INHERITANCE OF DEFORMED LEAVES
IN PHARBITIS NIL

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(WITH ONE FIGURE)

Pharbitis Nil, the Japanese morning glory, exhibits several types of variegation. Usually the variegated leaves are more or less deformed by the insertion of white tissues which develop poorly. Another deformed leaf, called "deficient," is a peculiar type of irregularity. The affected leaves are so deformed that they might be mistaken for leaves affected either by disease or an attack of insects. The abnormality, however, is transmitted quite in a Mendelian ratio. The writer will attempt later to discuss the genetic behavior of the variegated and the deficient leaves.

Two types of variegation

COMMON VARIEGATION

The recessive nature of the variegated leaf (fig. 1 A, B) to the wholly colored condition has been successively proved by several writers, including TAKEZAKI (4), HAGIWARA (1), IMAI (2), and MIYAKE and IMAI (3). In raising an F_2 from the self-colored F_1, the alternative characters were segregated in the usual ratio of 3:1. Table I gives the total data obtained in the writer's breeding experiments during the past ten years.

The raising of the F_3 generation did not give any novel results beyond expectation. The F_3 data of two crosses are summarized in table II. This table gives quite the expected results of monohybrid inheritance.

"GEJIGEJI" VARIEGATION

In several crosses of the variegated and the wholly colored leaves, however, unexpected results were obtained. The parents of these specimens of the crossing having no unusual feature, I naturally expected to obtain therefrom the simplest segregation of 3:1 in the F_2 generation. Actually, however, the results were contrary to ex-

pectation, giving the addition of a new type of variegation among the variegated segregates. As the novelty assumes fine markings of variegation, it can be differentiated clearly from the common type.
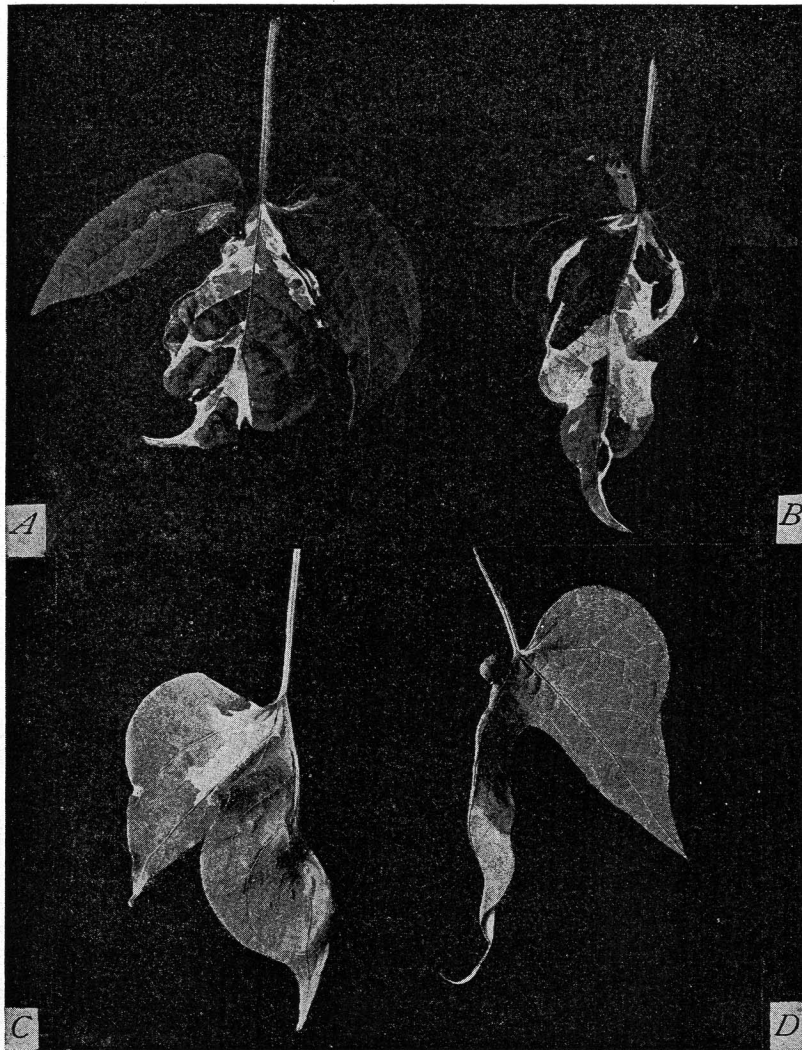

Fig. 1.—*A, B*, ordinarily variegated leaves, but much deformed; *C, D*, deficient leaves; *C*, variegated, *D*, self-colored.

