

## LINKAGE-STUDIES IN PISUM. II

by

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### I. INTRODUCTION

In Linkage-Studies I the results of three crosses, involving ten genes, were dealt with. Seven independent genes or groups of linked genes were found.

The present paper gives the results of three more crosses, involving four more factors. Among the fourteen genes nine independent ones

were found, although *Pisum* possesses seven bivalent chromosomes. The recent literature on linkage in *Pisum* will be discussed later in connection with my own results.

## II. MATERIALS AND METHODS

### § 1. *The factors*

The ten factors which were already studied in Linkage-Studies I are:

- (1)  $A$  : flowers colored (recessive: white).
- (2)  $B$  : flowers purple (rec.: pink).
- (3)  $D^w$ : leaf-axil-spot double (the recessive allelomorph in the present crosses is  $D$  which makes the axil-spot single).
- (4)  $Fa$  : stems normal (rec.: fasciated).
- (5)  $Gp$  : pods green (rec.: yellow).
- (6)  $P$  : membrane in the pod-wall (rec.: no membrane).
- (7)  $V$  : strong membrane in the pod-wall (rec.: thin membrane in the presence of  $P$ ).
- (8)  $Pl$  : hilum black (rec.: not black).
- (9)  $M$  : seed-coat marbled (rec.: not marbled).
- (10)  $F$  : seed-coat stippled (rec.: not stippled).

Factors  $B$ ,  $D^w$ ,  $M$  and  $F$  are only active in the presence of  $A$ ;  $V$  is only active in the presence of  $P$ .

The four factors, added to the above ten in the present study, are:

- (11)  $I$  : cotyledons yellow (rec.: green).
- (12)  $Le$  : internodes long (rec.: short).
- (13)  $W_2^w$  : glaucous stems and foliage (rec.: emerald).
- (14)  $S$  : seeds free in the ripe pod (rec.: seeds adhering "chenille"-like).

$I$  and  $Le$  are most probably two of MENDEL's factors (4).  $W_2^w$  was recently described by WELLENSIEK (10); speaking in terms of relative amounts of wax,  $W_2^w$  stands for "much" and  $w$  for "little" in the present material.  $S$  was first found by DE VILMORIN (8).

Another factor, called  $Q$  and causing a dominant abortion, hitherto unreported in literature, is also present in the material described here. Since all relations to the above fourteen genes are not known yet, it will not be discussed in the present paper. However, in the summarized results of cross 41, given in the appendix, the segregation of the pair  $Qq$  is mentioned; this will prevent duplication in a later publication.

§ 2. *The parents and the crosses*

The new parental line is "Pois à brochettes", originally obtained through the kindness of Mr. A. MEUNISSIER at Verrières-le-Buisson, France. It carries the four new genes in recessive form. The genotypes of the four parental lines — using single symbols for simplicity's sake — are:

1-2-56-1 :  $A B D^w \underline{fa} \underline{Gp} P v \underline{pl} m F I \underline{Le} W_2^b S$   
 P. thebaicum :  $A b D \underline{Fa} \underline{Gp} P V \underline{Pl} M f I \underline{Le} W_2^b S$   
 P. à cosse jaune :  $a B D^w \underline{Fa} \underline{gp} p V \underline{pl} m F I \underline{Le} W_2^b S$   
 P. à brochettes :  $a B D^w \underline{Fa} \underline{Gp} P V \underline{pl} m F i \underline{le} w_2^b s$

P. à cosse jaune and P. à brochettes carry  $B$ ,  $D^w$  and  $F$  cryptomerically; moreover  $V$  in the former line is cryptomeric.

The new crosses, with the factors involved in each of them, are:

Cross 38. 1-2-56-1 × P. à broch. :  $A-\underline{Fa}-V-I-\underline{Le}-W_2^b-S$ .

Cross 40. P. à broch. × P. thebaicum:  $A-B-D^w-\underline{Pl}-M-F-I-\underline{Le}-W_2^b-S$ .

Cross 41. P. à c. jaune × P. à broch. :  $\underline{Gp}-P-I-\underline{Le}-W_2^b-S$ .

The following table gives the numbers of individuals in the crosses.

	sown	succumbed	% succumbed	germ plants	full-grown plants
Cross 38	1184	20	1.7	1164	1112
Cross 40	1140	3	0.3	1137	1107
Cross 41	1176	33	2.8	1143	1072

§ 3. *Growing-methods, notes, methods of publishing, calculations*

The material was treated in exactly the same way as described in details in Linkage-Studies I. However, the preparation of a summarizing  $F_2$ -table of cross 40 was too complicated, because so many genes were studied in this cross. The table would have become too long and this method, desirable as it is when not too many genes are studied, would be inefficient in this case. Consequently only summarizing  $F_2$ -tables of crosses 38 and 41 are given in the appendix and the figures for cross 40 are immediately taken from the field-notes.

Classification after cotyledon-color was of course made before sow-

ing and the earlier it is done, the better the two classes can be distinguished.

In crosses 38 and 40 a colored seed-coat masked the cotyledon-color; therefore a small part of the skins had to be cut away in order to make classification after cotyledon-color possible. Together with flower-color also axil-spot, stem-form, internode-length and wax were taken. A little later pod-color and pod-membrane were observed and, when the plants were dry, the seed-characteristics (hilum, marbling, stippling, chenille) were noted.

