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Further Investigations on the Genetics of Flower Colours in Mimulus cardinalis, Hort.

The first preliminary reports about the genetics of flower-colours in *Mimulus cardinalis*, Hort. were published by the writer in 1929 and 1930 (see 1 and 2). It was already shown in these papers that the different colours of the petals were dependent on the capacity or non-capacity for development of anthocyan in the cell sap and of xanthophyll in the chloroplasts respectively. It was also proved by breeding tests that the respective genes of these characters were members of 2 different pairs of allelomorphs and that they undoubtedly followed a Mendelian segregation. From this point of view we could suppose that the genes were carried by the chromosomes. Therefore the first part of our new investigations in 1928 till 1931 was devoted to the study of the chromosomal behaviour of this plant species. The detailed results will be published, but elsewhere. In this short commentary we want to merely state that the diploid number of chromosomes in metaphases of the somatic cells has been found to be 16 (=2n) and that the chromosomes in this cardinalis species are relatively small in number and large in their size, in contradistinction to the chromosomes of quinquevulnerus, tigrinus and tigrinoides varieties, in which we worked previously (see 3), where the chromosomes were found to be numerous, about 64 in number (diploid), kidney-shaped and very small-bodied. The chromosomes of the cardinalis species are on the other hand quite simple and broad U-shaped. They can be easily seen in the cells of tips of roots, which were regenerated from stem-cuttings under the base of the leaves. There is no difficulty in counting them after using *Nawaschin*'s fixative and staining them with *Heidenhain*'s iron haematoxylin.

The relatively small number of chromosomes in Mimulus cardinalis is indeed one of the most important and interesting facts, because it is in connection with this that we may expect that the linkage of genes of different pairs of allelomorphs can be, in general, more easily met with in cardinalis species than in any other species with a high number of chromosomes as, for instance, is the case with garden varieties, mentioned above, containing approximately 64 chromosomes. Also the genes for development and non-development of anthocyan may indeed show a linkage with the genes for development and non-development of xanthophyll, or not, and on this basis may be included into the same pair of chromosomes or carried by two different pairs of chromosomes, respectively. Experiments, which will now be referred to, may, as we hope, certainly prove one of both just mentioned possibilities, if they be but worked out on a large scale. Otherwise, as is the case in our investigations up to the present, they may at least afford some ideas about experiments, which may be fittest to solve the above mentioned problems and at the same time secure the greatest accuracy in distinguishing the different colour-types in a given progeny.

But first before we begin with a description of our new experiments, we must recapitulate in short our previous results and especially those which concern the realization of colours in the flowers of each of our three cardinal races. First it is important to mention that it is quite easy to show by means of microscopical examination that the first of our pure parental races, the scarlet race (CCRR), homozygous for development of anthocyan (RR) and xanthophyll (CC), contains in the cells of the petals not only a deep coloured cell sap with a bluish red anthocyan but also deep yellow chloroplasts, coloured again with xanthophyll. On microscopical examination also the second parental race, the yellow one (CCrr), homozygous for development of xanthophyll (CC) and non-development of anthocyan in the cell sap (rr), is seen to contain deep yellow plastids and colourless cell sap. In the same way the third of our main parental races, the dull magenta race (ccRR), homozygous for non-development of xanthophyll (cc), but homozygous for development of anthocyan (RR), has on the other hand colourless plastids and deep coloured sap, as was the case with the scarlet race.

The fourth possible race with colourless plastids and colourless cell sap, probably therefore »white«, the race of ccrr genic constitution, was not in our hands before the year 1928, but it has now been produced by our breeding work and preserved for next investigations. We know already now that this race has not quite white petals, but white flowers with a delicate tint of ivory and rouge. The light pink tint is more strongly developed mainly round the opening of the corolla's tube and sometimes spreads considerably over the upper surface of the petals. Sometimes this tint is very faint, so that it seems to be most probably inherited as an independent character and its presence or absence brought about most likely by an extra pair of allelomorphs. This circumstance gives to our »white« race a great resemblance to some light pink hybrids of ccRr constitution, so called »pink-1.« hybrids in our previous investigations. Meanwhile this resemblance is strengthened by a development of a dark brown carmine-magenta spot round the opening of the corolla's tube, a so called »star«, a character which is again undoubtedly inherited through the action of a quite independent gene. We may suppose in this case such a gene, because we know »white« plants, in which such a »star«-character is absent, this being probably caused by an allelomorph producing a non-coloured area on the spot, where ordinarily such a »star« appears.