TABLE I

Cross	Self-colored	Variegated	Total
RA×21-2	62	17	79
RA×M1	80	20	100
RA×IH	90	16	106
β×318	148	56	204
A×71-2	102	37	139
β×73	140	31	171
RA×71-2	219	70	289
SG×RA	53	22	75
319×170	127	43	170
α×65	81	27	108
170×A2	244	73	317
170×77	50	13	63
M2×58-2	99	30	129
M2×M4	105	37	142
S3×S5	50	13	63
65×505	302	84	386
WS×AG	37	10	47
HT×229-1	89	20	109
N113×HT	44	22	66
A4×86-2	73	20	93
65×A2	124	45	169
22-2×A2	28	12	40
22-1×A2	124	42	166
106×A2	57	21	78
22-1×A5	56	20	76
11-1×A4	105	18	123
318×13-3	85	32	117
FZ×A2	133	29	162
NT×A2	196	59	255
M3×A2	14	6	20
A1×A2	106	28	134
50×A2	279	94	373
A4×A2	151	40	191
324×316	245	69	314
319×321B	189	61	250
326×A5	490	149	639
Total	4605.00	1398.00	6003
Expected	4502.25	1500.75	6003

TABLE II

Cross	Character of F2	Total of Pedigrees	Self-colored	Variegated	Total
65×505	Self-colored	9	902		902
		35	2150	656	1806
	Variegated	14		780	780
324×316	Self-colored	20	646		646
		40	900	297	1197
	Variegated	9		89	89

This peculiar pattern of variegation reminds one of a Japanese saying "the marks left by the creeping Gejigeji" (a kind of myriapod). We might therefore call this type of variegation "Gejigeji." The observed number of such F_2 specimens was composed of three forms, the wholly colored, the commonly variegated, and the "Gejigeji" leaves, which appeared practically in the proportion of 12:3:1, a

TABLE III

CROSS	SELF-COLORED	ORDINARILY VARIEGATED	"GEJIGEJI" VARIEGATED	TOTAL
314×A2 1.............	65	18	4	87
2.............	27	2	2	31
3.............	52	22	3	77
4.............	63	17	10	90
RA×314.............	37	2	2	41
314×T5.............	24	6	3	33
Total.............	268.00	67.00	24.00	359
Expected.............	269.25	67.31	22.44	359

$x^2 = 0.11$; P = practically 1.

TABLE IV

F_2 DATA OF CROSS 314×A2

CHARACTER OF F_2	PEDIGREE NUMBER	SELF-COLORED	ORDINARILY VARIEGATED	"GEJIGEJI" VARIEGATED	TOTAL
Self-colored.............	A	18	5	....	23
	B	8	3	1	12
	C	30	7	1	38
Ordinarily variegated....	D	....	8	....	8
	E	....	15	4	19
	F	....	20	8	28
"Gejigeji" variegated....	G	....	....	5	5
	H	....	....	6	6

modified dihybrid ratio (table III). To explain this result factorially the following two allelomorphic pairs may be assumed: (1) V, v.—v is a factor for the variegation, while its large letter is responsible for the wholly colored condition; (2) V_g, v_g.—This allelomorphic pair is connected with the "Gejigeji" marking; the v_g factor produces its effect on the vv-carrying plants, while on the wholly colored leaves it has no visible effects.