The chenille-characteristic is highly modifiable. A well developed adherence among the seeds is only found in emerald plants. Glauous plants, genetically chenille, usually have only two or three seeds adhering and many pods of these plants may have nothing but free seeds. It is therefore necessary to examine all the pods, before concluding to absence of chenille. Owing to this modifiability it may be questioned whether the classification free-chenille is always correct. A very strong linkage between glauous and free exists, however, and therefore a parallelism between the segregations involving  $W_2^b$  and S makes us feel more sure about the correctness of the free-chenille classification, since glauous and emerald plants are distinguished quite easily. Such a — not absolute — parallelism was found in all cases.

### III. EXPERIMENTAL RESULTS AND CONCLUSIONS

#### § 1. *The monofactorial $F_2$ -segregations*

In tabulating the monofactorial  $F_2$ -segregations, the theoretical expectation is put in parentheses, while c indicates actual deviation divided by standard deviation of expectation. The original segregation-numbers for cotyledon-color are given, so that the totals in these cases are larger than the totals for the other segregations. As will be seen below all segregations fit the expectations, except the last one. Evidently modification has caused the large deviation (see above).

(1) A.	Cross 38.	837	:	275
		(834)	:	(278)
		c 0.2		
	Cross 40.	809	:	298
		(830.25)	:	(276.75)
		c 1.5		

- (2) *B.* Cross 40. 616 : 193  
(606.75) : (202.25)  
c 0.7
- (3) *D<sup>w</sup>.* Cross 40. 582 : 227  
(606.75) : (202.25)  
c 2.0
- (4) *F<sub>a</sub>.* Cross 38. 857 : 255  
(834) : (278)  
c 1.6
- (5) *G<sub>p</sub>.* Cross 41. 807 : 265  
(804) : (268)  
c 0.2
- (6) *P.* Cross 41. 824 : 248  
(804) : (268)  
c 1.4
- (7) *V.* Cross 38. 870 : 242  
(834) : (278)  
c 2.5
- (8) *Pl.* Cross 40. 834 : 273  
(830.25) : (276.75)  
c 0.3
- (9) *M.* Cross 40. 607 : 202  
(606.75) : (202.25)  
c 0.02
- (10) *F.* Cross 40. 607 : 202  
(606.75) : (202.25)  
c 0.02
- (11) *I.* Cross 38. 869 : 315  
(898) : (296)  
c 2.0  
Cross 40. 832 : 308  
(855) : (285)  
c 1.6  
Cross 41. 884 : 292  
(882) : (294)  
c 0.1

(12) <i>Le.</i>	Cross 38.	807	:	305
		(834)	:	(278)
		c=1.9		
	Cross 40.	831	:	276
		(830.25)	:	(276.75)
		c=0.05		
	Cross 41.	813	:	259
		(804)	:	(268)
		c=0.7		
(13) <i>W<sub>2</sub><sup>h</sup></i>	Cross 38.	829	:	283
		(834)	:	(278)
		c=0.3		
	Cross 40.	797	:	310
		(830.25)	:	(276.75)
		c=2.3		
	Cross 41.	831	:	241
		(804)	:	(268)
		c=1.9		
(14) <i>S.</i>	Cross 38.	824	:	288
		(834)	:	(278)
		c=0.7		
	Cross 40.	798	:	309
		(830.25)	:	(276.75)
		c=2.2		
	Cross 41.	865	:	207
		(804)	:	(268)
		c=4.3		

### § 2. *The factor-relations*

The factorial relations are published below in the same way as before. The first column gives the actually obtained  $F_2$ -numbers  $AB : Ab : aB : ab$  and, on the next row, the numbers expected in case of independence in parentheses. The second column gives the actual and the expected ratios  $(AB + ab) : (Ab + aB)$  which means sum of original combinations : sum of recombinations or, in the repulsion phase, sum of recombinations : sum of original combinations. Below the cross-number the type of combination is indicated in parentheses. The value of  $c$ , again, represents the quotient of actual

deviation and standard deviation. Theoretical expectations are calculated according to KAPPERT's method and in case of linkage — indicated by a larger value for  $c$  than 3.0 — the gametic ratio and the percentage of crossing-over are calculated after WELLENSIEK's method just as in Linkage-Studies I. When there is a 9 : 3 : 4 expectation, only the first two terms can be used in linkage-studies; independence of the factors in question means that these terms correspond to a 3 : 1- expectation.

Those combinations of factors which were already studied in Linkage-Studies I and occur again in the present material, will be numbered as they were before. The 46 new combinations will be numbered 46, 47 . . . ., 91.

