Linkage studies in *Pharbitis Nil.* II

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(With 1 Text-figure)

(Received for publication, November 4, 1930)

Contents

Introduction

In *Pharbitis Nil*, the linkage value has been estimated from the data obtained by selfing the hybrids in the majority of cases. The recombination frequency due to crossing over is the same in macrosporogenesis and in microsporogenesis (Imai and Tabuchi in press). Therefore, the observed F_2 or F_3 data showing linkage can logically be analyzed on the basis of the general calculation, by squaring the gametic numbers of linked distribution. By the dependent and independent relations of the genes, the writer has found ten linkage groups in this plant (Imai 1929, in press, a). The haploid chromosomes being 15 in number, we expect still five other linkage groups or independent genes. In this paper, the writer describes the present state of knowledge in the linkage studies of *Pharbitis*, adding some facts newly obtained.

Variegated linkage group

The v L. G.[1] was known to include seven genes, variegated (v), crumpled-1 (c1), Blown-1 (B1), fasciated-3 (f3), brown (br), faded (fd)

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[1] "L. G." is an abbreviation of "linkage group".

and couple (cu) (Imai in press, a and b). The frequency of recombination is known to be 17·5 per cent for v and cl, 29·3 per cent for v and Bl, roughly 20—25 per cent for v and f3, about 31·0 per cent for v and fd and about 37·7 per cent for v and cu. The locus br is situated not far from those of v and cl. The gene faded constitutes a triple system of allelomorphs with normal and smeary (fd^s). By a rescent investigation (Imai and Tabuchi in press), the relative loci of variegated, crumpled-1 and Blown-1 were determined by backcross experiments. These three genes are located in the variegated chromosome, with the arrangement v—cl—Bl, at a distance of 14·9 units between v and cl, and 17·8 units between cl and Bl. These are the most accurate figures obtainable at present to indicate the relative distance between them. In 1930, the writer raised a number of F_2 from different crosses, with the intension of showing linkage relations between these genes. Fifteen in all from the cross of variegated crumpled-1 Blown-1 and normal were observed. Six of them, however, were not recorded on crumpled-1, and their linked data of variegated and Blown-1 are shown in Table 1.

Table 1. F_2 showing linkage for variegated and Blown-1

Cross	Bl	+	Bl v	v	Total
MN × RF	159	75	62	9	305
H15 × RF	173	73	77	7	330
190 × RF......	127	51	56	2	236
S6 × RF	91	54	40	2	187
RF × SM	76	46	42	2	166
Total	626	299	277	22	1224
Expected	641	277	277	29	1224

$\chi^2 = 3{\cdot}788 \qquad P = 0{\cdot}29$

If the similar data, which were published in the previous paper (Imai 1929), are added to those given in Table 1, we have the following segregates:

	Bl	+	Blv	v	Total
Observed . . .	1048	504	458	42	2052
Expected . . .	1075	464	464	49	2052

$\chi^2 = 5{\cdot}204 \qquad P = 0{\cdot}16$

The expected numbers of both tables were calculated on the basis of 30·8 per cent of recombination for variegated and Blown-1, the figure of which was determined by backcross experiments. The F_2 data of the other nine crosses are shown in Table 2.

Table 2. F_2 showing linkage for variegated, crumpled-1 and Blown-1

Cross	Bl	+	Bl v	v	Bl cl	cl	Bl v cl	v cl	Total
422 × RF...	107	56	16	3	16	0	42	1	241
356 × RF...	129	65	18	4	20	1	41	1	279
ID × RF...	93	62	6	5	15	0	37	1	219
220 × RF...	145	68	18	6	19	1	54	0	311
YG × RF ..	104	57	17	2	17	2	49	1	249
410 × RF...	81	58	15	2	9	0	41	1	207
465 × RF...	152	71	17	2	21	1	42	2	308
455 × RF...	62	33	5	2	7	0	25	0	134
ML × RF...	111	53	9	5	13	0	27	2	220
Total	984	523	121	31	137	5	358	9	2168
Expected	989	489	112	36	147	2	377	16	2168

