FURTHER STUDIES ON THE GENETICS OF THE WHITE MARGINED FLOWER OF THE JAPANESE MORNING GLORY

by

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The detection of a factor inhibiting the formation of the white margin on the corolla of the Japanese morning glory, *Pharbitis Nil*, was first made by TAKEZAKI (1916), and the case was repeatedly proved by HAGIWARA (1926) and myself (1926) in the course of his experiments and mine. I designated this factor as F^h; it behaves as a dominant to its mate in inheritance. The function of the factor is the complete suppression of the white margin, thus causing the flower to be self-colored. Another inhibitor, detected by me (1926) and symbolized as F^f, suppresses the formation of the white pattern either completely or greatly, and consequently often produces self-colored flowers. The manifestation, however, is subject to considerable fluctuation, which renders the detection of the factor in the breeding analysis somewhat difficult. In my recent experiments, I detected another *semi*-inhibitor, which has a slighter effect than the factor F^f and is closely linked with a diluting factor for the flower color. In my previous paper, I have pointed out the fact that F^a, one of the complementary factors for the formation of the white margin, is closely linked with *contracta*, whereas F^b, the other complementary factor, is strongly linked with *Nandina*. Now I have met with a case, in which self-colored or poorly white-margined flower was almost exclusively accompanied by willow leaf.

AN INHIBITOR CLOSELY LINKED WITH A DILUTING FACTOR FOR THE FLOWER COLOR

The flower of D 366, which was grown as a „parental stock", was dilute magenta (RIDGWAY'S „Magenta Violet") in color and had a white-margin pattern of the „half" degree [1]. In the next generation an unexpected segregation took place regarding the relation between white-margin and color intensity, as is shown in table I.

TABLE I. THE DATA OF THE FIRST SEGREGATING GENERATION OF D 366, SHOWING THE SEGREGATION OF FLOWER COLOR AND WHITE MARGIN

	Dilute magenta flower					Magenta flower					Total
	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	
Observed . .	3	60	6	—	—	20	6	—	—	—	95

As to the flower color we observed that magenta („Rood's Violet") is segregated as a recessive according to the 3 : 1 ratio. Notwithstanding the fact that the magenta flower is one of the varieties, which are commonly found in our gardens, hardly any investigator recognized and distinguished this type in the studies on the flower color, which fact is probably due to its resemblance to a red flower. The red flower, however, is of a vivid red, while the magenta is more or less darkish. This difference is sufficient to identify them. The quantitative variation in the degree of white margin ranges from „complete" to „slight". It is noteworthy, however, that there is a remarkable difference in the variation of degree between the dilute magenta and the magenta flowers; the former have few „complete" but many „half"-margined flowers, while in the latter we observe just the reverse. The average pattern value [2] of the dilute magenta flowers is 2.96, while that of the

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[1] In my studies the quantitative variation of the degree of white margin has been classified into four standards, „complete", „half", „slight" and „dotted".

[2] A quantitative estimation of the pattern value is attempted by the application of figures, 4 for „complete", 3 for „half", 2 for „slight", 1 for „dotted" and zero for its absence.

magenta flowers attains 3.77, which must be considered as remarkably high. From the simple segregation as to flower color, D 366 is considered to be heterozygous for the factor for color intensity, or D_1d_1 in constitution. Considering the pattern inheritance, however, the determination is not easy, as the segregation is concerned with a quantitative character. Roughly speaking, the incomplete patterns, from „half"

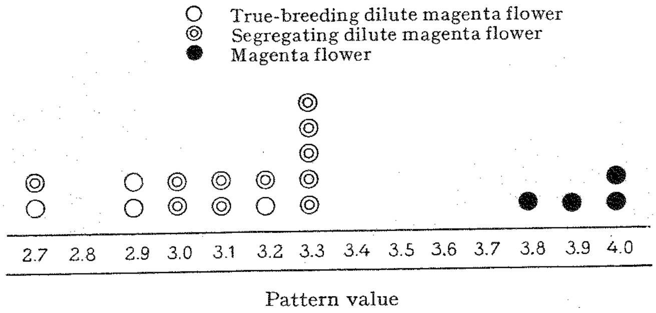
TABLE II. THE DATA OF THE SECOND SEGREGATING GENERATION OF D 366, SHOWING THE SEGREGATION OF FLOWER COLOR AND WHITE MARGIN

The figures printed in heavy type indicate the flower color and degree of white margin of the mother plants.