(1) $A-B.$	616	:	193							
Cross 40.	(606.75)	:	(202.25)							
( $a B \times A b$ )	$c=0.7$									
(2) $A-D^w.$	582	:	227							
Cross 40.	(606.75)	:	(202.25)							
( $a D^w \times A D$ )	$c=2.0$									
(3) $A-\underline{Fa}.$	644	:	193	:	213	:	62	706	:	406
Cross 38.	(645)	:	(192)	:	(212)	:	(63)	(708)	:	(404)
( $A \underline{fa} \times a \underline{Fa}$ )								$c=0.1$		
(6) $A-V.$	641	:	196	:	229	:	46	687	:	425
Cross 38.	(655)	:	(182)	:	(215)	:	(60)	(715)	:	(397)
( $A v \times a V$ )								$c=1.8$		
(7) $A-\underline{Pl}.$	619	:	190	:	215	:	83	702	:	405
Cross 40.	(609)	:	(200)	:	(225)	:	(73)	(682)	:	(425)
( $a \underline{pl} \times A \underline{Pl}$ )								$c=1.2$		
(8) $A-M.$	607	:	202							
Cross 40.	(606.75)	:	(202.25)							
( $a m \times A M$ )	$c=0.02$									
(9) $A-F.$	607	:	202							
Cross 40.	(606.75)	:	(202.25)							
( $a F \times A f$ )	$c=0.02$									
(10) $B-D^w.$	448	:	168	:	134	:	59	507	:	302
Cross 40.	(443)	:	(173)	:	(139)	:	(54)	(497)	:	(312)
( $B D^w \times b D$ )								$c=0.7$		

(15) $\underline{B-Pl}$ .	462 : 154 : 157 : 36	498 : 311
Cross 40.	(471) : (145) : (148) : (45)	(516) : (293)
( $B \underline{pl} \times b \underline{Pl}$ )		c=1.3
(16) $\underline{B-M}$ .	457 : 159 : 150 : 43	500 : 309
Cross 40.	(462) : (154) : (145) : (48)	(510) : (299)
( $B \underline{m} \times b \underline{M}$ )		c=0.7
(17) $\underline{B-F}$ .	562 : 54 : 45 : 148	710 : 99
Cross 40.	(462) : (154) : (145) : (48)	(510) : (299)
( $B \underline{F} \times b \underline{f}$ )		c=14.6
Gametic ratio is 12.5 : 2.0 : 1.7 : 12.2; crossing-over is 13.0 %		
(22) $\underline{Dw-Pl}$ .	445 : 137 : 174 : 53	498 : 311
Cross 40.	(445) : (137) : (174) : (53)	(498) : (311)
( $D^w \underline{pl} \times D \underline{Pl}$ )		c=0.0
(23) $\underline{Dw-M}$ .	428 : 154 : 179 : 48	476 : 333
Cross 40.	(437) : (145) : (170) : (57)	(494) : (315)
( $D^w \underline{m} \times D \underline{M}$ )		c=1.3
(24) $\underline{Dw-F}$ .	445 : 137 : 162 : 65	510 : 299
Cross 40.	(437) : (145) : (170) : (57)	(494) : (315)
( $D^w \underline{F} \times D \underline{f}$ )		c=1.2
(27) $\underline{Fa-V}$ .	671 : 186 : 199 : 56	727 : 385
Cross 38.	(670) : (187) : (200) : (55)	(725) : (387)
( $f a \underline{v} \times F a \underline{V}$ )		c=0.1
(31) $\underline{Gp-P}$ .	613 : 194 : 211 : 54	667 : 405
Cross 41.	(620) : (187) : (204) : (61)	(681) : (391)
( $G \underline{p} \underline{P} \times g \underline{p} \underline{p}$ )		c=0.9
(43) $\underline{Pl-M}$ .	466 : 153 : 141 : 49	515 : 294
Cross 40.	(464) : (155) : (143) : (47)	(511) : (298)
( $\underline{pl} \underline{m} \times \underline{Pl} \underline{M}$ )		c=0.3
(44) $\underline{Pl-F}$ .	461 : 158 : 146 : 44	505 : 304
Cross 40.	(464) : (155) : (143) : (47)	(511) : (298)
( $\underline{pl} \underline{F} \times \underline{Pl} \underline{f}$ )		c=0.4
(45) $\underline{M-F}$ .	427 : 180 : 180 : 22	449 : 360
Cross 40.	(455) : (152) : (152) : (50)	(505) : (304)
( $\underline{m} \underline{F} \times \underline{M} \underline{f}$ )		c=4.1
Gametic ratio is 4.7 : 9.5 : 9.5 : 4.7; crossing-over is 33.1 %.		
(46) $\underline{A-I}$ .	617 : 220 : 204 : 71	688 : 424
Cross 38.	(618) : (219) : (203) : (72)	(690) : (422)
( $A \underline{I} \times a \underline{i}$ )		c=0.1