$\chi^2 = 13{\cdot}008 \quad P = 0{\cdot}07$

The expected number was calculated on the basis of recombination of 14·9 per cent for v and cl, 17·8 per cent for cl and Bl, and 30·8 per cent for v and Bl (coincidence, 0·35), which were obtained by backcross experiments (IMAI and TABUCHI in press). The "goodness of fit" is not high enough, or only one chance for every about fourteen samplings, and this is due partly to the low viability of Blown-1. In the backcross, the normal (against Blown-1) segregates appeared 1·09 times the Blown-1 segregates in number, where we expect the same number for both. In F_2, Blown-1 and normal were segregated into a 2·79: 1 ratio, where we expect 3 Blown-1: 1 normal. These figure definitely show the low viability of Blown-1.

Cordate linkage group

The co L. G. was noted as including three genes, cordate (co), feathered (fe) and semi-contracted (sc) (IMAI 1929). The linear arrangement of the three genes in the cordate chromosome is probably co—fe—sc, with an interval of 1·2 units between co and fe, and 17·7 units between fe and sc. This conclusion was drawn from the data showing the recombination frequency of 1·2 per cent for co and fe, 18·6 per cent for sc and co, and 17·7 per cent for fe and sc. Later experiments added the other three genes, precocious (pc), palmate (pl) and crêpe (cp), which were linked to each other, and in turn, linked to this linkage group (IMAI in press, a). Precocious is found to be linked with co, the recombination frequency being about 24·6 per cent. Crêpe is known to be almost independent or sometimes slightly linked with cordate. Both crêpe and feathered are respectively in a triple system of allelomorphs; namely, the former with normal and reversed (cp^r), and the latter with normal and creased (fe^c). The linkage data for creased and cordate were perfectly covered by an expectation made on the basis of 1·2 per cent of recombination (IMAI in press, b), the value of which was calculated from linkage for feathered and cordate (MIYAKE and IMAI 1925). Crossing crêpe (strain SU) with creased (strain RJ), the writer obtained an F_2 showing repulsion between crêpe and creased, the data collected being 137 normal, 55 crêpe, 47 creased and 5 crêpe creased. As formerly stated, linkage between cordate and crêpe occurs in a very slight degree, and the former is closely linked with feathered, or in 1·2 per cent of recombination. Therefore, creased (or feathered) may be linked slightly with crêpe, more or less, compared with the linkage value for cordate and crêpe. From the data shown above, the recombination frequency for creased and crêpe is calculated to be 33·8 per cent, which is rather higher than expectation. Owing to the fact that the figure is based on the repulsion data, the writer will reserve the determination of the frequency value for the future. The data, however, furnish an additional proof for the fact that the loci of the group including pc, pl and cp are situated in the cordate chromosome together with the group including co, fe and sc.

Contracted linkage group

In the former report (IMAI in press, a), the conclusion drawn in regard to the ct L. G. was as follows: "The contracted linkage group includes eleven genes, interaxil-green (ig), contracted (ct), Margined-1 (Mr1), tube-white (tw), intense (i), Margined-reduced (Mr-r), shrubby (sh), Rayed (Ry), cream (cr), duskish (dk) and purple (pr). Experimental data giving rough material make possible the drawing of a preliminary chromosome map of the following relations: Ry (0), cr (± 1·2), ig (10·3), sh (10·3 ± 3·1), ct (15·8), Mr1 (16·8), tw (23·9), i (48·5), Mr-r (48·5 ± 3·0). The loci of the two genes, duskish and purple, seem to be in the right part of the chromosome." Of these genes, both contracted and Margined-reduced constitute respectively a triple series of allelomorphs, the former with normal and star (ct^s), and the latter with normal and margined-slight (mr-r^s). Later experiments, however, nullified the previous conclusion that purple is included in this group; namely, pr is independent of ig, ct, Mr1, Ex (Expanded) and dk, which are located in the contracted chromosome. Therefore, purple may be separated from this linkage group. Two new members, flecked and Expanded, being added to the ct L. G. by a rescent investigation, the group now includes twelve genes, constituting the largest linkage group. In the following, some results newly obtained will be presented. Owing to the fact that we had no Margined-1 tube-white in the linked segregation, the writer regarded it as a fact that white margin cannot manifest itself in flowers with white tubes (tw), due to gene interaction. In crossing experiment made rescently, however, Margined-1 tube-white was actually produced, contrary to expectation. The Mg flowers of an F$_2$ from the cross of Margined-1 (strain RF) and tube-white (strain SM) included 97 Margined-1, 2 Margined-1 tube-white, 4 normal and 27 tube-white, indicating linkage between Margined-1 and tube-white. The recombination frequency is 5·0 per cent. The failure to produce Mr1 tw in the previous crosses cannot be accounted for at present, and must be regarded as mere chance deviation, however unsatisfactory this may be. Formerly the recombination for Mr1 and tw is calculated to be 7·1 per cent, which nearly corresponds to the newly obtained figure. At the present state of knowledge, the recombination for Mr1 and tw may be regarded roughly as 5·0 per cent, adopting the new value.

