PRESLIA Věstník Československé Botanické Společnosti v Praze Bulletin de la Société Botanique Tchécoslovaque à Prague VOL. XI., 1932.

Dr. A. BROŽEK:

Mendelian analysis of the "red-orange-yellow" group of flower-colours in Mimulus cardinalis hort.

Institute of Plant-Physiology, Charles University, Department of Genetics, Prague, Czechoslovakia.

There is an interesting fact, both from the theoretical and the horticultural point of view, that the number of various flower-colours in recently cultivated garden populations of *Mimulus cardinalis* Hort., as we find them for instance at the present time in VILMORIN-ANDRIEUX and Co.'s establishments in Verrières-Le-Buisson near Paris, was highly increased in contradistinction to the number of colours which was known some 30 years ago (in about 1894) in "old" cultures of this plant (1). Really the modern garden populations of *Mimulus cardinalis* Hort. show a most remarkable range of colours, running from nearly white ones with a delicate touch of pink and yellow to different pinks, magentas and red-magentas in one direction, and to pale yellow, canary yellow, orange, scarlet and red colours in another direction, whereas the "old" cultures show plants with various red, orange and yellow blossoms, or more precisely, according to VILMORIN's own French terminology, plants with "rouge pourpre", "coccciné", "orange" and "jaune" flowers. According to horticulturists' experiences (1, 2) and even according to our own experience and our recent genetical analysis (3, 4, 5) of flower-colours in *Mimulus cardinalis* Hort., there is no doubt that this great variability of flower-colours with the increased number of colours in modern cultures are facts, which can be readily explained only through hybridization, if the different colours mentioned above are characters which are relatively constant and can be only slightly modified through external, environmental influences. There is now no doubt that they can be explained as results of very complicated processes of hybridization between various garden types, most of them of hybrid origin or directly hybrids, but according to GRANT's (2, pg. 347) suppositions in some way related to wild *cardinalis* and *Lewisii* species from the section *Erythranthe* and *Paradanthus*, or, according to our suppositions, as results of recombination processes of two main pairs of genes, introduced from yellow and magenta parental races — these races having originated as *gene mutations* in pure red plants of the *cardinalis* species — into the hybrid constitution of double hybrid types with yellowish pink blossoms (3, 4, 5). Both these theories are indeed admissible, also GRANT's theory accounts for possibility of crossings between different *species* in contradistinction to our theory, which accounts for crossings of two *varieties* of the same plant species and therefore for crossings of two plants with the same number of chromosomes genetically related. GRANT's theory therefore seems to be of less advantage.

