A GENETIC ANALYSIS OF WHITE-MARGINED FLOWERS IN THE JAPANESE MORNING-GLORY

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INTRODUCTION

Some races of the Japanese morning-glory (*Pharbitis Nil*) bear flowers with white peripheral margins[1] on the corollas. Notwithstanding the fact that the white-margined flower is a variation recognized rather recently, it now enjoys great popularity in our gardens. The pattern is commonly transmitted as a simple dominant to the self-colored flower (TAKEZAKI 1916, MIYAZAWA 1918, IMAI 1919, 1925, HAGIWARA 1922a, 1922b). TAKEZAKI (1916) found cases, however, in which the white-margined flower behaves as a recessive to the self-colored condition, owing to the presence of an inhibitor. When such dominant and recessive white margins were concerned in the segregation of F_2, it was found that there were 13 self-colored and 3 white-margined flowers. The fact was later confirmed by HAGIWARA (1922b, 1926).

The writer's further study showed the occurrence of complemental factors concerned in the formation of the white-margined flower, besides the presence of the inhibitor above cited. As was already pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926), the common factor for the white-margined flower is linked quite closely with the *contracta* factor,

[1] This condition is called "Fukurin" in Japanese.

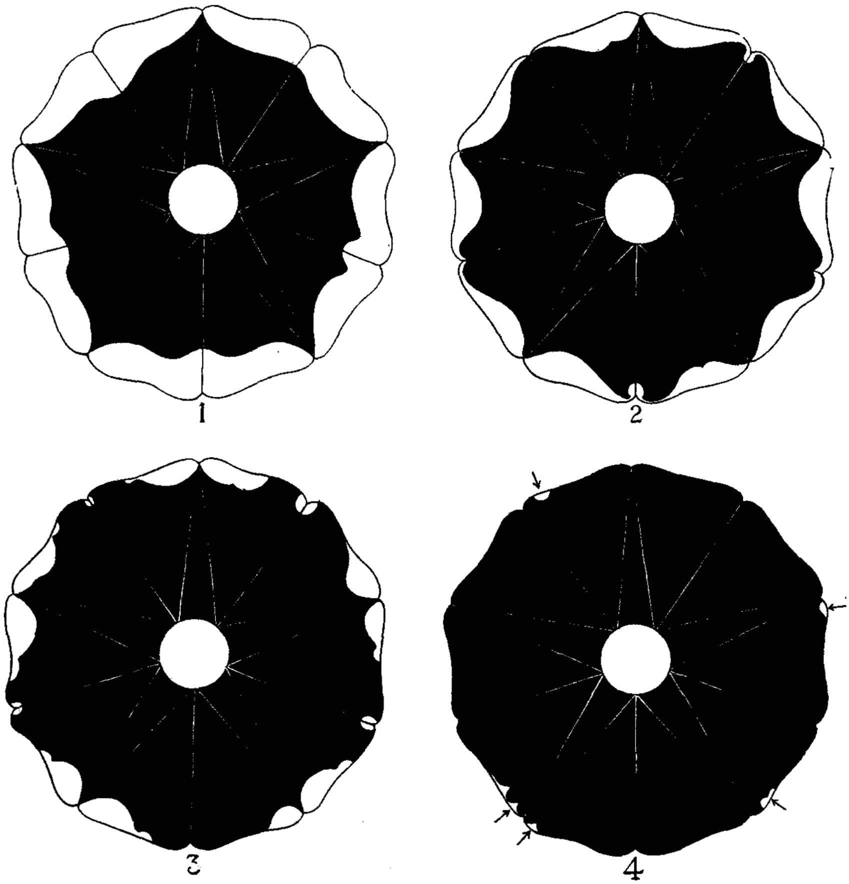
the former being one of the complemental factors. Another complemental factor is that of being linked very closely with the factor for the *Nandina* leaf,[2] which is uncommon in our gardens. The fact that the development of the white margin was somewhat variable according to its environment, under which the plant is raised, was repeatedly shown by these Japanese investigators. Some cases of the writer's experiment, however, revealed themselves to be so extremely variable that they could hardly be considered as ordinary cases, and genetic analysis showed that such conspicuous variations are, at least, due mainly to the segregations of a factor, which reduces the quantity in development of the corolla pattern, and is very susceptible of fluctuation in its manifestations. As a result, an apparently self-colored flower is frequently produced in the hybrid progeny, but it gives rise to white-margined flowers making a large part of the members in the subsequent generation. And further, this factor is moderately linked with the factor *c* (IMAI 1921), which is responsible for the white flower with a colored stem.

THE QUANTITATIVE VARIATION OF THE WHITE MARGIN

The development of the white margin on the corolla depends somewhat upon the environment under which the plants were grown and upon the time of observation at which the record of the characters was taken. Even in one individual the white margin varies generally in such a manner that the development of pattern, however perfect it may be, is inclined to diminish, as a rule, little by little in later stages of plant growth. MIYAZAWA (1918) observed a case in which the production of the self-colored flower in some F_2 families conspicuously exceeded all expectations, and he attributed this result to the dry conditions of his pot culture. The manifestation of the common white-margined flower, which acts as a dominant to the self-colored condition, is due to the occurrence of the factor F^a (see a later section). In the homozygotic white-margined flower the development of the pattern is almost complete, though sometimes it may diminish to some extent, while it becomes invariably imperfect in the heterozygotic flower. According to the writer's experience variation in the development of the corolla pattern is not so conspicuous even in the heterozygotic flower when the plant is raised on open ground and the record is taken in a suitable period from normally grown individuals. But when both complemental factors are segregated or a partial inhibitor is concerned (see the explanation of a later section) we can see a remarkable series of

[2] This peculiar form of the leaf somewhat resembles that of *Nandina domestica*, an evergreen shrub. The genetic data concerning this leaf will be given in another paper.

variations ranging almost continuously from a very meagre pattern to perfection. In the latter case, variation frequently leads to a self-colored flower without its manifestation. Such a flower cannot be distinguished from the ordinary constant type without a breeding test. Thus the quantitative variation of the pattern runs sometimes in a continuous series of



FIGURES 1 to 4. The artificial standards in the variation of white margin.

gradation from completeness to total absence. In order to measure the quantity of the pattern, the writer has, for convenience, classified the pattern into four classes; namely, "complete," "half," "slight" and "dotted" the standard of each degree being given in figures 1 to 4.

THE SIMPLEST SEGREGATION

The F_1 of a cross performed between a white-margined flower and a self-colored one usually represents a white peripheral pattern on the corolla to the degree of "half" or nearly so. In this case, the simplest segregation takes place generally at the ratio of 3:1. The data representing this simplest segregation of two alternative characters are collected in table 1.

TABLE 1

F_2 data making simplest segregation.

CROSS	WHITE-MARGINED	SELF-COLORED	TOTAL	SEGREGATING PERCENT OF RECESSIVE PLANTS
α×65	67	24	91	26.37
324×α	43	12	55	21.82
α×314	80	22	102	21.57
320×A5	30	17	47	36.17
50×M4	238	88	326	26.99
323×316	36	9	45	20.00
323a×316	66	15	81	18.52
220×316	243	71	314	22.61
220×65	127	36	163	22.09
65×220	265	105	370	28.38
224×220	30	15	45	33.33
65×324	93	30	123	24.39
Total	1318	444	1762	25.20
Expected	*1321.5*	*440.5*	*1762*	*25.00*

With some of these F_2, the F_3 rearing was made, but no new result was found beyond expectation. We shall now tentatively set down the summarized data of F_3 plants derived from two crosses in table 2.

TABLE 2

Summarized data of F_3 progenies from two crosses 324×316 and 65×326.

CROSS	PHENOTYPE OF F_2 AND NEXT GENERATION		NUMBER OF PEDIGREES	WHITE-MARGINED	SELF-COLORED	TOTAL	SEGREGATING PERCENT OF RECESSIVE PLANTS
324×316	White-margined	breeding true	19	669		669	0.00
		segregating	33	622	213	835	25.51
	Self-colored	breeding true	17		428	428	100.00
65×326	White-margined	breeding true	27	683		683	0.00
		segregating	33	795	266	1061	25.07
	Self-colored	breeding true	13		389	389	100.00
Total	White-margined	breeding true	46	1352		1352	0.00
		segregating	66	1417	479	1896	25.26
	Self-colored	breeding true	30		817	817	100.00

Upon comparison of genotypes of white-margined F_2 flowers, after impartially omitting those pedigrees which contained only a few individuals (below ten in number), we found the result of table 3, in which the number observed quite satisfies the expected ratio of 1:2.

TABLE 3

Comparison of genotypes of white-margined F_2 flowers.

CROSS	NUMBER OF HOMOZYGOTIC PEDIGREE	NUMBER OF HETEROZYGOTIC PEDIGREE	TOTAL
324×316	12	29	41
65×326	20	29	49
Total	32	58	90
Expected	*30*	*60*	*90*

Two crosses given in table 1, $\alpha\times314$ and $\alpha\times65$, were composed of segregating numbers just in accordance with the 1:2:1 ratio of "complete" margined flowers, imperfect ones varying from "slight" to "half," and self-colored, as indicated in table 4. We have no accurate knowledge as to how such a result was obtained particularly in these crosses, but it may be

TABLE 4

F_2 result of two crosses segregating into a 1:2:1 ratio.

CROSS	"COMPLETE"-MARGINED	"HALF"-MARGINED	"SLIGHT"-MARGINED	SELF-COLORED	TOTAL
$\alpha\times314$	20	42	18	22	102
$\alpha\times65$	27	40		24	91
Total	47	100		46	193
Expected	*48.25*	*96 50*		*48.25*	*193*

due to the fact that the record was taken in a favorable time to note the genotype in its manifestation. The individuals in the hybrid progeny may have varied during the time of flowering, so it is a matter of a little difficulty to obtain the record of each member at a favorable period on a somewhat larger scaled experiment.

THE OCCURRENCE OF COMPLEMENTAL FACTORS

The result of cross 26×UN

Since the complemental factors were discovered by BATESON and PUNNETT in a classical experiment, quite a number of similar instances have been reported. The writer also detected such a case of manifestation in the white-margined flower of the Japanese morning-glory, in the hybrid progeny of a cross 26×UN, the former bearing flowers with "perfect" white margins, while the latter had self-colored corollas. The F_1 plants obtained by this cross bore white-margined flowers to the degree of "slight" on an average, and they segregated numerically into 276 white-margined and 159 self-colored individuals, or 1.74:1 in ratio, in the subsequent generation. The investigation of the cause, by which such a phenotypic ratio is developed, makes it advisable to consider the segregation of the other characters at the same time, because the white-margined and the self-colored flowers represented special relationship to *contracta and Nandina* leaf in their segregation. The actual segregation of the F_2 result is shown in table 5.

TABLE 5

F_2 data of cross 26×UN representing a complicated segregation.

PEDIGREE	NORMAL					*Nandina*					TOTALS
	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-Colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	
1	15	12	32	5	1				1	14	80
2	9	12	18	1						8	48
3	3	14	18	6					2	12	55
4	10	20	32	2	2				1	18	85
5	5	15	41	8	2					18	89
Total	42	73	141	22	5	0	0	0	4	70	357
	278				5	0			74		357

PEDIGREE	*Contracta*					Contracted *Nandina*					TOTALS
	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-Colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	
1			1		26					4	31
2					8					3	11
3					8					3	11
4			1		13					5	19
5			2		13					1	16
Total	0	0	4	0	68	0	0	0	0	16	88
	4				68	0			16		88
Grand Total											445

F_2 data of cross 26×UN.