The recombination frequency for interaxil-green and Margined-1 was found to be 6·5 per cent (Imai 1929). Table 3 shows new data collected from coupling segregation for ig and Mr1, with the available results cited in the former report.

Table 3. Data showing linkage for interaxil-green and Margined-1

Family	Mr1	Mr1 ig	+	ig	Total
S$_1$ from 375-DA	65	2	3	17	87
S$_2$ from 375-DA	458	21	22	153	654
F$_2$ from ID × SW	123	5	7	38	173
Total	646	28	32	208	914
Expected	657	29	29	199	914

$\chi^2 = 0{\cdot}617 \qquad P = 0{\cdot}88$

The frequency of recombination for Mr1 and ig is 6·6 per cent, which is at present the most reliable figure for the linkage value of this relation.

According to the expectation made on the basis of the newly determined relations, interaxil-green may be linked with contracted at about 5·6 per cent of recombination. In Table 4 is shown an F_2 presenting repulsion segregation for ig and ct.

Table 4. F_2 showing linkage for interaxil-green and contracted

Cross	+	ct	ig	ct ig	Total
SW × H180....	134	61	58	2	255
Expected	129	62	62	2	255

$\chi^2 = 0{\cdot}468$ P = 0·92

Owing to repulsion, the reliable figure of recombination cannot be calculated on the basis of the data shown in Table 4. Therefore, the expected number calculated on the basis of 5·6 per cent of recombination was applied to the data, showing nearly perfect accordance between expectation and observation.

From rescent experiments, flecked (fl), a recessive gene resulting in a white flower with fine flecks, is found to be added to the ct L. G. The proofs obtained are presented as follows: In F_2 of the cross from flecked and Margined-1, very few normal flowers appeared, the abnormality being due to close linkage between flecked and Margined-1. The actual data obtained are shown in Table 5.

Table 5. F_2 showing linkage for Margined-1 and flecked

Cross	Mr1	+	fl	Total
465 × RF......	229	1	78	308
465 × 405	159	2	62	223
Total	388	3	140	531
Expected	395	3	133	531

$\chi^2 = 0{\cdot}492$ P = 0·81

The white margins being difficult to detect on the flecked flowers, the class fl may contain some Margined-1 flecked, though few in number, which are produced by crossing over. From the relative frequency of the Margined-1 and normal segregates, the recombination is calculated to be about 1·2 per cent. Therefore, flecked is distanced 1·2 units from Margined-1 on the right side or on the left in the contracted chromosome. If this is the case, flecked should be again closely linked with contracted, which is linked with Margined-1 at about 1 per cent. Table 6 contains F_2 data proving this.

Table 6. F_2 showing linkage for contracted and flecked

Cross	+	ct	fl	ct fl	Total	w2
SU × 465	101	49	56	0	206	71

No double recessive segregates are obtained in this cross, indicating close linkage between flecked and contracted. Interaxil-green being linked with Margined-1 at 6·6 per cent of recombination, it should be again linked with flecked, the recombination being expected to be 6·6 ± 1·2 per cent. In Table 7 is presented an F_2 showing repulsion segregation for interaxil-green and flecked.

Table 7. F_2 showing linkage for interaxil-green and flecked

Cross	+	ig	fl	Total
465 × SW	100	39	43	182

Interaxil-green cannot be phenotypically identified in the case of flecked segregates. The data indicate rather weak linkage, and considerably different from expectation, probably due to chance deviation.

