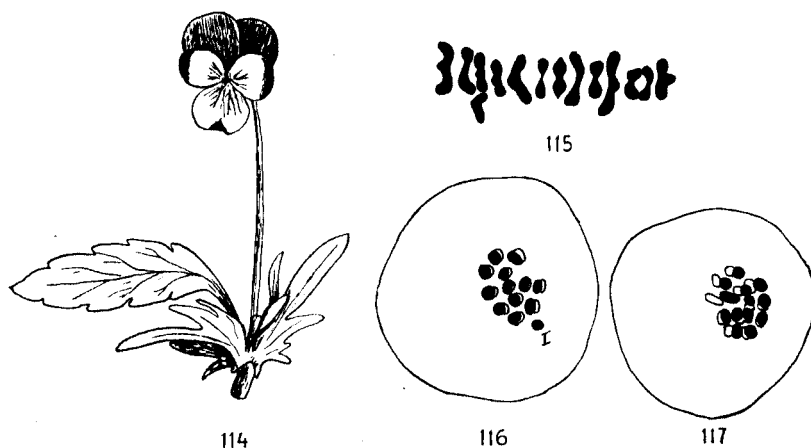
It was surprising that of the 171 *F*<sub>2</sub>-plants (see diagram, fig. 112) no one was of *Orphanidis*-type. This applies to flower shape, direction of stem (*F*<sub>2</sub> mainly erect), hairiness and shape of stipules. *F*<sub>2</sub> was of a glabrous appearance, no ones were hirsute as *Orphanidis*, although

several were puberulent; stipules of many plants showed influence from *Orphanidis*, but there were no true *Orphanidis*. No sufficiently reasonable explanation of this phenomenon can be offered. The crossing was not done reciprocal, and the  $F_2$  plants resembled their *tricolor* grandmother as to type but showed segregation for many characters. The possibility is not excluded that it may be due to plasmatic influence from *tricolor*. Reciprocal crossing will decide this question. But there are other possibilities also, for instance that the two extra *tricolor* chromosomes exercise an essential influence upon the determination of type (most plants seem to have the extra chromosomes) or that the *Orphanidis* type is very recessive.

Some of the  $F_2$ -plants were noted to have very thick stems and were suspected of being tetraploid or at least with increased chromosome number. It will be remembered that some  $F_1$ -pollen mother cells showed almost no conjugation, but this was not connected with much splitting of univalents. Two  $F_2$ -plants of the thick-stemmed type were selfed and gave many seeds (compare the pedigree on fig. 113); the seeds germinated comparatively well, but none of the two plants were tetraploid; one of them, V. 1227—1, had about 13 bivalent chromosomes, another one, V. 1227—2, had  $2n = 28$ , some-

Fig. 113. Pedigree of *Viola crassicaulis*.

times forming  $14_{II}$  (fig. 115), sometimes  $13_{II} + 2_I$ . The offspring of these two plants (V. 1349 and 1350 in fig. 113) were mainly of the thick-stemmed type, being an instance of *gigas* growth without tetraploidy. In V. 1350 appeared two unexpected *velutina* aberrants with upper petals faint velvety, one of them, V. 1350—1, also had a faint velvet spot on the lower petal. This plant was extremely thick-stemmed and very fertile, giving abt. 800 seeds; the chromosome number was 25 ( $12_{II} + 1_I$ , fig. 116). About half of these seeds were sown and gave a total of 327 plants (V. 1463), a very vigorous culture. The flower colour ranged from pure yellow (intense or more pale) to dark



Figs. 114—117. *Viola crassicaulis*. 114: Flower, leaf and stipules; 115: V. 1227—2 ( $= F_2$ ), het. met.,  $14_{II}$  (probably one IV), other plates of this plant showed  $2_I$ ; 116:  $F_3$ , V. 1350—1, het. met.,  $12_{II} + 1_I$ ; 117:  $F_4$ , V. 1463—1, het. met.,  $13_{II}$ .

violet. A large number of the plants had more or less velvety petals and at least one of them was of the recessive *velutina* 3 type with a velvet border on the lateral petal and a triangular velvet blotch on the lower petal. This plant, V. 1463—1, was selfed and gave abt. 2000 seeds; it had 13 bivalent chromosomes (fig. 117). Fig. 118 shows in the upper row the two parental species and in the lower row some flower types of their descendant, V. 1463, the flower most to the right being of V. 1463—1.

For convenience sake this line of population is named

*Viola crassicaulis*,

referring to its very thick and vigorous stems. Fig. 114 is reproduced from a line drawing of this type; the leaves are correlated with the stems and are large and rather thick. The stipules remind somewhat

about those of *Orphanidis*. The flowers are rather large but as compared with the leaves they are small; the petals are of a very broad shape, reminding about the cultivated pansies.

There is no doubt that this vigorous type of a fertility as large as in *arvensis* would have a chance in nature. Curiously enough it has stopped at the chromosome number of 13, that of its *tricolor* parent. If a type like this was found somewhere on the Balcan Peninsula, it would no doubt be described as a new species; a number of the types from this area in the neighbourhood of *V. Orphanidis*, *elegantula* and the Balcan *alpestris* type, *V. macedonica* BOISS. et HELDR., probably arose in a similar way.

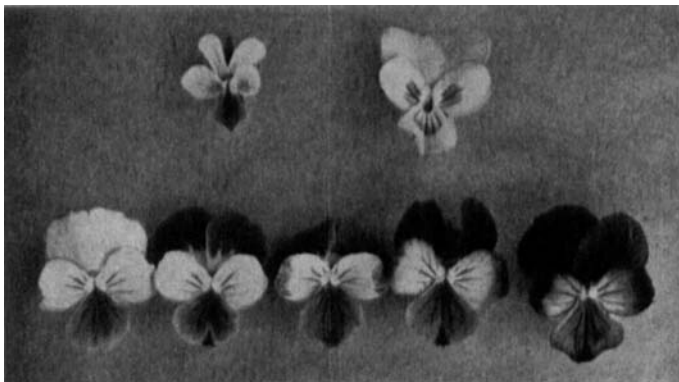
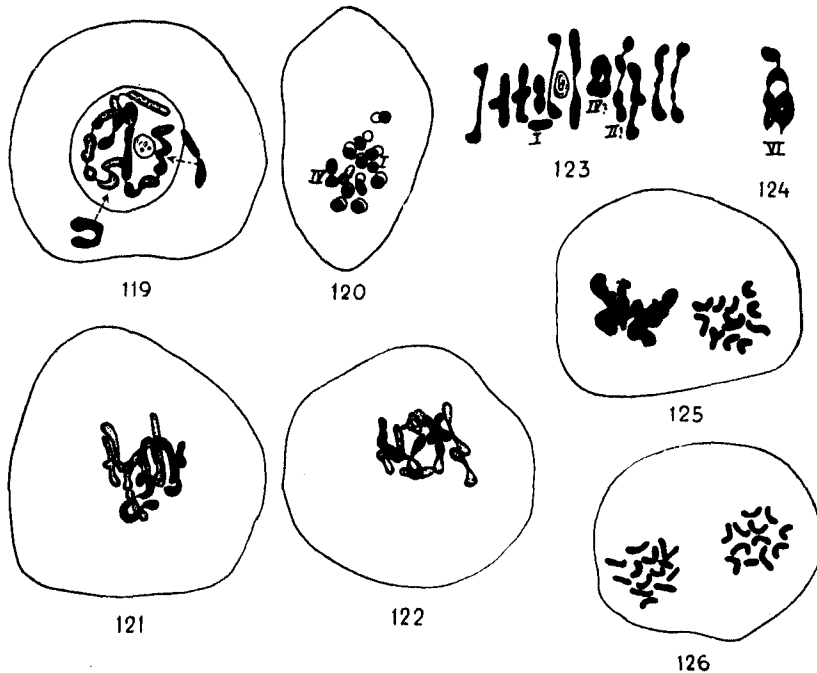


Fig. 118. Upper row, left: *tricolor alba*-yellow; right: *Orphanidis*. Lower row: flower types of *V. crassicaulis*, V. 1463 ( $F_4$ ); different degrees of velvety violet, the left one pure yellow, the right one is V. 1463—1 (*velutina* 3 and probably constant).

Another point of interest in Cross 7 are the changes which take place with the chromosomes during later generations of some of its types. There is no doubt that a more close following up of the behaviour of chromosomes in offspring of known hybrids would clear up many problems and shake some theories at their base.

$F_1$  of Cross 7 did not show much multivalent association of chromosomes (figs. 40—44), but some of the later generations show much more. Probably the conditions in hybrids favour a segmental interchange between non-homologous chromosomes. Figs. 119—126 illustrate meiosis in two violet flowered  $F_3$ -plants, one of V. 1457, another of V. 1462 (see the diagram, fig. 112). In diaphase of the first one, fig. 119, the chromosomes were connected in two long chains with some 10—11 in each and two free pairs; in the chains there appeared

to be a paired arrangement, but the pairs were connected end to end with short strands; (in one of the chains two pairs are seen parallelly connected). This arrangement in chains was not kept so strictly through all heterotypic divisions (fig. 120 shows a fairly regular heterotypic metaphase with only one chain of four, an uneven pair and one univalent), but most of them were irregular and impossible to disentangle; the somatic chromosome number of this plant was 25.



Figs. 119—126. Unexpected chromosomal types and associations in  $F_3$  of Cross 7, 119—120 are from one plant, 121—126 from another one, and they came from different  $F_2$ -parents. 119: Chains of chromosomes in diaphase; 120: a comparatively regular het. meta-anaphase in polar view,  $1_{IV} + 10_{II} + 1_I$ . 121—122: Early het. metaphases, multivalent association of chromosomes in irregular chains; 123: a less intricate het. met., but chromosomes very long, not as ordinary *Viola*-chromosomes, probably  $1_{IV} + 10_{II} + 1_I$  (and one nucleolus); 124: a peculiar hexasomic association; 125: hom. metaphase,  $n = 12$ ; 126: hom. anaphase,  $n = 13$ , 12; long, bent chromosomes as in *Polemonium* and in many *Compositae*.

In the plant of V. 1462 (figs. 121—126) a change as to meiotic chromosome shape had taken place and the chromosomes were also in this one connected to long, somewhat irregular chains even in early heterotypic metaphase (figs. 121—122); the arrangement was never in regular zig-zag chains or rings as in *Oenothera*. It is surprising, still,

how the chromosomes are able to disentangle themselves during meiosis in such a manner that they become distributed into two almost equal groups. As far as could be seen the diploid chromosome number was 25 (fig. 126), and the countings in homotypic metaphase ran as follows:

n =	11	12	13	14
number of nuclear plates	1	4	3	2, total: 10.

Most heterotypic metaphases maintained the multivalent association between the chromosomes, and the associations could appear in peculiar shapes as the hexasome seen in fig. 124 or in still more complicated connections. Some of them, indeed, could be fairly regular as that shown in fig. 123 with one quadrivalent, 10 bivalents and one bivalent; two of the pairs here resemble tetrasomes, but the total chromosome number of the plant indicates that at least one of them was only a disome.

The bivalent chromosomes were changed to long rods, and one or two of them could assume the shape of crosses (fig. 123); in the homotypic division they were bent in the shape of V's similar to somatic chromosomes. This shape of meiotic chromosomes is extraordinary for Violets, but other large plant groups exist, in which it is the normal one, for instance *Crepis* and many other *Compositæ*. It was also noted in *Polemonium* (J. CLAUSEN 1931 a). MANN LESLEY and FROST (1927) described a *Matthiola*, which deviated from normal *Matthiola* in a similar manner by having long chromosomes. By courtesy of Dr. FROST the writer had the opportunity of having this type in culture and was able to verify the statements of the two authors as to chromosome shape; in heterotypic and homotypic divisions it corresponds fully to the type of the long chromosomes in the genera mentioned. MANN LESLEY and FROST found the long chromosome type to be recessive, probably depending upon only one gene in their case. It is interesting to note that such shape or such conduct of chromosomes, which seemingly is of an essential nature as characterising large taxonomic units, may arise suddenly after crossing in part of the offspring, not in all of it and that it may probably arise by a single mutation also. This does not speak against the taxonomic value of such characters, it only shows that they may arise sporadic, when a proper combination of genes is realised, and it is a hint about the way, in which such differences arose.

The polysomic arrangement of chromosomes of the two plants mentioned resembles very much the illustrations recently published by

KATTERMANN (1931) of multivalent association between chromosomes of *Anthoxanthum odoratum* and *Bromus erectus*, regarded as good species.