Pedigree number	NORMAL					*Nandina*					*Contracta*					CONTRACTED *Nandina*					TOTAL	REFERENCE
	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-Colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted" margined	Self-colored		
32	14*	12																			26	A
34	21																				21	
39	27	0																			27	
40	24	5																			29	
44	23	11																			34	
68	13	0																			13	
5	15	19	4						1	8											47	B
7	5	7	5							4											21	
8	18	2							2	3											25	
13	26	6	6						4	4											46	
16	16	2	3						5	4											30	
21	26	7	1						5	12											51	
22	20	1							4												25	
29	26	4	1						1	6											38	
47	20	8	2		1				2	4											37	
56	13	5	1						2	6											27	
59	47	11	2		1				3	10											74	
62	27	12	1						12	4											56	
63	73	9							3	13											98	
65	18	20	7		1				4	6											56	
14	5	4	10	2	7					9											37	C
49	1	1	4	3	3					4											16	
58	8	17	6	2	7				6	13											59	
3	13	8	1												4						26	D
20	4	10	17		1							1			3						36	
27	4	2	8												2						16	
28	4	1	2												2						9	
38	4	7	7												3						21	
71	5	10	3												7						25	
1	8	13	12		1				3	8					7					4	56	E
9	1	7	19	3	2					4					9					3	48	
10	14	17	33	1	1				7	17			1		16					1	108	
12	4	6	12						1	11					5					4	43	
15	3	6	6						2	2					2						21	
17	10	14	46	4	1				2	18					16					7	118	
30	6	12	8		1				1	3					5					1	37	
31	11	11	24	3	1				1	9			1		13					2	76	
36	1	6	30	10					1	21					8					5	82	
45	4	4	6	2					2	3					10						31	
46	15	15	18						2	15					6					2	73	
50	6	17	23	2	1					14					10						73	
52	2	3	13	4						13					8					1	44	
53	15	11	8		2				4	6					10					3	59	
64	11	16	14	3						14					14					2	74	
67	6	12	15	3	1				1	11			1		16					1	67	
11									8	6											14	F
43										2											2	
48										6											6	
54										5											5	
18									2	7										4	13	G
24									6	7										2	15	
51									4	10										3	17	
60										5										1	6	
61									6	14										5	25	
66									4	3										4	11	
Total of 7 pedigrees															132						132	H
Total of 9 pedigrees															148					40	188	I
Total of 2 pedigrees																				12	12	J

* Bold face figures indicate the character of the respective parental F_2.

The fact that the factor for *contracta* links with that for the white-margined flower was pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926), the frequency of crossovers being only about 1 percent. If the same factor of the white-margined flower is concerned in segregation in the present cross, there should naturally be found some data indicating a special relation to the segregations of the margined condition and of *contracta*. The F_2 results observed, as was shown in table 5, were complicated by simultaneous segregation of another factor of the white-margined flower, so the exact nature of the segregation cannot clearly be realized. But it can be roughly said from the results obtained that the majority of the segregating *contracta* bloomed into self-colored flowers, while a large part of the normal, with the exception of the normal *Nandina*, bore flowers with white margins. Concrete evidence, therefore, may be sought in the F_3 results shown in table 6. In an actual trial, 6 pedigrees in class D of table 6 were segregated in a dihybrid fashion in regard to the factors for *contracta* and the white-margined flower. The segregation of these pedigrees are regarded to be the same in nature, so by adding them together a total sum is obtained as shown in table 7. The segregating numbers of the normal and *contracta* are 120 and 22 respectively, indicating a some-

TABLE 7

Summarized data of 6 pedigrees in class D of table 6.

	NORMAL					*Contracta*					TOTAL
	"Complete"-margined	"Half"-margined	"Slight" margined	"Dotted"-margined	Self-colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	
Total observed	34	38	38	0	1	0	1	0	0	21	133
	110				1	1				21	
Expected	*99.095*				*0.655*	*0.655*				*32.595*	133

$\chi^2 = 5.676$ P = 0.131

what larger discrepancy in the ratio (deviation $= \pm 13.5$, while probable error $= \pm 3.48$). Being probably affected by such deviation, the value of goodness of fit in table 7 was still no more than 0.131. This consideration makes an explanation necessary for in the present cross we are concerned also with the segregation of the factor for the white-margined flower linked with that of *contracta* at about 1 percent of the crossover frequency. But with the evidence of this special relation only we cannot understand the whole aspect of the F_2 segregation. Upon inspecting the items in the normal *Nandina* of table 5, we are struck by the fact that they all bear self-colored flowers, excepting a few "dotted"-margined flowers, somewhat similar to the case of *contracta*. Fourteen pedigrees in class B of table 6 are considered to make dihybrid segregation of the factors for the white-margined flower and *Nandina*. Table 8 contains the summarized data of the numbers thus observed. Many plants with *Nandina* leaves bore self-

TABLE 8

Summarized data of 14 pedigrees in class B of table 6.

	NORMAL					*Nandina*					TOTAL
	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	
Total observed	350	112	34	0	3	0	0	0	48	84	631
	496				3	0			132		
Expected	*471.75*				*1.5*	*1.5*			*156.25*		*631*

$\chi^2 = 8.010$ P=0.046

colored flowers, including a considerable number of "dotted"-margined flowers. From classes F and G of table 6 we may conclude that there exists a close relation between the manifestations of the "dotted" margin and full coloration in the normal *Nandina*. The "dotted" margin frequently fails to develop in different flowers of a plant which is recorded as having that characteristic. As the result of this fluctuation the record may contain some errors, and for this reason, we cannot attach much importance upon the observed numbers of the "dotted"-margined and self-colored flowers in the normal *Nandina* leaves. In accounting for the appearance of "dotted"-margined flowers in the normal *Nandina* type, attention may be called to the probable occurrence of a modifier, which affects the development of the white margin to a slight degree. Whether this is the case or not, we may be permitted to recognize such a "dotted"-margined flower to be a modified form in the self-colored condition, and so this form may be added to the self-colored flowers of the normal *Nandina*, when consideration is made in regard to the segregating aspect of the factor for the white-margined flower, which holds a close relation to that of *contracta*. Thus we can take the quantitative estimation in intensity of the linkage from 496 normal leaves with white-margined flowers, 3 normal leaves with self-colored flowers, no *Nandina* leaves with white-margined flowers and 132 *Nandina* leaves with self-colored flowers (the last including "dotted"-margined flowers from the allowance above-designated) as indicated in table 8. The gametic ratio estimated from these is 209.03:1, the crossovers being 0.48 percent or nearly 0.5 percent in frequency. Here the value of P, as indicated in table 8, is not so high as desirable, due mainly to the influence of a discrepancy observed in the segregation of the normal *versus Nandina* allelomorphs. From the segregating numbers of 273 normals, 74 *Nandinas*, 72 *contractas* and 16 contracted *Nandinas* (total 435), it can be recognized that the factors concerned in the present data segregated freely, without linkage ($\chi^2=9.713$, $P=0.022$).[3] Consequently it may be clear that the factor, which governs the formation of the white margin on the corolla and links with that for *contracta*, differs from that which is linked with the factor for *Nandina*, indicating the occurrence of two working factors for the manifestation of the white margin. As a result, there was no simple segregation of the corolla pattern in F_2 of the present cross. Almost all members that were found in the classes of *Nandina*, *contracta* and contracted *Nandina* of table 5 (with actually only 4 exceptions among 162 individuals), had self-colored flowers, or they consisted of those regarded practically as self-colored flowers in their factor relationship, so that the total obtained by adding the observed numbers of these three classes corresponds to seven-sixteenths of the total F_2. Then the ratio of white-margined and self-colored flowers is expected to be 9:7, indicating the occurrence of two complemental factors for the formation of the corolla pattern. In practice, however, those plants carrying a recessive foliage factor or factors, were somewhat weak in their growth, and they gave rise to some discrepancy in the ratio of segregation; the actual deformity in number of the self-colored flowers may not be regarded to be of any serious source in factor analysis. In an attempt to realize the genetic relationship of the present cross, on such a basis, it will be necessary to establish the following factors:

1. F^a, f^a—The F^a factor concerns the formation of the white margin on the corolla, and it holds 1 percent of the crossovers with the factor for *contracta*.

2. F^b, f^b—In the coexistence of F^a, the factor F^b completes the manifestation of the white margin. Neither factor, acting by itself, produces the trait, but both work in complemental cooperation. The factor f^b is linked closely with that for *Nandina*. In the combination of F^a and f^b in general, no white margin is produced on the corolla, but when they enter into *Nandina* leaves, they often manifest themselves in "dotted"-margined flowers.

[3] The value of P is low, but we cannot attribute this, as far as the data show, to the result of any factorial complexity beyond the recognition of a superficial deviation.

Now we shall attempt to settle the genetic composition of the parents of the present cross. In addition to the complemental factors above-represented, it may be necessary to give those for *contracta* (d) and *Nandina* leaf (n_n) in this consideration. The genetic composition of 26 is considered to be a quadruple dominant from the fact that the specimen is a normal-leafed *non-contracta* with white-margined flowers. Then its partner, UN, is regarded as one carrying a quadruple recessive constitution. In the cross of such a parentage, the F_1 should be heterozygous for

TABLE 9

Theoretical expectation in F_2 of cross 26×UN.

CHARACTER	RATIOS	
	Calculated on the basis of such gametic ratios: Between d and f^a, x:1 Between n_n and f^b, y:1	Applied such real figures: x=100 y=200
Normal with white-margined flower	$9x^2y^2+12x^2y+12xy^2+16xy+6x^2+8x+6y^2+8y+4$	3672622404 (or 45565)
Normal with self-colored flower	$6x^2y+6xy^2+20xy+3x^2+10x+3y^2+10y+5$	36553005 (or 454)
Nandina with white-margined flower	$6x^2y+8xy+3x^2+4x+4y+2$	12191202 (or 151)
Nandina with self-colored flower	$3x^2y^2+6xy^2+4xy+2x+3y^2+2y+1$	1224200601 (or 15188)
Contracta with white-margined flower	$6xy^2+8xy+4x+3y^2+4y+2$	24281202 (or 301)
Contracta with self-colored flower	$3x^2y^2+6x^2y+4xy+3x^2+2x+2y+1$	1212110601 (or 15038)
Contracted *Nandina* with white-margined flower	$4xy+2x+2y+1$	80601 (or 1)
Contracted *Nandina* with self-colored flower	$x^2y^2+2x^2y+2xy^2+x^2+y^2$	412050000 (or 5112)
Total	$16x^2y^2+32x^2y+32xy^2+64xy+16x^2+32x+16y^2+32y+16$	6626089616 (or 81810)

four sets of allelomorphs, and produce the following sorts of gametes: (Here the gametic ratio in the linkage of d and f^a is denoted by x:1 (x>1), and that of n_n and f^b by y:1 (y>1)).

$$\mathrm{xy}DN_nF^aF^b+\mathrm{x}DN_nF^af^b+\mathrm{y}DN_nf^aF^b+\mathrm{x}Dn_nF^aF^b+\mathrm{y}dN_nF^aF^b+1DN_nf^af^b$$
$$+\mathrm{xy}Dn_nF^af^b+1dN_nF^af^b+1Dn_nf^aF^b+\mathrm{xy}dN_nf^aF^b+1dn_nF^aF^b+\mathrm{y}Dn_nf^af^b$$
$$+\mathrm{x}dN_nf^af^b+\mathrm{y}dn_nF^af^b+\mathrm{x}dn_nf^aF^b+\mathrm{xy}dn_nf^af^b$$

As formerly worked out, the numerical relations are about x=100 and

$y=200$, so if we apply these actual figures the above formula will be changed into:

$20000DN_nF^aF^b + 100DN_nF^af^b + 200DN_nf^aF^b + 100Dn_nF^aF^b$
$+ 200dN_nF^aF^b + 1DN_nf^af^b + 20000Dn_nF^af^b + 1dN_nF^af^b + 1Dn_nf^aF^b$
$+ 20000dN_nf^aF^b + 1dn_nF^aF^b + 200Dn_nf^af^b + 100dN_nf^af^b + 200dn_nF^af^b$
$+ 100dn_nf^aF^b + 20000dn_nf^af^b$

On fertilization with free combinations of these 16 different gametes, the F_2 members may be produced at an unusual ratio as indicated in table 9. If a calculation is made with this ratio as a basis, the theoretical numbers applied to the actual F_2 total will be as shown in table 10.