Expanded (Ex) is a dominant gene and manifests a white margin broadly bordering the corolla (Fig. 1, pag. 324). Expanded is found to be linked with contracted, the data being collected in Table 8.

Table 8. F_2 showing linkage for contracted and Expanded

Cross	Ex	+	Ex ct	ct	Total
9T × NF	98	44	38	0	180
SE × E7	46	23	19	0	88
Total	144	67	57	0	268

Thus Expanded is closely linked with contracted, but, owing to repulsion, there appeared no double recessives, without which we cannot determine the linkage value. Expanded seems to be a qualified form of the Margined character. Therefore, we have two possible cases, one is that Expaneded is allelomorphic to Margined-1, and the other—that Expanded is a modifier working on the Margined character and linked closely with Margined-1. If the former is the case, Margined-1 constitutes a triple series of allelomorphs with Expanded and normal; and if the latter is the case, Expanded, Margined-1, contracted and flecked are located very close together in the contracted chromosome. The key to the problem, however, has not been obtained as yet.

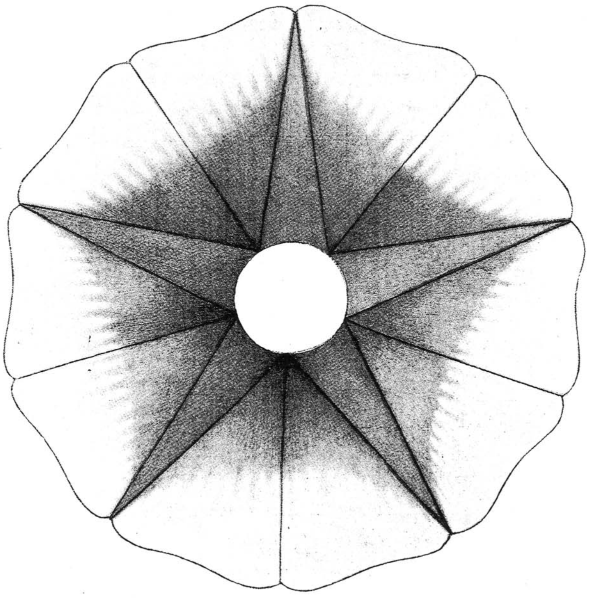

Fig. 1. Expanded flower

The extent of independence in the ten linkage groups

At present we have ten linkage groups in *Pharbitis Nil*, containing the following loci:

1. Variegated linkage group . . v, c1, B1, f3, br, fd (fd^s), cu.
2. Cordate linkage group . . . co, fe (fe^c), sc, pc, pl, cp (cp^r).

3.	Yellow linkage group	y (y^{i}), dy, lt1, de, sp-r.
4.	Acuminate linkage group	ac, Mr2, mg.
5.	Contracted linkage group	Ry, cr, ig, sh, ct (ct^{s}), Mr1, Ex, fl, tw, i, Mr-r ($mr\text{-}r^{s}$), dk.
6.	Speckled linkage group	sp, w1, Mr-f.
7.	Delicate linkage group	dl, c2.
8.	Pear linkage group	p, f1, f2, B2.
9.	Duplicated linkage group	dp, st, D, w2a, dg, e.
10.	Retracted linkage group	r, fo.

These ten linkage group include 50 loci, with the distribution of 7 loci in the v L. G., 6 loci in the co L. G., 5 loci in the y L. G., 3 loci in the ac L. G., 12 loci in the ct L. G., 3 loci in the sp L. G., 2 loci in the dl L. G., 4 loci in the p L. G., 6 loci in the dp L. G., and 2 loci in the r L. G. Of these, the six loci, faded, feathered, crêpe, yellow, contracted and Margined-reduced, each being composed of triple allelomorphs, the ten linkage groups include 56 genes. In the above calculation, Expanded is tentatively regarded as located in the locus different from Margined-1, being placed very closely together.

The relations between these groups have been found to be independent, so far as the writer's studies are concerned. The extent of independence is shown below:

Cordate linkage group. For the v L. G., co to v, c1 and B1; fe (fe^{c}) to v, c1, B1 and fd; sc to v, c1 and B1; pc to c1; cp (cp^{r}) to v, c1 and B1.