Cross 8: *V. tricolor*  $\times$  *alpestris* and reciprocal.  
(*ddl*  $\times$  *DDLL*.)

The yellow flower colour of the *alpestris* types applied is not so intense as in yellow flowered *tricolor*, apparently due to the action of some faintly bleaching gene.  $F_1$  of violet *tricolor*  $\times$  yellow *alpestris* is therefore violet, not whitish violet as  $F_1$  of violet *tricolor*  $\times$  yellow



Fig. 127. Cross 8 C, *tricolor alba*  $\times$  *alpestris velutina*, flowers. Upper row from left: *alpestris* and *tricolor alba*; under them  $F_2$ -types. Middle row from left: violet, yellow, yellow *velutina* 1, and the rare, maculate, *alpestris* parental type; lower row: two flowers with local mutations to *velutina* in upper petals, one of them also petaloid; dilute mauve and *alba*.

*tricolor* (compare the flower colours on plate I, J. CLAUSEN 1926). Correspondingly, yellow of the *alpestris* cross has to be classified as a recessive, although in yellow plants the violet no doubt is still present, but concealed by the action of homozygous yellow. The violet will appear at spring time and when local mutations occur, as in the two flowers to the left in lower row of fig. 127.

Taken in this way the classification as to yellow lower petal was reliable. Table 11 shows this segregation for three different crosses



(A, B and C). The yellow from *alpestris* yellow-*velutina* (= *polychroma*) seems to give a more vital type (Crosses B and C) than the yellow from *alpestris typica* (Cross A), because in Crosses B and C

TABLE 11. Segregation of flower colour in  $F_2$  of *tricolor*  $\times$  *alpestris* (all three crosses reciprocal):  $ll \times LL$ .

Flower colour	Cross 8 A: <i>tricolor</i> violet $\times$ <i>alpestris</i> yellow		Cross 8 B: <i>tricolor</i> violet $\times$ <i>alpestris</i> <i>polychroma</i>		Cross 8 C: <i>tricolor</i> alba $\times$ <i>alpestris</i> <i>polychroma</i>	
	observed	calculated	observed	calculated	observed	calculated
light to dark violet, $ll + Ll$	512	503, <sub>3</sub>	819	885, <sub>7</sub>	507	600, <sub>7</sub>
yellow, $LL$ ...	159	167, <sub>7</sub>	362	295, <sub>3</sub>	294	200, <sub>3</sub>
total	671	671, <sub>0</sub>	1181	1181, <sub>0</sub>	801	801, <sub>0</sub>
		non velvety	1120		723	
		velvety	61		78	
		total	1181		801	

yellow is considerably in excess of its calculated proportion. Among those classified as violet there were a large scale of lighter and darker shades.

The velvety blotch on the two upper petals of *alpestris polychroma* is a peculiar character in its inheritance, but it is subject to phenotypical modification. It was apparent that very few velvety plants occurred in  $F_2$ , and most of them were only faintly velvety, very few had the typical blotch on the upper petals, rarely they would be total *velutina* on the two upper petals; fig. 127 shows flowers of parents and  $F_2$  of Cross 8 C.

Cross C showed that *alpestris* has two polymeric (multiple) genes for anthocyanin similar to *tricolor*, but in addition it contains the gene *D* for dilute mauve similar to *arvensis* as will be seen from the enumeration below:

	observed	calculated
anthocyanous..... A . . . . .	801	
dilute mauve ..... $aaD$ . $\left\{ \begin{array}{l} ll \\ Ll \end{array} \right.$	18	23, <sub>6</sub>
alba..... $aadd$ $\left\{ \begin{array}{l} ll \\ Ll \end{array} \right.$	12	7, <sub>9</sub>
alba-yellow ..... $aa$ . . $LL$	12	10, <sub>5</sub>

The crosses stress the position of *V. alpestris* as being specific different from *tricolor*, partly by the minor irregularities in meiosis of  $F_1$  and partly by aberrant types occurring in  $F_2$ , especially the petaloid types (see fig. 127 and table 17 in Chapter VI). But apparently it stands just on the verge of being specific different and is therefore an interesting type illustrating that the conception of species must be conventional in some cases.

Taxonomists have sometimes thought that *alpestris* is more related to *arvensis* than *tricolor* is, and the genetical analysis verifies this assumption. In common with *arvensis* it has the genes *L* for yellow and *D* for dilute mauve, which are absent from typical *tricolor*, and it segregates petaloid and *velutina* aberrants, when crossed with *tricolor*, just as *arvensis* does, but it gives no semisterile types as the cross *tricolor*  $\times$  *arvensis*.

*Cross 9: V. tricolor*  $\times$  *arvensis*.

This cross has been dealt with previously (J. CLAUSEN 1926), but there are some additions to be made with regard to the character *dilute mauve* and to later generations of some constant types illustrating the origin of new species.

TABLE 12. *Inheritance of dilute mauve (D), extracted from arvensis, introduced into tricolor and crossed with different tricolor-varieties (all reciprocal).*

Flower colour, anthocyanin in stems		for- mula	dilute mauve $\times$ rose-velutina [ $a_1a_1a_2a_2DDRR(II) \times$ $A_1A_1A_2A_2ddrr(II)$ ]		dilute mauve $\times$ <i>alba</i> ( $aaDD \times aadd$ )		dilute mauve $\times$ <i>alba</i> -yellow ( $aaDDII \times aaddLL$ )	
			obser- ved	cal- culated	obser- ved	cal- culated	obser- ved	cal- culated
anthocyanous		A...	713	705,0	—	—	—	—
green <i>aa</i>	dilute mauve	<i>D. II</i>	28 <sup>1</sup>	35,3	391	390,7	54	45,2
	mauve-yellow	<i>D. LL</i>	—	—	—	—	76	90,4
	<i>alba</i> .....	<i>ddLL</i>	11	11,7	130	130,3	50	45,2
	<i>yellow</i> .....	<i>ddll</i>	—	—	—	—	61	60,2
total			752	752,0	521	521,0	241	241,0
ratio				60 : 3 : 1		3 : 1		3 : 6 : 3 : 4

<sup>1</sup> 8 of these dilute rose.

The gene, *D*, for dilute mauve was first proved to be present in *Viola arvensis* as shown by the Cross *V. tricolor alba*  $\times$  *arvensis* (J. CLAUSEN 1926, Cross XIV, p. 107). Via a backcross of  $F_1$  to *tricolor alba* and selfing through two successive generations a type was selected, which was a true *tricolor* as to morphological characters and with 13 bivalent chromosomes as *tricolor*, but with the *D*-gene introduced from *arvensis*. This type was crossed with some *tricolor* varieties, before it was homozygous for the *D*-gene, and  $F_1$ -plants with *D* were selected for  $F_2$ . Table 13 shows these segregations, which are not too far from expectation. Dilute mauve in a single dose is seen to be epistatic to

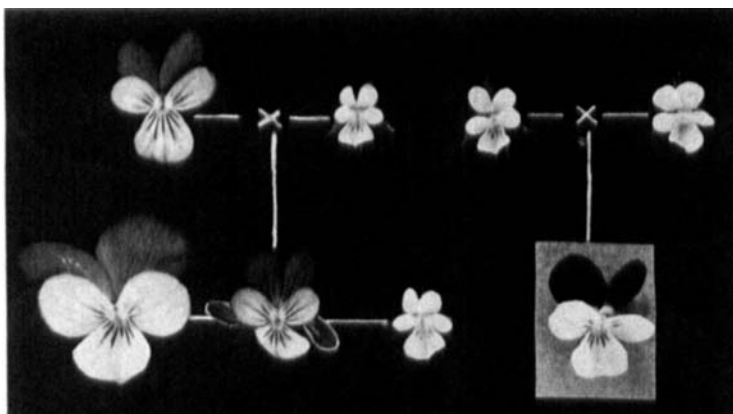


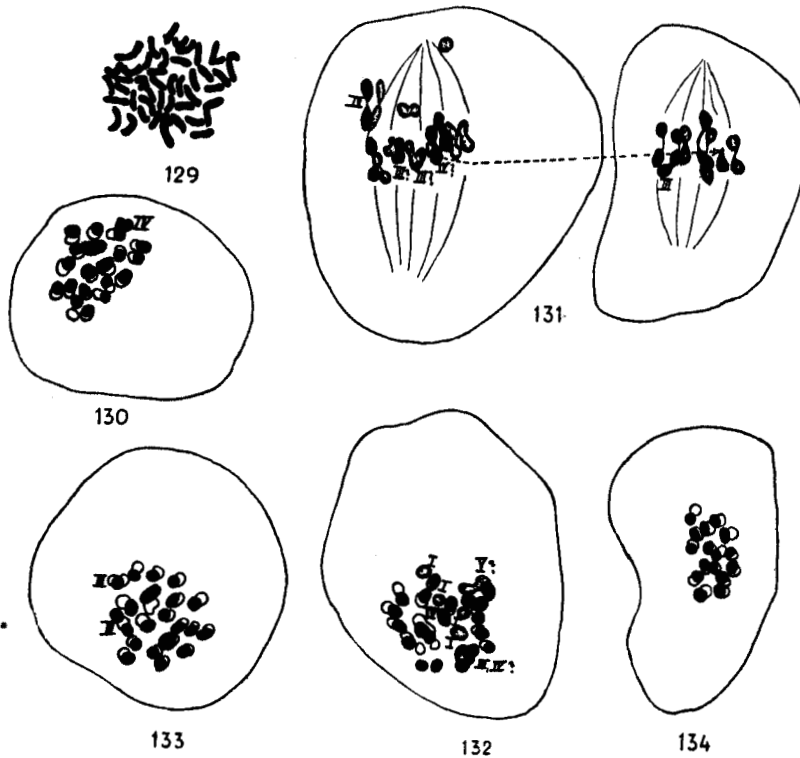
Fig. 128. New and constant types produced by crossing of *tricolor* and *arvensis*. Left part: *tricolor typica* and *arvensis* Line 52 with three types, namely from left: *V. hyperchromatica* ( $n = 21-23$ ), *V. petaloidea* ( $14_{II}$ ) and a constant segregated *arvensis* ( $16_{II}$ ). Right part: *arvensis*, Line 52 and *arvensis*, Line C, both with 17 bivalents; under them their aberrant, *V. velutina*,  $n = 16$  (loss of one chromosome).

heterozygous yellow, *Ll*, but homozygous yellow is epistatic to dilute mauve, as well *DD* as *Dd*.

Of the constant types described in the 1926-paper were some few selected to be propagated generation after generation in order to try them for constancy and find out the chromosome number at which they would stop, if changes took place. Flowers of these types are shown in fig. 128 together with their parent types (see explanation under the figure).

*Viola hyperchromatica* is described on pp. 126—130 of the cited paper. It arose from a partial duplication of the chromosomes in meiosis of a single  $F_1$ -plant, which formed gametes with chromosome number ranging from abt. 15 to 25, giving plants with a somatic

chromosome number of abt. 39 to 46. Under ordinary conditions are 11 to 13 *arvensis*-chromosomes capable of conjugation with the same number of *tricolor*-chromosomes; it is therefore evident that a duplication of chromosomes as in *hyperchromatica* may create some possibility for the formation of tetrasomes and trisomes. Such are actually found and most nuclei, indeed, contain tetrasomes and trisomes and con-



Figs. 129—134. Chromosomes of *V. hyperchromatica* (129—133) and *V. velutina* (134). 129—131:  $F_7$  of the original cross, 129 shows a somatic chromosome plate,  $2n = 42$ ; 130:  $18_{II} +$  at least one IV; 131: het. met., abt.  $2_{IV} + 3_{III} + 12_{II} + 1_I$ . 132:  $F_8$ , het. met.,  $2n$  between 40 and 43, a pentasome, tetrasomes, monosomes and probably also trisomes; 133:  $F_8$ , het. met.,  $2_{III} + 18_{II} = 42$ . 134: *V. velutina*, het. met.,  $16_{II}$ , not entirely regular in their arrangement ( $F_5$ ).

sequently also monosomes. The chromosome number varies a little from plant to plant, although it generally lies about  $2n = 42$ . But the morphological type has been maintained unaffected by the slight changes in chromosome complement. Fig. 128 shows its appearance in fall, when it attains its fullest development as compared with *tricolor*. Figs. 129—131 show chromosome complement of an  $F_7$ -plant, 132 of

will be remembered, is the same for both species, viz.  $n=17$ .  $F_1$  was whitish to pale violet flowering with rather small flowers (fig. 143); it had a very faint labellum (almost none) under the stigma, a dark spot on the style and was rather hispid. The fertility was good, as it gave abt. 1500 seeds from five plants, and 542  $F_2$ -plants were raised from them.