Cross 40.	596 : 213 : 215 : 83	679 : 428
( <i>a i</i> × <i>A I</i> )	(593) : (216) : (218) : (80)	(673) : (434)
		<i>c</i> =0.4
(47) <i>A-Le.</i>	616 : 221 : 191 : 84	700 : 412
Cross 38.	(607) : (230) : (200) : (75)	(682) : (430)
( <i>A Le</i> × <i>a le</i> )		<i>c</i> =1.1
Cross 40.	609 : 200 : 222 : 76	685 : 422
( <i>a le</i> × <i>A Le</i> )	(607) : (202) : (224) : (74)	(168) : (426)
		<i>c</i> =0.2
(48) <i>A-W<sub>2</sub><sup>b</sup>.</i>	624 : 213 : 205 : 70	694 : 418
Cross 38.	(624) : (213) : (205) : (70)	(694) : (418)
( <i>A W<sub>2</sub><sup>b</sup> × a w<sup>q</sup></i> )		<i>c</i> =0.0
Cross 40.	588 : 221 : 209 : 89	677 : 430
( <i>a w<sup>b</sup> × A W<sub>2</sub><sup>b</sup></i> )	(582) : (227) : (215) : (83)	(665) : (442)
		<i>c</i> =0.7
(49) <i>A-S.</i>	621 : 216 : 203 : 72	693 : 419
Cross 38.	(620) : (217) : (204) : (71)	(691) : (421)
( <i>A S</i> × <i>a s</i> )		<i>c</i> =0.1
Cross 40.	588 : 221 : 210 : 88	676 : 431
( <i>a s</i> × <i>A S</i> )	(583) : (226) : (215) : (83)	(666) : (441)
		<i>c</i> =0.6
(50) <i>B-I.</i>	448 : 168 : 148 : 45	493 : 316
Cross 40.	(454) : (162) : (142) : (51)	(505) : (304)
( <i>B i</i> × <i>b I</i> )		<i>c</i> =0.9
(51) <i>B-Le.</i>	454 : 162 : 155 : 38	492 : 317
Cross 40.	(464) : (152) : (145) : (48)	(512) : (297)
( <i>B le</i> × <i>b Le</i> )		<i>c</i> =1.5
(52) <i>B-W<sub>2</sub><sup>b</sup>.</i>	456 : 160 : 132 : 61	517 : 292
Cross 40.	(448) : (168) : (140) : (53)	(501) : (308)
( <i>B w<sup>b</sup> × b W<sub>2</sub><sup>b</sup></i> )		<i>c</i> =1.2
(53) <i>B-S.</i>	456 : 160 : 132 : 61	517 : 292
Cross 40.	(448) : (168) : (140) : (53)	(501) : (308)
( <i>B s</i> × <i>b S</i> )		<i>c</i> =1.2
(54) <i>Dw-I.</i>	416 : 166 : 180 : 47	463 : 346
Cross 40.	(429) : (153) : (167) : (60)	(489) : (320)
( <i>D<sup>w</sup> i</i> × <i>D I</i> )		<i>c</i> =1.9

(55) $Dw-Le$ .	447 : 135 : 162 : 65	512 : 297
Cross 40.	(438) : (144) : (171) : (56)	(494) : (315)
( $D^w le \times D Le$ )		c=1.3
(56) $Dw-W_2^b$ .	425 : 157 : 163 : 64	489 : 320
Cross 40.	(423) : (159) : (165) : (62)	(485) : (324)
( $D^w w^b \times DW_2^b$ )		c=0.3
(57) $Dw-S$ .	425 : 157 : 163 : 64	489 : 320
Cross 40.	(423) : (159) : (165) : (62)	(485) : (324)
( $D^w s \times D S$ )		c=0.3
(58) $Fa-I$ .	633 : 224 : 188 : 67	700 : 412
Cross 33.	(633) : (224) : (188) : (67)	(700) : (412)
( $fa I \times Fa i$ )		c=0.0
(59) $Fa-Le$ .	632 : 225 : 175 : 80	712 : 400
Cross 38.	(622) : (235) : (185) : (70)	(692) : (420)
( $fa Le \times Fa le$ )		c=1.2
(60) $Fa-W_2^b$ .	631 : 226 : 198 : 57	688 : 424
Cross 38.	(639) : (218) : (190) : (65)	(704) : (408)
( $fa W_2^b \times Fa w^b$ )		c=1.0
(61) $Fa-S$ .	627 : 230 : 197 : 58	685 : 427
Cross 38.	(635) : (222) : (189) : (66)	(701) : (411)
( $fa S \times Fa s$ )		c=1.0
(62) $Gp-I$ .	613 : 194 : 200 : 65	678 : 394
Cross 41.	(612) : (195) : (201) : (64)	(676) : (396)
( $Gp i \times gp I$ )		c=0.1
(63) $Gp-Le$ .	615 : 192 : 198 : 67	682 : 390
Cross 41.	(612) : (195) : (201) : (64)	(676) : (396)
( $Gp le \times gp Le$ )		c=0.4
(64) $Gp-W_2^b$ .	629 : 178 : 202 : 63	692 : 380
Cross 41.	(626) : (181) : (205) : (60)	(686) : (386)
( $Gp w^b \times gp W_2^b$ )		c=0.4
(65) $Gp-S$ .	650 : 157 : 215 : 50	700 : 372
Cross 41.	(651) : (156) : (214) : (51)	(702) : (370)
( $Gp s \times gp S$ )		c=0.1
(66) $P-I$ .	618 : 206 : 195 : 53	671 : 401
Cross 41.	(625) : (199) : (188) : (60)	(685) : (387)
( $P i \times p I$ )		c=0.9