Yellow linkage group. For the v L. G., y (y^{i}) to v, c1, B1, fd and cu; dy to v, c1, B1, fd and cu; lt1 to v, c1, B1 and cu; sp-r to v, c1 and B1. For the co L. G., y to co, fe, sc and cp; dy to co, sc and cp; lt1 to sc and cp; sp-r to co, pc and cp.

Acuminate linkage group. For the v L. G., ac to v, B1, fd and cu; mg to v, c1, B1, fd and cu. For the co L. G., ac to co, sc and cp; mg to co, sc and cp. For the y L. G., ac to y and dy; mg to y, dy and sp-r.

Contracted linkage group. For the v L. G., Ry to c1 and fd; ig to v and c1; ct (ct^{s}) to v, c1, B1 and fd; Mr1 to v, c1 and B1; Ex to c1; fl to v, c1 and B1; tw to v, c1 and B1; i to v, c1 and B1; Mr-r ($mr\text{-}r^{s}$) to v; dk to v and c1. For the co L.G., Ry to co and fe; ig to co, pc, pl and cp; ct to co, fe, sc and cp; Mr1 to co, sc and cp; Ex to co; fl to co and cp; tw to co and cp; i to co and cp; Mr-r to co and cp; dk to co, sc and cp. For the y L. G., Ry to y; ig to y; ct to y, dy and sp-r; Mr1 to y, dy and lt1; fl to y; tw to y; i to y, dy and sp-r; dk to y, dy and lt1. For the ac L. G., Ry to ac; ig to mg; ct to ac and mg; Mr1 to mg and Mr2; tw to mg; i to mg; dk to ac and mg.

Speckled linkage group. For the v L. G., sp to v, c1, B1 and fd; w1 to v and c1. For the co L. G., sp to co, pc and cp; w1 to sc and cp. For the y L. G., sp to y and sp-r; w1 to y. For the ac L. G., sp to mg; w1 to mg. For the ct L. G., sp to Ry, ig, ct, Mr1, i and dk; w1 to Ry, ig, ct, Mr1, i and dk.

Delicate linkage group. For the v L. G., dl to v, c1 and B1. For the co L. G., dl to co and cp. For the y L. G., dl to y and dy. For the ac L. G., dl to ac and mg. For the ct L. G., dl to Ry, ct, Mr1, tw, i, Mr-r and dk. No data have been obtained as yet for the sp L. G.

Pear linkage group. For the v L. G., p to v, c1 and B1. For the co L. G., p to co, sc and cp. For the y L. G., p to y and dy. For the ac L. G., p to ac and mg. For the ct L. G., p to Ry, ig, ct, Ex, fl, tw, i, Mr-r and dk. For the sp L. G., p to sp and w1. For the dl L. G., p to dl.

Duplicated linkage group. For the v L. G., dp to v, c1 and fd; D to c1; w2a to v, c1, B1, fd and cu; dg to v, c1 and B1; e to v, c1 and B1. For the co L. G., dp to co and fe; w2a to co, fe, sc, pc and cp; dg to co, fe, sc and cp; e to co and cp. For the y L. G., dp to y; D to mg; w2a to ac and mg; dg to ac and mg. For the ct L. G., dp to ig, ct, Mr1, i and dk; D to i; w2a to Ry, ct, Mr1, i and dk; dg to ig, ct, Mr1, fl and dk; e to ct, Mr1, fl, i and dk. For the sp L. G., dp to sp; w2a to sp and w1; dg to w1; e to w1. For the dl L. G., dp to dl. For the p L. G., w2a to p; dg to p; e to p.

Retracted linkage group. For the v L. G., r to v, c1, B1 and fd. For the co L. G., r to co and fe. For the y L. G., r to y, dy and sp-r. For the ac L. G., r to mg. For the ct L. G., r to Ry, ct, Mr1, fl, tw and dk. For the sp L. G., r to sp and w1. For the dl L. G., r to dl. For the p L. G., r to p. For the dp L. G., r to dp, w2a, dg and e.