$F_2$  showed fairly clear segregations for several characters (see segregation for size of flowers, for labellum and for spot on style in Chapter VI). As for flower colour, dark violet types (as *rothomagensis*)

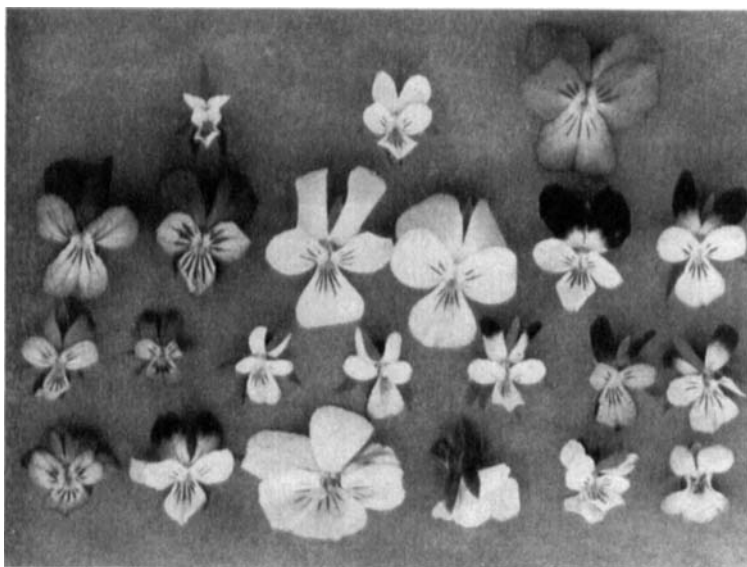


Fig. 143. Flowers of Cross 16, *arvensis*  $\times$  *rothomagensis*. Upper row from left: *arvensis*,  $F_1$  and *rothomagensis*; under them a series of  $F_2$ -types. Second row from above: large flowered types, two violet, two whitish and two *velutina* aberrants; third row: small flowered and intermediate types, two violet, two whitish and two violet *velutina* aberrants; bottom row: aberrants, namely violet petaloid, *velutina* petaloid, peloric and petaloid type in frontal and in side view, a type with four sepals petaloid, and finally a small peloric type.

were separable from the other range of variation covering all transitions from yellowish white (similar to *arvensis*) to pale violet; the enumeration is given below:

	observed	calculated	ratio
(WW) <i>L.vv</i> , yellowish white	435	516	13
(WW) <i>L.V.</i> + (WW) <i>llvv</i> , pale violet			
(WW) <i>llV.</i> , violet . . . . .	81	96,7	3

plants. These plants were just enough to get one yellow flowered  $F_2$ -plant, showing that *Kitaibeliana* contains the gene *L* for yellow similar to *arvensis*. The segregation is given below:

	$F_2$	$F_1 \times \text{tricolor alba}$
anthocyanous	$\left. \begin{array}{l} \text{violet} \dots\dots\dots 8 \\ \text{whitish to pale violet} \dots\dots\dots 22 \\ \text{yellow} \dots\dots\dots 1 \end{array} \right\} 31$	$\left. \begin{array}{l} 8 \\ 12 \\ - \end{array} \right\} 21$
green, <i>alba</i>	$\left. \begin{array}{l} \dots\dots\dots 1 \end{array} \right\}$	$\left. \begin{array}{l} 1 \end{array} \right\}$

There were no *alba* plants in  $F_2$  but one in the backcross, suggesting that at least three polymeric, basal genes for anthocyanin are present in *Kitaibeliana*. All  $F_2$ -plants were small flowering and of *Kitaibeliana*-type, while in the backcross four large flowering plants were found in addition to 17 intermediate. This may suggest that the small flower size of *Kitaibeliana* as compared with *arvensis* is due to the presence of one pair more of principal inhibiting genes, *F*, for flower size.  $F_2$  was largely sterile. There were 26 plants with spot on style in  $F_2$  and five without.



135  
Fig. 135. Flower, leaf and stipules of *tricolor*  $\times$  *rothomagensis*,  $F_1$ .

Cross 13. *V. tricolor alba*-yellow  $\times$  *rothomagensis* (violet).  
[ $a_1a_1a_2a_2 \dots (vv)LLwvss \times A_1A_1A_2A_2A_3A_3(VV)llWWSS$ .]

The formulæ for this crossing written above are only tentative as regards the *V*-, *L*- and *W*-genes. If consideration had not to be taken to Cross 16, which indicates that *rothomagensis* is homozygous *WW*, and to some later generations of the present cross, the segregations could be explained in a less complicated manner.

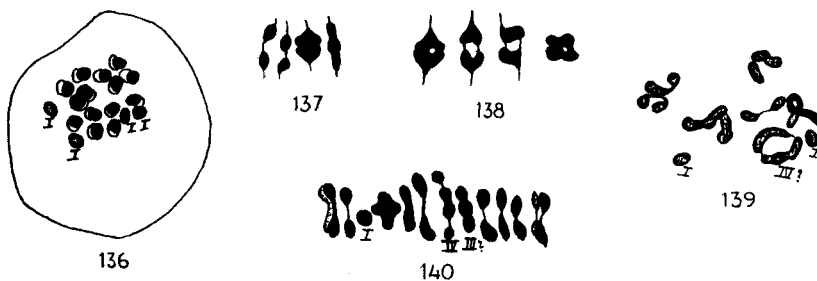
$F_1$ , 34 plants from 47 seeds, was a very long- and thin-stemmed type with stiff and straight, ciliate hairs (fig. 135) reminding much about *rothomagensis* itself. The young flowers were whitish but changed to light or pale violet. It was extremely sterile, and the few seeds obtained germinated very poorly as seen below:

	seeds per capsule	total of seeds	full-grown plants
$F_1$ selfed	3—4	99	15
$F_1 \times \text{tricolor alba}$ -yellow	5—7	414	41

The plants obtained were too few for a proper explanation as to the complicated segregation of flower colour, but violet and yellow and a series of pale violet to yellow were obtained as seen below:

	$F_2$	$F_1 \times \text{tricolor alba-yellow}$
anthocyanous	$\left\{ \begin{array}{l} \text{violet} \dots\dots\dots 4 \\ \text{pale violet to whitish} \dots\dots\dots 10 \\ \text{yellow} \dots\dots\dots 1 \end{array} \right\} 15$	$\left\{ \begin{array}{l} 5 \\ 19 \\ 13 \end{array} \right\} 41$
green	$\left\{ \begin{array}{l} \text{alba} \dots\dots\dots - \\ \text{alba-yellow} \dots\dots\dots - \end{array} \right\}$	$\left\{ \begin{array}{l} 2 \\ 2 \end{array} \right\}$

It is noteworthy that real violet plants were segregated in the backcross  $F_1 \times \text{tricolor yellow}$ ; a corresponding cross but with *tricolor* supplying the violet in  $F_1$  would never give violet plants in backcrosses to yellow; similarly, in  $F_4$ , a dark violet flowered plant segregated 26 dark violet to eight yellow. These segregations suggest that *rothomagensis* contains the epistatic V-gene for violet flower colour, and this gene is not much inhibited by the bleaching W-gene. As for the



Figs. 136—140. Chromosomal conditions in later generations of Cross 13. 136—138:  $F_2$ -plant with a very large chromosome (probably aggregation of two); 136: het. met.,  $13_{II}$  (one large) +  $4_I$ ; 137: the large and two ordinary chromosomes in side view; 138: the large chromosome from four different pollen mother cells, the one most to the right in polar view. 139—140: an  $F_3$ -plant, 139 shows chromosomes from a diaphase with a large, ringshaped tetrasome; 140: het. met., abt.  $11_{II} + 1_{IV} + 1_{III} + 1_I = 30$ .

segregation of the characters anthocyanin and dark style spot, see Chapter VI.

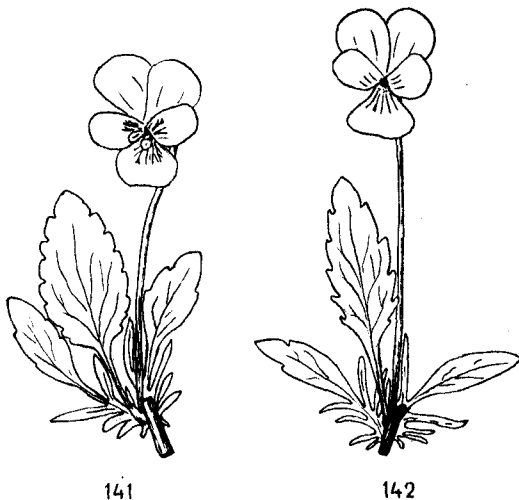
A large chromosome probably of double nature was observed in an  $F_2$ -plant (figs. 136—138), a constant pale yellow plant giving in  $F_3$  66 pale yellow flowered plants. The big chromosome was twice the size of the others and somewhat bipartite, and in polar view it often showed a curious perforation in the middle (fig. 138, right). It may have arisen through a close union of two and two chromosomes, for a similar chromosome is not seen in any of the parents. It suggests that chromosomes may sometimes aggregate. Figs. 139—140 show diaphase and heterotypic metaphase from a dark violet  $F_3$ -plant, which seg-

regated yellow flowered plants in  $F_4$ ; a real tetrasome was almost invariably present here. Sometimes this tetrasome would resemble a bivalent, but very large, ringshaped chromosome; in other cells, however, the four were attached end to end (fig. 140).

Cross 14: *V. tricolor hortensis*  $\times$  *lutea*.

[*ll(pal pal)*, no spot  $\times$  *LL(Pal Pal)*, with spot.]

The *Viola lutea* type applied is not intense yellow on the lower petal, but it is not whitish either, as in *V. arvensis*. In  $F_2$  of the crossing were plants segregated, which were much more intense yellow than the *lutea* parent, and this suggests that *lutea* possesses and *tricolor* lacks a bleaching gene of minor order than *W*; it has been named *Pal*.



Figs. 141—142. Flower, leaf and stipules of  $F_1$ . 141: *tricolor*  $\times$  *lutea*, trace of doubleness in flower; 142: *tricolor*  $\times$  *Battandierii*.

A total of 90 hybrid seeds were obtained and 36  $F_1$ -plants were grown from two crosses; 14 of these belonged to the reciprocal cross *V. tricolor hortensis*  $\times$  *lutea* and were good and vigorous plants showing characters from both parents; the leaves were larger and more long ovate than in *V. lutea* (see fig. 141), an

influence from the very vigorous *tricolor hortensis*. The other 22  $F_1$ -plants represented the weak hybrid *tricolor alba*  $\times$  *lutea*, of which almost all plants died young (by »black leg»); only four  $F_2$ -plants of this last cross reached flowering stage. The  $F_1$ -flowers of the cross *tricolor hortensis*  $\times$  *lutea* were larger than in *lutea* but of similar shape and also streaked as in this species. The colour of the lower petal was pale yellow as in its *lutea* parent, that of the upper ones faint mauve. The fertility was not good, 3—4 seeds per capsule by self pollination; the richly flowering plants could produce as many as abt. 300 seeds, but only a little more than 10 % germinated, giving in all 91  $F_2$ -plants from abt. 800 seeds.



The segregation for flower colour is shown below:

lower petal	observed	calculated	
pale yellow <i>L Pal</i> .....	33	68	68,3
intense yellow <i>L pal</i> .....	35		
violet <i>ll Pal + ll pal pal</i> .....	23	22,7	total: 91

The upper petals of the yellow types may have some violet in them.

It was unexpected that segregation did not take place at all regarding spot on style; all 91  $F_2$ -plants had a very distinct spot. This may be due to polymery (multiple genes, *V. lutea* is octoploid!), but spot on style did not show true polymery in any other cross. More probably it is due to duplication of univalents by splitting as mentioned before in chapter IV, perhaps in connection with differential viability.

As regards other characters segregation was evident, also as to the vegetative type, whether similar to *tricolor* or to *lutea*. Two  $F_3$ -cultures with 85 and 41 plants, respectively, were constant with spot as  $F_2$ . The first one was constant yellow, the last also constant yellow but segregating *velutina*. The general appearance of the  $F_3$  cultures was very uniform and similar to *lutea*.

*Cross 15: V. tricolor hortensis*  $\times$  *Battandierii*.