(67) $\underline{P}\text{-}\underline{L}e$ .	617 : 207 : 196 : 52	669 : 403
Cross 41.	(625) : (199) : (188) : (60)	(685) : (387)
( $\underline{P}le \times \underline{p}Le$ )		$c=1.0$
(68) $\underline{P}\text{-}\underline{W}_2^b$ .	635 : 189 : 196 : 52	687 : 385
Cross 41.	(639) : (185) : (192) : (56)	(695) : (377)
( $\underline{P}w^b \times \underline{p}W_2^b$ )		$c=0.5$
(69) $\underline{P}\text{-}\underline{S}$ .	658 : 166 : 207 : 41	699 : 373
Cross 41.	(665) : (159) : (200) : (48)	(713) : (359)
( $\underline{P}s \times \underline{p}S$ )		$c=0.9$
(70) $\underline{V}\text{-}\underline{I}$ .	634 : 236 : 187 : 55	689 : 423
Cross 38.	(642) : (228) : (179) : (63)	(705) : (407)
( $\underline{v}I \times \underline{V}i$ )		$c=1.0$
(71) $\underline{V}\text{-}\underline{L}e$ .	570 : 300 : 237 : 5	575 : 537
Cross 38.	(631) : (239) : (176) : (66)	(697) : (415)
( $\underline{v}Le \times \underline{V}le$ )		$c=7.6$
Gametic ratio is 2.4 : 15.3 : 13.4 : 2.2; crossing-over is 13.8 %.		
(72) $\underline{V}\text{-}\underline{W}_2^b$ .	651 : 219 : 178 : 64	715 : 397
Cross 38.	(649) : (221) : (180) : (62)	(711) : (401)
( $\underline{v}W_2^b \times \underline{V}w^b$ )		$c=0.25$
(73) $\underline{V}\text{-}\underline{S}$ .	645 : 225 : 179 : 63	708 : 404
Cross 38.	(645) : (225) : (179) : (63)	(708) : (404)
( $\underline{v}S \times \underline{V}s$ )		$c=0.0$
(74) $\underline{Pl}\text{-}\underline{I}$ .	614 : 220 : 197 : 76	690 : 417
Cross 40.	611 : 223 : 200 : 73	684 : 423
( $\underline{pl}i \times \underline{Pl}I$ )		$c=0.4$
(75) $\underline{Pl}\text{-}\underline{L}e$ .	630 : 204 : 201 : 72	702 : 405
Cross 40.	(626) : (208) : (205) : (68)	(694) : (413)
( $\underline{pl}le \times \underline{Pl}Le$ )		$c=0.5$
(76) $\underline{Pl}\text{-}\underline{W}_2^b$ .	600 : 234 : 197 : 76	676 : 431
Cross 40.	(600) : (234) : (196) : (77)	(677) : (430)
( $\underline{pl}w^b \times \underline{Pl}W_2^b$ )		$c=0.1$
(77) $\underline{Pl}\text{-}\underline{S}$ .	600 : 234 : 198 : 75	675 : 432
Cross 40.	(601) : (233) : (197) : (76)	(677) : (430)
( $\underline{pl}s \times \underline{Pl}S$ )		$c=0.1$
(78) $\underline{M}\text{-}\underline{I}$ .	446 : 161 : 150 : 52	498 : 311
Cross 40.	(447) : (160) : (149) : (53)	(500) : (309)
( $\underline{m}i \times \underline{M}I$ )		$c=0.1$

(79) $M\text{-}Le.$	460 : 147 : 149 : 53	513 : 296
Cross 40.	(457) : (150) : (152) : (50)	(507) : (302)
( $m\text{ }le \times M\text{ }Le$ )		$c=0.4$
(80) $M\text{-}W_2^b.$	446 : 161 : 142 : 60	506 : 303
Cross 40.	(441) : (166) : (147) : (55)	(496) : (313)
( $m\text{ }w^b \times M\text{ }W_2^b$ )		$c=0.7$
(81) $M\text{-}S.$	446 : 161 : 142 : 60	506 : 303
Cross 40.	(441) : (166) : (147) : (55)	(496) : (313)
( $m\text{ }s \times M\text{ }S$ )		$c=0.7$
(82) $F\text{-}I.$	449 : 158 : 147 : 55	504 : 305
Cross 40.	(447) : (160) : (149) : (53)	(500) : (309)
( $F\text{ }i \times f\text{ }I$ )		$c=0.3$
(83) $F\text{-}Le.$	471 : 136 : 138 : 64	535 : 274
Cross 40.	(457) : (150) : (152) : (50)	(507) : (302)
( $F\text{ }le \times f\text{ }Le$ )		$c=2.0$
(84) $F\text{-}W_2^b.$	430 : 177 : 158 : 44	474 : 335
Cross 40.	(441) : (166) : (147) : (55)	(496) : (313)
( $F\text{ }w^b \times f\text{ }W_2^b$ )		$c=1.6$
(85) $F\text{-}S.$	430 : 177 : 158 : 44	474 : 335
Cross 40.	(441) : (166) : (147) : (55)	(496) : (313)
( $F\text{ }s \times f\text{ }S$ )		$c=1.6$
(86) $I\text{-}Le.$	600 : 221 : 207 : 84	684 : 428
Cross 38.	(596) : (225) : (211) : (80)	(676) : (436)
( $I\text{ }Le \times i\text{ }le$ )		$c=0.5$
Cross 40.	619 : 192 : 212 : 84	703 : 404
( $i\text{ }le \times I\text{ }Le$ )	(609) : (202) : (222) : (74)	(683) : (424)
		$c=1.2$
Cross 41.	619 : 194 : 194 : 65	684 : 388
( $i\text{ }le \times I\text{ }Le$ )	(617) : (196) : (196) : (63)	(680) : (392)
		$c=0.3$
(87) $I\text{-}W_2^b.$	612 : 209 : 217 : 74	686 : 426
Cross 38.	(612) : (209) : (217) : (74)	(686) : (426)
( $I\text{ }W_2^b \times i\text{ }w^b$ )		$c=0.0$
Cross 40.	587 : 224 : 210 : 86	673 : 434
( $i\text{ }w^b \times I\text{ }W_2^b$ )	(584) : (227) : (213) : (83)	(667) : (440)
		$c=0.4$