TABLE 10

Comparison of observed and expected numbers in F_2 of cross 26 × UN.

	NORMAL		*Nandina*		*Contracta*		CONTRACTED *Nandina*		TOTAL
	White-margined	Self-colored	White-margined	Self-colored	White-margined	Self-colored	White-margined	Self-colored	
Observed	268	5	0	74*	4	68	0	16	435
Expected	*242.278*	*2.414*	*0.803*	*80.758*	*1.600*	*79.960*	*0.005*	*27.182*	*435*

$\chi^2=16.843$ $P=0.019$

* This includes "dotted"-margined flowers.

The value of P is very low, but we can hardly attribute this to any genetic speciality, because the discrepancy may be regarded mainly as having been influenced by a deficiency of the recessive classes, as formerly stated.

Next we may compare the actual F_3 results with our theoretical expectations. Class A of table 6 contains the pedigrees which bred true to the normal and white-margined flowers, so it includes those having the constitution of $DDN_nN_nF^aF^aF^bF^b$. The fact that these families carry both the dominant factors for the white margin in a double dose, gives reason for the expectation of all flowers to be "complete"-margined in their progeny. But the practical result was that they often contained a number of the "half"-margined flowers. This, however, is probably due to a mere fluctuation. The parental plants of Nos. 39 and 68 were recorded to be "half" in the development of its white margin on the corolla, but all offspring of these pedigrees grew up to bear "complete"-margined flowers. Number 40 showed almost the same result. These data may endorse the fact that the homozygotic white pattern sometimes expresses fluctuation to such an extent. Class B contains the pedigrees segregated in the factor for *Nandina* and one of the factors for the formation of white-margined flowers, as formerly cited, their genetic composition consequently being considered as $DDN_nn_nF^aF^aF^bf^b$. In this factor composition a linked assortment should occur in the subsequent generation. Class C includes three pedigrees. The individuals with white-margined flowers (from normal) and those with self-colored corollas (total of those from the normal and of all *Nandina*, the latter containing specimens with "dotted"-margined flowers) being 63 and 49, respectively, in the total of this class, the result coincided perfectly with the segregating ratio of 9:7. From this ratio the pedigrees are regarded to have segregated into two complemental factors. Here segregation took place in the *Nandina* allelomorphs, but bred true to the normal habit (*non-contracta*). Consequently their genetic constitution may be $DDN_nn_nF^af^aF^bf^b$. This is the only class containing the pedigrees produced by crossover gametes+non-crossover gametes, as far as the trial breeding tests were concerned; namely, they are the result of $DN_nF^af^b+Dn_nf^aF^b$ or $Dn_nf^af^b+DN_nF^aF^b$, the former in each union being a crossover gamete, while the latter is a non-crossover gamete. In table 11, the observed total of these three pedigrees and the expectation are given in comparison. Here the expected numbers of the normals with white-margined and with self-colored flowers, *Nandina* with white-margined and with self-colored flowers are calculated from a ratio of 362406:122406:1203:160401, respectively.

TABLE 11

Summarized data of 3 pedigrees in class C of table 6.

	NORMAL					*Nandina*					TOTAL
	"Complete"-margined	"Half"-margined	"Slight"-margined	"Dotted" margined	Self-colored	"Complete"-margined	"Nalf"-margined	"Slight"-margined	"Dotted"-margined	Self-colored	
Total observed	14	22	20	7	17	0	0	0	6	26	
	63				17	0			32		112
Expected	62.792				21.208	0.208			27.792		112

$\chi^2=2.250$ P=0.527

The value of P is 0.527, indicating about one occurrence with such deviation in every two trials. The pedigrees assembled in class D are considered to be $DdN_nN_nF^aF^aF^bf^b$, as their segregation in the F_3 generation took place in both allelomorphic sets of F^b, f^b and D, d. Class E, however, contains the pedigrees making tetrahybrid segregation as F_2 did.

As to the segregation of the corolla pattern in this class, we count, in total, 612 white-margined flowers (the sum of those plants found in the normal and *contracta*) and 398 self-colored flowers (the sum of those having "dotted"-margined flowers and self-colored ones of *Nandina*, and all individuals with self-colored flowers found in *contracta* and contracted *Nandina*); the result having considerably deviated from the theoretical numbers of 568 and 442 calculated from the ratio of 9:7 (here deviation = ±43.875, probable error = ±10.63), though in the main we may accept the occurrence of the ratio in this case. Both recessive *Nandina* and *contracta* individuals were fewer in number than expected, and especially the discrepancy of the ratio in the segregation of the latter was evident. These properties of deviation may have been employed somewhat remarkably, in obtaining such a departure in the segregating numbers with respect to the corolla pattern, as a close association of the characteristics took place. Table 12 shows an application of the theoretical numbers to the observed ones of those 16 pedigrees.

TABLE 12

Summarized data of 16 pedigrees in class E of table 6.

	NORMAL		*Nandina*		*Contracta*		Contracted *Nandina*		TOTAL
	White-margined	Self-colored	White-margined	Self-colored	White-margined	Self-colored	White-margined	Self-colored	
Total observed	609	11	0	196	3	155	0	36	1010
Expected	*562.531*	*5.605*	*1.864*	*187.506*	*3.716*	*185.654*	*0.012*	*63.111*	*1009.999*

$\chi^2 = 28.340$ P = 0.0002

The goodness of fit is remarkably low, but here we cannot make any consideration for further steps than the recognition of such a superficial cause as above-described.

The foregoing statements were concerned with the examination of the offspring of normal F_2. In attempting a further discussion on the results of *Nandina* F_2, we may ascertain that class F can be considered as $DDn_nn_nF^aF^af^bf^b$ from its results, and class G as $Ddn_nn_nF^af^af^bf^b$. In regard to *contracta* F_2, we presume the genetic compositions to be $ddN_nN_nf^af^aF^bF^b$ and $ddN_nn_nf^af^aF^bf^b$ for classes H and I, respectively. Lastly, the pedigrees of contracted *Nandina*, of which the data were gathered in class J, are considered to be propagated in quadruple recessive homozygotes.

Tracing back the F_2 individuals from the F_3 data, an attempt can be made to analyze the nature of the origin of the gametes, whether produced by crossover mechanism or not, which made up the F_2 plants after fertilization. The classes from F to J are all self-colored flowers produced by the lack of at least one dominant factor of the two complemental ones necessary for the production of the white-margined pattern, so it is impossible to determine precisely what genotypes they are in respect to the factors for this character. For this reason, it seems advisable to omit these from our consideration in making the following statement with the data furnished by the classes from A to E. As the first consideration, it will be necessary to try to demonstrate the relation between the allelomorphic set, *d* and f^a. Classes A and B being double dominant heterozygotes in these factors, the parental plants of 20 pedigrees of these classes are noted as being produced by the union of two non-crossover gametes, and the same statement will hold good with the origin of 22 pedigrees collected in classes D and E, because these pedigrees produced coupling segregations. The genetic composition of the three pedigrees tabulated in class C, however, being regarded as DdF^af^a, their development should start from the union of DF^a, a non-crossover gamete, and Df^a, a crossover one. Summing up these considerations we have:

Non-crossover gametes × non-crossover gametes	42 pedigrees
Crossover gametes × non-crossover gametes	3 pedigrees
Total	45 pedigrees

To produce 45 parental plants of these F_3 pedigrees, the gametes needed for their development will be duplicated with the total of 45. Of these pedigrees, however, 3 being derived, each from the union of a non-crossover gamete and a crossover one, the items of the above total are:

Non-crossover gametes	87
Crossover gametes	3
Total	90

In this calculation, the frequency of crossoversis 3.33 percent,practically within 1 percent of the expected value. The same treatment is made as to the relationship between n_n and f^b allelomorphic set, but no crossover gametes could be detected.

A word may be added here in respect to the variation in degree of the white margin. The cases in which "dotted"-margined flowers appeared among the normals in the hybrid progeny were confined to the families segregated into both complemental factors of white-margined flowers. Thus the F_2 families and classes C and E of F_3 contained a considerable number of "dotted"-margined flowers. They all segregated as dihybrid, as to the corolla pattern (see class E of table 6). From this, it is quite certain that the "dotted"-margined flower of the normal carries the factors of the white margin in a double heterozygous condition. The parental plants of the pedigrees collected in classes C and E carry double heterozygotic constitution for the corolla pattern, but their white margins were "slight" in degree, with a few exceptions of those having "half"-margined and "dotted"-margined flowers. So we can safely say that the plants double-heterozygotic for the factors of the corolla pattern have the "slight" margins, which, however, vary perceptibly at times into its neighboring degree; that is, from the "dotted" margin to the "half" one. In class E, 11 normal individuals of the self-colored flower were counted, the number somewhat exceeding the expected. It cannot be denied that these self-colored flowers contain some false individuals, carrying double heterozygotic constitution for the corolla pattern, as the result of an extreme fluctuation in their manifestation. We had no case in our trial breeding experiments of normals bearing self-colored flowers, so the evidence to prove this occurrence is still wanting; but such possibility cannot be completely rejected, as far as the writer's experience is concerned. If the case should actually occur, the variation of the white margin in the double heterozygotic plants will be extended practically to the degree of absence or self-colored flower, one of the furthest extremities of the quantitative variation of this pattern.

Now we proceed to discuss the problem as to what degree of development in the corolla pattern corresponds to the flowers single-heterozygotic to the factor F^a or F^b. In regard to the effect of the factor F^a, we stated already in the former section that its single heterozygotic expression is "half" or "slight." The parental F_2 plants of class D in table 6 carry a constitution of $F^af^aF^bF^b$ for the corolla pattern and they had practically their corresponding degree of white margin. The factor F^b, however, does not appreciably reduce the amount of manifestation of the corolla pattern in its single heterozygotic state. This evidence may be accepted by looking over the contents of class B in table 6. In most cases the parental plants of these pedigrees had practically the white pattern in a "complete" degree, but they contained some "half" ones. As stated before, the factor f^b working with F^a frequently produces "dotted"-margined flowers in *Nandina* plants. In this, we have an opportunity of seeing what difference there may be in the proportion of the manifestation of the "dotted" pattern if they are homozygotic or heterozygotic as to the factor F^a. The results contained in classes B, C, E, F and G will give something in such a comparative study. Of these, classes B and F are composed of pedigrees homozygotic to F^a, while the rest, C, E and G, are heterozygotic to it. The total of the former *Nandina* plants consisted of 35.22 percent "dotted"-patterned flowers, but in the latter *Nandina* only 18.58 percent were "dotted." In this calculation, we cannot neglect the interference of fluctuating variation, which, although slight in quantity, may to some extent affect the values so that it will be almost impossible to rely absolutely on them. The difference between these two values, however, is too much to be regarded as the result of a simple variation; namely, the former value amounts to nearly twice the latter. So we conclude that the chance of the appearance of "dotted"-margined flowers in *Nandina* is considerably large in the homozygotes for the factor F^a, when compared with its heterozygotes. In summing up the results, the modes of manifestation of various genotypes in regard to the factors for corolla marking are (table 13):

TABLE 13

The corresponding manifestations of quantitative development in the corolla pattern to respective genotypes.

GENOTYPE	PRESENCE AND ABSENCE OF WHITE MARGIN AND ITS DEGREE	
	In normal	In *Nandina*
$F^aF^aF^bF^b$	"Complete"	white-margined (degree not clear)
$F^af^aF^bF^b$	"Half"—"slight"	
$F^aF^aF^bf^b$	"Complete"	
$F^af^aF^bf^b$	"Half"—"slight"—"dotted"	
$f^af^aF^bF^b$	Absent	Self-colored*
$f^af^aF^bf^b$	"	" "
$F^aF^af^bf^b$	"	"Dotted"—absent
$F^af^af^bf^b$	"	" †
$f^af^af^bf^b$	"	Absent

* See discussion in next section.