Genes located in other chromosomes

So far as the data gathered up to the present time are concerned, two genes, maple and polymorphic, are found to be independent of the known ten linkage groups, and they may be located in the other chromosomes. The extent of proofs is as follows: Maple (m), which is allelomorphic to willow (m^w), is independent of v, c1, B1 and fd of the v L. G.; co, fe, sc and cp of the co L. G.; y and dy of the y L. G.; ac and mg of the ac L. G.; ig, ct, Ex, fl, tw, i, Mr-r and dk of the ct L. G.; sp and w1 of the sp L. G.; dl of the dl L. G.; p of the p L. G.; dp, w2a, dg and e of the dp L. G.; r of the r L. G. Polymorphic (py) also to v, B1 and fd of the v L. G.; co, sc and cp of the co L. G.; y and dy of the y L. G.; ac and mg of the ac L. G.; ct and Mr1 of the ct L. G.; w1 of the sp L. G.; dl of the dl L. G.; p of the p L. G.; w2a and dg of the dp L. G.; r of the r L. G.

Other cases showing linkage

Purple (pr) is independent of v, c1, B1 and cu of the v L. G.; co, sc and cp of the co L. G.; y, dy and lt1 of the y L. G.; ac and mg of the ac L. G.; ig, ct, Mr1 and Ex of the ct L. G.; w1 of the sp L. G.; dl of the dl L. G.; p of the p L. G.; and m, py, df (deficient) and cd (contorted). Crisscrossed (cs) also shows no linkage with c1, B1 and fd of the v L. G.; co and cp of the co L. G.; mg of the ac L. G.; ig, ct, Mr1, i and tw of the ct L. G.; dl of the dl L. G.; and m. In an F_2 obtained by crossing purple (strain NF) with crisscrossed (strain ID), the writer got 74 normal, 28 crisscrossed, 37 purple and 2 crisscrossed purple, indicating repulsion segregation. The frequency of recombination for purple and crisscrossed is 19·0 per cent. The figure, however, being based on repulsion data, is not very reliable.

Light-1 (formerly light) was found to be linked closely with yellow and dusky, and the gene is regarded as located in the yellow chromosome (Imai in press, a). Light-2 (lt2), however, shows no linkage with dusky of the y L. G. (Imai 1930), and also with v, c1 and B1 of the v L. G.; co and fe of the co L. G.; ct, Mr1 and i of the ct L. G.; p of the p L. G.; e of the dp L. G.; and pr, m and wr (wrinkled). Ivory, wich is probably allelomorphic to white-3, is independent of fe of the co L. G.; y of the y L. G.; Ry and ct of the ct L. G.; w2a of the dp L. G. If ivory is allelomorphic to white-3, additional information of the extent of independence will be furnished from the data of white-3. White-3 is already known to be independent of v, c1 and fd of the v L. G.; co, sc and cp of the co L. G.; w1 of the sp L. G.; D, w2a and dg of the dp L. G.; r of the r L. G.; and m, Mr-s (Margined-suppressed), bf (buff) and t (terminal). In an F_2 segregation obtained by crossing light-2 (strain 190) with ivory (strain RJ), light-2 is linked with ivory, the data being 84 normal, 45 light-2 and 30 ivory. The plants bearing ivory seeds always show green stems and white flowers, and therefore light-2 has no effect on ivory. The deficit of the ivory segregates is evidently due to their low viability.

The segregation above cited is nearly 2:1:1 in proportion, exhibiting close repulsion.

As stated elsewhere (Imai 1930), Lined (Ln) is linked with striated (sa), the recombination being roughly 16 per cent. Lined is independent of v, c1 and B1 of the v L. G.; fl of the ct L. G.; dg of the dp L. G.; and m.

We have now ten linkage groups and two independent genes. The haploid chromosomes of *Pharbitis Nil* being 15 in number, we should expect still, the three other gene-groups located in the different chromosomes. The three linkage groups above shown, however, are incomplete in proofs of independence to the known ten linkage-groups as well as to the two independent genes, maple and polymorphic, and therefore we must reserve this information for the future.