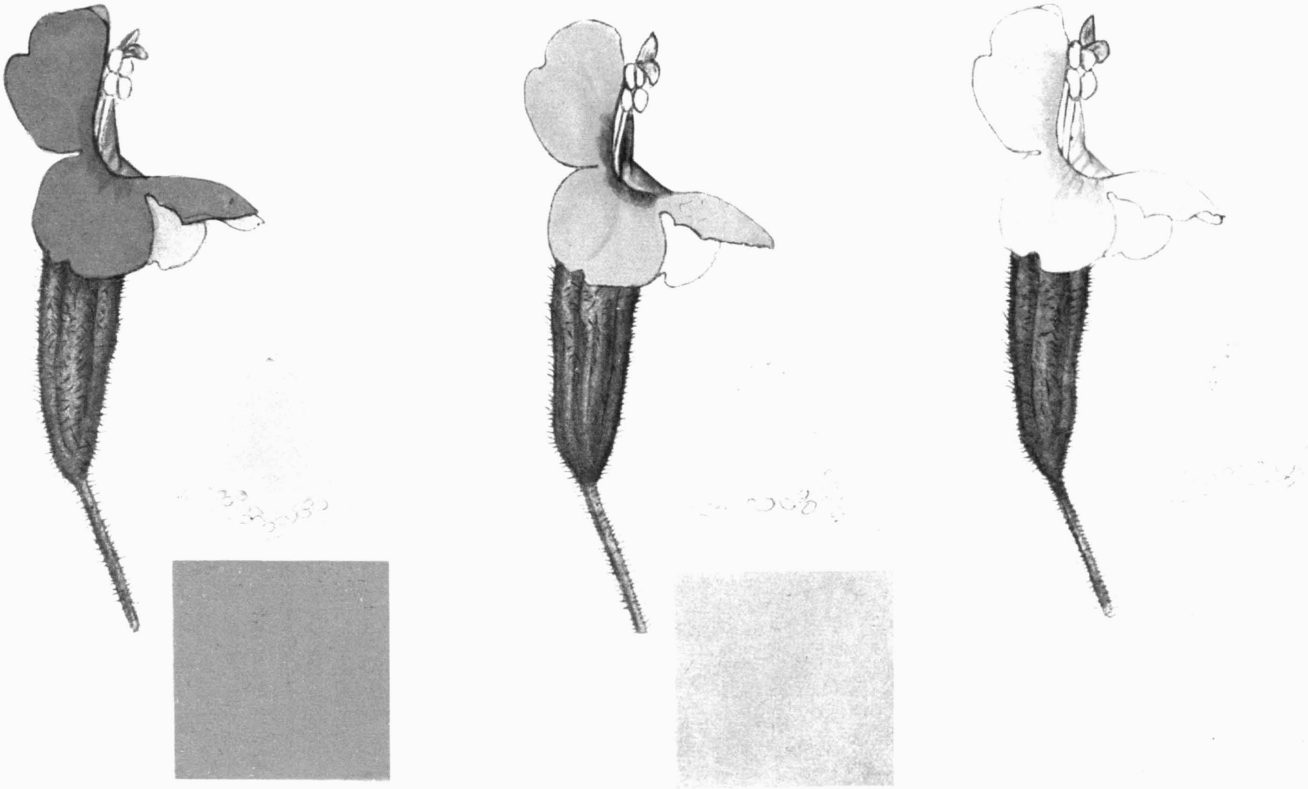
But in any case the variability of colours in itself as well as the increase of same in modern *Mimulus* cultures are more complicated phenomena than the colour variability in "old" populations of this species, if the "red-orange-yellow" group of colours be common both for wild *cardinalis* types, spread from Utah and Arizona, west to Oregon and Lower California (2, pg. 139) as well as for "old" horticultural *cardinalis* types, as they were cultivated by VILMORIN (1, pg. 638, 639). According to VILMORIN we know for instance that these cultures of *cardinalis* horticultural types were derived by selection of different plants of open garden populations and originated by crossings and recrossings of various garden types, but especially (according to VILMORIN) such as *M. cardinalis Hudsoni* Hort., with brilliant scarlet and red flowers ("rouge cramoisi clair"), *M. cardinalis aurantiacus* Hort. with orange flowers ("orange"), *M. cardinalis atrosanguineus* Hort. with colour of blood ("coleur de sang") and *M. cardinalis maculatus* Hort.; and further that also these parental types were hybrids, so that it was impossible by selection of single plants with blossoms of certain colour (and probably by open pollination) to rear, by means of sexual reproduction and seed selection, different pure strains of colours, because such selected plants gave in their particular progenies not only types similar to themselves, but also types with other colours in various and inconstant ratios. A constant progeny with the same colour of flowers as in selected plant could therefore only be secured in succeeding generations by asexual reproduction of selected plants from cuttings, and especially cuttings from shoots of rootstocks, or, in short, through cultivation in clones.*) This indicates clearly that the "old" VILMORIN types of *cardinalis* horticultural varieties were more or less of hybrid constitution and in open cultures and by means of free pollination could therefore only give inconstant progenies, even by repeated selection of certain types. We hope that the present investigation will throw more light upon these facts and bring some evidences that the "red-orange-yellow" group of colours in *cardinalis* horticultural varieties and most probably too in wild ones (2, pg. 139) is brought about by one main pair of Mendelian genes only, of which one gene (R) influences the development of a bluish red anthocyan in the cell sap of the petals and the other (r) again the non-development of the same pigment. Along these lines we have experimentally proved that the scarlet types are always homozygous (RR) in respect to bluish red or magenta cell sap, the yellows homozygous again, but for colourless cell sap (rr), whereas the oranges are always hybrids (Rr), and, in connection with this hybrid constitution, there are types with less intensively coloured (i. e. pale) magenta cell sap. Indeed the more or less coloured or colourless cell sap is not the only cause which influences the resulting colour in flowers of different *cardinalis* varieties, for we proved by microscopical examination and by breeding experiments that the resulting colour in different *cardinalis* varieties depends too on coloured or colourless plastids or chromoplasts, according as these small bodies are coloured or not by means of a yellow carotinoid. Consequently the development or non-development of this yellow pigment points to action of the respective genes, of which one (C) governs the development and the other (c) the non-development of this pigment. In conformity with these suppositions we really found in all three *cardinalis* varieties, in scarlets, oranges and yellows, that their plastids were always deeply yellow and that therefore the plants could be