† The proportion of appearance of "dotted"-margined flower should be less than that of the above.

It must be understood that the table represents only an outline of the various manifestations of the pattern factors under different combinations.

The result of cross M3×WN

Another case in which F_2 made dihybrid segregation by the occurrence of two complemental factors for the white-margined corolla, was observed in the hybrid progeny of M3, normal (*non-contracta*), bearing "Rangiku" leaves[4] and blue flowers with a complete white margin on the corolla, and WN, normal, bearing *Nandina* leaves and white flowers with a colored tube. The F_1 plants grew up to the *non-contracta* bearing normal, three-lobed leaves and blooming colored flowers with a "slight" white margin. They gave rise to a population of F_2 as will be seen in table 14.

TABLE 14

F_2 data of cross M3×WN.

PEDIGREE	NORMAL			*Nandina*			TOTAL
	White margined	Self-colored	White Flower	White margined	Self-colored*	White Flower	
1	53	15	23	0	22	5	118
2	29	9	15	0	9	3	65
Total	82	24	38	0	31	8	183
Expected	*76.948*	*25.989*	*34.313*	*0.255*	*34.058*	*11.438*	*183.01*

$\chi^2=2.443$ P=0.783

* This contains "dotted"-margined flowers.

Since this paper is chiefly to represent the data concerning the segregation of both corolla pattern and *Nandina*, the results in regard to that of other characters are omitted for brevity. And as the field record of white corolla pattern contained some points to be dissatisfied with the classification of degree, they are put together under "white"-margined without being classified, as usual. The white flowers, however, are taken in the segregating number, because we cannot make direct observation on these flowers, as to whether they have white margin or not.

The individuals having a white-margined flower and a self-colored one counted 82 and 55 respectively, besides 46 individuals of white flowers. The white flower behaves as a simple recessive to the colored condition, due to the segregation of the *R,r* allelomorphs, of which the writer has made a genetic statement elsewhere (IMAI 1921). If the factors for white-margined flowers and that for white flowers are freely assorted—the case is practically true as will be stated later—we can analyze the factors concerned in the present case by omitting the actual number of the white flower segregants. The observed numbers above described of 82 white-margined flowers and 55 self-colored ones correspond almost to the expected numbers of 77.06 and 59.94, respectively, calculated on the basic ratio of 9:7. So here we have also the segregation of two complemental

[4] "Rangiku" is an irregular leaf form having sharp pointed lobes, and transmitted as a recessive to the normal.

factors for the corolla pattern as already met with in the former cross. The present cross, however, is somewhat different, as far as we can see, from the result of the former in this respect that, two parents being both homozygotic to the normal habit, we had no segregation concerning the *contracta*. As a result of such a factor relation, one of the factors for the corolla pattern has no linked factor, and so it is expected to segregate freely in the hybrid progeny of this cross. Thus the allelomorphs concerned may be considered to be four sets: $N_n{:}n_n$, $R{:}r$, $F^a{:}f^a$ and $F^b{:}f^b$. The genetic constitution of M3, one of the parents in this cross, then, must be a quadruple dominant homozygote, $N_nN_nRRF^aF^aF^bF^b$, which is a normal-leafed one bearing colored flowers with a "complete" white margin. Its partner, WN, however, is regarded as a quadruple recessive homozygote, because it is a *Nandina*-leafed one with white flowers, and because the segregation of the corolla pattern occurred in a dihybrid scheme in this cross. In the F_2 segregation, however, the actual result was somewhat complicated by the occurrence of linkage between the factors, n_n and f^b. Thus the theoretical numbers of the F_2 segregation are calculated in table 15.

TABLE 15

Theoretical ratio of F_2 in cross M3×WN.

CHARACTER	FORMULA	THEORETICAL RATIO
Normal with white-margined flower	$27y^2+36y+18$	1087218
Normal with self-colored flower	$9y^2+36y+18$	367218
Normal with white flower	$12y^2+24y+12$	484812
Nandina with white-margined flower	$18y+9$	3609
Nandina with self-colored flower	$12y^2+6y+3$	481203
Nandina with white flower	$4y^2+8y+4$	161604
Total	$64y^2+128y+64$	2585664

The application of the above ratio to the actual total is represented at the bottom of table 14, showing a high rate of possibility of about eight for every ten trials.

In looking over the F_3 table (table 16), we see a perfect agreement, in the main points, between expectation and observation. We will pass over the explanation as to how our factor hypothesis holds good in each pedigree, but give some discussions on several remarkable points, which may need a statement.

1. The factor r assorts itself freely from the *Nandina* factor and both complemental factors for the corolla pattern. This evidence can be seen from the segregating contents of the F_2 offspring and the F_3 classes of

TABLE 16
F_2 data of cross M3×WN.

CHARACTER OF PARENTAL F_1	REFERENCE	GENETIC CONSTITUTION	PEDIGREE NUMBER	NORMAL "Complete"-margined	NORMAL "Half"-margined	NORMAL "Slight"-margined	NORMAL "Dotted"-margined	NORMAL Self-colored	NORMAL White Flower	*Nandina* "Complete"-margined	*Nandina* "Half"-margined	*Nandina* "Slight"-margined	*Nandina* "Dotted"-margined	*Nandina* Self-colored	*Nandina* White Flower	TOTAL
Normal with white-margined flower	A	$N?R?F^a f^a F^b?$	35		1			1								2
	B	$NNRrF^aF^aF^bF^b$	4	1	11	4			4							20
	C	$NNRrF^a f^a F^bF^b$	12	1	2	7	2	6	3							21
			17	2	2	8		2	5							19
	D	$NnRRF^aF^aF^bf^b$	5	14	22	3	1						2	5		47
	E	$NnRRF^a f^a F^b f^b$	8	1	2	14	10	16					1	16		60
			16	2	2	8		6					2	3		23
			36		5	9	1	4					1	6		26
	F	$NnRrF^aF^aF^b f^b$	1	1	10	17	1		10				4	1	2	46
			19	1	12	7			11				7	4	1	43
			30		6	7			11					8	1	33
	G	$NnRrF^a f^a F^b f^b$	3	1	7	14	7	12	6				4	9	4	64
			6	3	5	8	2	5	10				3	5	2	43
			14			4		4	4					1		13
			34	1	7	11		2	7				3	2	2	35
Normal with self-colored flower	H	$NNRr f^a f^a(F^bF^b)$	total of 2 pedigrees					46	16							62
	I	$NnRR f^a f^a(F^b f^b)$	9					11						2		13
	J	$NnRr f^a f^a(F^b f^b)$	total of 6 pedigrees					81	31					30	6	148
Nandina with self-colored flower	K	$nnRrF^a? \ (f^b f^b)$	18										5	10	5	20
			22										4	19	5	28
			24										1	5	2	8
Normal with white flower	L	$NNrr? \ (F^bF^b)$	total of 3 pedigrees						35							35
	M	$Nnrr \ ? \ (F^b f^b)$	total of 5 pedigrees						97						30	127
Nandina with white flower	N	$nnrr \ ? \ (f^b f^b)$	total of 2 pedigrees												15	15

C, F, G and J. There may be found considerable deviation in the segregating numbers in some cases, probably to be attributed to a superficial disturbance.

2. It is interesting that all flowers of the *Nandina*-leafed individuals, in the classes I and J of the F_3 pedigrees, had self-colored corollas, not a single plant revealing itself to bear "dotted"-margined flowers. From this evidence it may be said that the factor F^b cannot manifest a "dotted" margin on its corollas of *Nandina* individuals when its complemental factor is recessively homozygotic. The absence of the corolla pattern on these flowers indicates a conspicuous difference in nature between the factors, F^a and F^b, because the former frequently produces a "dotted"-margined flower in the f^bf^b plants as was already shown. In class K, we cannot make an accurate calculation of the behaviors of the F^a, f^a allelomorphs, directly from these data, but the appearance of "dotted"-margined flowers will tell, at least, that the pedigrees of this class are either homozygotic to the factor F^a or its heterozygotes, though the factor f^b is in a double dose. From the fact that the factor f^b links very closely with the factor n_n, one may deduce that these *Nandina* pedigrees naturally contain the linked recessive factor in a homozygous condition as the cross was a coupling case.

TABLE 17

Total data of linked dihybrid segregation of n_n and f^b, contained in this paper.

SOURCE	NORMAL WITH WHITE-MARGINED FLOWER	NORMAL WITH SELF-COLORED FLOWER	*Nandina* WITH WHITE-MARGINED FLOWER	*Nandina* WITH SELF-COLORED FLOWER	TOTAL
26×UN-F_3(class B)	496	3	0	132	631
M3×WN-F_3(class D)	40	0	0	7	47
-F_3(class F)	62	0	0	24	86
Total	598	3	0	163	764
Expected	*571.5*	*1.5*	*1.5*	*189.5*	*764*

$\chi^2=7.935$ P=0.048

3. The pedigrees under class D made a dihybrid segregation in regard to the factors for *Nandina* and white-marginedness, and the segregation occurred in high linkage. In this connection, the dependent assortment shows that it was the allelomorphs of F^bf^b that were concerned with the segregation of the flower pattern in the present case. Class F also made a similar segregation as far as we understood the appearance of white-flowered segregants. If these data are added to class B, table 6, of the cross 26×UN, we should have the total results of linked dihybrid segregation of n_n and f^b, as indicated in table 17. From

these segregating numbers the frequency of crossovers is calculated to be 0.39 percent.

4. As already described, the development of the white margin on the corolla differs in a degree in a single heterozygotic condition, according as it rests on f^a or f^b. This is also the case in the present cross. The white margin in this cross, however, is weaker in development than in the previous cross, the margin generally becoming considerably smaller in quantity, probably due to the fact that the record was taken somewhat late. As a result of this condition, the impression in the difference of manifestation between the two cases above cited is practically weakened when compared with that of the cross, 26×UN, but we must recognize this underlying fact. Table 18 may serve to make this matter plainer, for in it the actual degree and value of the white margin, in every genotype, are given.

TABLE 18

Quantitative evidence of the difference in manifestation of single heterozygote between factors f^a and f^b in cross M3×WN.

REFERENCE	GENOTYPE O PARENTAL F_2	CLASS	NUMBER OF F_3 INDIVIDUAL	AVERAGE PATTERN VALUE*	GENOTYPE OF SEGREGATING WHITE-MARGINED FLOWER AND ITS RATIO
(1)	$F^aF^aF^bF^b$	B	16	2.81	all $F^aF^aF^bF^b$
(2)	$F^a f^aF^bF^b$	C	24	2.33	$1F^aF^aF^bF^b+2F^a f^aF^bF^b$
(3)	$F^aF^aF^b f^b$	D & F	106	2.78	$1F^aF^aF^bF^b+2F^aF^aF^b f^b$
(4)	$F^a f^aF^b f^b$	E & G	124	2.19	$1F^aF^aF^bF^b+2F^a f^aF^bF^b+2F^aF^aF^bf^b$ $+4F^af^aF^bf^b$

* The value is calculated on the following basis:

Degree	Value
"Dotted"-margined flower	1
"Slight"-margined flower	2
"Half"-margined flower	3
"Complete"-margined flower	4

The value of double homozygote (1) is expected to be 4, while its actual value is only 2.81. Ref. 3 is single heterozygotic to the factor f^b, so it must contain two-thirds of the plants carrying $F^aF^aF^bf^b$ among the white-margined flowers. Practically, however, no considerable diminution in degree is recognizable, because the factor f^b in a heterozygotic condition does not apparently affect the development of the white margin, as stated in a former page. Actually, the average value of the F_3 progeny of such plants was 2.78, which is very close to (1). The value of (2), on the contrary, ought to be somewhat lower, when compared with those of (1) and (3), for it contains the factor f^a in a heterozygotic state. The actual value of (2) was 2.33, and thus confirmed our expectation. The factor f^b does not diminish the development of white margin in a marked quantity in the F^aF^a composition, but somewhat in the F^af^a constitution. The value of (4), therefore, is a step lower, in comparison with the other three classes, as (4) carries double-heterozygotic factors. Actually 2.19 was the average value of (4), and was the lowest development.