Miscellaneous

Some fragmentary data for the independent segregation of some genes have been gathered and are shown below: Buff (bf) to v of the v L. G.; cp of the co L. G.; i of the ct L. G.; w1 of the sp L. G.; p of the p L. G.; w2a and e of the dp L. G. Deficient (df) to c1 of the v L. G.; co and cp of the co L. G.; ig and i of the ct L. G.; w2a of the dp L. G. Terminal (t) to v and c1 of the v L. G.; co of the co L. G.; dk of the ct L. G.; w1 of the sp L. G.; w2a and dg of the dp L. G.; r of the r L. G. Glabrous (g) to v and c1 of the v L. G.; mg of the ac L. G.; ct and Mr1 of the ct L. G.; dg of the dp L. G.; r of the r L. G. Wrinkled (wr) to v, c1 and B1 of the v L. G.; co of the co L. G.; y and dy of the y L. G.; mg of the ac L. G.; Mr1 of the ct L. G.; and g. Contorted (cd) to c1 of the v L. G.; cp of the co L. G.; dy of the y L. G.; mg of the ac L. G.; ct, i and Mr1 of the ct L. G.; p of the p L. G.; w2a of the dp L. G.; r of the r L. G.; and m and py. Petaloid (pt) to v of the v L. G.; co, fe, sc and cp of the co L. G.; y of the y L. G.; ig, i and dk of the ct L. G.; w2a and dg of the dp L. G.; and m. Half-white (hw) to v and c1 of the v L. G.; cp of the co L. G.; Mr1 and tw of the ct L. G.; p of the p L. G.; and m. Tinged (tg) to c1 of the v L. G.; D and w2a of the dp L. G. Variegated-reduced (v-r) to v of the v L. G.; sc of the co L. G.; y and lt1 of the y L. G.; Mr1 of the ct L. G.; dg of the dp L. G. Side-reduced (sr) to y of the y L. G.; ct of the ct L. G.; r of the r L. G. Blizzard-1 (Bz1) to v and B1 of the v L. G.; Mr1 of the ct L. G. Blizzard-2 (Bz2) to v and B1 of the v L. G.; Mr1 of the ct L. G.; and Bz1.

Conclusion

The present state of knowledge in regard to the linkage groups of *Pharbitis Nil* is as follows:

1. The variegated linkage group is composed of seven genes, variegated (v), crumpled-1 (c1), Blown-1 (B1), fasciated-3 (f3), brown (br), faded (fd) and couple (cu). The relative disfance of the loci, variegated, crumpled-1 and Blown-1, on the chromosome is 14·9 between v and c1 and 17·8 between c1 and B1, in the order of arrangement v—c1—B1, with the recombination of 30·8 per cent for v and B1. The frequency of recombination is roughly 20—25 per cent for v and f3, about 31·0 per cent for v and fd, and about 37·7 per cent for v and cu. The locus br seems to be situated not far from the loci, v and c1.

2. The cordate linkage group includes six genes, cordate (co), feathered (fe), semi-contracted (sc), precocious (pc), palmate (pl) and crêpe (cp). The former three genes seem to be located in the cordate chromosome, in the linear arrangement, as probably being co—fe—sc, with a distance of 1·2 between co and fe, and 17·7 between fe and sc. Precocious is linked with co, the recombination being about 24·6 per cent. The recombination percentage is about 34·6 for pl and cp, about 31·3 for pc and pl, and about 38·8 for pc and cp.

3. The yellow linkage group is composed of five genes, yellow (y), dusky (dy), light-1 (lt1), deformed (de) and speckled-reduced (sp-r). The frequency of recombination for y and dy is 1·0 per cent, and therefore they are located close together in the yellow chromosome. Light-1 is linked rather closely with y and dy. Speckled-reduced is linked with y at about 28·5 per cent of recombination, and with de at about 15 per cent of recombination.

4. The acuminate linkage group is composed of three genes, acuminate (ac), Margined-2 (Mr2) and magenta (mg). The recombination percentage is about 0·5 for ac and Mr2, and 21·2 for ac and mg.