*) „Quelques soins que l'on présume pour récolter séparément les graines de ces variétés ou des autres, qui d'ordinaire ne manquent pas de se produire dans la culture le semis ne les reproduit qu'en partie, en sorte qu'il faudra recourir au bouturage pour celles qu'on voudra perpétuer. Les boutures se font sous cloches, a la fin de l'été, en pôts ou en terrines que l'on hiverne sous châssis; ou bien encore au printemps, sur couche et sous cloches, au moyen de jeunes rameaux pris sur des plantes conservées sous verre." (*1.* I. Vol. pg. 638.)

estimated as homozygous types (CC) in respect to this character of having yellow plastids, and that furthermore the scarlet, orange and yellow colours in their flowers were in fact the result due to a yellow background (formed by yellow plastids) permeating through magenta, pale magenta and colourless cell sap, acting as different screens respectively. (See Plate I.) Consequently the scarlet-homozygous plants of *cardinalis* horticultural varieties are of *RRCC* constitution, the yellow-homozygous plants of *rrCC*, and the orange-hybrids of *RrCC*, constitution. The orange types as simple intermediate hybrids could therefore not be reared in subsequent generations through autogamy or crossing between themselves as pure strains, strains constant in their orange flower-colour, because their colour is based on interaction of two genes of the same pair of allelomorphs (*R* and *r*). Therefore we always got out of them, through autogamy or intercrossing between themselves, progenies which were composed of scarlet-homozygous, orange-hybrids and yellow-homozygous plants in 1:2:1 ratio. It is of interest that the orange *cardinalis* types in the "red-orange-yellow" group of colours present to us in horticultural history the same feature as the "blue andalusians" in poultry breeding. Of course the orange *cardinalis* plants can be easily reared in uniform progenies only by means of cuttings or in clones, as was the old experience of horticulturists, mentioned above. In our case the genes for development of yellow carotinoid could be supposed without any doubt, because we succeeded in isolating homozygous races with colourless (*RRcc*) plastids and consequently with dull magenta flowers in accordance with their magenta coloured cell sap. But the question of the inheritance of colours in the "red-orange-yellow" group of colours in *cardinalis* species is not such a simple matter as would be thought at first sight, because there are some characters, also inherited, which, by their development, may more or less strongly change the three main colours just mentioned, but especially the yellow and orange colours. First we must point out the reddish magenta tint, which is spread around the corolla-tube over the upper surface of the petals. This tint may extend considerably, mainly on the surface of the two upper petals of the upper corolla-lip, from their bases up to their middle parts. This character is inherited without any doubt and can therefore be developed in different degrees, with more or less intensity, and change more or less the yellow flowers into oranges and oranges into yellows, phenotypically indeed, so that it is sometimes very difficult to distinguish precisely at first sight between these two types of flowers. On the other hand there is not such trouble with scarlet types, in distinguishing them from oranges and yellows, because in them this tint, if present, dissapears for direct observation. Of less importance in this direction is the development of a dark purple spot, spread around the bases of the petals. This character or so called "star" is again undoubtedly inherited. It seems to be alternately inherited with a system of dark magenta purple stripes, running down and parallel with the corolla-tube, on its inner surface.

Material and methods.

Mimulus cardinalis Hort. was brought to our culture from VILMORIN's garden establishments at Verrières-Le-Buisson near Paris, France, in 1925. Since then plants have been reared in our experimental garden at the Plant Physiological Institute, Charles University, Prague, Czechoslovakia, and have shown a relatively very small variability in respect to vegetative parts, but an extraordinarily large variability in flower-colours. Nevertheless it is of importance to know other main characters. *Mimulus cardinalis* Hort. is a perennial plant, villous on main stems, lateral branches, leaves, pedicels and calyces. (See fig. 1.) In all these