This cross was reciprocal and no difference could be observed between the two reciprocals. In fact,  $F_1$  is very uniform, although the chromosome number of the *Battandierii* type oscillates. 31 crossed seeds gave 15 vigorous and very rich flowering plants.  $F_1$  has a large end lobe of stipules and flower shape similar to *Battandierii*, but its size is smaller (fig. 142). KRISTOFFERSON (1923) gave a description of this specific hybrid, of which he cultivated  $F_1$  and  $F_2$ . Similar to *Battandierii* are the peduncles of  $F_1$  jointed with the stem. 79  $F_2$ -plants from 313 seeds were all violet flowering as expected.

*Cross 16: V. arvensis*  $\times$  *rothomagensis*.

$$\left[ \begin{array}{l} \text{yellowish white } vvLLWW \times \text{violet } VVllWW, \\ BBFFss \times bbffSS. \end{array} \right]$$

As mentioned under Cross 13 the formula for flower colour of *rothomagensis* is tentative only. The *W*-gene has been attributed to it in order to explain that no yellow plants are segregated in  $F_2$  of the present cross.

The reciprocal cross will not germinate, while 80 seeds of the present combination gave 63  $F_1$ -plants. The chromosome number, if

will be remembered, is the same for both species, viz.  $n = 17$ .  $F_1$  was whitish to pale violet flowering with rather small flowers (fig. 143); it had a very faint labellum (almost none) under the stigma, a dark spot on the style and was rather hispid. The fertility was good, as it gave abt. 1500 seeds from five plants, and 542  $F_2$ -plants were raised from them.

$F_2$  showed fairly clear segregations for several characters (see segregation for size of flowers, for labellum and for spot on style in Chapter VI). As for flower colour, dark violet types (as *rothomagensis*)

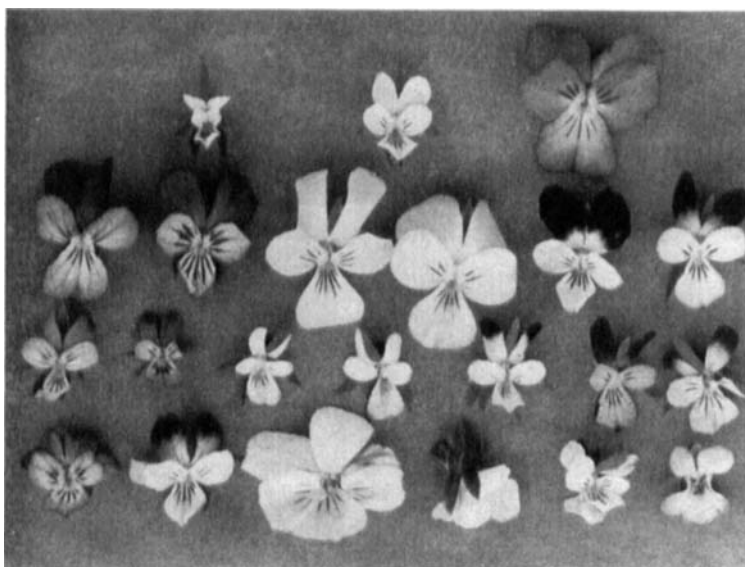


Fig. 143. Flowers of Cross 16, *arvensis*  $\times$  *rothomagensis*. Upper row from left: *arvensis*,  $F_1$  and *rothomagensis*; under them a series of  $F_2$ -types. Second row from above: large flowered types, two violet, two whitish and two *velutina* aberrants; third row: small flowered and intermediate types, two violet, two whitish and two violet *velutina* aberrants; bottom row: aberrants, namely violet petaloid, *velutina* petaloid, peloric and petaloid type in frontal and in side view, a type with four sepals petaloid, and finally a small peloric type.

were separable from the other range of variation covering all transitions from yellowish white (similar to *arvensis*) to pale violet; the enumeration is given below:

	observed	calculated	ratio
(WW) <i>L.vv</i> , yellowish white	435	516	13
(WW) <i>L.V.</i> + (WW) <i>llvv</i> , pale violet			
(WW) <i>llV.</i> , violet . . . . .	81	96,7	3

Fig. 143 gives an impression of the variation in  $F_2$  from yellowish white to violet, and from small to large flowers. It shows also the flowers of some of the aberrant types, which appeared in great number (see Chapter VI): *velutina*, petaloid, and also peloric types with spur on lateral petals. Sterile or semisterile plants were frequent in  $F_2$  (see Chapter VI, aberrant types). They were just of the type described from Cross 9, *tricolor*  $\times$  *arvensis* (J. CLAUSEN 1926, 1927 a), the leaves were entire but often necrotic and variegated due to local absence of green palissade tissue (see fig. 144 and compare it with figures in the

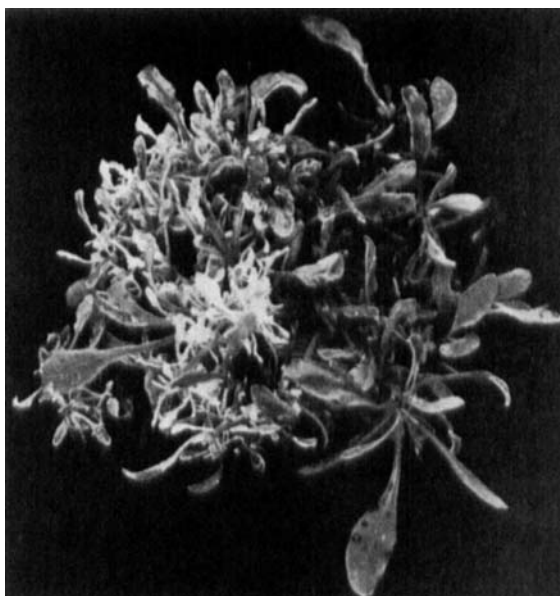


Fig. 144. Sterile type of Cross 16 (with entire leaves); white-variegated by degeneration of palissade tissue.

paper cited above and with fig. 146 of Cross 19 in the present paper). *Viola rothomagensis*, when crossed with *arvensis*, gives accordingly the same aberrant types as the cross *tricolor*  $\times$  *arvensis*. This should indicate some genic or constitutional similarity between *rothomagensis* and *tricolor*, but the fertility test shows *rothomagensis* to be much more related with *arvensis* than with *tricolor*.

There was found a similar linkage between the inhibitors for flower size and for labellum as previously noted in the cross *tricolor*  $\times$  *arvensis*, but this is not surprising as the inhibitors in both cases came

from *arvensis*; the numbers of the four combinations were the following:

flower size	labellum	observed
small ( <i>FF</i> ) + intermediate ( <i>Ff</i> )	no labellum ( <i>B.</i> )	343
	with labellum ( <i>bb</i> )	33
large ( <i>ff</i> ) . . . . .	no labellum ( <i>B.</i> )	24 <sup>1</sup>
	with labellum ( <i>bb</i> )	116

516

Plants with large flowers and without labellum were generally yellowish white flowering, and the sepals of such plants were also large, almost of the same length as the petals.

*Cross 17: V. Kitaibeliana*  $n = 7 \times$  *arvensis*, Line 52.

Except as regards chromosome number these two species are very alike. The most striking difference is in shape and size of flower as seen from fig. 5.  $F_1$  in this respect resembled its *arvensis* parent, while the characters of the leaves were more like *Kitaibeliana*.

About 1100 seeds from two  $F_1$ -plants yielded no more than 197  $F_2$ -plants, and these had all without exception the open, flattened shape of corolla similar to their *arvensis* parent. For numerous small characters were segregations noted: mode of branching, length of nodes (1.5—4 cm.), shape of stipules, dentation of leaves (crenate, serrate, somewhat incised), shape of leaves (ovate, spatulate, lanceolate; broad, narrow, leaves of most plants were narrow as in *Kitaibeliana*), thickness of stem, size of flower (varied somewhat, but mainly as in *arvensis*), corolla-limb (broad or narrow, more or less flattened, never of the closed cup-shaped type as in *Kitaibeliana*) and finally the colour of the flower (yellowish white to faint mauve). There were also great variation as to fertility, ranging from absolute sterility to abt. 200 seeds on rather small plants.

The relative constancy of some *arvensis* characters may depend upon the behaviour of the chromosomes in  $F_1$ , where many univalents (mainly *arvensis* chromosomes) split. The average number of chromosomes in  $F_2$  appears to be above that of  $F_1$  ( $2n = 24$ ), tending to go in direction towards the *arvensis* parent ( $2n = 34$ ) or even above it. In nine  $F_2$ -plants the chromosome numbers were the following:

$2n =$  abt. 21, 24, 26, 27 or 28 (two plants), 28, 29, 31, abt. 36.

A semi-sterile plant with entire leaves (cf. Crosses 16 and 19) was segregated. Neither of the parent species had spot on style, and spot was not syntethizised through the crossing.

<sup>1</sup> 21 of these were yellowish white!

Cross 19: *V. Kitaibeliana*  $n = 7 \times$  *Kitaibeliana*  $n = 18$ .

This cross was made in both directions, and 13  $F_1$ -plants were secured from 93 crossed seeds. Fig. 145 shows the  $F_1$  between its two



Fig. 145. Cross 19; from left: *Kitaibeliana*  $n = 7$ ,  $F_1$ , *Kitaibeliana*  $n = 18$ .

parents. It was extremely sterile, and as the seeds were very few and *Kitaibeliana* otherwise is a good selfpollinator, it was thought safe to use also seeds from some few capsules set before the plants were removed to the insect-proof green house. A total of abt. 500 seeds were collected from five  $F_1$ -plants. This gave only 33  $F_2$ -plants and of these abt. 15 were apparently vicinists, triple hybrids from open pollination. 18 plants apparently came from selfpollination and had small flowers with closed, not flattened limbs and yellowish white or faintly mauve colour of flowers.

Almost all hybrid, vicinistic seeds may have germinated, while of the real  $F_2$ -seeds only abt. 3.6 % were able to do so. The experiment thus shows that when a highly sterile hybrid under natural conditions is exposed to open pollina-



Fig. 146. Sterile type with entire leaves, segregated in  $F_2$  of Cross 19.

tion, then formation of triple hybrids or backcrosses will be favoured more than selfpollination. This is because the pollen from the hybrid is not so good as from a foreign pure species, and seeds containing at least one complete, balanced set of chromosomes germinate much better than do seeds containing only recombined and exchanged chromosomes from two specific different sets. As well the supposed vicinistic plants as the true  $F_2$ -plants were almost completely sterile. One plant of the non-flowering sterile type with entire leaves appeared in  $F_2$  (fig. 146).

Cross 22: *V. lutea*  $\times$  *Battandierii*.

[pale yellow,  $LL(Pal\ Pal) \times ll(pal\ pal)$ , violet.]

These species are doubtless somewhat related, and crossing is easily effected. 40 seeds giving 22  $F_1$ -plants were obtained from it.  $F_1$  reminds vegetatively about *Battandierii* (also in its flower shape) but the flower colour is more pale violet and the lower petal is faintly yellowish.

About 250 seeds were obtained by selfpollination, and these gave 131  $F_2$ -plants. Many gradations as to colour of flowers were noted, but a number of different shades can also be produced by the interaction of the two genes suggested above. Some of the yellow types were yellow on the lower petal only, faint violet on the upper ones; others were pale yellow on all petals, and one was noted, which was intense yellow on all five petals as the cultivated *V. lutea grandiflora*. The enumerations as to flower type of  $F_2$  are given below:

flower colour	observed	ratio	calculated
violet . . . . . $ll$	43	1	98,3
$\pm$ pale violet . . $Ll$	52	2	
yellow, $\pm$ intense $LL$	36	1	32,7

A wide range of variation was noted in  $F_2$  as to growth type (cespitose or with very long side branches), size of flowers, length and shape of spur (straight or curved) and length of peduncle (one plant had peduncles as long as 15 cm.). Ten plants had abnormal style, either a bilateral or even a three-sided, actinomorphic one.

Cross 24: *V. (tricolor alba-yellow*  $\times$  *Orphanidis)*  
 $\times$  (*cornuta*  $\times$  *elegantula*).

Although *V. tricolor* cannot be crossed directly with *cornuta*, this present quadruple hybridisation was easily effected. HERIBERT NILSSON (1930) had similar experiences in his multispecific hybrids of *Salix*.