The circumstance of our possessing only three races, scarlet, dull-magenta and yellow, rendered it possible for us to get only one double-hybrid plant with the constitution cR. Cr. This was a yellowish-pink hybrid plant, yellowish mainly on the lower surface of its petals, named in our previous papers »pink-II«. We got this hybrid by means of crossing the dull-magenta race ccRR with a yellow one of the CCrr constitution, or vice versa. By microscopical examination we proved that this plant had light yellow plastids and light red cell sap in the petals, which fact points to the presence of R-r and C-c allelomorphs acting together, once in the direction of light yellow plastids and once in the direction of light red cell sap. Through self-pollination of blossoms of this hybrid we got several F_2 generations in 1930 and 1931, in which we found not only all expected genotypes and colours, as we described in our previous investigations (1, 2), but also the »white« race described above. We found among F_2 plants not only both the pure parental races, dull-magenta (ccRR) and yellow (CCrr), but also a newly combined scarlet race (CCRR) and the »white« race (ccrr) just mentioned. Moreover we got yellowish pink hybrids, yellowish mainly on the lower surface of their petals, the so-called »pink-II« types of double hybrid constitution CcRr, then pinks of a simple hybrid constitution ccRr, pinkish-whites on the lower surface of their petals, the so-called »pink-I« types, then pale yellow simple-hybrids with Ccrr constitution, then oranges with CCRr combination of genes and finally bright-magentas of CcRR constitution.

These bright magenta hybrids as well as the pale yellow hybrids Ccrr could not be satisfactorily recognized in our cultures before the year 1928. First during the period 1928—1931 they could be found among plants of different F_2 generations derived by autogamy from pink-II. hybrids, or among plants of generations, which were produced by reciprocal crossings of pink-II. hybrids with pure scarlet, dull-magenta and yellow races respectively. The bright-magenta hybrids were also produced in the same period by direct experiments, in which the dull-magentas (ccRR) were reciprocally crossed with the pure scarlet race CCRR. In this way we could produce at last F_1 generations, which were all composed uniformly of bright-magenta hybrid plants only. Although the qualitative analysis of colour inheritance in Mimulus cardinalis was, by the above experiments, proved satisfactorily before the year 1931, we were not able up to that time to trace this inheritance on a quantitative basis. But the experiments in this direction are now under way.

But the interaction of genes in colour inheritance in Mimulus cardinalis was tested further by the three back-crossings, in which the pink-II. double hybrid plants, all of the cR. Cr constitution, were reciprocally crossed with scarlet (CCRR), dull-magenta (ccRR) and yellow (CCrr) races respectively, and in all these experiments we got only types of plants and colours according to our expectations. Thus in crossings of scarlet homozygous plants (CCRR) with pink-II. hybrids and vice versa, we got progenies, which were composed only of scarlets (CCRR), oranges (CCRr) bright-magentas (CcRR) and pink-II. hybrids (CrRr). The result of this experiment agreed fully with expectations, as is to be seen from scheme no. 1.

	CR	Cr	cR	cr
CR	CR CR	Cr CR	cR CR	cr CR
	scarlet	orange	bright magenta	pink-II

Scheme no. 1.

Four quite different types of colours were secured again in cases where homozygous yellow races CCrr were crossed reciprocally with pink-II. double hybrid plants. We got out of these crossings plants with orange (CCRr), yellow (CCrr), pink-II. (CcRr) and pale yellow (Ccrr) blossoms. Also this result was found to be in accordance with our expectations, as is to be seen from scheme no. 2.

	CR	Cr	cR	cr
Cr	CR Cr	Cr Cr	cR Cr	cr Cr
	orange	yellow	pink-II	pale yellow

Scheme no. 2.

The third reciprocal cross of dull magenta race ccRR with pink-II hybrids produced again four different types of colours. In this experiment there were produced bright-magenta hybrids (CcRR), pink-II double hybrid plants (CcRr), dull-magenta pure race (ccRR) and pink-I simple hybrids (ccRr), so that the result of this experiment agreed again fully with our expectations, as is seen from scheme no 3.

	CR	Cr	cR	cr
cR	CR cR	Cr cR	cR cR	cr cR
	bright magenta	pink-II	dull magenta	pink-I

Scheme no. 3.

Although the four expected classes of colours do appear in all these three experiments without doubt, yet we must point out that in the case of experiments 2 and 3 it was not so easy to get such a precise distinction in colour types as in experiment 1, in which the scarlet races were crossed with pink-II hybrids reciprocally. In experiment 1. we could safely separate the bright-magenta types directly from scarlets or pink-II types, whereas we were not able to do so in the other two experiments, in which yellow races with pinks-II (see the 2nd experiment) were reciprocally crossed and dull-magenta races with pinks-II (see the 3rd experiment), and in which it was not possible to draw a line between pale yellow, yellow and orange types on the one hand and between bright-magentas, dull-magentas and pinks-II on the other hand. But if we have not the possibility of separating the single types of plants according to their single progenies, cultivated out of them by autogamy, we have in progenies, which are results of back-crossings of scarlets with pinks-II and vice versa, the simplest and fittest method for stating whether the four types of plants are here represented with different frequencies or not, and in connection with this, whether we have here linkage of C-c and R-r pairs of allelomorphs or not! Of course in case of linkage we must then in this experiment suppose that among the scarlet, orange, bright-magenta and pink-II plats the scarlet (CR.CR) and pink-II plants (cr.CR) are cross-overs, in which recombination of chromosomes of the same pair took place by means of crossing-over process. Evidently we shall finally be able, only from the 1.rst experiment, to estimate, without tracing single progenies of each single plant, the linkage degree of both respective pairs of genes. But this must be reserved for further investigations.

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