parts it is slightly viscid and consequently has a faint musk smell. If cultivated in small pots, it reaches a height of about 20 to 50 *cm*, has only few lateral branches from its lower parts of main stem, but develops into a strong plant if cultivated in free rich garden soil. It has then many lateral branches, first somewhat procumbent, but afterwards erect and reaches a height of about 50 to 80 *cm*. Towards the end of the season we see that the plant develops at its base many small shoots from rootstocks by means of which it may easily be preserved for next season or propagated by cuttings. At the end of the season it must be kept in sheltered places and protected against frost and cold. Leaves are obovate, 2 to 8 *cm* long and 1 to 3 *cm* wide, auriculate and sessile, 3 to 5 nerved from the bases of their blades, in pairs, opposite and simple. Their edges are irregularly serrate, so that they look, as if bitten out on their edges. Pedicels are as long as the leaves, sometimes a little longer. Calyces are tubular, about 2—3 *cm* long. They are dotted with red brown or dark purple. The ribs are more or less also coloured with red brown or purple. They terminate in teeth 4 to 5 *mm* long, equal, broadly ovate and acute. This coloration of calyces is sometimes very strongly developed and spreads over the whole calyx except the teeth. But most often this coloration is of less intensity and spreads only over the middle parts and bases of the calyx, in such cases being again often more developed in upper parts of the calyx. In other cases we find again the calyces entirely green. But these cases are again relatively rare. We suppose therefore that these different coloration grades are inherited, but in a way unknown up to the present time. The corolla is strongly bilabiate, about 4 *cm* long and 2½ to 3 *cm* wide. The upper lip is divided into two erect lobes, which are more or less turned back. The lower lip is more flat, or, in older blossoms has also lobes more or less turned back. It is divided into three lobes (petals), two of them are lateral and one is central. The latter is somewhat convex, the two lateral ones more or less flat. The margins of the lobes (or petals) are practically always entire, though even we found in some cases that they were slightly notched. But they always have in the middle of their margin a small incision. At the sinuses we always find cylindrical, silky, small and unicellular, somewhat pointed hairs. Otherwise the margins of the petals is hairless. Furthermore we see another type of hairs running in two broad rows, parallel with and down through the corolla-tube on its lower surface. These hairs are about twice as long and thick as those in the sinuses and are blunt at the tips. There is a peculiar fact that the cell sap of these two kinds of hairs is always colourless even in races where all epidermal cells have coloured cell sap. Therefore the hairs are always coloured only according to their chromoplasts. The tube of the corolla is slightly exerted from the calyx and slightly contracted at its base up to the point where the filaments are inserted. As is usual in the Scrophulariaceae family, there are two middle filaments longer than two outer ones, but both no longer than the style, so that their anthers are placed close under the lips of the stigma and a little sidewise. The anthers are villous with silky, flat, unicellular, acuminate and white hairs. They open by means of a longitudinal split and then contain pollen grains of sulphur-yellow colour. The style terminates in a bilabiate stigma. The stigma lips are oblong, ciliate at their edges and sensitive. The upper lip is plain and erect, or slightly turned up, the lower one more or less strongly turned back and down. The stigma-lips are pale green, whereas the style is nearly white. The lips in open blossoms are widely open, but in buds they are tightly closed together and turned downwards. After having been irritated by some mechanical impulse, the lips close together in a relatively short time, in 2 to 5 seconds. Then the stigma remains closed for about 10 to 30 minutes, but afterwards it opens again. These movements take place without res-

pect to the stigma's having been pollinated or not during the time of irritation. The style and the anthers are protected by the upper lip of the corolla, even if the anthers and the stigma are relatively much exerted from the tube. The closed stigma in buds secures of course an easy castration. The same is with the anthers. These, in buds, are also closed so that they can be readily removed without the danger that the stigma could be contaminated through their pollen. Both the stigma lips and the anthers open at the time when the blossoms spread out their petals. After the pollination, both artificial or natural, the style fades in 2 or 3 days and afterwards becomes brown and dark. Finally it dries and the corolla falls off. On the other hand the calyx remains during this period and in the next ripening period also, and dries slowly, just as pediecls do. When capsules are formed, the calyx becomes erect and remains so till the dry capsules are opened by means of longitudinal splits. Capsules are oblong and acuminate. Seeds are very small, oblong and numerous. Their testa is reticulate, light to dark brown or nearly black. The castration of the buds is then performed in such a way that the calyx is first opened by a sharp scalpel and then the corolla-tube too. This is done by a lateral incision along the tube, beginning at the point where the filaments depart from the wall and ending nearly at the place, where the tube finishes. The stamens are then extracted by means of sharp pincers. This procedure is harmless in respect to development of the corolla itself, and it is easy to control, by a strong lens, whether the stigma really remained free from contamination of pollen during the castration process. In one or two days after the castration has been performed, the stigma can be artificially fertilized. Both after the castration and artificial pollination the flower is enclosed in a muslin cover and left so till the moment when the style is about to become dry and the capsule to develop. After this time of 10 or 14 days the covers can be removed. Also in case of autogamy the same procedure is to be used. The bud is enclosed in a muslin cover before autogamy has been performed, and put into it again afterwards. The transferring of pollen is done in each case by means of a small piece of wood, cut flat and newly prepared, of course, for every fresh experiment. The seeds are then sown in April, seeds form each particular capsule separately in a single pot. In May the germinated seedlings are then transplanted again into pots in an open culture, one plant to one pot. Naturally the plants are then in this culture protected against a too strong sun, rain and wind. Our experiments commenced in 1926 with a selecting of particular plants with certain blossom-colours out of a mixed garden population. Up to 1927 we succeeded, and since that time we have succeeded, — in isolating pure scarlet, yellow and magenta (dull) strains respectively, so that already in 1929 and onwards crossings could be done. The first artificial orange hybrid, i. e. the result of the cross between pure yellow and pure scarlet strain, was secured in 1930.