On the assumption that these factors are present, the genetic composition of the F_1 plants may be considered to be VvV_gv_g. Then we should expect the following combinations of the factors in the F_2:

$$(VV_g + Vv_g + vV_g + vv_g)^2 =$$
$$\underbrace{1VVV_gV_g + 2VvV_gV_g + 2VVV_gv_g + 4VvV_gv_g}_{\text{9 self-colored}} + \underbrace{1vvV_gV_g + 2vvV_gv_g}_{\substack{\text{3 commonly} \\ \text{variegated}}} + \underbrace{1vvv_gv_g}_{\substack{\text{1 "Gejigeji"}}}$$

The segregating ratio of the actual numbers corresponds rather closely to the theoretical expectation. The F_3 specimens were raised in considerable numbers, but the majority of them were damaged by wandering dogs, which destroyed the seedlings by digging in the field and disturbing the labels. Only a part of them, therefore, which may assist the formulation of the above hypothesis, are given in table IV.

The F_3 data thus corresponded quite closely with the expectation. The Gejigeji variegation, due to the combined representation of two recessive factors, was thus fixed at its first appearance. In the F_2 segregation of the hybrids of the variegated and the wholly colored leaves, there was commonly the simplest ratio, as was stated in the former section, while in a few cases there appeared the Gejigeji form of variegation in addition to the two parental types. Why should we obtain such different results in apparently the same sort of matings? In considering this problem, it must be remembered that the exceptional crosses were performed invariably by utilizing 314, a pure green leafed specimen, as one of the parents. It was assumed that the factor v_g has no influence on the wholly colored leaf, and its presence cannot be detected by phenotypically examining the self-colored, VV-carrying leaves. The pedigree 314 bears the wholly colored leaf, but genetically it carries the v_g factor in the full condition; namely, the genetic composition is VVv_gv_g. On crossing this strain with the common variegated one (vvV_gV_g), we ought to obtain the self-colored leaves carrying the VvV_gv_g factors as the F_1 hybrid, and they actually do result in an F_2 consisting of three forms in the $12:3:1$ ratio.

Deficient leaf

APPEARANCE

From a packet of seeds furnished by a seedsman several plants were raised having cordate leaves. On self-fertilization they produced families which bred true to the cordate leaf. Examining the seedlings in the bed, the writer was struck with the unexpected occurrence of a few deformed cotyledons, each forming a certain peculiar shape with fine white splashes on the margin of the affected parts. Encouraged by this discovery, I transplanted all of the seedlings of the abnormal family, and watched their behavior throughout the season. This deformity occurred more frequently on the leaves, but in general its appearance was confined to only a part of the leaves of each individual. On account of the deformed condition of the leaves, this abnormality is called "deficient."[1] This deficient leaf suggests a disease appearance (fig. 1 *C, D*). The types of the deficient leaf are not confined to any particular shape, but it is possible for any part to be missing. As the deficiency does not appear in all leaves on one individual, however, observation must be made of every leaf throughout the plant growth, from the cotyledons to the upper leaves. The result of such an aberrant family is shown in table V. The deficient leaves thus appeared in only 10.6 per cent, where 25 per cent would be expected in the Mendelian inheritance. The deviation exceeds three times the standard error; so some statement would seem to be needed to account for the source of such an abnormal segregation.

EXPERIMENTAL DATA

One of the deficient leaves thus obtained, no. E225, was crossed with a normal leaf (M10). The F_1 plants of this reciprocal mating were quite normal, showing no deformed feature. In the following generation there occurred again some deficient leaves. The actual segregation is given in table VI.

The proportion of the deficient leaves is 12.3 per cent, the value being not more than half of that expected. The deviation again exceeds three times the standard error. Such repeated deviations

[1] The deficient leaf is not a novelty in the Japanese morning glory; this type has figured at times in literature, even in our classical books such as *Asagao Fu* (1830).

cannot be attributed to the result of mere chance sampling. For the
sake of convenience of statement, the discussion on this subject will
be postponed to later pages. Here let us consider the F_3 data. The
raising of this generation was made from 62 F_2 plants, out of which
56 were normal and the remaining 6 were deficient. The results of
these families are given in table VII. When the families including
less than 10 individuals are omitted from calculation, the homozy-
gotic and the heterozygotic normals are 13 and 20 respectively, where
we should theoretically expect 11 and 22; while 4 normals (nos. 19,
25, 26, and 40), which were previously excluded from this calcula-
tion, gave entirely unexpected results. They were apparently quite

TABLE V

	Normal	Deficient	Total
Observed.........	93 (89.4 per cent)	11 (10.6 per cent)	104
Expected.........	78	26	104