5. Considerable difference observed in the frequency of occurrence of "dotted"-margined flowers in the *Nandina* individuals between the pedigrees of homozygotes for the factor F^a and of its heterozygote has been dealt with in the results of the former cross. In the present cross, this conclusion also holds good throughout the data obtained. The frequencies are 41.94 percent and 25 percent in the homozygotes and heterozygotes, respectively. The former is nearly twice as many as the latter, the quantitative relation being almost the same as that observed in the previous cross. From these figures, we can ascertain that about twice as many "dotted"-margined flowers are produced, when both the factors, F^a and f^b are homozygotic, compared with the $F^af^af^bf^b$ in *Nandina* plants. If the factor f^a is homozygous, the plant, however, will remain invariably as bearing self-colored flowers, whatever the constitution of its complemental allelomorphs, F^b:f^b, may be.

6. Owing to the occurrence of close linkage between the factors n_n and f^b the arrangement of the genotypic ratios of F_2 must be considerably modified. In an attempt to inspect the items in regard to the gametes which contributed to the formation of F_2 zygotes, we obtained a result as follows: As far as the breeding shows, 14 individuals (classes below H were omitted from the calculation for the reason that in these we cannot accurately detect the behavior of the allelomorphs, F^b:f^b, without making a test breeding), which were used in the F_3 trial, are all the products of the union of non-crossover gametes. If the number thus tested in the previous cross, 26×UN, is added to this, we have 59 in total, and still all of them are the zygotes of non-crossover gametes+non-crossover gametes. The frequency of crossovers between the factors n_n and f^b being considered to be at about 0.4 percent, which tells us the expectation that one plant derived from the union of non-crossover and crossover gametes is obtainable in testing about 125 F_2. So it will be natural to have no zygote having such an origin in the test of such a small number as 59 against 125.

7. The genetic ratio of the white-margined F_2 is expected to be

$1F^aF^aF^bF^b:2F^af^aF^bF^b:2F^aF^aF^bf^b:4F^af^aF^bf^b$. The data are shown in table 19 together with those of 26×UN.

TABLE 19

Genotypic comparison of white-margined F_2 flowers of crosses 26×UN and M3×WN.

GENOTYPE	26×UN	M3×WN	TOTAL*	EXPECTED	RATIO
$F^aF^aF^bF^b$	6	1	7	6.444	1
$F^af^aF^bF^b$	5	2	7	12.889	2
$F^aF^aF^bf^b$	14	4	18	12.889	2
$F^af^aF^bf^b$	19	7	26	26.778	4
Total	44	14	58	58.000	9

$\chi^2=4.767$ P=0.193

* The pedigrees containing below ten individuals are not counted.

The value of P is so high that one case takes place in every five trials.

SEGREGATION OF THREE FACTORS CONCERNING THE PRODUCTION OF WHITE-MARGINED FLOWER

The F_1 plants obtained by the cross of 500, a pedigree of green-stemmed white flowers producing white seeds, and 65, a pedigree flowering with a purple corolla and "complete" margin, bore colored flowers, but, contrary to expectation, they lacked the corolla pattern. In F_2 we found a small number of white-margined flowers among self-colored offspring, besides

TABLE 20

F_2 data of cross 500×65.

PEDIGREE	"COMPLETE"-MARGINED	"HALF"-MARGINED	"SLIGHT"-AND "DOTTED"-MARGINED	SELF-COLORED	WHITE FLOWER	TOTAL	WHITE-MARGINED/SELF-COLORED
1	3	3	9	66	25	106	0.23
2	1	1	2	26	15	45	0.15
3	1	2	5	76	26	110	0.11
4		2	6	25	8	41	0.32
5	2	6	13	153	65	239	0.14
6	1	2	5	61	29	98	0.13
7		2	5	94	32	133	0.07
8	2	2	13	117	47	181	0.15
Total	10	20	58	618	247	953	0.14
		78					
Expected	*11.17*	*89.34*		*614.24*	*238.25*	*953*	*0.16*

$\chi^2=1.909$ P=0.593

the segregants of white flowers. Marginal white of the F_2 flowers varied in degree from "dotted" to "complete," as represented in table 20.

The proportion in number of white-margined flowers in the total data of F_2 is 0.14:1, as is shown at the bottom of the table, or, in other words, the latter goes over seven times as many as the former in number. These data admit of no hasty analysis. By the progress of the experiment, however, it is revealed that three factors contributed to bring about these results; namely, two complemental factors, as detected in the previous experiment, for the formation of the corolla pattern and an inhibitor for the production of the characteristic. The third factor, as studied by TAKEZAKI (1916) and HAGIWARA (1922b, 1926), entirely suppresses the manifestation of the complemental factors, resulting in a self-colored flower which cannot be distinguished from an ordinary recessive one. We shall designate this factor by a symbol of F^h. In the f^hf^h constitution, however, the effect of the complemental factors is visible. The genetic constitution of 65, therefore, can be readily considered to be $F^aF^aF^bF^bf^hf^h$ from the fact that it bears "complete"-margined flowers. To give self-colored flowers in F_1, and follow a trihybrid segregation in the subsequent generation, its partner, 500, must be $f^af^af^bf^bF^hF^h$ in constitution. The latter parent being practically a white flower, the present cross thus also

TABLE 21

Theoretical F_2 ratio expected in the segregation of three white-margin factors.

GENETIC COMPOSITION	ITS RATIO	CHARACTER	ITS RATIO
$XF^aF^bF^f$	81		
$Xf^aF^bF^f$	27		
$XF^af^bF^f$	27		
$Xf^af^bF^f$	9	self-colored flower	165
$Xf^aF^bf^f$	9		
$XF^af^bf^f$	9		
$Xf^af^bf^f$	3		
$XF^aF^bf^f$	27	white-margined flower	27
$xF^aF^bF^f$	27		
$xf^aF^bF^f$	9		
$xF^af^bF^f$	9		
$xF^aF^bf^f$	9	white flower	64
$xf^af^bF^f$	3		
$xf^aF^bf^f$	3		
$xF^af^bf^f$	3		
$xf^af_bf^f$	1		
Total	256		256

concerns the segregation of allelomorphs of colored flowers producing black seeds (strictly speaking, these should be considered as "colored seeds" against white ones) *versus* white flowers producing white seeds. Now we shall represent this allelomorphic set by X and x. The actual segregation in F_2, therefore, took place in these four pairs of factors, wherein the result indicated in table 21 is expected. For brevity, the genetic composition in this table is represented in a simplex form, instead of the duplex indication. Then the ratio in number of the self-colored, white-margined and white flowers is 165:27:64. Taking the former two into

TABLE 22

Genotypes of white-margined F_2 flowers and expectation on their behavior in the next generation.

CHARACTER OF F_2	GENOTYPE	ITS RATIO	TOTAL	RESULT OF F_3
	$F^aF^aF^bF^bF^fF^f$	1		
	$F^a f^aF^bF^bF^fF^f$	2		
	$F^aF^aF^b f^bF^fF^f$	2		
	$F^a f^aF^b f^bF^fF^f$	4		
	$F^aF^a f^b f^bF^fF^f$	1		
	$F^a f^a f^b f^bF^fF^f$	2		
	$F^aF^a f^b f^bF^f f^f$	2		
	$F^a f^a f^b f^bF^f f^f$	4		
Self-colored flower	$f^a f^aF^bF^bF^fF^f$	1	37	breed true to self-colored
	$f^a f^aF^b f^bF^fF^f$	2		
	$f^a f^aF^bF^bF^f f^f$	2		
	$f^a f^aF^b f^bF^f f^f$	4		
	$f^a f^a f^b f^bF^fF^f$	1		
	$f^a f^a f^b f^bF^f f^f$	2		
	$f^a f^aF^bF^b f^f f^f$	1		
	$f^a f^aF^b f^b f^f f^f$	2		
	$F^aF^a f^b f^b f^f f^f$	1		
	$F^a f^a f^b f^b f^f f^f$	2		
	$f^a f^a f^b f^b f^f f^f$	1		
	$F^aF^aF^bF^bF^f f^f$	2	2	segregate into 3 self-colored and 1 white-margined
	$F^a f^aF^bF^bF^f f^f$	4	8	segregate into 13 self-colored and 3 white-margined
	$F^aF^aF^b f^bF^f f^f$	4		
	$F^a f^aF^b f^bF^f f^f$	8	8	segregate into 55 self-colored and 9 white-margined
	$F^aF^aF^bF^b f^f f^f$	1	1	breeds true to white-margined
White-margined flower	$F^a f^aF^bF^b f^f f^f$	2	4	segregate into 3 white-margined and 1 self-colored
	$F^aF^aF^b f^b f^f f^f$	2		
	$F^a f^aF^b f^b f^f f^f$	4	4	segregate into 9 white-margined and 7 self-colored

consideration, the proportion is 1:0.16, very close to the actual ratio of 1:014. Next we shall make the statements by neglecting the segregation of white flowers. The expected ratio of self-colored and white-margined flowers in F_2 is 55:9, their F_2 genotypes and hereditary behavior in the F_3 progeny being indicated in table 22. The F_3 table (table 23) contains the results of 19 white-margined and 78 self-colored F_2 flowers, the total being

TABLE 23

F_3 data of cross 500 × 65.

PEDIGREE NUMBER	"COMPLETE" MARGINED	"HALF" MARGINED	"SLIGHT" MARGINED	"DOTTED" MARGINED	SELF-COLORED	WHITE FLOWER	TOTAL	WHITE MARGINED/ SELF-COLORED
Total of 14 pedigrees					643*		643	0.00
Total of 38 pedigrees					1216	343	1559	0.00
15				2	21		23	0.10
28	1	1	3	1	48		54	0.13
50	3	1	8	11	102		125	0.23
59			1	1	5		7	0.40
67				1	3		4	0.33
72				2	6		8	0.33
75		1	1	2	7		11	0.57
77		1	2	1	23		27	0.17
83	3	2	17	8	54		84	0.56
85			4	6	20		30	0.50
3		1	1	1	19	9	31	0.16
5	1	3	1	7	30	4	46	0.40
13	3	2	3	9	45	23	85	0.38
14			1	5	31	17	54	0.19
19		1	1	7	60	30	99	0.15
29		4	2	3	41	14	64	0.22
47	2	2	2	4	48	19	77	0.21
51				3	12	3	18	0.25
60	1		1	2	15	3	22	0.27
69	2	5	7	6	31	13	64	0.65
71				1	9	2	12	0.11
73				1	6	1	8	0.17
81		1	1	2	11	2	17	0.36
82		1	4	3	39	10	57	0.21
92	1	3	6	11	40	15	76	0.53
94			1	1	17	2	21	0.12

TABLE 23—*Continued.*

PEDIGREE NUMBER	"COMPLETE" MARGINED	"HALF" MARGINED	"SLIGHT" MARGINED	"DOTTED" MARGINED	SELF-COLORED	WHITE FLOWER	TOTAL	WHITE-MARGINED SELF-COLORED
9	5		**13**	15	37		70	0.89
18	2	6	**3**	4	14		29	1.07
44		2	2	**2**	7		13	0.86
62	3	6	27	**28**	73		137	0.88
96	2	2	3	**5**	11		23	1.09
7	3	9	7	**21**	33	14	87	1.21
17	6	5	**2**	8	25	21	67	0.84
38		3	**4**	6	11	8	32	1.18
55	2	3	**2**	1	11	7	26	0.73
66	10	16	**8**	19	38	22	113	1.39
12	3	7	**10**	3	13		36	1.77
30	8	**15**	22	23	21		89	3.24
64	1	4	5	**6**	2		18	8.00
90	1	3	**13**	3	9		29	2.22
20	2	1	5	**1**	4	10	23	2.25
32	2	3	**5**	2	2	2	16	6.00
35	5	4	**6**	4	8	15	42	2.38
53	2	2	**3**	1	4	7	19	2.00
63	3	3	4	**6**	2	4	22	8.00

* Bold face figures indicate the character of the respective parental F_2.