Pedigree number	Dilute magenta flower					Magenta flower					Total	Pattern value
	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent		
2		12	**5**								17	2.71
5		**7**	1								8	2.88
8		**36**	3								39	2.92
10	10	**25**	3								38	3.18
3		**3**	1			1					5	3.00
4		**9**	7	1		2	4				23	2.70
6		**16**	4			5					25	3.04
9		**16**	6			9	1				32	3.09
11	3	**28**	2			11	1				45	3.27
12	1	**38**	2			16					57	3.26
15	2	**34**				11	1				48	3.27
16	1	**15**				4					20	3.25
17		**14**				6					20	3.30
18	4	**25**				9					38	3.34
19		**19**	3			6					28	3.11
1						**14**	3				17	3.82
7						**8**					8	4.00
13						**15**					15	4.00
14						27	**2**				29	3.93

to „slight", are segregated together as a simple dominant to the „complete" condition. If the partial inhibitor, working in the segregation, is designated as F^p, the genetic composition of the parental plant is regarded to be $D_i d_i$ $F^p f^p$, as far as the present consideration is concerned. The actual segregation may be sufficiently explained by the additional acceptance of a linkage-relation between these pairs of allelomorphs. The proof for this supposition was furnished by an examination of the second segregating generation obtained in cultivating progenies of 19 segregates, as is indicated in table II. To make the result visible at a glance, the frequency distribution of the variation in the average pattern values of the pedigrees is shown in table III.

TABLE III. THE FREQUENCY DISTRIBUTION OF THE VARIATION IN THE AVERAGE PATTERN VALUES OF 19 PEDIGREES IN THE SECOND SEGREGATING GENERATION OF D 366



From this table we see that the ● pedigrees have invariably a high pattern value, contrary to the ○ and ◎ pedigrees, the pattern value of which is always low. This considerable difference between the magenta and dilute magenta pedigrees is, of course, due to the close linkage of the segregating factors. As the ● pedigrees carry the $f^p f^p$-constitution, the white margin is formed in the „complete" degree by the action of the ground-factors, which as a rule are present in all members without being counteracted by inhibitory factors. In such cases the development of the white margin is only subject to some fluctuation. The average pattern value of the four ● pedigrees is 3.94. On the other hand all plants of the ○ pedigrees, or at least most of them, are expected to be of the $F^p F^p$-constitution and actually all four proved to be really so, with one exception. Though pedigree No. 10 bred true to the dilute magenta flower color, it consisted of 10 „complete”, 25 „half” and 3 „slight” individuals. To acccount for this segregation the motherplant of this pedigree is considered to have originated from the union of a D_iF^p-gamete, a non-cross-over, and a D_if^p-gamete, a cross-over. The one ○ plant, occurring in the 3.2 column of table III, represents the pedigree in question. As to the ◎ pedigrees, they all possess pattern values corresponding to the theoretical ones, as will be seen from table III, with one doubtful exception in the 2.7 column (No. 4). One should expect most of the ◎ pedigrees to be double heterozygotes as a consequence of close linkage. The pattern development of the individuals of No. 4 was throughout weaker than that of other similar pedigrees, as appears from table II. One might be inclined to regard this as a consequence of homozygosity for the inhibitor, F^p. If this were the case, a parallel fluctuation in the quantitative variation of the white margin should be expected in both groups, dilute magenta and magenta flowers. Actually, however, the degree of the white pattern in the magenta flowers is so much higher throughout than that in the dilute magenta ones that it makes the above suggestion unacceptable. In my opinion the pattern value of the individuals of this pedigree was estimated generally lower than was their actual value 1). If my above-mentioned view is correct, the dilute magenta and magenta pedigrees, nineteen in all, are to be regarded as follows:

Non-crossover + non-crossover	18
Crossover + non-crossover	1
Total.	19

From these figures, the frequency of crossovers is calculated to be 2.63%. Anyhow, the number on which the calculation was made being meagre, we cannot calculate the exact linkage value, which is but roughly determined by the above estimation.