Cross 41.	631 : 182 : 200 : 59	690 : 382
( $i w^b \times I W_2^b$ )	(630) : (183) : (201) : (58)	(688) : (384)
(88) $I$ - $S$ .	609 : 212 : 215 : 76	$c=0.1$ 685 : 427
Cross 38.	(608) : (213) : (216) : (75)	(683) : (429)
( $I S \times i s$ )		$c=0.1$
Cross 40.	588 : 223 : 210 : 86	674 : 433
( $i s \times I S$ )	(585) : (226) : (213) : (83)	(668) : (439)
		$c=0.4$
Cross 41.	655 : 158 : 210 : 49	704 : 368
( $i s \times I S$ )	(656) : (157) : (209) : (50)	(706) : (366)
		$c=0.1$
(89) $Le$ - $W_2^b$ .	598 : 209 : 231 : 74	672 : 440
Cross 38.	(602) : (205) : (227) : (78)	(680) : (432)
( $LeW_2^b \times lew^b$ )		$c=0.5$
Cross 40.	599 : 232 : 198 : 78	677 : 430
( $lew^b \times LeW_2^b$ )	(598) : (233) : (199) : (77)	(675) : (432)
		$c=0.1$
Cross 41.	626 : 187 : 205 : 54	680 : 392
( $lew^b \times LeW_2^b$ )	(630) : (183) : (201) : (58)	(688) : (384)
		$c=0.5$
(90) $Le$ - $S$ .	594 : 213 : 230 : 75	669 : 443
Cross 38.	(598) : (209) : (226) : (79)	(677) : (435)
( $LeS \times le s$ )		$c=0.5$
Cross 40.	600 : 231 : 198 : 78	678 : 429
( $le s \times LeS$ )	(599) : (232) : (199) : (77)	(676) : (431)
		$c=0.1$
Cross 41.	662 : 151 : 203 : 56	718 : 354
( $le s \times LeS$ )	(656) : (157) : (209) : (50)	(706) : (366)
		$c=0.8$
(91) $W_2^b$ - $S$ .	821 : 8 : 3 : 280	1101 : 11
Cross 38.	(614) : (215) : (210) : (73)	(687) : (425)
( $W_2^b S \times w^b s$ )		$c=25.5$
	Gametic ratio is 16.2 : 0.3 : 0.1 : 16.7; crossing-over is 1.2 %	
Cross 40.	796 : 1 : 2 : 308	1104 : 3
( $w s \times W_2^b S$ )	(575) : (222) : (223) : (87)	(662) : (445)
		$c=27.1$
	Gametic ratio is 15.6 : 0.1 : 0.1 : 17.5; crossing-over is 0.6 %	

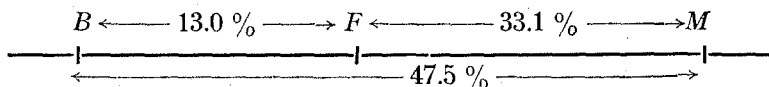
Cross 41.	822	:	9	:	43	:	198		1020	:	52
$(w^b s \times W_2^b S)$	(671)	:	(160)	:	(194)	:	(47)		(718)	:	(354)
									$c=19.6$		

Gametic ratio is 16.8 : 0.4 : 1.5 : 14.0; crossing-over is 5.8 %

### § 3. Conclusions

The different values for  $c$  are either considerably smaller than 3.0 — in most cases — or considerably larger, so that there is no doubt about independent inheritance or linkage.

Let us first discuss those relations which are a replication of part of Linkage-Studies I.  $B-F$  and  $M-F$  were found to be linked with crossing-over percentages of 13.0 % and 33.1 %. No direct linkage between  $B-M$  was found, but linkage may have escaped observation due to a crossing-over of about 50 %. If the gametic ratio for  $B-M$  is computed, it would mean a crossing-over of 47.5 %. Consequently the following mapping of  $B-F-M$  in one of the chromosomes is evident:



This means absence of double crossing-over. In Linkage-Studies I a method was used for the calculation of double crossing-over from the trihybrid gametic ratio. If this method is applied to the present material, quite astonishing results are obtained, for one of the gametic values is found to be negative and so is the percentage of double crossing-over. The gametic ratio is as follows:

$$\begin{array}{cccccccc}
 BFM : BFm : BfM : Bfm : bFM : bFm : bfM : bfm \\
 5.4 : 7.1 : 1.5 : 0.5 : -0.7 : 2.4 : 8.0 : 4.2
 \end{array}$$

Since the parental combinations are  $BFm$  and  $bFM$ , the double cross-overs are  $Bfm$  and  $bFM$  and the percentage of double crossing-over would be  $-0.7\%$ . This means of course nothing but that the numbers in the present material are too small for the method of calculation, or that there is no double crossing-over at all. But the occurrence of the phenotypic classes  $Bfm$  and  $bFM$  cannot be explained unless double crossing-over is supposed to have occurred.