Fig. 147 shows the pedigree of Cross 24.  $F_1$ , V. 1205, consisted of 39 plants and due to its being  $F_1$  of a quadruple hybrid it showed a considerable variation (see fig. 148). This applies to flower shape (remininding about *cornuta*, *elegantula*-*Orphanidis* or even *Battandierii*), direction of lateral petals (horisontal or turned upwards, never downwards), colour of flowers (dark violet to very light or pale violet and reddish violet), length and shape of spur (straight or curved), extension of the yellow eye present in all plants, shape of style, shape of stipules

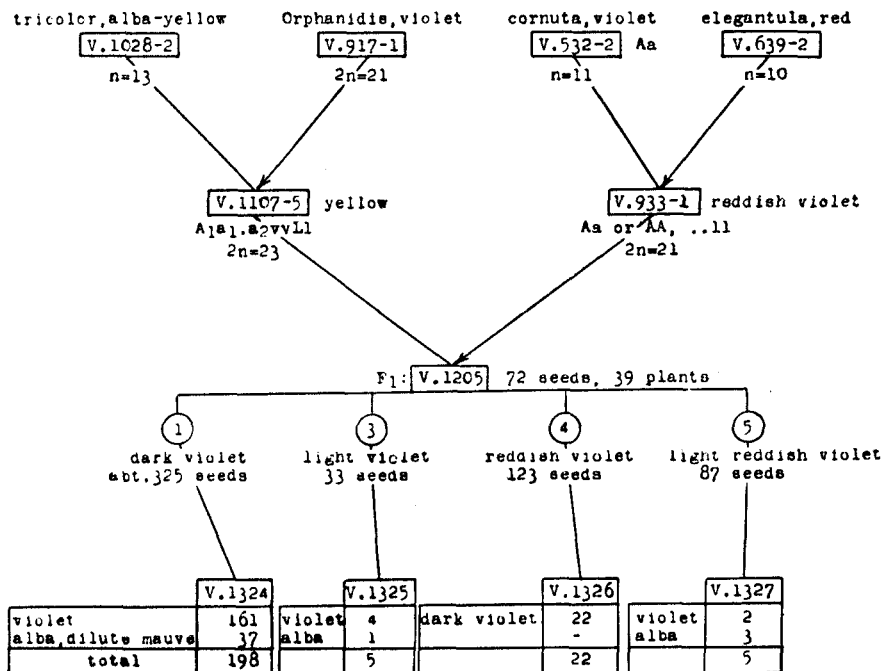


Fig. 147. Diagram of Cross 24 (quadruple).

(triangular, lyrate-pinnatifid, palmate; degree of division also varying) and the character of the ramification, whether cespitose or more open. The fertility was also extremely variable.

Different types of segregation were also obtained in the individual  $F_2$ -sowings (V. 1324—1327, fig. 147) according to from which of the four grandparental species the chromosomes were derived, which constituted the corresponding  $F_1$ -plant in V. 1205.

Fig. 149 shows flowers of V. 1324. This  $F_2$ -sowing contained 198 plants; the general appearance of them was similar to *cornuta*; this applies decidedly to leaves and stipules. The shape of flowers

corresponded to Cross 5 (*Orphanidis*  $\times$  *cornuta*), but some plants suggested a *tricolor* parent. The erect growth was derived from *tricolor*.

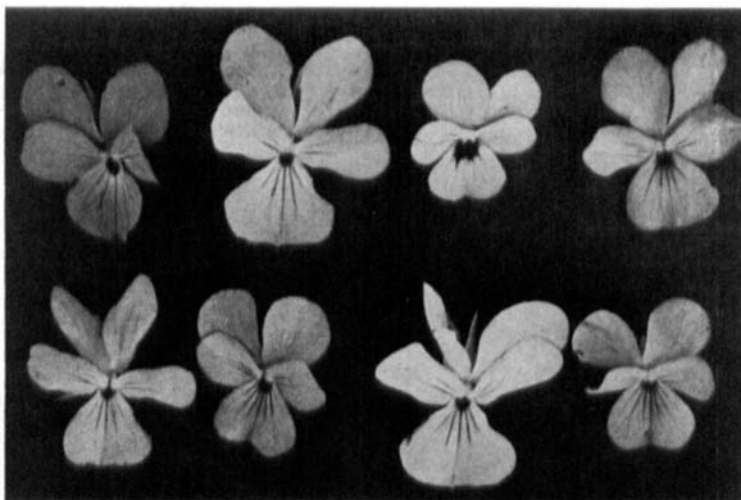


Fig. 148. Types of flowers in Cross 24,  $F_1$ , V. 1205 (portrait film).

The colour of the violet flowers was somewhat darker than in the *Orphanidis*  $\times$  *cornuta* cross and more reddish (influence from *ele-*

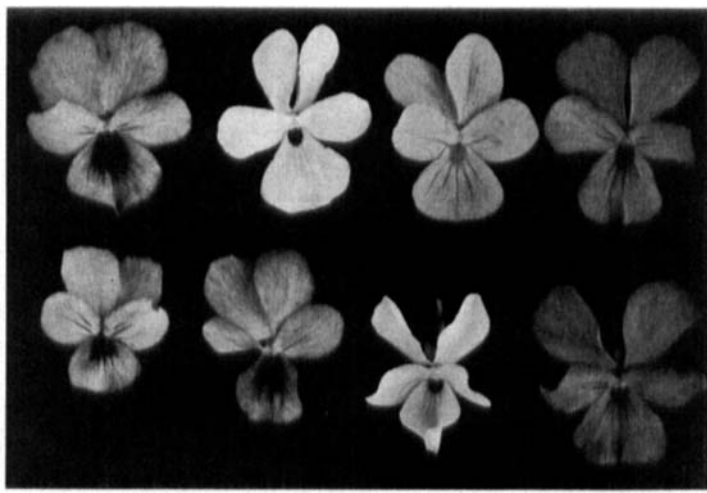


Fig. 149. Types of flowers in V. 1234,  $F_2$  of quadruple hybrid. Note difference as to yellow eye, direction of lateral petals and shape of petals (portrait film).

*gantula*?). The yellow eye could originate from any of three parents. There were large variation as to extent of yellow in the eye, and one



plant had a totally white eye (fig. 149, a *cornuta*-character). The segregated green-stemmed, non-anthocyanous plants were not *alba*-flowering but with a tinge of dilute mauve; this colour is not noted from any of the parents before; the non-anthocyanous condition could be inherited from either *tricolor* or *cornuta*. The direction of the lateral petals varied from horizontal or slightly turned upwards to downwards turned as in *cornuta*. The flower size was very variable, the spur of all plants was thin and straight, but in no case it attained a length as in *cornuta*. Some were hirsute as *Orphanidis*, others almost glabrous. Apparently, therefore, this sowing showed characters from all four parent species.

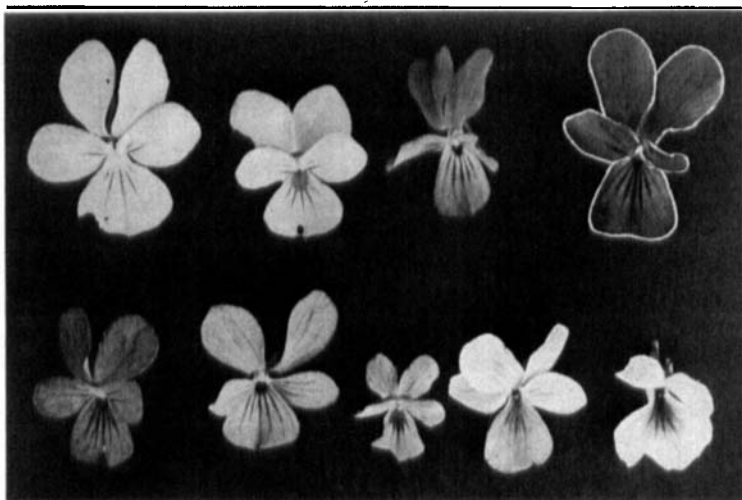


Fig. 150. Cross 24,  $F_2$ -types of flowers. Upper row from left: two flowers of V. 1325; the two to the right and the three left ones in lower row are of V. 1326; right in lower row: two *alba* flowers of V. 1327, the right one is peloric (portrait film).

V. 1325 resembled *tricolor* as to its flower shape (fig. 150); two of a total of five plants had flowers with white eye (no yellow); the stipules were as in *cornuta* or as in *elegantula*.

V. 1326 contained only dark reddish violet flowering plants, two of them were much darker than any of the parents (fig. 150); four plants were caespitose, non-flowering dwarfs (in addition to 22 flowering plants); the flower shape reminded about *elegantula* and *Orphanidis* and the stipules varied from a shape similar to those of *elegantula* to a triangular shape as in *cornuta*. Most characters in this sowing point towards *elegantula*.

V. 1327 contained three pelories among a total of five flowering

plants (fig. 150); these pelories were all *alba*-flowering. The stipules were of *elegantula* type with some influence from *cornuta*.

It appears as if the presence of chromosomes from four different sets do not cause any serious inflictions.

## VI. SEGREGATION OF SOME CHARACTERS.

While the flower colour shows such complicated inheritance that it was thought better to deal with it under the individual crossings, some of the other characters may more practically be dealt with for more hybrids simultaneously.

### THE BASAL GENES FOR ANTHOCYANIN COLOURS (A-SERIES).

Table 13 shows that *elegantula*, *Orphanidis* and *cornuta* all carry one basal gene for anthocyanin. Cross 7 gives too few *alba*, but there

TABLE 13. Segregation of A-genes.

being analysis of		<i>elegantula</i> n = 10				<i>Orphanidis</i> n = 11, 10'				<i>cornuta</i> n = 11	
Cross no.		Cross 1: <i>corn. alba</i> × <i>elegant.</i>		Cross 6: <i>tric. alba-y.</i> × <i>elegant.</i>		Cross 5: <i>Orphan. ×</i> <i>corn. alba</i>		Cross 7: <i>tric. alba-y.</i> × <i>Orphanidis</i>		<i>cornuta</i> , violet × <i>alba</i>	
formulae		$\frac{a_1}{A_1}$		$\frac{a_1 a_2}{A_1}$		$\frac{A_1}{a_1}$		$\frac{a_1 a_2}{A_1}$		$\frac{A_1}{a_1}$	
		obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
$F_2$	antho- cyanous	42	42,7	6	4,5	113	106,5	163	158,2	106	105,0
	<i>alba</i>	15	14,3	—	1,5	29	35,5	15	19,8	34	35,0
	total ratio	57	57,0	6	6,0	142	142,0	178	178,0	140	140,0
		3 : 1		3 : 1?		3 : 1		8A : 1a?		3 : 1	
backcrosses		antho- cyanous		17	16,0	—	—	95	94,7		
		<i>alba</i>		15	16,0	—	—	47	47,3		
		total ratio		32	32,0	—	—	142	142,0		
				1 : 1				2A : 1a?			
						$F_3$		antho- cyanous	160	144,0	
								<i>alba</i>	32	48,0	
								total ratio	192	192,0	
										3 : 1?	

are also too many for a 15 : 1-ratio. The same *Orphanidis*-parent, viz. V. 917—1, was applied for both Cross 5 and Cross 7. Cross 5 gives a 3 : 1 ratio, and as it is a less wide cross than 7, it is more conclusive than this latter; Cross 5 refers to five  $F_1$ -plants. In Cross 7 would a gametic ratio of  $2A : 1a$  (by gametic sublethality) explain the segregation as well in  $F_2$  as in the backcrosses. But other characters show almost normal segregation (for instance violet : yellow), and zygotic sublethality of the *alba* type may therefore be more probable. The ratio is bettered in  $F_3$ .

Wild growing types of *tricolor* and *alpestris* have two basal genes for anthocyanin, while the old garden type of *tricolor* (as well *velutina* 3, as *tricolor nigra*) has three genes and segregates 63 : 1 (as *arvensis*). It will be remembered that *alpestris* and *arvensis* both contain the *D*-gene for dilute mauve.