$D = \pm 15.00; \; SE = \pm 4.42.$

TABLE VI

	Normal	Deficient	Total
Observed.........	138 (87.7 per cent)	18 (12.3 per cent)	146
Expected.........	109.5	36.5	146

$D = \pm 18.5; \; SE = \pm 5.23.$

normal, yet the greater part of their offspring were composed of
deficient leaves, the proportion of the abnormal individuals being
87.6 per cent. Such aberrant results are quite similar to those ob-
served in the progeny of the deficient leaves. Six deficient leaves
were taken for raising the F_3 generation, and the resultant offspring
contained a few normals among the deficient leaves. With reference
to each deficient family, some of them bred true to the abnormality,
while the others produced some normal rogues. On the average,
the abnormal plants appeared in 86.15 per cent of the cases, the
other 13.85 per cent being normal.

OCCURRENCE OF FALSE NORMALS

The abnormal features in the experimental data will be arranged
according to four points: (1) the extracted deficient leaves of the

TABLE VII

F_3 DATA OF CROSS M10×E225

Character of F_2	Total of Pedigrees	Normal	Deficient	Total
	22	511	....	511
	1.........	15	4	19
	2.........	76	14	90
	3.........	43	5	48
	4.........	30	5	35
	5.........	16	10	26
	6.........	31	11	42
	11.........	2	1	3
	17.........	37	11	48
	18.........	44	7	51
	20.........	5	1	6
	21.........	4	1	5
	22.........	9	3	12
	23.........	10	4	14
	27.........	15	10	25
	29.........	4	2	6
	31.........	18	6	24
Normal	32.........	28	6	34
	38.........	17	6	23
	41.........	8	1	9
	42.........	26	5	31
	45.........	9	2	11
	46.........	33	5	38
	47.........	52	7	59
	51.........	18	5	23
	52.........	33	6	39
	53.........	22	4	26
	54.........	3	1	4
	55.........	43	3	46
	57.........	20	2	22
	60.........	3	1	4
	Total......	674.00	149.00	823
	Expected...	617.75	205.25	823
	19.........	7	13	20
	25.........	4	28	32
False normal	26.........	3	48	51
	40.........	2	24	26
	Total......	16	113	129
	Expected...	0	129	129
	10.........	0	15	15
	30.........	2	6	8
	34.........	7	36	43
Deficient	39.........	0	1	1
	50.........	0	2	2
	59.........	9	52	61
	Total......	18	112	130
	Expected...	0	130	130

segregating families were fewer than expected; (2) a few plants, which were noted as normal, gave progenies which included deficient leaves for the large part; (3) the deficient leaves sometimes did not breed true to the abnormality, producing a few individuals which appeared quite normal; (4) the results of (2) and (3) are fundamentally the same, although the phenotype of the parental plants was not common, the one being normal, while the other was deficient.

The following statements attempt to explain these unusual results. As already stated, the deficient feature is not displayed on all leaves of the individual plant; its occurrence is confined to a few leaves only. In such an abnormal state we might expect the occurrence of the exceptional plants which failed to manifest the deficient feature on the leaves throughout their plant growth. As the Japanese morning glory is an annual, dying in the autumn, its life duration is specially limited. If the species were a perennial and made practically unlimited growth, such a false normal might not be obtained. As the so-called false normals are the result of the false representation of the deficient factors throughout their development, they should produce offspring with the same characteristics as those of the deficient plants, frequently producing some false normals among the deficient descendants. In the segregating generation of the normal parents we should also expect the occurrence of false normals. This fluctuating representation should lower the value of the percentage of the deficient leaves in the segregating families.

Failing frequency

To estimate the failing frequency of the deficient representation, all available results are collected in table VIII, where the data of the progeny of the false normals are added on the assumption that they are genetically the same. Thus the estimate shows that the failing frequency on an average is 13.13 per cent.