The next problem to be discussed is that of the degree of dominance of the factor F^p. To recapitulate the other known inhibitors: the domi-

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1) The estimation of the white margin, late in the flowering period, will generally result in a lower pattern value, while the early estimation will give a higher value. A pedigree consisting of specimens having on an average an early flowering time should be estimated somewhat lower than their observed value, because the records on all pedigrees, originated from the same cross, were usually taken at the same time or in the course of a few days.

nance of F^h is perfect, while that of F^f is incomplete, the white margin character of the heterozygotes showing a range of variation, which is intermediate between those of the F^fF^f and the f^ff^f plants. From the motherplants of the nineteen pedigrees in question, three are regarded to be of the F^pF^p-constitution and twelve of the F^pf^p-constitution, whereas the 4 ● pedigrees are considered to have the f^pf^p-constitution. The average pattern value of the former three is 2.84, which roughly indicates the action of the inhibiting factor F^p in a double dose. The other twelve, however, also segregated as to the flower color, with the exception of No. 10. Therefore their dilute magenta segregates will probably consist of F^pF^p and F^pf^p plants in a definite ratio, and all their magenta segregates should carry f^pf^p, on account of the existence of a close linkage. If we calculate the average pattern values of the dilute magenta and magenta flowers of these eleven pedigrees on the strength of these suggestions the result will be as follows:

Dilute magenta flowers	2.90
Magenta flowers	3.92

In comparison with the average pattern value calculated before, this 3.92 almost equals the 3.94 of the f^pf^p-pedigrees and this 2.90 closely approaches 2.84 of the F^pF^p-pedigrees. Although the former agreement is rather a matter of course, it provides of itself an indirect proof for the close linkage. The latter consistency tells us that the dominance of the factor F^p is almost complete.

THE SEGREGATION OF THE SELF-COLORED FLOWER AND ITS ACCOMPANIMENT WITH THE WILLOW LEAF

Another „parental stock", D 366-I, having the same origin as D 366, with which we were concerned in the foregoing pages, had cordate leaves and dilute magenta flowers with a white margin pattern of the „half" degree. On selfing it segregated as is shown in table IV.

From the data contained in this table, we see the segregation of the linked factors, D_l and F^p, but the data are complicated by the appearance of an additional assortment, characterized by willow leaf, accompanied by flowers which are either self-colored or have a white margin pattern of a very slight degree. The quantity of the white margin in flowers of the willow-leaf type varied from its absence to

TABLE IV. THE DATA OF THE FIRST SEGREGATING GENERATION OF D 366-I, SHOWING THE SEGREGATION AS TO LEAF FORM, FLOWER COLOR AND WHITE MARGIN

	Cordate leaf with dilute magenta flower					Cordate leaf with magenta flower					Cordate-willow leaf with dilute magenta flower					Cordate-willow leaf with magenta flower					Total
	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent.	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	
Observed	31	130	5	—	—	42	11	—	—	—	—	—	—	22	25	—	—	5	17	—	288

„slight" (Fig. 1), the majority of cases being „dotted" or „absent". This reduces the average pattern of these 288 plants to 2.62. As was shown in my previous paper (1926), F^a, one of the formative factors for the white margin, can frequently produce the white pattern in a slight degree in *Nandina* flowers, carrying a f^bf^b-constitution, notwithstanding the acting of F^a as a complementary factor to F^b in the production of white margin in the ordinary flowers. Seemingly a similar phenomenon occurred in the segregation of the willow-leaf. Although I have no critical data at my disposition, enabling an identification of the segregating pattern factor strongly accompanying the willow-leaf with either f^a or f^b, or with any other known or unknown factor, we may expect that the segregation, recorded in table IV, is partly due to heterozygosity for one of the ground factors for white margin, tentatively designated here as F vs. f, and besides to an inhibitor which partly suppresses its full action. If this suggestion holds true the factor is very closely linked to m′, and then the unbalanced distribution of the segregates in table IV is due to this linkage and to that between

D_i and F^p. If this be the case the genetic constitution of the „parental stock" D 366-I has to be considered as $Mm'D_idF_ifF^pf^p$. The segregation of a heterozygote of this constitution will closely resemble that of

FIG. 1. Cordate willow bearing a narrow-split magenta flower with „slight" white margin.

a dihybrid on account of the two systems of linked factors. Consequently the following distribution should be expected:

$1MMDiDiFFF^pF^p + 2Mm'DiDiFfF^pF^p + 2MMDidiFFF^pf^p + 4Mm'DidiFfF^pf^p$	9 cordate leaves, dilute magenta flowers with incomplete white-margin
$1MMdidiFFf^pf^p + 2Mm'didiFff^pf^p$. . .	3 cordate leaves, magenta flowers with complete white-margin
$1m'm'DiDiffF^pF^p + 2m'm'DidiffF^pf^p$.	3 cordate-willow leaves, dilute magenta flowers with or without white-margin of very slight degree
$1m'm'didifff^pf^r$	1 cordate-willow leaf bearing magenta flower with white-margin of small or very slight degree (or with self-color, cf. table V).