TABLE 13 (continued). Segregation of *A*-genes.

being analysis of		<i>tricolor</i> n = 13				<i>alpestris</i> n = 13		<i>arvensis</i> n = 17		<i>Kilaib- eliana</i> n = 18	<i>rothom- agensis</i> n = 17
		wild types		<i>hortensis</i>							
Cross no.		<i>tricolor</i> , coloured × <i>alba</i>		<i>tricolor</i> , <i>hortensis</i> × <i>alba</i>		Cross 8: <i>tric. alba</i> × <i>alpestris</i>		Cross 9: <i>arvensis</i> × <i>tric. alba</i>		Cross 11:	Cross 13:
formulae		$A_1A_2$ $a_1a_2$		$A_1A_2A_3$ $a_1a_2a_3$		$a_1a_2$ $A_1A_2$		$A_1A_2A_3$ $a_1a_2$		$A_1A_2A_3(?)$ $a_1a_2$	$a_1a_2$ $A_1A_2A_3$
		obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	obs.
$F_2$	antho- cyanous	5200	5132,8	2147	2147,9	801	790,3	1054	1050,3	31	15
	<i>alba</i>	275	342,2	35	34,1	42	52,7	13	16,7	—	—
	total ratio	5475	5475,0	2182	2182,0	843	843,0	1067	1067,0	31	15
			15 : 1		63 : 1		15 : 1		63 : 1	63 : 1?	63 : 1?
		backcrosses				anthocyanous <i>alba</i>		376	371,9	20	37
						total ratio		49	53,1	1	4
								425	425,0	21	41
								7 : 1	7 : 1	7 : 1?	7 : 1
								$F_3$		anthocyanous <i>alba</i>	30
										1	1
										total ratio	31
											63 : 1?

Abbreviations: *alba-y.* = *alba*-yellow; *corn.* = *cornuta*; *Orphan.* = *Orphanidis*; *tric.* = *tricolor*; calc. = calculated; obs. = observed.

The numbers are too small in Crosses 11 and 13 for an exact analysis of *Kitaibeliana*  $n = 18$  and *rothomagensis*, respectively; but the few segregated *alba* individuals seem to indicate at least three *A*-genes in both species, just as in the third hexaploid species, viz. *arvensis*.

Provided the distribution of univalent chromosomes in  $F_1$  is at random and free (that is to say: surplus chromosomes from the species with the largest chromosome number do not unite autosyndetic, nor polysomic, and are not eliminated to any considerable degree) the segregation of dominant genes present in the univalent chromosomes should not deviate considerably from the ordinary Mendelian ratio, because only the dominant genes count and recessive genes count equal to absence of the corresponding chromosome. When a species shows a recessive character, say green stem, it can have no dominant genes (*A*) at all for this character present in any of its chromosomes, all its chromosomes are, so to say, recessive as to *A*, being *a*.

When the species with the highest number of chromosomes is that with the dominant *A*-genes, elimination of univalent chromosomes with any of these genes may bring the segregation down to a seemingly lower degree of polymery; and if univalent chromosomes containing any *A*-genes split, or if autosyndesis takes place, the hybrid may simulate a segregation according to a higher degree of polymery or eventually behave as constant.

It appears as if there is a close coincidence between the degree of polymery and the degree of polyploidy within the *Melanium* section, when it is remembered that *elegantula*, *cornuta* and *Orphanidis* are regarded as diploid members of a 10-series, while *tricolor* and *alpestris* are tetraploid, and *arvensis*, *Kitaibeliana* 18 and *rothomagensis* are hexaploid members of a 6-series.

Not many analyses have been made of the distribution of genes in hybrids between species differing as regards chromosome number and as regards polymery. One of the most interesting is the cross *Euchlæna perennis*,  $n = 20 \times$  *Zea Mays*,  $n = 10$  (R. A. EMERSON 1929). Crosses were made in such a way that comparison could be made between segregations, where Maize (the diploid parent) carried the dominant genes, *Euchlæna* (tetraploid) the recessive ones, and, on the other hand, such segregations, where the tetraploid *Euchlæna* carried the dominant genes. *Euchlæna*- and Maize-chromosomes appear to be close homologous; the test showed, in fact, that Maize carried one set of genes, *Euchlæna* at least two sets for each of the four dominant

characters investigated. When Maize was used as the dominant parent, the backcross of  $F_1$  to recessive Maize showed a true monohybrid segregation, giving 341 of the dominant type and 401 of the recessive one (a 1 : 1 ratio). On the other hand, when *Euchlæna* was the dominant parent and the  $F_1$ -hybrid similarly was backcrossed to recessive Maize, only very few recessives were segregated, namely 29 recessives as compared with 375 dominants; this is even far below the expected dihybrid ratio 3 : 1 and also far below a trisomic ratio 5 : 1, being more like a 15 : 1 segregation. The ratio probably indicates a certain percentage of autosyndesis between dominant *Euchlæna* chromosomes. Such autosyndesis has no visible effect, when the *Euchlæna* chromosomes carry the recessive genes.

The segregations in *Viola* indicate in almost all cases a free assortment of the chromosomes carrying the polymeric (multiple) genes, but in those cases, where the dominant species has the largest number of chromosomes, one of the polymeric genes may be without a mate.

#### FLOWER SIZE AND PRESENCE OF LABELLUM.

These two characters are by taxonomists regarded as taxonomic significant ones and justly so. It is therefore of some interest that also specific characters segregate after laws similar to varietal characters. This is dealt with previously for one hybrid (J. CLAUSEN 1926). Here is only given a survey on these segregations (see table 14).

Several genes are no doubt responsible for flower size and for labellum, but the segregations seem to indicate at least one gene with a superior effect of inhibiting the flower size. The segregation is clearest, when the genes of minor importance modifying the effect of the inhibiting gene are absent. Cross 11 suggests at least two inhibiting genes for flower size and for labellum in *Kitaibeliana*  $n = 18$ , which is very small flowered. There is linkage between the inhibitors for flower size and for labellum (J. CLAUSEN 1926 and Cross 16).

#### YELLOW EYE.

It has been mentioned that yellow eye is a character of superior taxonomical value. The segregation of this character is shown in table 15.

It is obvious from the types segregated, of which some are shown in figs. 148—150, that genes also for the extension of the eye are present, and the entire genetical basis for this character may be a very complicated one.

TABLE 14. *Segregation of flower size and labellum in different crosses.*

Cross		Cross 9: <i>tricolor</i> × <i>arvensis</i> (XI, 1926)		Cross 11: <i>tricolor</i> × <i>Kitaibeliana</i> n = 18		Cross 16: <i>arvensis</i> × <i>rothoma-</i> <i>gensis</i>			
flower size		obs- erved		calc- ulated	obs- erved		obs- erved	calc- ulated	
small + intermediate		$F_2$ +	243	236,3	$F_2$	31	$F_2$	376	387,0
large		$F_4$	72	78,7		—		140	129,0
total ratio		315		315,0 3 : 1	31 15 : 1?		516	516,0 3 : 1	
backcross, $F_1$ × large flowered	flower size	(XII, XIV, XV 1926)							
	intermediate	538		479,5	17				
	large	421		479,5	4				
	total ratio	959		959,0 1 : 1	21 3 : 1?				
labellum									
without		$F_2$	215	219,0	backcross to <i>tricolor</i>	16	$F_2$	367	387,0
with		+	77	73,0		5		149	129,0
		$F_4$							
total ratio		292		292,0 3 : 1	21 3 : 1?		516	516,0 3 : 1	

TABLE 15. *Segregation of yellow eye. ( $F_2$ ).*

Crosses	Cross 1: <i>cornuta</i> × <i>elegantula</i>		Cross 5: <i>Orphanidis</i> × <i>cornuta</i>	grand total
	obs- erved	calc- ulated	observed	
yellow .....	73	66,0	127	200
white .....	15	22,0	15	30
total	88	88,0	142	230

Back cross (*cornuta* × *elegantula*) × *elegantula* gave 27 plants, all with yellow eye.

## STYLE SPOT.

See notes on the inheritance of this character on p. 242. Table 16 shows its segregations.

TABLE 16. *Segregation of the character style spot in the individual crosses (all showing a 3 : 1 ratio).*

Crosses formulae:	Cross 8: <i>alpestris</i> <i>Ss</i> × <i>tricolor</i> <i>SS</i>		Cross 11: <i>Kitaibeliana</i> 18 × <i>tricolor</i> <i>s</i> <i>S</i>		Cross 13: <i>tricolor</i> × <i>rothoma-</i> <i>gensis</i> <i>s</i> <i>S</i>		Cross 14: <i>tricolor ss</i> × <i>lutea</i> <i>SS</i>			Cross 16: <i>arvensis</i> × <i>rothoma-</i> <i>gensis</i> <i>s</i> <i>S</i>	
generation	<i>F</i> <sub>2</sub>		<i>F</i> <sub>2</sub>		<i>F</i> <sub>2</sub> + <i>F</i> <sub>3</sub> + <i>F</i> <sub>4</sub>					<i>F</i> <sub>2</sub>	
	obs- erved	calc- ulated	obs- erved	calc- ulated	obs- erved	calc- ulated	<i>F</i> <sub>2</sub>	<i>F</i> <sub>3</sub>	total	obs- erved	calc- ulated
with spot	143	156,0	26	23,3	82	85,5	91	127	218	385	387,0
no spot ...	65	52,0	5	7,7	32	28,5	—	—	—	131	129,0
total	208	208,0	31	31,0	114	114,0	91	127	218	516	516,0

## ABERRANT TYPES.

It is striking that there are certain aberrant types, which segregate again and again from different specific crosses. Table 17 gives a survey on their segregation.

TABLE 17. *Aberrant types segregated in F<sub>2</sub> of different specific crosses.*

Crosses	Cross 6: <i>tricolor</i> × <i>elegantula</i> backcross		Cross 8: <i>alpestris</i> × <i>tricolor</i> <i>F</i> <sub>2</sub>		Cross 9: <i>tricolor</i> × <i>arvens.</i> 6 crosses	Cross 16: <i>arvens.</i> × <i>rotho-</i> <i>mag.</i>	Cross 17: <i>Kitaib.</i> 7 × <i>arvens.</i>	Cross 19: <i>Kitaib.</i> 7 × <i>Kitaib.</i> 18	Cross 22: <i>lutea</i> × <i>Battand.</i>
types	<i>F</i> <sub>1</sub> × <i>tric.</i>	<i>F</i> <sub>1</sub> × <i>eleg.</i>	<i>A</i>	<i>B</i> + <i>C</i>	<i>F</i> <sub>2</sub>	<i>F</i> <sub>2</sub>	<i>F</i> <sub>2</sub>	<i>F</i> <sub>2</sub>	<i>F</i> <sub>2</sub>
normal	34	67	461	1971	4423	436	196	19	119
sterile	24	23	—	—	75	26	1	1	—
dwarfs	—	—	—	53	70	22	—	—	2
petaloids	—	—	—	—	88	23	—	—	10
pelorics	—	—	—	—	52	35	—	—	—
<i>velutina</i>	—	—	2	—	—	—	—	—	—
total	58	90	463	2024	4708	542	197	20	131

Cross 7. In *F*<sub>3</sub> of *tricolor* × *Orphanidis* appeared two *velutina* aberrants.

The aberrants from different crosses were often so similar in type, that they would be difficult to tell apart, if being mixed with each others. The sterile dwarfs in Cross 6, only, were different from other dwarfs, inasmuch as they were not of the necrotic-variegated type; some of them were very minute, cespitose dwarfs without any signs of flowering at all.

No doubt the chromosomes of the *Melanium* section are largely very homologous as to genetic structure, and certain losses, therefore, create similar aberrants. Some of the aberrants are probably due to losses of entire chromosomes, but deficiencies and real mutations may also have taken place, although they never occur in the pure species cultivated.

## VII. CONCLUSIONS AND SUMMARY.

In the following the general biological facts substantiated and dealt with in some detail in the foregoing will be extracted and summarized.

### HOMOLOGY OF CHROMOSOMES.

It is characteristic for the *Melanium* violets that all specific hybrids show some amount of chromosome conjugation. There is a tendency that so many bivalents are formed as corresponds to the haploid chromosome number of the parent with the smallest number of chromosomes; that is to say, such number of bivalents is maximum, but in most cells one or two pairs less are formed. Some specific hybrids vary so largely as to chromosomal conjugation that cells are observed without any conjugation of chromosomes at all, but they show a great range of variation in the number of univalents from cell to cell (Cross 7: *tricolor*  $\times$  *Orphanidis* and Cross 11: *tricolor*  $\times$  *Kitaibeliana*  $n = 18$ ). Cross 18 (*nana*  $\times$  *arvensis*,  $n = 24 \times 17$ ) shows comparatively the smallest amount of conjugation of any of the hybrids.