It must be remembered that the registration of flower colours, of nearly each particular plant of the respective progenies, has been done by means of coloured chalks. In this way we have collected up to the present a considerable number of colour-copies of the respective blossoms, these being a part of our extensive breeding books concerning our plants. Such a collection of many hundreds of colour-samples is of very great advantage for a theoretical and horticultural work, as it is not only the one possible means by which the breeder may see at a glance all individuals of the respective progenies, just cultivated, but also the means by which progenies grown two, three or even more years before can be seen. The comparison of flower-colours of parental plants with those of their progenies is, of course, by this method of registration by means of coloured chalks much facilitated.

Cytology.

We have determined the diploid (= 2n) chromosome number of *Mimulus cardinalis* Hort. as 16. (See fig. 2.) But the chromosomes may be easily found and counted only in cells of tips of roots which were regenerated from the nodes of the stems, and also in roots, which are not more than 1 or 2 *mm* long. The main roots of germinating seeds, reared on moistened blotting paper were found too small for this purpose, because giving relatively a too small number of cells with equatorial plates of chromosomes. The same was with root-tips which could be found in well developed plants, in root-tips adjacent

Fig. 1. *Mimulus cardinalis* Hort. General view of the plant.

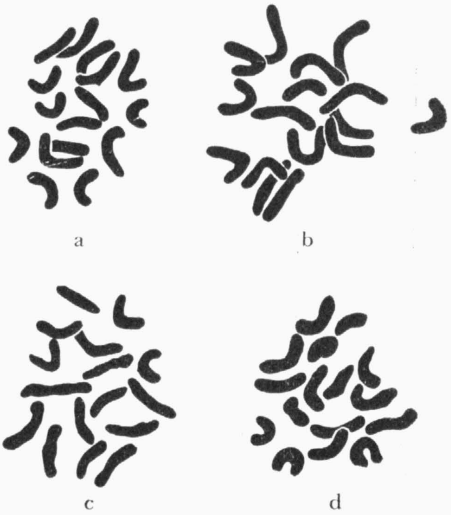


Fig. 2. *Mimulus cardinalis* Hort. Four metaphases plates of chromosomes in cells of root-tips. Each plate contains a diploid number of 16 chromosomes. Two of the plates of chromosomes (*a* and *b*) are taken from the cells of dermatogen of one and the same section, the other two (*c* and *d*) from cells of periblem, but out of roots of two different plants. $-^{1}/_{12}$ *mm* Reichert Apoch. Oil.-Immers; Zeiss Comp. Ocul. × 30.

to the wall of the pot in which the plant was grown. Also such old roots are too thin and therefore give no suitable material. These adventitious roots were cut off and killed immediately by a NAVASHINE fixative solution (i. e. 4 *ccm* commerc. formaline, 1 *ccm* conc. acetic acid, 15 *ccm* of 1% chrom. acid, time: 24 hours). The microscopical slides were then stained in a HEIDENHAIN iron haematoxylin, or also very successfully (7) by means of a modified method according to R. Y. CAJAL (i. e. 3 minutes in conc. magenta, 1 to 2 minutes in running water, 10 to 15 minutes in conc. picro-indigo-carmine, 5 to 10 seconds in water with a mere trace of conc. acetic acid in it; differentiation by means of 80% alcohol for a very short time). We found the chromosomes, stained by means of magenta, with a shining scarlet purple colour and remaining transparent, which was of advantage especially in cases where the chromosomes were too densely accumulated and had to be followed and counted under such circumstances. Also

a green screen, if desirable, may be used successfully, when the magenta staining method has been used. The cytoplasm remains slightly pinkish blue, whereas the nucleoli in resting nuclei are slightly sky-blue. Furthermore we must remember that these splendid colours can be seen on slides, if only a strong electric lamp and a weak blue screen are used. Otherwise by means of haematoxylin the chromosomes are, as usual, opaque. The chromosomes are undoubtedly of different size, although the size differences are not so clear, because the chromosomes are, compared with those of other plants, very small. Nevertheless they are considerably larger than, for instance, are those in garden varieties of the *Mimulus* with diploid number of about 64 chromosomes (as is the case in varieties of *M. quinquevulnerus*, *tigrinus* and *tigrinoides*, Hort.). Therefore the size and shape differences in them are not quite clear. Nevertheless most of them have the

Table I.