97. The F_3 expectation of the white-margined F_2 flowers is (1) one breeding true, (2) four throwing one-fourth self-colored flowers, (3) four making segregation into 9 white-margined and 7 self-colored flowers, in every 16 trials. Nineteen F_2 used for F_3 treatment had all flowers with an incomplete margin. Consequently their F_3 was expected to be either (2) or (3), and the results actually proved the case. Though the ratio of white-margined flowers *versus* self-colored ones in every pedigree, as indicated in table 23 is, to some extent, variable, still it can be divided into two categories; one the segregating ratio of 3:1 and the other the segregating one of 9:7.

TABLE 24

Summarized data of 9 pedigrees expected to have segregated in the 3:1 ratio.

	WHITE-MARGINED	SELF-COLORED	TOTAL
Total	191	65	256
Expected	*192*	*64*	*256*

In a tentative attempt, 9 pedigrees of numbers 12, 20, 30, 32, 35, 53, 63, 64 and 90 may be regarded to have segregated in the former ratio. If we omit the segregants of white flowers in this calculation, the total data will be as shown in table 24.

Thus the result will perfectly accord with the expected numbers calculated on the basis of a 3:1 ratio, if only one individual would occur in the other column. Those pedigrees, however, which are considered to have segregated in a 9:7 ratio are numbers 7, 9, 17, 18, 38, 44, 55, 62, 66 and 96, ten in number, and their total data are shown in table 25 with the white-flowered segregants omitted.

TABLE 25

Summarized data of 10 pedigrees expected to have segregated in the 9:7 ratio.

	WHITE-MARGINED	SELF-COLORED	TOTAL
Total	265	260	525
Expected	*295.31*	*229.69*	*525*

Thus the pedigrees segregating in a 3:1 ratio and of those assorting in a 9:7 one are 9 and 10, respectively, in number. This almost completely coincides with the expectation, where an equal ratio was foresighted. In regard to the "complete"-margined flowers, two F_2 plants were examined with their progeny, which were allowed to grow in the same seedling bed, together with those of white flowers, but no self-colored flowers were found among them, all flowers that came out day after day having completely white-margined corollas.

Out of 78 self-colored F_2, 26 threw some white-margined flowers in the subsequent generation, but the remaining 52 bred true to the type. On this occasion, the expectation is 37 pure pedigrees against 18 segregating ones, or, in other words, about a 2:1 ratio is expected, and the result was actually so. According to the expectation, the items of the segregating families should be 2:8:8 or 1:4:4 of (1) segregating into self-colored and white-margined flowers in a 3:1 ratio, (2) segregating into the same phenotypes in 13:3, and (3) segregating in the same classes in 55:9 as in the case of F_2. In these three segregating types, the proportion of the white-margined flowers to the self-colored ones is 0.33, 0.23 and 0.16,respectively. These figures practically so resemble one another that we can hardly draw a distinct line among them by looking at the F_3 data. But evidence in favor of this occurrence can be obtained in another way of estimation. Thus, if 26 self-colored F_2, which threw some white-margined flowers, are of these three segregating types in the ideal ratio, the white-margined flowers to the self-colored ones is estimated to be 0.212, in the total proportion, and the actual average ratio calculated on the basis of the total results of 26 pedigrees is 0.296, which is a confirmation, and not the rejection, of our hypothesis.

In the foregoing pages, the white flower segregants were often omitted in statement. But if a linkage occurs in the relation between the factor x and one of the three factors concerning the white margin, we should have a more or less misleading result for accurate discussion. Such, however, was not the case, the data supporting the view of independent inheritance.

THE OCCURRENCE OF A SPECIAL FACTOR WHICH VARIES THE QUANTITY OF THE WHITE MARGIN

The result of cross 324×71–2

In a cross between 324, a pedigree having white margin on its corolla, and 71–2, a pedigree of white flowers with a colored stem, white-margined flowers were produced as F_1. The degree in development of the white margin was "slight" in these hybrids. And we have the following record for F_2:

TABLE 26

F_2 data of cross 324×71–2.

PEDIGREE	"COMPLETE"-MARGINED	"HALF"-MARGINED	"SLIGHT"-MARGINED	"DOTTED"-MARGINED	SELF-COLORED	WHITE FLOWER	TOTAL
1	0	1	5	14	22	10	52
2	1	1	12	16	23	19	72
3	1	1	4	8	11	8	33
4	2	5	18	35	50	35	145
5	5	8	18	21	10	24	86
6	1	5	13	11	11	10	51
Total	10	21	70	105	127	106	439

Of six families, four, from 1 to 4, were cultivated in our experimental garden of the Agricultural College, TOKYO IMPERIAL UNIVERSITY, while the other two, 5 and 6, were grown on a private farm at Komaba. They all segregated about one-fourth of white flowers, and still contained a number of self-colored flowers. In regard to the contents of the colored flowers, the ratio of white-margined flowers to the self-colored ones varies considerably in different families; namely:

TABLE 27

Varied proportions of white-margined flowers to self-colored ones in the F_2 families.

PEDIGREE	WHITE MARGINED FLOWER : SELF-COLORED FLOWER		
1	0.91:1	1.17:1	1.62:1
2	1.30:1		
3	1.27:1		
4	1.20:1		
5	5.20:1	3.90:1	
6	2.73:1		

The ratios of the families, 1 to 4, are relatively low, being close upon one another, while those of families, 5 and 6, however, are very high, the average being 1.17:1 and 3.90:1 respectively. From the point that these ratios correspond almost exactly to 9:7 (=1.29:1) and 3:1, respectively, some may accept the occurrence of different segregating ratios in different families which are derived from the same parents. But this is quite incorrect. It can hardly be thought that these unexpected results are due to the heterozygotic nature of the parents, because both parental pedigrees have been selfed through generations, and are considered each as a constant race, though one parent, 71–2, being a white flower, the behavior of the factors for the corolla pattern cannot be detected with its phenotype, which fact might admit of doubt. The variability of the ratio is more evident in F_3. The average ratio of the white-margined flowers to the self-colored ones in 18 F_3 pedigrees, (see table 28), which produced some self-colored flowers, is 11.51, being much larger than 1.62, the average value of F_2. The F_3 plants were raised on a private farm leased at Yoyogi, a neighboring division of Komaba, in 1923. The conspicious difference in the average values of F_2, therefore, is attributable to the difference of the plots on which the pedigrees were grown. This fact may throw light on the special nature of a factor now dealt with. An unexpected result also came out from an examination of the progeny of self-colored F_2, which, according to our experience, should either breed true or throw some white-margined flowers in the subsequent generation. Actually their offspring, without an exception, consisted of a great number of white-margined flowers. The test was made with 15 F_2 plants, and the results were quite clear of the unusual behavior, due to the fluctuating manifestation of a corolla pattern. Then, another question will arise as to whether all variations witnessed here in degree of the white margin are also due to a mere fluctuation or not. Though there may have existed a considerable fluctuation in this connection, the variation is attributed, to some extent, to the segregation of a definite factor as the basis of the whole variability.

TABLE 28

F_3 data of cross 324×71-2.

PEDIGREE NUMBER	"COMPLETE"-MARGINED	"HALF"-MARGINED	"SLIGHT"-MARGINED	"DOTTED"-MARGINED	SELF-COLORED	WHITE FLOWER WITH COLORED STEM	TOTAL	AVERAGE PATTERN VALUE	WHITE-MARGINED/SELF-COLORED
23	18	12*	6				36	3.33	100.00
4	6	18	**2**	2			28	3.00	100.00
6			**1**				1	2.00	100.00
9	7	8	**0**				15	3.47	100.00
17	16	12	**5**				33	3.33	100.00
45	29	15	**3**	2			49	3.45	100.00
8	6	12	**5**	2	4	12	41	2.48	6.25
26	13	21	**28**	6	3	20	91	2.49	22.67
41	4	12	**15**	5	1		37	2.35	36.00
19	15	13	10	**0**			38	3.13	100.00
21	2	14	1	**1**			18	2.94	100.00
27		4	2	**1**	1		8	2.13	7.00
28		2	5	**1**			8	2.13	100.00
30	20	8	3	**1**			32	3.47	100.00
39	8	21	3	**0**			32	3.15	100.00
1	1	4	3	**5**	2	3	18	1.80	6.50
2	6	3	3	**3**		7	22	2.80	100.00
12			3	**4**		1	8	1.43	100.00
14	8	13	9	**3**	1	6	40	2.71	33.00
22	1		4	**1**		2	8	2.17	100.00
33	2	5	7	**1**		3	18	2.53	100.00
25		6	13	3	**3**		25	1.88	7.33
29		9	5	1	**0**		15	2.53	100.00
35	1	1	3	6	**9**		20	0.95	1.22
38	11	6	15	20	**9**		61	1.83	5.78
46		1	3		**0**		4	2.25	100.00
47			1	3	**2**		6	0.83	2.00
3			1	1	**1**	1	4	1.00	2.00
5	2	6	4	3	**1**	8	24	2.31	15.00
11	3	11	15	8	**5**	14	56	1.98	7.40
13	1	10	9	8	**1**	4	33	2.07	28.00
18	1	1	4	3	**1**	5	15	1.80	9.00
20	4	8	16	17	**11**	18	74	1.59	4.09
31	4	8	16	19	**4**	20	71	1.78	11.75
40	1	3	4	11	**9**	9	37	1.14	2.11
44	4	6	16	4	**0**	11	41	2.33	100.00
Total of 11 pedigrees						**312**	312		

* Bold face figures indicate the character of the respective parental F_2.

A comparison is made between the degree of the parental F_2 and the average value of their progeny in table 29.

TABLE 29

Average value of the F_3 pedigrees in each pattern.

DEGREE AND VALUE OF WHITE MARGIN OF F_2	NUMBER OF F_3 PEDIGREE	AVERAGE VALUE
Absence (0)	15	1.82
"Dotted" (1)	12	2.53
"Slight" (2)	8	2.82
"Half" (3)	1	3.33
"Complete" (4)	..	..

A definite correlation exists between them, and their condition tells us of the occurrence of a factor which plays some rôle in the variation of degree of the white margin. Consequently, the self-colored flowers, which appeared in F_2, are not the results of a mere fluctuation of the ordinary white-margined flowers, but they are due to the failure in manifestation of the corolla pattern as a result of fluctuation of the plants having the genetic constitution for a little quantity of white margin. The little quantity of the corolla pattern here is an effect of a special factor, which is novel to us. The above table indicates a tendency that the F_2 flowers, which have a large quantity of white margin, have generally the corresponding genetic constitution. But the fact that the white-margined flowers are much higher in their proportion to the self-colored ones in F_3 than to that of F_2, and that each F_3 pedigree is generally higher in the average value than its corresponding parental F_2, represents how strongly the environment affected on the general manifestation of the factor in question. Such conspicuous variability is also found in the pattern value of the F_2 families as is given in table 30.

TABLE 30

Varied pattern values of F_2 families.