In three crosses were more bivalents formed than the species with the smallest chromosome number allowed; these were Cross 3 (*lutea*  $\times$  *elegantula*,  $24 \times 10$ ), Cross 12 (*tricolor*  $\times$  *nana*,  $13 \times 24$ ) and Cross 15 (*tricolor*  $\times$  *Battandierii*,  $13 \times 26-30$ ). A species with a high number of chromosomes enters in all these crosses, and excess bivalents are no doubt formed by autosyndetic union of chromosomes from the species with the high chromosome number. But these species do not always give autosyndesis. Cross 14 (*tricolor*  $\times$  *lutea*) shows the number of univalents stipulated by the difference between the numbers of the two species; Cross 20 (*nana*  $n = 24 \times$  *Kitaibeliana*  $n = 7$ ) does not



show any autosyndetic union of the 17 extra *nana* chromosomes, although they unite in Cross 12; and in Cross 18 (*nana*  $\times$  *arvensis*,  $n = 24 \times 17$ ) the *nana* chromosomes are obviously neither capable for conjugation with themselves nor with those of *arvensis*.

The polysomic arrangement of chromosomes is mainly found in the hybrids, in which *elegantula* enters as one parent (Crosses 1, 2 and 6 and also in Cross 3). The hybrids of *rothomagensis* show a tendency, though not so striking, for formation of trivalents and, rarely, quadrivalents (Crosses 13 and 16), and the hybrids with *lutea* contain often irregular chains of chromosomes (Crosses 3, 14 and 21).

If these phenomena should be interpreted in the manner current in recent cytological philosophy, one would say that the chromosome complements of the *Melanium* species are built up regularly from the base in a manner that one set of chromosomes is present in all species, and the set added in tetraploids is present also in all hexaploids and octoploids; the extra set added in the hexaploids should furthermore be present twice in the octoploids. The following scheme gives a picture of the chromosomal complements of the species belonging to the subsection *Tricolores*, and each letter indicates a set of approximately six chromosomes:

diploid:  $\frac{A}{A}$  (*Kitaibeliana*  $n = 7$ ),  
 tetraploid:  $\frac{AB}{AB}$  (*tricolor*, *alpestris*,  $n = 13$ ),  
 hexaploid:  $\frac{ABC}{ABC}$  (*arvensis*, *rothomagensis*,  $n = 17$ ; *Kitaibeliana*  $n = 18$ ),  
 octoploid:  $\frac{ABCC}{ABCC}$  (*nana*, *lutea*,  $n = 24$ ).

The repeated set in the octoploid species should account for the autosyndesis observed in some of their hybrids. The cytological construction of the *Melanium* subgenus is different from that of *Nicotiana* (R. E. CLAUSEN 1928, KOSTOFF 1930) and from *Triticum* (AASE 1930, WATKINS 1930 and BLEIER 1930); these two genera do not have one basal set of chromosomes present in all of their species. Although some variation occurs in the amount of conjugation in meiosis of hybrids in these two last named genera, the operation of the chromosomes in the hybrids largely suggests definite sets of chromosomes present, in some cases homologous, in other non-homologous or both together. A very well established case of repetition or duplication of at least one set of chromosomes in a polyploid species is the hexaploid *Solanum*

*nigrum*, the haploid type of which shows abt. 12 bivalents and 12 univalents with a tendency even to the formation of one or two trisomes (JØRGENSEN 1927).

The *Melanium* species with 10 and 11 chromosomes apparently contain at least the same basal set of chromosomes as the *Tricolores* have, and perhaps also a part of the second set (B). But the chromosome set of *elegantula* should furthermore represent a partial reorganisation by segmental interchange between non-homologous chromosomes, not any systematic and regular interchange but a more irregular one. Some of the chromosomes of *rothomagensis* should have undergone a similar reorganisation. This will account for the polysomic association of chromosomes met with in the hybrids of these species with the other ones.

The polymeric (multiple) genes proven to be present in *Viola* species in accordance with the degree of polyploidy of the species (in first line the A-genes for anthocyanin) suggest a repetition of a single set of chromosomes in the polyploid species. But many other genes are not repeated in each set, and there is no doubt that many differences exist, also as regards genes, between the individual sets of chromosomes present in the polyploid species. Probably only genes, which are very widespread and perhaps homozygotic present in almost the entire section, and vital significant genes are repeated in each set of chromosomes. Many processes may have furthered the differentiation of chromosomal sets from possibly one original set. Addition, subtraction, duplication and exchange of entire chromosomes or parts of them may account for many deviations from an identical behaviour by chromosomes of species with the same chromosome number.

But although there seems to be some reality behind the theory about homology of chromosomes as indicated by their ability or non-ability for mutual conjugation, such facts are known, which cause great difficulties for this winning and plain theory.

The chromosomes of *arvensis* show their homology with those of *tricolor* by conjugation with *tricolor*'s chromosomes, at least the 12—13 first ones do so. When *tricolor* next is crossed with *V. nana*  $n = 24$ , 16—18 bivalents are formed, proving the homology of *tricolor*'s chromosomes with a similar number of *nana* chromosomes; but in addition a certain number of *nana* chromosomes must conjugate mutually, which after the theory of homology should prove that *nana* had two homologous sets of chromosomes at least. The same *nana* was crossed with *arvensis* and almost no bivalents at all were formed in this hybrid.

By homology at least 12—13 *arvensis* chromosomes could be expected to be capable for conjugation with a similar number of *nana* chromosomes, but only 2—6 bivalents were observed, and these may just as well be *nana* chromosomes conjugating mutually, as they did in the *tricolor*  $\times$  *nana* hybrid. How are we to look upon such discrepancies?

Conjugation of chromosomes no doubt indicates a kind of homology between them (perhaps some identical arrangement of certain genes and their alternatives), but non-conjugation does not necessarily always mean non-homology. Other factors may prevent the conjugation. Non-conjugation in pollen mother cells of pollensterile *V. Orphanidis* and complete conjugation in embryosac mother cells of the same plant (J. CLAUSEN 1930 b) indicated that environment conditions of the cells may prevent conjugation, even if the chromosomes themselves are homologous. The poor conditions in the male archespor was probably due to a certain constellation of genes or chromosomes. The similar asynaptic condition without conjugation of chromosomes in the pollen mother cells of a *Zea Mays* may in its realisation have been produced even by the action of a single recessive pair of genes (BEADLE 1930). The chromosomes conjugate, *if* the individual chromosomes are homologous, and *if* conjugation is not prevented by some unbalance in the total complement of chromosomes of the individual in question. Such unbalance is frequent in specific hybrids.

The present investigation shows how prudent we have to be in drawing conclusions about the origin of chromosome numbers. DARLINGTON and MOFFETT (1930) conclude that the 17-chromosome *Pyrus* belong to a 7-series and have two sets plus one partial set of seven chromosomes. They base this assumption upon the presence of hexasomes and other polysomic associations observed in the types investigated. Their figures afford no conclusive evidence about hexasomes and tetrasomes in the pure species as *Pyrus floribunda* and *P. Ringo*, but they do so for the cultivated types, which are very complicated hybrids. Specific or even varietal hybrids, whose parents had normal conjugation of chromosomes, can produce very complicated polysomic associations of their chromosomes. This was proven to be the case with OSTENFELD's *Polemonium* hybrids (J. CLAUSEN 1931 a), and more hybrids described in the present paper show the same. The polysomic association of chromosomes in the cultivated types of *Pyrus* may therefore just as well have arisen secondary through more or less remote crossings between types belonging to the 17-series.

## TAXONOMIC RELATIONSHIP OF SPECIES; SPECIES CONCEPT.

The diagram, fig. 1, of the crossing possibilities of the species subject of this paper shows that *Viola cornuta-orthoceras* and *Viola calcarata* occupy isolated positions in the section; *cornuta* was capable of crossing with *Orphanidis* and *elegantula* only, and *calcarata* likewise with *Battandierii*. Assumed hybrids of *calcarata* with *alpestris* are noted from nature, but these are sterile.

*V. tricolor* and probably also *arvensis* occupy a central position in the *Melanium* section; at least the first named species forms hybrids with almost all groups of species; it was extensively used for crossing, that is true, but one reason for this extensive use was its ability for crossing. The distribution of the two species and their ability for dissemination over large parts of the world suggest that they are not too specialised, as the alpine species of this section are, and this is perhaps the reason why their genic complement harmonizes with almost all species of their section; they act, so to say, as common denominators of the entire section.

*V. Kitaibeliana* seems to be more specialised, but the types forming this species are largely incompatible with each others, although the morphological uniformity no doubt also suggests a certain uniformity as to genes determining the morphological type. But their genic complement must contain other elements causing the incompatibility. It is paradoxal, indeed, that one member of this collective species, viz. *nana*, is much more compatible with a morphologically very different species as *Viola tricolor* than the types belonging to the collective species *V. Kitaibeliana* are mutually.

*Viola lutea* and *Battandierii* are compatible as shown by their hybrid, and the author suggests that *rothomagensis* belongs in their relationship; the crossing *rothomagensis*  $\times$  *lutea* did not succeed, but the reciprocal one was unfortunately not tried; keeping in mind the difficulty encountered by the crossing *rothomagensis*  $\times$  *arvensis* but not by the reciprocal one, there is a possibility that the cross *lutea*  $\times$  *rothomagensis* would succeed. Spontaneous crossings show *rothomagensis* to be very compatible with *Battandierii*.

The position of *Battandierii* has been somewhat unclear. BECKER (1925) draws it to *Calcarate*, but its possibilities for crossings with *tricolor* and *arvensis* (KRISTOFFERSON 1923), with *rothomagensis* and with *lutea* place it among the *Tricolores*, because *calcarata* itself is not compatible with these species. The correction as to its chromosome

number in the present paper also stresses its position in the neighbourhood of *lutea* rather than near *calcarata*. But of the *Tricolores* it is the species, which shows the closest relationship to *calcarata*.

It becomes more and more difficult to maintain a criterium for specific difference and specific relationship. The old claim, morphological difference combined with more or less intersterility between specific different types, is not answered to by nature in all cases. Groups of types exist, which on practical grounds by taxonomists must be kept into one species (e. g. *Kitaibeliana*—*nana*), but which are incompatible by crossing, giving almost sterile hybrids, while one of the types in this collective species (viz. *nana*) is fully fertile with a morphologically very different species, having a widely different chromosome number. FEDERLEY (1928) observed in the generic hybrid *Metopsilus porcellus*  $\times$  *Charocampa elpenor* complete conjugation of the chromosomes combined with full fertility and Mendelian segregation in  $F_2$ , while the specific hybrid *Pygara curtula*  $\times$  *P. pigra* was sterile and did not show any conjugation of chromosomes. This shows that a classification based upon morphological difference alone may often need a correction based upon a cyto-genetic study.

The chromosome number itself often is a support in the botanical taxonomy and classification, but in some cases it fails entirely. The morphological appearance of the species does not depend upon the chromosome number itself. The highly different types belonging to *tricolor*—*alpestris* (fig. 5, the two right flowers in the upper row and the entire middle row) all have 13 chromosomes, while the uniformly appearing types of *arvensis*—*Kitaibeliana*—*nana* (fig. 5, lower row) have widely different numbers of chromosomes, viz. 17, 7, 8, 18 and 24. It is the content of genes, not the manner in which these genes are aggregated, which determines the morphological type. The homology of chromosomes may in some cases be a help in classification, but as previously shown this is not either an unfailing one, when secondary interchange between primarily non-homologous chromosomes must be supposed to have taken place.

It will be impossible to give any definition on the conception of species covering all cases, as attempted by several authors (DU RIETZ 1930, TURESSON 1929, partially also HERIBERT NILSSON 1930). Nature is infinitely much richer than our termini and classifications allow, and the points of view advocated by BABCOCK (1930) without any attempt at a definition of the species seem to the present author to be most in accordance with the conditions shown by nature itself. It might be

convenient and also correct to maintain a merely theoretical conception of species together with one for the practical taxonomy based upon easily observable and safe morphological characters. According to the theoretical conception of species the different chromosomal types of *Kitaibeliana* should be treated as specific different, while the practical taxonomy should treat them as one species. The chromosomal types need no specific names of their own, but may in the present case be named *V. Kitaibeliana* with the chromosome number added just as practised in the present paper. But the *delimitation* and *classification* of species acknowledged in practical taxonomy, as a matter of course have to be established on investigations starting from as many points as possible in order to arrive at the most natural classification and delimitation; such kinds of investigations have to cover variation in field and its relation to ecological conditions and geographical distribution, chromosomal morphology and complement, crossing possibilities and fertility of hybrids, degree and nature of chromosomal conjugation in hybrids and genic accordance.

The *Melanium* violets afford a very good illustration to the difficulties encountering any definition of species concept. We cannot even use phylogenetic principles, for the species may have arisen from crossings similar to those described and shown diagrammatically in fig. 1, and they have probably collected their genes from very different sources. Phylogeny in the *Melanium* section shall probably not be illustrated in the manner of a branching tree, but sooner as an intricate network.