Year	Yellow lines	Scarlet lines
1929	No. 1: 3a, 12a, (1—24) No. 2: $13a_1$, 3b, (1—10)	No. 3: 3a, 11a, (1—30)
1930	No. 4: 3a, 5a, 9a, (1—10)	No. 5: 3a, 11a, 17b, (1—10)
1931	No. 6: 3a, 5a, 9a, 5a, (1—10) No. 7: 3a, 5a, 9a, 1a, (1—10)	No. 8: 3a, 11a, 17b, 3a, (1—10) No. 9: 22a, 3b, 8b, 5b, (1—10)

shape of a flat *U* and seem to have two almost equally long arms. Often we find the chromosomes, even if they are arranged in equatorial plates, in various positions, rarely in pairs, and often more or less irregularly contorted. This is seen, for instance, on four (a—d) metaphases plates in cells of root-tips, two of which (a and b) are taken from the cells of dermatogen of the same section of the root, whereas the other two (c and d) are again from cells of periblem, but out of different plants. The drawings were made at table level by means of camera lucida at $^1/_{12}$ *mm* REICHERT apochromatic oil immersion and ZEISS comp. ocular, × 30. The chromosomes in meiosis have not been found up to the present. The two main pigments, on which the colour of the petals depends is a red-purple anthocyan and a yellow carotinoid. Whereas the first is dissolved in the cell sap, the latter is contained again in the plastids (chromoplasts). These small oval bodies are always to be found in the cytoplasm of the cell. In epidermal cells of the upper surface of the petals we find them accumulated only on the bottom of the cells, whereas the upper part of the cells we see filled up with coloured (or colourless) cell sap. (See coloured Plate I.) Just the same condition we find in epidermal cells of the lower surface. But here the cells are slightly convex in contradistinction to those of the upper surface, which are conical. In cells of the mesophyll of the petals the plastids are scattered irregularly. Probably the slightly convex shape of the lower epidermal cells and consequently a not so great layer of cell sap, as is the case in conical cells of the upper surface, causes the colour of the petals on their lower surface to be not so bright as it is on the upper surface.

Experiments.

Among the many different yellow, scarlet and magenta pure lines of *Mimulus cardinalis* Hort. cultivated in our experimental garden up to the present

Table II.

Year	F_1-generations	Type of crossing	Type and number of plants observed
1930	No. 10: (3a, 11a, 17e × $13a_1$, 3b, 1g), 1—10	scarlet × yellow	9 orange
	No. 11: (3a, 12a, 1e × 3a, 11a, 17e), 1—10	yellow × scarlet	8 orange
1931	No. 12: (3a, 5a, 9a, 5b × 3a, 11a, 17b, 3a), 1—10	yellow × scarlet	8 orange
1932	No. 13: (22a, 3b, 8b, 5b, 5d × 3a, 5a, 9a, 1a, 1e), 1—17	scarlet × yellow	16 orange
	No. 14: (3a, 5a, 9a, 1a, 1d × 3a, 11a, 17b, 3a, 4b), 1—22	yellow × scarlet	21 orange

time, there are only 5 yellow and 4 scarlet lines, reared in 1929, 1930 and 1931, of special importance in connection with the genetics of flower-colours in the "red-orange-yellow" group. (See the list of lines on Table I.) All these lines, just as the others, were derived from plants selected from a mixed population grown in 1926; but autogamy was performed on their blossoms first in the next year 1927. All these lines originate from two hybrid plants (3 and 13) with orange blossoms, except one line only (the line No. 9), the mother plant of which (22) was a pure plant in respect to its red flower-colour. The relationship of particular scarlet and yellow lines can be easily found from their respective symbols, as we see them in the Table I., if we only remember that each individual Arabian numeral, contained in them, denotes the particular plant, symbols a, b, . . . denote individual blossoms of the main axis, and symbols a_1, a_2 . . ., or b_1, b_2 . . . and so on, blossoms of the lateral branches; and finally figures in brackets denote

Table III.