Next we shall attempt to find the failing value from the data of the segregating normal families. When the value is represented by x, the extracted deficient leaves must be $1-x$, and consequently the normal sisters are $3+x$. From this relation we establish the following formula:

$$\frac{\text{number of normal leaves}}{\text{number of deficient leaves}} = \frac{3+x}{1-x}.$$

If we apply the F_3 result to this formula, the value of x is 0.4324, or about 43 per cent. Comparing this value with that obtained with the deficient families, the former is more than three times the latter, the difference being too great to be accounted for by mere chance sampling. Then how can we explain it? For the sake of brevity we shall call the failing proportion calculated from the data in the deficient families the "direct value," and that estimated from the results of the segregating progeny of the normals the "indirect value." This designation shows that the segregating aspect of the deficient leaves is different one from the other. In the calculation of the direct value we are dealing with the pure families for the deficient leaf, and as the operation on these was made immediately, the value may not need any qualification. The indirect value, how-

TABLE VIII

	NORMAL	DEFICIENT	TOTAL	PERCENTAGE OF NORMAL
Observed {from "false normal"	16	113	129	12.40
{from "deficient"	18	112	130	13.85
Total	34	225	259	13.13

ever, depends upon the difference of the theoretical and the observed numbers; so it might include the deviation caused by unequal mortality, or any other such cause as might be present. Strictly speaking, the so-called indirect value of failing frequency, then, might be of absolute frequency plus a. As to the reason why such a difference between the direct and the indirect values cannot be attributed to mere chance of deviation, the following statements may be added. The indirect values of the F_2 data and the segregating family of unknown origin were 70 and 57.69 per cent respectively. Comparing these frequencies with 43 per cent of the F_3 data, the respective difference is not small, and might be accounted for partly by the chance deviation due to the small number of samplings. We cannot neglect the growing condition of the plants in the different years, however, for the influence of the deficient feature may depend largely upon the plant growth. So far as the writer's data showed, the indirect value was always higher than the direct by a consider-

able percentage; this fact may be related to the actual occurrence of unequal mortality between the normal and the deficient leaves, the latter being more damaged than the former in the course of their growth. The problem as to the time when such unequal mortality was introduced (after germination or at gametic or embryonic development), and the problem as to cause (weakness in the struggle or lethal fate) must be left undecided here. If the difference between the direct and the indirect values can be regarded as the result of the influence of unequal mortality, it may represent the actual frequency of the mortality of the deficient leaves. Such a difference amounted to 30.11 per cent in the F_3 data, showing that about 30 per cent[2] of unequal mortality occurred in the deficient members of that generation. The reason why only the F_3 data are cited for the comparison is that only these were grown in the same year and produced under about the same treatment as was given to the families from which the direct value was calculated.

Summary

Under the heading of "Deformed leaves" the writer dealt with the genetic behavior of the variegated and the deficient leaves. Summing up, the results are:

1. The variegation is transmitted as a simple recessive to the wholly colored condition.

2. In some crosses of the variegated and the wholly colored leaves there appeared unexpectedly a new type of variegation, called "Gejigeji," a faint variegated pattern.

3. The Gejigeji type is caused by a recessive factor, its dominant allelomorph being a normal variegation on the common basis of the double v.

4. The representation of the Gejigeji pattern is limited to the variegated individuals, the effect not being apparent in the self-colored leaves.

5. The ratio in the dihybrid segregation is a $12:3:1$ with reference to the wholly colored, the commonly variegated, and the Gejigeji leaves.

[2] From the fact that the homozygotic and the heterozygotic normals were obtained in the usual $1:2$ ratio, this value might not be related to any gametic cause.

6. The effect of the deficient leaf factor appears in both coty-
ledons and leaves.

7. The deficient leaf looks just like a symptom of some disease,
yet it is caused by a genetic factor. The abnormality behaves as a
simple recessive to the normal.

8. The representation of the deficient feature is not manifested
on all leaves of the plant, only a few leaves in one individual being
affected.

9. For this reason there may sometimes be found deficient leaves
which are quite normal, failing to show the proper characteristic
throughout their plant growth.

10. The false normals may either breed true or produce again a
few normals among the deficient offspring.

11. The failing proportion of the deficient leaves is about 13 per
cent in the direct calculation, while it attains about 40–70 per cent
in the indirect process.

12. The difference of two values may perhaps be mainly due to
the unequal mortality of the deficient leaves. If this is the case, the
so-called indirect value is of absolute frequency plus a.

13. We expect the value to fluctuate depending on the condition
of the plant growth.

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