MÜNTZING's very interesting synthetisation of *Galeopsis Tetrahit* from the *pubescens*  $\times$  *speciosa* cross (MÜNTZING 1930) strikingly shows how prudent we have to be as regards phylogenetic relationship. The artificial *Tetrahit* arose from a group, which should be supposed to be a phylogenetic different one, as its two parent species are neither capable of crossing with spontaneous *Tetrahit* nor with the artificial one, while artificial *Tetrahit* crosses with as well the spontaneous *Tetrahit* as its near relative *bifida*; in its chromosome number ( $n = 16$ ) the artificial *Tetrahit* corresponds with the two latter and not with its own parents ( $n = 8$ ). Crossing possibilities do not necessarily imply phylogenetic relationship but probably only a certain kind of genic affinity; the morphological relationship dealt with in taxonomy is probably for the main part contingent upon genic conformity, not always on a joint phylogenetic development.

The *Melanium* section and in first line the *Tricolores* subsection

gives the impression of being a young group still in full development, as its species are not yet delimited by the boundaries of intersterility.

The present paper in itself has the character of being a summary of the investigations. A real summary implying all the facts will, therefore, not be given, but the results of the individual crossings are summarized in tables 1 and 2 (pp. 224—227). Apart from the main conclusions drawn on the foregoing pages some concrete and general results will here briefly be abstracted:

(1). *Viola diffusa* is most naturally classified in the *Melanium* section, not as before in the *Nomimum* section (p. 235).

(2). The Botanic Garden type of *V. Battandierii* shows an oscillating number of chromosomes,  $n = \text{abt. } 26-30$  (p. 233).

(3). The chromosome number in itself does not determine the morphological type; dominance is contingent upon the action of the genes, not upon the chromosome numbers of the parents (p. 236).

(4). Almost all the *Melanium* hybrids show variable conjugation of their chromosomes. Many of them show polysomic association of the chromosomes, although the parents have normal disomic conjugation.

(5). The species of the *Melanium* section constitute a complete series of transitions as regards intersterility, morphological differences and conjugation of chromosomes in their hybrids. It is therefore impossible to draw any sharp line between differences of specific and of subspecific order (instances: Cross 4 and Cross 9).

(6). Types belonging to one and the same species, morphologically spoken, show the heaviest degree of intersterility in any *Melanium* hybrid (Cross 19, p. 259 and 287).

(7). There is no absolute coincidence between the degree of sterility and the amount of chromosomal conjugation.

(8). The specific significant differences are determined by Mendelian genes, but due to chromosomal irregularities they do not always segregate in regular Mendelian ratios. Even characters distinguishing taxonomically larger groups segregate.

(9). The wild growing species are characterised by a very intricate and complicated cooperation of genes (see for instance the inheritance of flower colours, pp. 239—242, table 4).

(10). The genetical analysis shows some accordance between *alpestris* and *arvensis* (Cross 8, p. 276).

(11). There is good accordance between the degree of polyploidy of the *Melanium* species and the degree of polymery (number of multi-

valent genes), which they show as to the basal genes for anthocyanin, the *A*-genes (table 13, p. 292—293).

(12). Chromosomes from species, which do not cross directly, may be brought together through quadruple hybridisation (Cross 24, p. 288).

(13). In some hybrids the later generations tend to increase the chromosome number above the number of  $F_1$  (often connected with doubling of some chromosomes); other hybrids tend to decrease the chromosome number as compared with  $F_1$  (elimination of single chromosomes).

(14). New constant, very fertile and vigorous species can be isolated in the offspring of the specific hybrids. The following types were named or mentioned: *V. phæno-elegantula* (p. 265), *V. crassicaulis* (p. 270), *V. hyperchromatica* (p. 277), *V. petaloidea* and *V. velutina* (p. 279). They seem to be just as characteristic as many wild growing species and show similar peculiarities. The species applied for the present crossings grow together in the same areas. Especially in the area of the Balcan Peninsula may such new species be formed.

(15). Single genes can be introduced from one species into another, irrespective of the difference in chromosome number. Thus was the gene *D* for dilute mauve flower colour introduced from *arvensis* into a 13-chromosome *tricolor* (p. 276—277, Cross 9).

(16). Crossing favours »segmental interchange» between non-homologous chromosomes through later generations, resulting in multi-valent association of the chromosomes into long chains (p. 271, figs. 119, 121—122).

(17). In  $F_3$  of Cross 7 was a plant segregated, which deviated from what is normal in *Viola* as to the shape of chromosomes in meiosis. The chromosomes were long as in certain *Compositæ* and *Gramineæ* (figs. 123—126, p. 272). An  $F_2$  plant of Cross 13 suggested an aggregation of two chromosomes into one (figs. 136—138, p. 281).

#### LITERATURE CITED.

1. AASE, HANNAH C. 1930. Cytology of *Triticum*, *Secale*, and *Aegilops* hybrids with reference to phylogeny. — Research Studies, State Coll. of Washington, 2, pp. 5—60.
2. BABCOCK, E. B. 1931. Cyto-genetics and the species-concept. — Am. Nat. 65, pp. 5—18.
3. BEADLE, G. W. 1930. Genetical and cytological studies of Mendelian asynapsis in *Zea Mays*. — Cornell Univ. Agric. Exper. Stat., Mem. 129, pp. 1—23.



4. BECKER, W. 1905. Die systematische Behandlung der Formenkreise der *Viola calcarata* und *lutea* (im weitesten Sinne genommen) auf Grundlage ihrer Entwicklungsgeschichte. — Beih. z. Bot. Centralbl. 18, Abt. 2, pp. 347—393.
5. — 1923. *Viola* Asiaticæ et Australenses. V., Gruppe *Melanium* GING. — Beih. z. Bot. Centralbl. 40, Abt. 2, pp. 69—102.
6. — 1925 a. *Viola* in ENGLER und PRANTL: Die natürl. Pflanzenfam. 21, pp. 363—376.
7. — 1925 b. *Viola pseudo-Munbyana* spec. nov. patriae ignotae. — Repert. spec. nov. regn. vegetab. 22, pp. 23—24.
8. BLEIER, H. 1930. Cytologie von Art- und Gattungsbastarden des Getreides. — Der Züchter, 2. Jahrg., pp. 12—22.
9. CLAUSEN, J. 1921. Studies on the collective species *Viola tricolor* L. (Preliminary notes). — Bot. Tidsskr. 37, pp. 205—221.
10. — 1922. Studies on the collective species *Viola tricolor* L. II. — Bot. Tidsskr. 37, pp. 363—416.
11. — 1926. Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* MURR. — Hereditas VIII, pp. 1—156.
12. — 1927 a. Non-Mendelian inheritance in *Viola*. — Hereditas IX (Festschrift für W. JOHANNSEN 19<sup>3</sup>/<sub>2</sub>27), pp. 245—256.
13. — 1927 b. Chromosome number and the relationship of species in the genus *Viola*. — Ann. of Bot. 41, pp. 677—714.
14. — 1929. Chromosome number and relationship of some North American species of *Viola*. — Ann. of Bot. 43, pp. 741—764.
15. — 1930 a. Inheritance of variegation and of black flower colour in *Viola tricolor* L. — Hereditas XIII, pp. 342—356.
16. — 1930 b. Male sterility in *Viola Orphanidis*. — Hereditas XIV, pp. 53—72.
17. — 1931 a. Genetic studies in *Polemonium*. III. Preliminary account on the cytology of species and specific hybrids. — Hereditas XV, pp. 62—66.
18. — 1931 b. *Viola canina* L., a cytologically irregular species. — Hereditas XV, pp. 67—88.
19. CLAUSEN, R. E. 1928. Interspecific hybridization in *Nicotiana*. VII. The cytology of hybrids of the synthetic species, *digluta*, with its parents, *glutinosa* and *tabacum*. — Univ. of Calif. Publ. in Botany, 11, pp. 177—211.
20. DARLINGTON, C. D. and MOFFETT, A. A. 1930. Primary and secondary chromosome balance in *Pyrus*. — Journ. Gen. 22, pp. 129—163.
21. EMERSON, R. A. 1929. Genetic notes on hybrids of perennial Teosinte and Maize. — Amer. Nat. 63, pp. 290—300.
22. FEDERLEY, H. 1928. Chromosomenverhältnisse bei Mischlingen. — Verh. V. int. Kongr. f. Vererbungswissenschaft, Berlin 1927. Suppl.-bd. I der Zeitschr. f. ind. Abst.- u. Vererb.-lehre, pp. 194—222.
23. GOODSPEED, T. H. and AVERY, PRISCILLA. 1930. Nature and significance of structural chromosome alterations induced by X-rays and radium. — Cytologia 1, pp. 308—327.
24. HAYEK, A. v. 1917. Beitrag zur Kenntnis der Flora des Albanisch-montenegrinischen Grenzgebietes. — Denkschr. Kaiserl. Akad. Wiss., Wien; mathem.-naturwiss. Klasse, 94, pp. 1—84.
25. JØRGENSEN, C. A. 1927. The experimental formation of heteroploid plants in the genus *Solanum*. — Journ. Gen. 19, pp. 1—79.

26. KATTERMANN, G. 1931. Über die Bildung polyvalenter Chromosomenverbände bei einiger Gramineen. — *Planta* 12, pp. 732—774.
27. KOSTOFF, D. 1930. Ontogeny, genetics, and cytology of *Nicotiana* hybrids. — *Genetica* 12, pp. 33—118.
28. KRISTOFFERSON, K. B. 1914. Über Bastarde zwischen elementaren Species der *Viola tricolor* und *V. arvensis*. — *Bot. Notiser för år 1914*, pp. 25—31.
29. — 1916. Om nedärvning av herkogami och autogami hos *Viola*. — *Bot. Notiser för år 1916*, pp. 113—120.
30. — 1923. Crossings in *Melanium*-Violets. — *Hereditas* IV, pp. 251—289.
31. LESLEY, MARGARET MANN and FROST, HOWARD B. 1927. Mendelian inheritance of chromosome shape in *Matthiola*. — *Genetics* 12, pp. 449—460.
32. MIYAJI, Y. 1929. Studien über die Zahlenverhältnisse der Chromosomen bei der Gattung *Viola*. — *Cytologia* 1, pp. 28—58.
33. MÜNTZING, A. 1930. Über Chromosomenvermehrung in *Galeopsis*-Kreuzungen und ihre phylogenetische Bedeutung. — *Hereditas* XIV, pp. 153—172.
34. NILSSON, N. HERIBERT. 1930. Synthetische Bastardierungsversuche in der Gattung *Salix*. — *Lunds Universitets Årsskrift*, N. F. Avd. 2. Bd. 27. Nr. 4, pp. 1—97.
35. DU RIETZ, G. E. 1930. The fundamental units of biological taxonomy. — *Sv. Bot. Tidskr.* 24, pp. 333—428.
36. THOMPSON, W. P. 1930 a. Shrivelled endosperm in species crosses in wheat, its cytological causes and genetical effects. — *Genetics* 15, pp. 99—113.
37. — 1930 b. Causes of differences in success of reciprocal interspecific crosses. — *Amer. Nat.* 64, pp. 407—421.
38. TURESSON, G. 1929. Zur Natur und Begrenzung der Arteinheiten. — *Hereditas* XII, pp. 323—334.
39. WATKINS, A. E. 1930. The wheat species: a critique. — *Journ. Gen.* 23, pp. 173—263.
40. WINGE, Ö. 1920. Über die Vererbung der Haarfarbe der Pferde. — *Zeitschr. f. ind. Abst.- u. Vererb.-lehre*, 24, pp. 1—32.
41. WITTROCK, V. B. 1896. *Viola* Studier. II. — *Acta Horti Bergiani* 2, No. 7, pp. 1—78.
42. — 1897. *Viola* Studier. — *Acta Horti Bergiani* 2, No. 1, pp. 1—142.

## CONTENTS.

	Page
I. Introduction .....	219
II. Specific material with a survey on hybridisations .....	222
III. Morphological notes on $F_1$ . Description of genic cooperation .....	234
IV. Notes on cytology and fertility of $F_1$ .....	243
V. Types of segregation in $F_2$ and later generations .....	261
VI. Segregation of some characters .....	292
VII. Conclusions and summary .....	298
Diagram of crossings, fig. 1 .....	222
List of hybridisations, tables 1 and 2 .....	224
List of gene symbols for flower colours, table 4 .....	242