5. The contracted linkage group contains twelve loci, Rayed (Ry), cream (cr), interaxil-green (ig), shrubby (sh), contracted (ct), Margined-1 (Mr1), Expanded (Ex), flecked (fl), tube-white (tw), intense (i), Margined-reduced (Mr-r) and duskish (dk). By the experimental data available at present, we can draw a preliminary map of the contracted chromosome showing the following relations: Ry (0), cr (± 1·2), ig (10·3), sh (10·3 ± 3·1), ct (15·9), Mr1 (16·9), Ex (16·9 ±), fl (16·9 ± 1·2), tw (21·9), i (46·5), Mr-r (46·5 ± 3·0). The locus dk appears to be present in the right part of the chromosome.

6. The speckled linkage group is composed of three genes, speckled (sp), white-1 (w1) and Margined-fluctuated (Mr-f), the recombination frequency being about 0·8 per cent for sp and w1, and roughly 20 per cent for w1 and Mr-f.

7. Two genes, delicate (dl) and crumpled-2 (c2), are known to constitute the delicate linkage group. The recombination frequency for dl and c2 is roughly 5 per cent.

8. The pear linkage group is found to contain four genes, pear (p), fasciated-1 (f1), fasciated-2 (f2) and Blown-2 (B2). The recombination frequency is roughly 20—25 per cent for f1 and f2, about 2·5 per cent for p and f1, and 23·5 per cent for p and B2.

9. Six genes, duplicated (dp), striped (st), Dilute (D), white-2a (w2a), dragonfly (dg) and extended (e) are found to constitute the duplicated linkage group. The recombination is 13·7 per cent for dp and st, 25·6 per cent for dp and D, 10·1 per cent for st and D, less than 1·4 per cent for D and w2a, 23·3 per cent for w2a and dg, and 38·3 per cent for dg and e. Their order in the chromosome may be dp—st—D±w2a—dg, with a distance of 13·7 units between dp and st, 10·1 units between st and D, and 23·3 units between w2a and dg. The locus extended is situated at a distance of 38·3 units in the right side or in the left of the locus dragonfly.

10. Only two genes, retracted (r) and foliate (fo), are known for the retracted linkage group. The two genes are closely linked.

11. Under the ten linkage groups are collected 50 loci, including 56 genes. So far as the writer's observations are concerned, the relations between these groups have been found to be independent.

12. The two genes, maple (m) and polymorphic (py), are segregated independently of the genes included in the ten linkage groups, so far as the writer's investigation is concerned. Therefore, the two loci seem to be situated in the other different chromosomes.

13. Three fragmentary cases showing linkage are found. Purple (pr) is linked with crisscrossed (cs), the recombination frequency being 19·0 per cent. Light-2 (lt2) is closely linked with ivory. Lined (Ln) shows linkage with striated (sa), the recombination being roughly 16 per cent. The proof for independence of these grouping genes from the known ten linkage groups as well as from the two independent genes (maple and polymorphic) is incomplete, and therefore they are treated as fragmentary cases of linkage.

Summary

The new facts presented in this paper are as follows:

1. The recombination frequencies, 14·9 per cent for v and cl, 17·8 per cent for cl and Bl and 30·8 per cent for v and Bl, which were determined by backcross experiments, are applied to the F_2 data, showing almost accordance between them.

2. Data showing weak repulsion between creased (allelomorphic to feathered) and crêpe are presented. This gives additional proof to the fact that crêpe is located in the cordate chromosome.

3. The recombination frequency for Margined-1 and tube-white is again calculated, and found to be 5·0 per cent. From available data, the recombination for interaxil-green and contracted is estimated to be 6·6 per cent. The 5·6 per cent of recombination for interaxil-green and contracted, calculated by the other linkage relations, covers perfectly the actual data showing repulsion. Flecked is linked closely with Margined-1 and contracted, the recombination in the former relation being 1·2 per cent. Flecked shows also linkage with interaxil-green. Expanded is closely linked with contracted.

4. Further data for the indepent relations of the ten linkage groups are given.

5. The two loci, maple (willow) and polymorphic, seem to be situated in the other chromosomes.

6. Two new cases showing linkage are detected. Purple is linked with crisscrossed, the recombination frequency being 19·0 per cent. Light-2 and ivory show close linkage in their segregation. The proof for independence of these grouping genes and the linked Lined-striated genes to the ten linkage groups and the two independent genes (maple and polymorphic) is incomplete.

7. New data giving proofs of independence for some genes are presented together with those previously obtained.

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