Practically, the manifestation of the white margin more or less fluctuates, and with this in mind, the above-mentioned expectation may be considered to agree with the data of table IV. Though the actual data seem to point to a weak linkage between D_i and M, the evidence is not sufficient to conclude whether this is the case or not. The factor f can frequently produce white margin in the willow-leaf type, though the degree is very slight. If we calculate the average value of the white margin for the four groups from the data contained in table IV we find:

Cordate leaf, dilute magenta flowers	3.15
„ „ , magenta flowers	3.79
Willow leaf, dilute magenta flowers	0.47
„ „ , magenta flowers	1.23

From 43 plants of this „parental stock" a second segregating generation was raised, the data of which are shown in table V. Table VI is concerned with the frequency distribution of the variation in the average pattern value of these 43 pedigrees. An examination of the latter table shows the fact that the result almost agrees with the theory. The ● pedigrees are of course the highest class in the pattern value, because they carry the f^pf^p-constitution on account of strong coupling. The pattern value of the ◉ pedigrees will be somewhat reduced by the influence of segregated willow-leaf plants, bearing the factors ff, but is not much affected. The ○ pedigrees, however, are of course low in their pattern value, as they are constituted F^pF^p; and the value will be gradually lower in the ◎ and ◎ pedigrees, on account of the segregation of willow-leaf plants. Actually, however, the ◎ pedigrees have generally higher values than the ○ ones, as will be seen in table VI.

TABLE V. THE DATA OF THE SECOND SEGREGATING GENERATION OF D 366-I, SHOWING THE SEGREGATION OF LEAF FORM, FLOWER COLOR AND WHITE MARGIN

Pedigree number	Cordate leaf with dilute magenta flower					Cordate leaf with magenta flower					Cordate-willow leaf with dilute magenta flower					Cordate-willow leaf, magenta flower					Total	Pattern value
	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent		
4		**7**	7	1																	15	2.40
9		**2**	4	1																	7	2.14
13		**0**	2																		2	2.00
22		**2**	11	1																	14	2.07
31		**3**	14	1																	18	2.11
38		**2**	7																		9	2.22

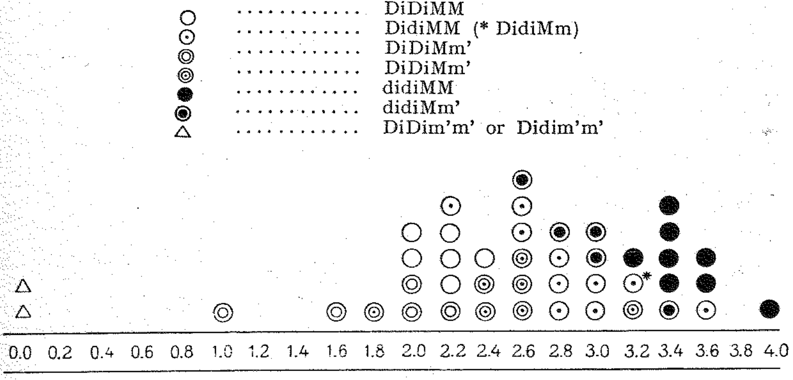
TABLE V (*Cont^d^*)

Pedigree number	Cordate leaf with dilute magenta flower					Cordate leaf with magenta flower					Cordate-willow leaf with dilute magenta flower					Cordate-willow leaf, magenta flower					Total	Pattern value
	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent	„Complete"	„Half"	„Slight"	„Dotted"	Absent		
1		**8**	5			1	3														17	2.76
3	**0**	7	2			3															12	3.08
6		**3**	3				1														7	2.57
7	1	**6**				1	1														9	3.67
30		**2**	19	1		1	5														28	2.29
32		**4**	3			2	2														11	2.91
39	**0**	7	7			3	5														22	2.82
40	**0**	3	8			5	1														17	2.82
41		**2**	4			1	2														9	2.67
10		**1**													2						3	1.00
14	1	**1**	2												1						5	2.20
15	1	**12**	6												6						25	2.08
18		3	**15**	1											*6						25	1.60
19		12	**16**												6						34	2.00
2		**5**	3			2	3	1							1				1		16	2.56
8	**2**	9				3									1						15	3.13
11	8	**14**				6	1						*1*		8		*1*			1	40	2.65
17	2	**14**	1			7	3								5					1	33	2.70
24	1	**5**	6				1	1							3					2	19	1.89
29	1	**6**	6			3	2								2					1	21	2.48
43		**8**	8	1		2	2						*1*		2		*2*			1	27	2.33
34		**9**	1			2						*3*	*1*			*1*	*1*				18	3.11
12						**8**	11														19	3.42
20						5	**7**														12	3.42
27						7	**4**														11	3.64
28						**5**	20														25	3.20
33						**1**	2														3	3.33
35						**13**	10														23	3.57
37						**4**	5														9	3.44
42						**6**															6	4.00
5						**15**	1												3		19	3.47
16						**8**	6	1											1	5	21	2.52
21						**15**	12											1		3	21	2.76
25						**3**														1	4	3.00
36						**12**	25										*1*			4	42	2.98
23															**1**						1	0.00
26															**2**						2	0.00