Year	F_2-generations	scarlet : orange : yellow ratio	
		observed	calculated[2])
1928	No. 15: 3a, (1—20)	4: 1: 3	(2·00± 3.1·22) : (4·00± 3.1·41) : (2·00± 3.1·22)
1929	No. 16: 3a, 10b, (1—80)	23:43:14	(20·00± 3.3·87) : (40·00± 3.4·47) : (20·00± 3.3·87)
1930	No. 17: 3a, 10b, 4b, (1—10)	3: 6: 1	(2·50± 3.1·37) : (5·00± 3.1·58) : (2·50± 3.1·37)
1931	No. 18: (3a, 11a, 27b× $13a_1$, 3b, 1g), 1b, (1—39)	7:17: 8	(8·00± 3.2·45) : (16·00± 3.2·83) : (8·00± 3.2·45)
	No. 19: (3a, 12a, 1e× 3a, 11a, 17e), 6f, (1—50)	11:26: 6	(10·75± 3.2·84) : (21·50± 3.3·28) : (10·75± 3.2·84)
1932	No. 20: (3a, 5a, 9a, 5b × 3a, 11a, 17b, 3a), 5f, (1—12)	2: 4: 2	(2·00± 3.1·22) : (4·00± 3.1·41) : (2·00 ± 3.1·22)
	No. 21: (3a, 12a, 1e× 3a, 11a, 17e), 6f, 4c, (1—59)	11:22:10	(10·75± 3.2·84) : (21·50± 3.3·28) : (10·75± 3.2·84)

[2]) According to JOHANNSEN (*8;* pg. 508—515).

the number of individuals in a particular progeny. We see for instance in this way that the yellow line No. 1. in 1929, i. e. the line 3a, 12a, (1—24) and the scarlet line No. 3 from the same year, i. e. the line 3a, 11a, (1—30) are "sister lines", because they were derived from two plants (12 and 11) of the same F_2-generation in 1928, this generation being a progeny of an original orange blossoming mother plant (3) of 1926/27. Analogous is the case, for instance, with the yellow lines No. 6 and 7, lines 3a, 5a, 9a, 5a, (1—10) and 3a, 5a, 9a, 1a, (1—10) from 1931, which are again "sister lines" in respect to yellow flowering individuals 5 and 1, both of these being again members of the same pure yellow line in 1930 (see line No. 4.), this again being derived from the same yellow homozygous mother plant 9, in 1929 and from its first blossom (a) on the main stem, — of course, by means of autogamy.

The first artificial orange hybrid *(RrCC)* was secured in 1930 by crossing the scarlet and yellow lines reciprocally. The respective F_1-generations Nos. 10 and 11 contained only plants with orange blossoms, 9 and 8 individuals respectively. (See Table II.) Similarly the next three experiments in 1931 and 1932 proved the same results, whilst the respective F_1-families, Nos. 12, 13 and 14 were composed again only of orange individuals, i. e. of 8, 16 and 21 orange

Table IV.

Year	Back-crossing generations	Type of crossings	Ratio of colour-types	
			observed	calculated[2])
1932	No. 22: [(3a, 5a, 9a× 3a, 11a, 17b), 10d× 3a, 5a, 9a, 5a, 10e], 1—25	orange × yellow.	or. : yel. 9 : 9	(9·00± 3.2·12) : (9·00± 3.2·12)
	No. 23: [(3a, 5a, 9a× 3a, 11a, 17b), 5e× 3a, 11a, 17b, 3a, 3c], 1—35	orange × scarlet.	or.: scl. 15:20	(17·50± 3.2·96) : (17·50± 3.2·96)

plants respectively. Therefore the uniformity in F_1-families in respect to orange flower-colour is clearly proved in all these 5 experiments, or in all, in 62 plants.

On the other hand we found in each F_2-generation types with scarlet, orange and yellow flowers (see Table III.), although there were only 7 F_2-families observed in 1928—1932, all derived by autogamy of blossoms of their respective orange parents. Some of them (see families Nos. 15, 16, 17 and 21) originated in this way from plants selected in the respective families, but some again from orange hybrids (see families Nos. 18, 19 and 20) directly produced through previous crossing-experiments. In all these 7 F_2-families the expected three types of colours appeared in ratios more or less close to the theoretical ratio 1 : 2 : 1. This was due, of course, not only to a small number of individuals in the respective generations observed, but mainly to difficulty in distinguishing the orange types from yellow ones. Nevertheless the theoretical ratio 1 : 2 : 1 of scarlet *(RRCC)*, orange *(RrCC)* and yellow *(rrCC)* types in progenies of orange hybrids *(RrCC)* holds true, and proves the supposition that the orange colour of blossoms is a simple hybrid character, which depends only on genes *(R* and *r)* of one and the same pair of allelomorphs, which then influence the development and non-development of the bluish-red anthocyan. But finally the progenies Nos. 15, 16 and 17 deserve our special attention, because they repeat the same process as was the case described by VILMORIN (1. pg. 638.) many years ago, where he selected orange hybrids in succeeding generations. Also in our experiments in 1927 a plant (3) with orange blossoms was selected, — in this case