Similar difficulties are found when the interference relations are calculated. We find namely:

crossing-over	<i>B-F</i>	among non-cross-overs	<i>F-M</i>	: 20.5 %
"	"	"	cross-overs	" : -2.1 %
"	"	<i>F-M</i>	non-cross-overs	<i>B-F</i> : 38.8 %
"	"	"	cross-overs	" : -5.4 %
"	"	<i>B-M</i>	non-cross-overs	<i>B-F</i> : 38.8 %
"	"	"	cross-overs	" : 105.4 %
"	"	"	non-cross-overs	<i>F-M</i> : 20.5 %
"	"	"	cross-overs	" : 102.1 %

Apart from the negative values the changes in the percentages of crossing-over are similar to those found before. Therefore we may conclude that also in the present material *B-F-M* form a linkage-group and are localized in the given order.

All the other relations, already studied before, resulted in independence and this is a confirmation of former results.

Among the new relations *V-Le* were found to be linked with 13.8 % crossing-over and so were *W<sub>2</sub><sup>b</sup>-S* with crossing-over percentages of 1.2 %, 0.6 % and 5.8 % in different crosses. The rather great variation in crossing-over may be due to genetic causes, probably also modification has played its part, especially in cross 41 where 5.8 % crossing-over was found. The  $F_2$ -ratio in this cross was 822 : 9 : 43 : 198 and very probably part of the third group (emerald, free) belongs to the fourth group (emerald, chenille). This modification was already discussed on p. 276.

No other cases of linkage were found and therefore the total results of the three crosses can be summarized as follows, if we put linked factors between brackets and separate independent ones by a dash.

Cross 38:  $A-\underline{Fa}-[V, \underline{Le}]-I-[W_2^b, S]$

Cross 40:  $A-[B, F, M]-D^w-\underline{Pl}-I-\underline{Le}-[W_2^b, S]$

Cross 41:  $\underline{Gp}-P-I-\underline{Le}-[W_2^b, S]$

In no cross the number of independent genes exceeds the number of chromosome-pairs which is seven. However, combining the above results with those, arrived at in Linkage-Studies I — namely:

$A-[B, F, M]-D^w-\underline{Fa}-\underline{Gp}-[P, \underline{Pl}]-V$   
gives:

$A-[B, F, M]-D^w-\underline{Fa}-\underline{Gp}-[P, \underline{Pl}]-[V, \underline{Le}]-I-[W_2^b, S]$ , and now the chromosome number is exceeded by two.

## IV. DISCUSSION

§ 1. *Comparison of results obtained with those of others*

The results of other investigators with regard to the mutual inter-relationships of the first ten of my factors were amply discussed in Linkage-Studies I. Therefore only the new relations are subject to discussion now, whereby it should always be kept in mind that identity between genes, acting similarly according to different authors, is never proven unless the work is done with the same material.

In 1917 WHITE (12) concluded to independence of  $A-B-\underline{Fa-I-Le}$ .

Independent inheritance of  $A-B-\underline{Fa-Pl-M-I-Le}$  was found by KAPPERT (3). I pointed before (11, p. 462) to the evidence that among KAPPERT's material  $B$  and  $M$  are linked with a crossing-over percentage not far from 50 %. The results, discussed in the foregoing part of this paper, offer new evidence to the existence of this linkage.

The linkage between  $W_2^b$  and  $S$  was found by WELLENSIEK (9) in 1925; he also obtained indications of independence between  $Le$  and  $W_2^b$ .

BREMER (1), in 1926, was the first to discover the linkage between  $Le$  and one of the genes  $P$  or  $V$ . Thru the kindness of Mr. BREMER I obtained samples of his material and I observed that his sugar-pea parent probably is a  $PP\ vv$ -type, so that his cross must have been  $PP\ VV \times PP\ vv$  and  $V$  is responsible for the segregation. Consequently the linkage, found by BREMER, is the same as one of the linkages in my material, namely between  $Le$  and  $V$ .

RASMUSSEN (5) considers  $P$ ,  $V$  and  $Le$  to belong to one linkage-group, in which the linkage between  $P$  and  $V$  escapes observation owing to about 50 % crossing-over. My material confirms RASMUSSEN's results as to the linkage  $V-Le$ , but I found  $P-Le$  independent. Another fact which speaks against combining  $P$ ,  $V$  and  $Le$  in one group is the linkage  $P-Pl$  as described in Linkage-Studies I, while both  $V$  and  $Le$  are independently inherited from  $Pl$ . In my material therefore  $P-Pl$  and  $V-Le$  form two separate linkage-groups and the different results of RASMUSSEN and me can hardly be explained, unless one or more of his genes are not identical with mine.

SVERDRUP (6) found independence between  $A-B-D-\underline{Fa-I-Le-W_2^b}$ ; at least, judging from her descriptions, the factors mentioned were among her material. Recently the TEDINS (7) found linkage between  $B-M$ , while they doubted about the relation  $M-F$  and unfortunately



did not study  $B-F$ . It is important with regard to my own results that the TEDINS concluded to linkage between  $B$  and  $M$ , because both were linked to a third gene;  $B$  and  $M$  themselves did not reveal a clear linkage due to 50 % crossing-over. These results confirm part of my results as to the linkage-group  $B-F-M$ . Moreover the TEDINS stated a number of cases of independence, namely  $A-B-D-Pl$ ;  $A-F$ ;  $A-I$ ;  $B-I$ ;  $D-M$ ;  $D-F$ ;  $Pl-M$ ; uncertain were  $A-M$  and  $M-I$ .