PEDIGREE	PATTERN VALUE		
1	0.64		
2	0.89		
3	0.92	0.83	
4	0.85		1.05
5	1.63	1.52	
6	1.37		

Here again a condiderable difference is recognized between the pedigrees of Nos. 1 to 4 and those of Nos. 5 and 6, which were raised on different farms as already observed in the ratio of white-margined flowers to self-colored ones. The average pattern value of the former is 0.83, while that of the latter 1.53, the difference between the two ratios being attributed to the diversity of the environments under which the plants were raised. And further, if we take the F_3 data the variability is more remarkable, the variation table as regards the frequency distribution of the pattern value of the F_3 pedigrees being indicated in table 31.

TABLE 31

Frequency distribution of F_2 in regard to the quantitative variation of white margin.

						●	□	●	●						
	●				○	●	□	□	○					●	
	□			○	○	□	○	○	○	○	●	●	●	●	
	○	○		○	○	○	○	○	○	○	●	●	●	●	
0.7	0.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7

Pattern value

In the above table, the symbol of ○ denotes F_2 segregated white flowers, while the one homozygous for the color producing factor is designated by ●. The F_2 represented by the symbol of □ gave only colored flowers, but the progeny is too small in number to ascertain whether it is homozygotic or heterozygotic in this respect. The frequency distribution represents a mountain with modal classes neighboring the mean value (2.32), and suggests the occurrence of a small mountain on each side. The result is thus no longer a single curve, but an aspect of frequency distribution resembling the variation curve developed on the basis of a 1:2:1 ratio of monohybrid. Such conspicuous variation cannot be attributed to a mere fluctuation, because the F_3 plants were raised on the same plot under the same treatment of cultivation. We may take this opportunity to explain the hereditary behavior of the corolla pattern by giving a factor, F^f, which reduces the quantity of the white margin in its development. The manifestation by this factor in the corolla pattern, however, is very variable in its nature, though, in the main, weakening in the development of the pattern when the plant contains the F^fF^f factors. The white margin of plants heterozygotic for this factor may fluctuate forward and backward, in a moderate degree, in the representation of their trait. Some fluctuation has been observed in the manifestation of the corolla pattern even in the $F^aF^aF^bF^bf^ff^f$[5] plants, according to the nature of the environments of the cultivation, the time of record, etc., the pedigrees

[5] The white-margined flowers usually carry such a genetic constitution.

making up the right mountain in the variation table are probably no more than the fluctuating representation of this genotype.[6] So the environment, in which F_2 was reared and recorded, greatly reduced by degrees the development of the corolla pattern. The self-colored F_2 flowers were not only $F^aF^aF^bF^bF^fF^f$ genotype, but they contained some $F^aF^aF^bF^bF^ff^f$, the F_3 data representing the mixture of the different genotypes in the self-colored flowers. In this connection, we may expect that the average pattern value of the pedigrees, in which appearance of the self-colored flowers is high in percentage, are lower, on the whole, than those of the low pedigree whether they are the same or not in their genetic constitution. Six F_2 families are the same in their factor complex, but they differ often very widely one from another. Notwithstanding their wide variability, there exists a good plus correlation between the frequency in appearance of self-colored flowers and the average pattern value. The same is true with the F_3 data, table 32 being given for this evidence.

TABLE 32

Data showing a fair plus correlation between pattern value and proportion of white-margined flowers to self-colored ones.

AVERAGE PATTERN VALUE	NUMBER OF PEDIGREE	AVERAGE PROPORTION OF WHITE-MARGINED FLOWER TO SELF-COLORED ONE
Below 1	3	1.74
1–2	7	6.66
2–3	8	19.41

In this case, we have again a plus correlation between them. An additional statement may be written in this connection. Of the F_3 pedigrees, those throwing no self-colored flowers are not so confined as to f^ff^f constitution, so their average pattern value may not be remarkably high, though higher, on the whole, than that of the pedigrees mixed with the self-colored flowers. In practice, we had the results noted in table 33.

TABLE 33

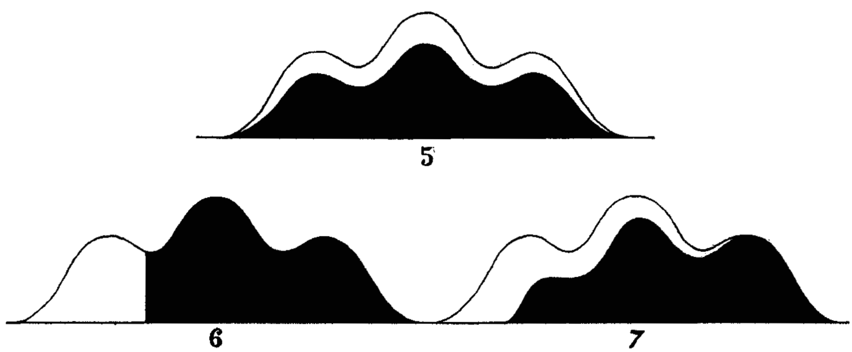
Average pattern values of white-margined pedigrees segregating self-colored flowers and those breeding true.

GENETIC TYPE OF WHITE-MARGINED PEDIGREE	NUMBER OF PEDIGREE	AVERAGE PATTERN VALUE
Pedigree breeding true	18	2.75
Pedigree segregating self-colored flower	18	1.90

The data prove this expectation. The evidence above-mentioned will serve to show the behavior of the factor F^f.

[6] We cannot deny the presumed occurrence of any modifier in this case.

The one fact that cannot be overlooked is a remarkable difference in the average pattern value between the F_3 pedigrees segregating white flowers and those giving only colored flowers. When the pedigrees containing below ten individuals were impartially omitted, the homozygotic and heterozygotic ones for the color producing factor were both 14 in number. The average pattern value of the former is 2.84, while that of the latter only 2.13, the difference seeming to be too large to be left unnoticed. Roughly speaking, this difference cannot be attributed to the effect of the environment which played an important rôle in different ways with different pedigrees, because they were raised in the same plot under uni-



FIGURES 5 to 7. The trimodal curves were diagrammatically figured by arranging the theoretical frequency of variation in the degree of the corolla pattern in the white-margined F_2 flowers based on a 1:2:1 ratio. The white part represents the distribution of the white flowers (in which the degree of the corolla pattern cannot be phenotypically recognized, but the figures are only theoretically attempted), while the black part is applied to that of the colored flowers. Figures 5, 6 and 7 show the cases of independent segregation, complete and partial repulsions, respectively.

form treatments and their record taken almost at the same time. This difference is recognized also by inspecting the variation table given on a former page. The frequency distribution of the homozygotic and heterozygotic colored flowers conspicuously differed one from the other. The members of the small mountain on the right-hand side supposed to have the $f^f f^f$ constitution consisted only of homozygotes for the factor X, while the contrary is the case with the members of the mountain on the left, though the latter is not so remarkable as the former. From such evidence, we may conclude that the factor f^f links with c (IMAI 1921), the factor for the white flower with a red stem. The F_2 plants, which were recognized to carry the $f^f f^f$ constitution, were all homozygous in regard to the factor C, and consequently the linkage in this case is a repulsion scheme, which is also expected by the parental constitution. The source of the factor F^f

must be searched after 71–2, because its partner 324 had a complete margin on its corolla.

When the factors f^f and c make an independent segregation, the distributive frequency of the white flowers in F_2 should be as represented in figure 5, where the members are equally distributed over a three-modal curve, and accordingly the variation curve of white margin in the colored flowers is not affected in its quality. When a complete repulsion takes place between them, the F_1 should give only two sorts of gametes, Cf^f and cF^f, so the result of F_2 is $1CCf^ff^f+2CcF^ff^f+1ccF^fF^f$. This formula, if shown graphically, will be as given in figure 6, where the distribution of the colored flowers is limited to two mountains, but the other is occupied by the

TABLE 34

Data for classification of results by which linkage calculation may be attempted.

	CC						Cc					
	F^fF^f		F^ff^f		f^ff^f		F^fF^f		F^ff^f		f^ff^f	
	Pedigree number	Pattern value	Pedigree number	Pattern value	Pedigree number	Pattern value	Pedigree number	Pattern value	Pedigree number	Pattern value	Pedigree number	Pattern value
	35	0.95	6*	2.00	4	3.00	3*	1.00	1	1.80		
	48*	0.85	25	1.88	9	3.47	12†	1.43	2	2.80		
			27*	2.13	17	3.33	40*	1.14	5	2.31		
			28*	2.13	19	3.13			8	2.48		
			29	2.53	21	2.94			11	1.98		
			38	1.83	23	3.33			13	2.07		
Frequency			41	2.35	30	3.47			14	2.71		
			46*	2.25	39	3.15			18	1.80		
					45	3.45			20	1.59		
									22*	2.17		
									26	2.49		
									31	1.78		
									33	2.53		
									44	2.33		
Total pedigrees containing many individuals	1		4		9		1		13		0	
Total pedigrees containing many individuals	1		4		0		2		1		0	
Grand total	..		..		..		3		14		0	

* Those containing a few individuals.

† This pedigree entered on the left side of middle mountain in the variation table, but it may be more properly put into this class by its segregating aspect.

members of the white flower. As a result, a quite asymmetrical curve in regard to the distribution of the colored flowers is obtained. While the linkage is moderately high, the frequency of the colored flowers may be somewhat asymmetrical, but it is represented by a trimodal curve or the like, as figure 7, due to the unbalanced distribution of the white flowers. To which of these does the result of the present cross correspond? As figured in table 31, the observed frequency of the corolla pattern in the white-margined F_2 flowers is represented by a somewhat trimodal curve but, strictly speaking, the left mountain is meagre, and resembles the figure 7. With a view to determining the value of linkage quantitatively, table 34 is arranged by inspecting the result of frequency of the white-margined F_2 flowers.

The classification cannot be as precise as wished for, owing to a conspicuous fluctuation in the manifestation of the factor F^f, but as it is based on the average pattern value of each pedigree and the segregating condition of the degree of the corolla pattern, the table will show an outline in this connection. Of 36 pedigrees, 19 segregated white flowers, while the rest did not. It may be a little premature to accept that all the latter are homozygous as to the color producing factor, because it contains some pedigrees which consisted of a few individuals. When these pedigrees (containing less than ten individuals) are omitted in calculation, we have 14 in number. In comparison of numbers between the homozygotic and heterozygotic pedigrees, such meagre ones must be omitted from the counting, also in the latter, for the sake of impartiality in this connection. Thus we have 14 pedigrees of both homozygotic and heterozygotic colored flowers. These numbers are deviated from the expected ratio of 1:2, but they may be regarded as a chance result. Owing to this considerable deviation, an examination in the contents of genotypes may be separately attempted. Of the 14 pedigrees homozygotic to the color producing factor, only one has the F^fF^f constitution, 4 the F^ff^f and the 9 remaining are considered to have the f^ff^f. In his case, the segregating scheme being a repulsion, four sorts of gamete of CF, Cf, cF and cf produced by F_1 should be formed in a ratio of 1:x:x:1 when the nature of unequal gametic production is represented by x ($x>1$). The pedigrees homozygotic to the factor C, therefore, are expected theoretically to be: F^fF^f 1, F^ff^f 2x and f^ff^f x^2. If the observed numbers cited above are applied to those figures, we shall have such values as $x=2$ from the relation of F^fF^f and F^ff^f, $x=3$ from F^fF^f and f^ff^f, and $x=4.5$ from F^ff^f and f^ff^f, or the average of $x=3.17$. If the same attempt is made with the pedigrees heterozygotic to the color producing factor, the result will be as follows: In this examination, however, our consideration is not prevented from being based on 17 pedigrees of the total quoted in the table, which contains some meagre pedigrees. Of these, F^fF^f is 3, F^ff^f 14 and f^ff^f 0, while, theoretically, the first is expected to be 2x, the second $2x^2+2$ and the last 2x. In these data, we have x=4.66 from the relation of the former two. If we venture to make an average between this value and the former, 3.17, the result is x=3.92 or the value of x is practically 4. This indicates that the gametic ratio between the factors *c* and f^f is 1:4 or, in other words, 20 percent of the crossovers. This linkage value does not necessarily indicate an accurate intensity of the real occurrence, because the estimation was not based on fully reliable data. So the value has to be accepted as a rough estimation of the linkage between *c* and f^f.