The figures printed in heavy type indicate the parental characteristics of each pedigree; those in italics represent the number of maple-leaf mutants. The asterisk denotes that in this group one plant occurred, producing a sport of the disguise-willow type.

This however, does not seem important, and is due partly to the comparatively small number of the willow segregates, partly to the appearance in small numbers of the maple-leaf mutant, the flowers of which have a white margin in the average degree of the cordate-leaf plants; their occurrence, however, was infrequent. On the contrary, the ⊙ pedigrees have a higher value in the dilute magenta group, because of the segregation of magenta flowered plants, which raise the average pattern value, whereas willow leaf, which has a tendency to decrease it, is not segregated.

TABLE VI. THE FREQUENCY DISTRIBUTION OF THE VARIATION IN THE AVERAGE PATTERN VALUES OF 43 PEDIGREES IN THE SECOND SEGREGATING GENERATION OD D 366-I



Pattern value

Willow-leaf generally produces no seeds on account of the abortion of the female organ. Quite exceptionally, however, I obtained three seeds from two willow-leaf plants. The pedigrees, which are designated by △ in table VI, have self-colored flowers, the pattern value of which is zero. Such a value is rather inevitable in a willow-leaf pedigree and it always will remain low, even if the development of the white pattern is incidentally favored.

Generally speaking, the majority of willow-leaf plants appearing in the second segregating generation bore flowers wanting white margin. In some cases, however, a „dotted" margin was observed, always coinciding with magenta flower-color. In comparing this result with that obtained in the foregoing generation, we find that the development of the white margin was generally poor in the latter generation. The main cause of this difference is difference in environmental circumstances.

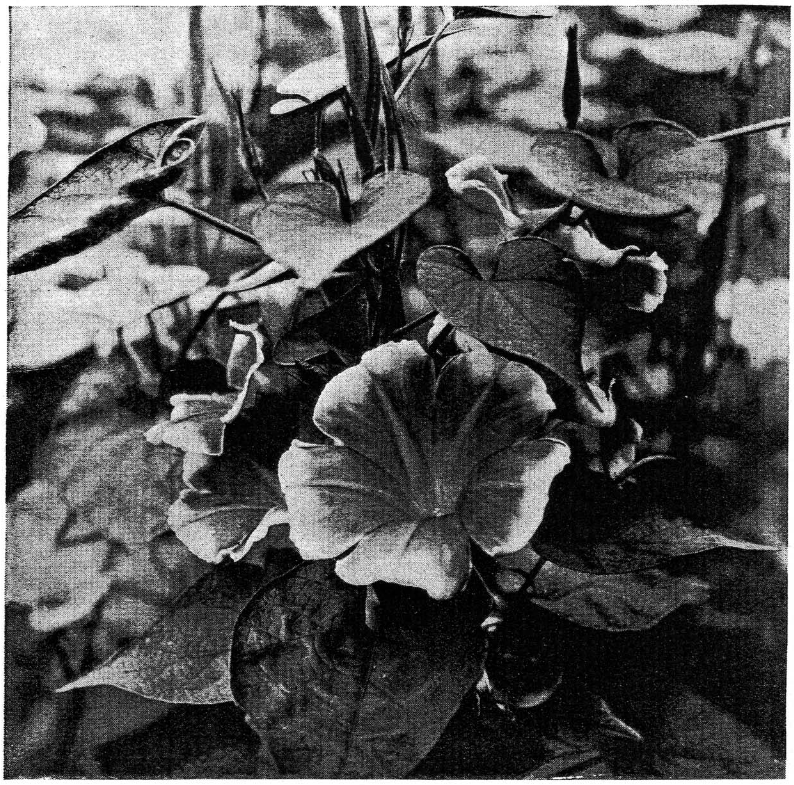


FIG. 2. Cordate-maple leaf bearing split magenta flowers with complete white-margin