In a paper which is in the press now (10) extensive evidence for independence between  $W_2^b$  and  $I$  is given.

### § 2. *The number of linkage-groups in Pisum*

Rather alarming statements about the number of linkage-groups in *Pisum* with regard to the chromosome-number are found in the recent literature on linkage in *Pisum*. Since the results, arrived at in the present paper, seem to be alarming also, it is worth while to discuss the situation critically.

WHITE's finding (12), in 1917, of seven independent genes — which number is the same as the haploid number of chromosomes — is not so very important, because WHITE studied the interrelations between 8 genes, two of which were known to be linked. Consequently WHITE had no chance of finding more independent genes than 7.

It was discussed before (11, p. 462) that evidently two of KAPPERT's (3) eight independent factors are linked with about 50 % crossing-over, so that his number of independently inherited factors is reduced to 7.

In 1925 HERZBERG-FRÄNKEL (2, p. 329—331) summarized some of the papers on linkage in *Pisum* and put up 10 "preliminarily independent" groups. Among these 10 groups 8 are those of KAPPERT. Apart from quite a few incorrect statements, not the least evidence was put forth that groups IX and X of HERZBERG-FRÄNKEL are independent from KAPPERT's 8 groups.

In the work of SVERDRUP (6), extended by DE WINTON (13), most of the relations between 15 genes were studied and 9 (SVERDRUP) or 10 (DE WINTON) independent genes or groups of linked genes were found. However, 14 relations were not studied and this leaves some doubt as to the correctness of the authors' final conclusions. If, for instance, we suppose that  $\underline{Fa}$  belongs to the group  $I-O$  ( $\underline{Fa-O}$  was not studied and  $\underline{Fa-I}$  may be linked with about 50 % crossing-over) and

if, furthermore, we suppose that  $W^a-D$  (not studied) are strongly linked and belong to the strongly linked  $R-Tl$  ( $Tl-D$  is not studied) on the opposite side of a chromosome, the number of independent genes is reduced to 7. This is, of course, a mere speculation, but it is only meant to illustrate that it is not allowed to draw final conclusions before all unknown relations are studied.

The same criticism can be offered with regard to the 9 independent groups which RASMUSSEN (5, p. 120—121) put up from data of different workers. There is not the least evidence that his groups 4 and 6 or 4 and 7 or 4 and 8 etc. are not the same, to which fact RASMUSSEN (5, p. 127) himself has pointed.

Consequently we see that disharmony between the number of independent genes and the number of chromosomes in *Pisum* has not definitely been proven yet. My own results, described in this paper, seem to be a proof, but assuming the possibility of linkage with frequent crossing-over between apparently independent factors, for instance between  $A-D^w$  and between  $Fa-Gp$ , the number of independent genes is reduced to seven. When we consider the fact that quite a few cases of linkage with percentages of crossing-over not far from 50 % have been demonstrated to exist by several workers, the above suggestion may not at all be considered as impossible. Even the finding of one more independent gene in addition to my nine independent ones would not be a final proof, but two or three more would make the immediate application to *Pisum* of MORGAN'S *Drosophila*-principles rather doubtful.

It might be that *Drosophila* offers an instance of an organism in which special and relatively simple principles are valid, while in *Pisum* somewhat different and more general principles hold true. The present state of the experimental work in *Pisum* does not allow to draw conclusions yet.

It is hoped to extend my linkage-studies in the next years by studying the relations of some more factors to the fourteen, about which the present paper has dealt with.

#### V. SUMMARY

1. In Linkage-Studies I the relations between ten factors —  $A, B, D^w, Fa, Gp, P, V, Pl, M, F$  — were described. In the

present study four more factors are added to these ten, namely:

$I$  : cotyledons yellow;  $i$ : green.

$\underline{Le}$  : internodes long;  $\underline{le}$ : short.

$\underline{W}_2^b$ : foliage and stem glaucous;  $w^b$ : emerald.

$S$  : seeds free in the ripe pod;  $s$ : seeds adhering "chenille"-like.

2. New evidence was obtained for the existence of a linkage-group  $B-F-M$ , in which  $B$  and  $M$  are linked with about 50 % crossing-over.
3. Among the relations, not studied before by the present author, linkage was found between  $V-\underline{Le}$  and between  $\underline{W}_2^b-S$ .
4. If we put linked factors between brackets and separate independent factors or groups by a dash, the total results are:  
 $A-[B, F, M]-D^w-\underline{Fa}-\underline{Gp}-[P, Pl]-[V, \underline{Le}]-I-[W_2^b, S]$
5. The number of bivalent chromosomes is exceeded by two by the number of independent genes or groups of linked genes.
6. From a critical examination of the recent literature and of own results, it is concluded that disharmony between the number of chromosomes and the number of linkage-groups in *Pisum* has not been finally demonstrated yet.

Wageningen, Dec. 12, 1928

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