[2]) According to JOHANNSEN (*8;* pg. 508—515).

a plant of a mixed garden population —, which afterwards produced in 1928 F_2-family (No. 15), which was composed of plants of three colour types, from which furthermore a new orange plant (10) was again selected, which in the next year, 1929, produced, of course by means of autogamy, the large F_2-generation No. 16. But again from this generation a new individual with orange blossoms (4) was selected, and this individual (4) developed in the next year, 1930, of course again by autogamy of its blossoms, a new F_2-generation (No. 17) with scarlet, orange and yellow plants. The inconstancy of orange colour is by these three successive generations undoubtedly very clearly proved.

Finally we must point to two experiments (see Table IV.), in which two orange hybrids, two "sister plants" (10 and 5) of the same family were crossed with their yellow or scarlet parents respectively. The results of these two experiments — both performed in 1932 — can be readily recognized from table IV, from which we see at once that the result of crossing an orange hybrid *(RrCC)* with a yellow homozygous plant *(rrCC)* was a progeny composed of oranges *(RrCC)* and yellows *(rrCC)* in a ratio 9:9, corresponding to the theoretical ratio 1:1, — and furthermore that the cross between orange hybrid *(RrCC)* and scarlet pure plant *(RRCC)* resulted in a progeny, which was composed of orange hybrids *(RrCC)* and scarlet pure types *(RRCC)* in a ratio 15:20 not far, indeed, from the theoretical expectation 1:1. In both these experiments the orange hybrids were taken as mother plants for breeding processes. Reciprocal experiments, in which orange hybrids would have been used as male parents, were not performed. But there is no doubt that they would also have given the same results. Of course the balance between orange hybrids and scarlet or yellow homozygous types in these two progenies is a fact which points clearly to a segregation of *R* and *r* genes in an orange plant and consequently to two kinds of gametes of *RC* and *rC* constitution, produced in equal numbers.

LITERATURE CITED.

1. VILMORIN-ANDRIEUX et Cie., Les Fleurs de Pleine Terre. I. Partie. Paris. 1894.
2. GRANT, A. L., A Monograph of the Genus Mimulus, Annals of the Missouri Botanical Garden. Vol. II, No. 2—3. 1924.
3. BROŽEK, A., Preliminary Report on the Genetic Constitution of Flower Colours in the Garden Population of the Mimulus cardinalis Hort., Acta Botanica Bohemica, Vol. VIII (1929). Prague.
4. BROŽEK, A., Remarks on the Genetic Constitution of Flower-colours in Mimulus cardinalis Hort., Reports of Proceedings of the Fifth International Botanical Congress, Cambridge, England, 1930.
5. BROŽEK, A., Further Investigations on the Genetics of Flower Colours in Mimulus cardinalis, Hort.; Preslia, Journal of the Czechoslovak Botanical Society, Vol. 10. 1931. Prague. 6.
6. BROŽEK, A., Nauka o dědičnosti (The Theory of Heredity). Rostlinopis. Vol. 5., 1930. Aventinum. Praha.
7. BÖHM, A.—OPPEL, A., Taschenbuch der mikroskopischen Technik. 7th. Ed. 1907. Berlin.
8. JOHANNSEN, W., Elemente der exakten Erblichkeitslehre. 2nd. Ed. 1913. Jena.

EXPLANATION OF PLATE I.

MIMULUS CARDINALIS Hort. A scarlet and yellow race with their orange hybrid (in the middle of the plate). The coloured squares are samples of typical colours of the flowers of the respective plant. Above these squares are drawn epidermal cells of the upper surface of the petals, with magenta, pale magenta and colourless cell sap and yellow chromoplasts. The flowers are nearly twice as large as their original size.

This plate was first published in Vol. 5. of "Rostlinopis" (Botany) and has been reprinted here by permission of the publishing firm *Aventinum*, Praha (Prague), Czechoslovakia.(6).