The result of cross 50×71–2

One of the parents used in this case being different from that of the former cross, while the other is similar in both cases, the data of the hybrid progeny gave another instance of a high fluctuation of the corolla pattern. Thus, in the present cross, 50 was used instead of 324, but the degree of the white margin in both races is the same; namely, a "complete" state. The F_1 plants came out to bear the "slight"-margined flowers, and the F_2 is composed of the following segregants:

TABLE 35

F_2 data of cross 50×71–2.

PEDIGREE	"COMPLETE"-MARGINED	"HALF"-MARGINED	"SLIGHT"-MARGINED	"DOTTED"-MARGINED	SELF-COLORED	WHITE FLOWER	TOTAL	PATTERN VALUE	WHITE-MARGINED/SELF-COLORED
1	2	12	18	2	0	16	50	2.33	100.00
2	2	18	19	12	1	9	61	2.15	51.00
3	6	26	34	14	2	30	112	2.24	40.00
4	5	10	36	3	0	17	71	2.31	100.00
5	2	2	17	4	1	9	35	2.00	25.00
6	6	6	29	13	0	23	77	2.09	100.00
7	10	10	22	14	0	24	80	2.29	100.00
8	5	5	48	28	4	27	131	1.93	25.00
9	6	6	26	19	0	29	92	2.10	100.00
Total	44	115	249	109	8	184	709	2.15	64.63

These F_2 plants were all reared on the ground of Yoyogi in 1922. Roughly speaking, the data of the nine families are the same. The segregation of one-fourth white flowers in every family was nothing but expectation, while the meagre appearance of the self-colored flowers is worthy of note. We used 71–2 as one of the parents, so the appearance of self-colored flowers may be naturally expected in this cross, owing to the countenance of the factor F^f. The frequency of the mixture, however, was very low, and the numerical ratio of the white-margined flowers to the self-colored ones is 64.63:1 in the total number. This is hardly comparable with 1.62:1, the corresponding F_2 ratio in the previous cross. The special nature of the factor F^f may account for such a result, but an additional assumption may be made with the occurrence of a modifier, which affects the function of the factor F^f. If the low frequency of the self-colored flowers is a hereditary tendency, the latter may be the case, while, if it is only a potential occurrence, the former concept is true.

The pattern value shows that the nine families are almost equally represented by fluctuating about 2.15. This is a little over twice as much as 1.05 of the corresponding value of 324×71–2. Thus the higher pattern value accompanies a higher ratio of white-margined flowers against self-colored ones. The actual frequency distribution of the F_2 plants in classes of the pattern degree occurred in a single curve (see the total number of table 35).

The results of crosses RA×71–2 and RB×71–2

In the previous crosses, in which 71–2 were used as one of the parents, the complemental factors for the corolla pattern were homozygous in regard to their constitution. On the contrary, each one (RA or RB) of the parents of the present crosses, taken as a partner with 71–2, had self-colored flowers and consequently they have to be regarded as $f^af^aF^bF^bf^ff^f$. In this state, F_1 had a very meagre quantity of white margin, and in F_2 they brought about the result found in table 36.

TABLE 36

F_2 data of crosses RA×71–2 and RB×71–2.

CROSS	WHITE-MARGINED	SELF-COLORED	WHITE FLOWER WITH COLORED STEM	TOTAL
RA×71–2	71	81	62	214
RB×71–2	40	51	25	116
Total	111	132	87	330

The record is not taken of the variation in quantity of the corolla pattern in the present crosses, and the table will not contain any classification in this respect.

In this connection, the genotypes expected in F_2 should be as indicated in table 37.

TABLE 37

Theoretical genotypes and their ratio in F_2.

REFERENCE	GENOTYPE	RATIO
A	$F^aF^aF^bF^bf^ff^f$	1 } 3
	$F^af^aF^bF^bf^ff^f$	2
B	$F^aF^aF^bF^bF^f f^f$	2
C	$F^aF^aF^bF^bF^fF^f$	1
D	$F^a f^aF^bF^bF^f f^f$	4
E	$F^a f^aF^bF^bF^fF^f$	2
F	$f^a f^aF^bF^b f^f f^f$	1 } 4
	$f^a f^aF^bF^bF^f f^f$	2
	$f^a f^aF^bF^bF^fF^f$	1

Of these classes, genotype A is invariably white-margined flowers, B is the same in the majority of the cases, though it sometimes assumes self-colored flowers, C and D are expected to mix together in a greater number of self-colored flowers than in the former case, and lastly E is most conspicuous in the production of self-colored flowers. The plants grouped in class F are certainly all self-colored flowers whatever their outer conditions may be. The proportion of the total numbers of this class is 25 percent, but the practical percentage may be enlarged by absorbing the number of the self colored flowers produced by the fluctuation of the classes B, C, D and E. A, however, is the only class that contains plants invariably blooming with a white-margined corolla, and their proportion to the total number is 18.75 percent. Practically the percentage of the mixture of the self-colored flowers should vary between 25, as its absolute minimum quantity, and 81.25 its maximum quantity, according to the nature of the environment, and the actual proportion was 54.32 percent which indicates nearly an intermediate value between the two limits. Of 54.32 percent, 25 percent is due to the results in segregation of the factor f^a, or of the plants carrying a constitution for blooming invariably self-colored flowers (corresponding to class F). Reducing this 25 percent, from the total, we obtain 29.32 percent, which is attributed theoretically to the result in occurrence of factor F^f. These F_2 were raised in the neighboring plot of F_2 pedigrees, 5 and 6, of 324×71–2 on the ground of Yoyogi under the same treatment. Roughly speaking, the two may be tentatively interpreted as due to the same chance fluctuation in regard to the development of the corolla pattern. The mixing proportion of the self-colored flowers in F_2 pedigrees, 5 and 6, of the cross 324×71–2 is 20.39 percent. For the reason that this figure represents the percentage of the failing manifestation of $F^aF^aF^bF^bF^fF^f$ and $F^aF^aF^bF^bF^ff^f$ (practically the latter concerns little in this case), the value may possibly be applied to the present cross. This 20.39 percent indicating the proportion of the false self-colored flowers among the $F^aF^aF^bF^b$ constitution, the value changed into 25 percent×25.39 percent = 15.29 percent in these present crosses, because in them the invariable mixture of 25 percent of the self-colored flowers was expected in the segregation of the factor f^a. The figure 15.29 percent represents the failing proportion in the manifestation of the white-margined flower to the total in the effect of the factor F^f. Practically, however, the subject is made more complicated by the segregation of the factor f^a. B and C are the only classes concerning this factor alone. The proportion of the plants having such a constitution corresponds to merely one-fourth of those in the cross 324×71–2. So the figure 15.25 percent must be divided by four, the result being 3.82 percent, which should be regarded as the failing proportion in the effect of the factor F^f alone. Thus the value concerning the classes B and C is tentatively determined. If we add this figure to 25 percent, the percentage of the properly self-colored flowers, we have 28.82 percent, the reduction of which from the total proportion of 54.32 percent leaves 25.50 percent, the corresponding proportionn of the co-working effect of the factors f^a and F^b. The items of the self-colored flowers in regard to their origin may be summed up in table 38.

TABLE 38

Classification of self-colored F_2 flowers in respect to the probable cause of their appearance.

PROBABLE CAUSE BY WHICH SELF-COLORED F_2 PLANTS WERE PRODUCED	RELATIVE PERCENTAGE	ABSOLUTE PERCENTAGE
Self-colored proper	25.00	46.02
Produced by the effect of F^f alone	3.82	7.03
Produced by the co-working effect of f^a and F^f	25.50	46.95
Total	54.32	100.00

Between the cases in which only the factor F^f is affected and in which both factors f^a and F^f are concerned, we have a conspicuous difference in the percentage of their quantity. This indicates a greater chance of production in the self-colored flowers heterozygotic to the factor f^a compared with the homozygotic F^aF^a. But we cannot directly compare the failing proportion of these two cases with their figures, because their mixing ratio is different in F_2. Theoretically the homozygotic and heterozygotic plants for the factor F^a are found in a 1:2 ratio among F_2. In comparison, we preliminarily divide 25.50 percent (the failing proportion of the latter) by two, and if the quotient 12.75 percent is compared with 3.83 percent (the failing proportion of the former), it proportionates in 3.38:1. This figure indicates an absolute difference in the failing proportion in the manifestation of the white-margined flower between the two classes above cited.

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SUMMARY

1. The white margin behaves, in most cases, as a simple dominant to the self-colored condition in inheritance. In F_2 of such crosses, segregation took place at a 3:1 ratio, and the results of the next generation were quite in accordance with expectation. The hybrid flowers have white margins to the degree of "half" or nearly so.

2. In most cases, the segregating ratio was recorded as 3:1, while in some crosses it occurred just in accordance with 1:2:1. In the latter cases, a sharp discontinuous segregation of homozygotic and heterozygotic white-margined flowers seems to occur in their manifestation.

3. The segregation of the self-colored flowers from the white-margined ones in the ordinary cases concerns the F^a, f^a allelomorphs.

4. The manifestation of the white margin, however, is not simple in some cases. The complete production of the white pattern is effected by two factors of F^a and F^b. Neither factor can produce by itself the marking on the corolla, acting complementally.

5. The fact that the white margin links very closely with *contracta* is already pointed out by IMAI (1919, 1925) and HAGIWARA (1922a, 1926). According to the estimation of linkage value the frequency of crossing over is about 1 percent. In this linkage, the factors concerned are f^a and d (*contracta*).

6. The other complemental factor F^b links with n_n, the factor for

Nandina. The frequency of crossing over between them is about 0.5 percent, or actually 0.39 percent.

7. In the combination of F^a and f^b the flower generally assumes full coloration, but when they enter into *Nandina* leaves their manifestation is often a "dotted"-margined corolla. So, the "dotted"-margined flowers of *Nandina* may be usually different from those of non-*Nandina* in their factor constitution.

8. The quantity of the white margin varies considerably according to the genetic constitution, whether homozygotic or heterozygotic for the pattern factor or factors. In a normal culture the manifestation of various factor combinations is estimated in table 13.

9. In a cross we met with the segregation of three factors, which concerned the production of the white margin; namely, two complemental factors and their inhibitor. The third factor F^h, as studied by TAKEZAKI (1916) and HAGIWARA (1922b, 1926), entirely suppresses the manifestation of the complemental factors, resulting in a self-colored flower. Under this circumstance, the ratio of white-margined and self-colored flowers in F_2 is 55:9. The results of F_2 and F_3 were quite in accordance with expectations developed by our hypothesis.

10. Our experience tells the fact that the self-colored flowers, which, produced in the hybrid progeny, should either breed true or throw some white-margined flowers in the subsequent generation, while we had quite exceptional cases, in which offspring, without an exception, consisted of a great number of white-margined flowers. This peculiar nature of inheritance is due to the fluctuating manifestation of a partial inhibitor F^f.

11. The factor F^f suppresses partially the formation of white margin, but the effect is very variable according to the environment under which the plants were raised. As the effect of this factor, the segregating ratio of white-margined and self-colored flowers varies in a considerable extent by the production of false self-colored flowers.

12. The partial inhibitor links moderately (about 20 percent) with c, the factor for the white flower with colored stem (IMAI 1921).

13. When the factor f^a is segregated simultaneously with the factor f^f the proportion of the self-colored flowers in F_2 should vary between 25 percent, as its absolute minimum quantity, and 81.25 percent, its maximum quantity, according to the nature of environment. In a cross, we actually obtained 54.32 percent of this proportion.

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