THE QUANTITY OF THE WHITE MARGIN OF MAPLE MUTANTS

The habitual mutability of the willow-leaf type is a well-known fact in the *Pharbitis*-genetics (IMAI 1925). In the progeny of D 366-I, I also obtained some maples in the cordate-willow-segregating pedigrees. The „parental stock'' was cordate-leaf, consequently the mutants appearing in its progeny were, as might be expected, cordate-maple leaves. It is a noteworthy fact that the maple-flowers (Fig. 2) have a white margin in the average degree of the cordate leaves, though they made their appearance by mutation from willows, which bear the factor f. The pedigrees, in which maple mutants appeared, are those of Nos. 11, 34, 36 and 43, table V, among which No. 34 is rather exceptional in segregating cordate and maple leaves, but no willows. The mother plant of this pedigree is, therefore, regarded as due to the union of a normal and a maple gamete, the latter being mutated from a willow-gamete. Be that as it may, all the maples appearing in the pedigrees must have originated in the same way, which may be symbolized by m'→m. We thus may discuss them together. In the present case, the factor m' accompanies f as a shadow on account of close linkage. We therefore expect that the maple mutants should carry it also. If this is the case, the white margin of the maples should on an average be of the same degree as that of the willows. Actually, however, the result was quite contrary to this, as we know from table V, 12 maple-leaved

FIG. 3. A willow-leaf plant with bud-sport of the disguise-willow type, which bears a dilute-magenta split flower. Note the occurrence of two leaf forms, disguise-willow (broader one) and willow (narrower one), on one plant. The plant on the left is cordate-leaf bearing dilute-magenta perfect flowers with „slight" white-margin.

plants being observed. Six of them had dilute magenta flowers, with an average pattern value of 2.50. The other six plants had magenta flowers, their pattern value averaging 3.17; these figures, however, are nothing but average standard values of the cordate segregates. From this fact we might conclude that the mutation of m'→m causes another factor variation, f→F, in the neighboring locus of the same chromosome. In his study on wheat, NILSSON-EHLE (1920) reported cases of double and triple mutations of linked factors, and FROST (1921) observed bud-variation involving two closely linked factors in a culture of *Matthiola annua*, adding apparently analogous examples from plants to the peculiar factorial or chromosomal phenomena observed in *Drosophila*. Recently NAGAI (1926) reported on an analogous case observed in *Oryza sativa*. It seems, however, to be too hasty to conclude that the present case observed by me is due to double mutations of m'→m and f→F, unless critical examinations are made. Another explanation for the phenomenon is that of an interaction of the factors considered, but I should like to leave the problem without any further fruitless arguments.

Lastly, I will make an additional note concerning the white pattern of the flowers of the disguise-willow, which appeared as a bud-sport on a willow-leaf plant (Fig. 3) in pedigree No. 18, table V; the original willow-leaf part as well as the mutated part bore self-colored flowers only. The mutation therefore does not influence the white margin.

At the end of the present paper, I wish to express my heartly acknowledgement to Professor K. MIYAKE and Mr. K. HASHIMOTO for their guidance and encouragement given in my investigation. The expenses needed for the work were defrayed in part by a grant from the Imperial Academy, to which my thanks are also due.

SUMMARY

1. The partial inhibitor, F^p, described in the present paper, is quite different in its behavior from F^f, discussed in a previous paper (1926). The factor F^p suppresses the complete development of the white margin in producing it in the „half"-„slight" degrees.
2. The dominance of F^p is almost perfect.
3. The manifestation of this partial inhibitor is subject to fluctuation, contrary to that of the other partial inhibitor (cf. IMAI 1926).
4. A strong linkage occurs between the allelomorphic pairs of F^p and f^p *versus* D_i and d_i, the latter controlling the intensity of the flower color, the cross-over value being about 3%.
5. The non-willow-leaf segregates bore flowers with white margin of a high degree, the willow-leaf ones, on the other hand, almost invariably had flowers which were either self-colored or with white margin pattern in a very slight degree. This may be due to another linkage relation.
6. Quite unexpectedly the maple-leaf mutants, produced by mutation of m' → m, have always the white margin in a degree averaging that of the flowers of the cordate-leaf plants.
7. The flowers of disguise-willow, which appeared as a bud-sport on a willow-leaf plant, however, had the average degree of the latter type.

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