THE OCCURRENCE OF MORE THAN 50 % CROSSING-OVER IN PISUM

by

S. J. WELLENSIEK
(Wageningen, Holland)

INTRODUCTION

In *Drosophila* all possible percentages of crossing-over occur, up to nearly 50 percent. MORGAN (2, p. 20), however, admits a theoretical possibility that " . . . . more than 50 percent crossing-over should be found", resulting in " . . . . a sort of inverted linkage . . . ., since the cross-over combinations would then be more frequent than the grand-parental types". Therefore SINNOTT and DUNN (4, p. 185) are "plus royaliste que le roi" when they positively state: "It is true that the amount of crossing-over between two genes never exceeds 50 per cent".

Theoretically the possibility of percentages of crossing-over higher than 50 % cannot be denied, but very few actual cases have been reported in literature thus far. WRIEDT and CHRISTIE (8, pp. 283-284) found 54.2 % crossing-over between two sex-linked genes in pigeons. Linkage between the two genes in question is only supposed to exist, because both of them are sex-linked. It is questionable whether this is an absolute proof of linkage, the more because the actually found numbers in the four back-cross classes, viz. 10 : 12 : 16 : 10, agree very well with the expectation of free combination, viz. 12 : 12 : 12 : 12. In a former paper (5, p. 241) I already cited CLAUSEN's *Viola* work (1, pp. 20-22), where rather strong indications for a linkage of about 85 % crossing-over were obtained; furthermore RASMUSSON (3, pp. 94, 99, 100) obtained results in *Pisum* which tend to point to linkage with a more frequent crossing-over than 50 %.

WELLENSIEK (5, pp. 236-239, 241) demonstrated linkage between two genes with more than 60 % crossing-over in *Pisum*. These results are based on F_2-material with not too many individuals. In the present paper the results of back-crosses will be reported. They confirm the former results that crossing-over with very high frequencies may occur [1]).

I wish to express my thanks to Mr. J. S. KEYSER, who very carefully made the back-crosses for me in 1928.

MATERIALS AND METHODS

Principally the relations between three genes were studied, viz.:

Gp: green pod; gp: yellow pod.
N :   thin pod-wall; n: thick pod-wall.
Ss :   straight pod; ss: curved pod.

Besides, notes were taken on the action of:

A :   colored flower; a: white flower.
P :   membrane at the innerside of the pod-wall; p: no membrane.
Q :   abortion of some of the seeds in the pods; q: no abortion.

Although violet and green pod (gene P_1 and its allelomorph p_1) were segregating in part of the material studied, no attention was paid to these characteristics, because classification of them takes much time owing to modification and because there was no special reason for studying them.

The above genes were brought in by three pure lines, already used in earlier studies, while three more lines at least recessive for gp, n and ss were isolated from the F_2 of cross 27, studied in 1927 (5). These latter lines are indicated as α, β and γ. The factorial composition of the parental lines — using single symbols for simplicity's sake — is:

Pois à cosse rouge :	gp	N	Ss	A	P	q
Pois à cosse jaune :	gp	N	Ss	a	p	Q
Reuzenboterpeul :	Gp	n	ss	a	P	q
α . . . . :	gp	n	ss	a	P	q
β . . . . :	gp	n	ss	a	p	?
γ . . . . . . :	gp	n	ss	a	p	q

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1) After having finished the present manuscript I found another instance of crossing-over of about 60% between two entirely different genes in entirely different material than described in the following. This new case will be treated in "Linkage-studies in Pisum III".

In so far as P and Q in lines α, β and γ are concerned, the genotype has been put up as a result of the back-crosses.

These back-crosses with the genes studied are:

14a   : (Reuzenboterp. × P. à c. rouge)   × α : Gp-N-S_s-A
27a (β) : (P. à c. jaune   × Reuzenboterp.) × β : Gp-N-S_s-P
27a (γ) : (P. à c. jaune   × Reuzenboterp.) × γ : Gp-N-S_s-P-Q

Mr. KEYSER made 126 back-crosses of 14a, 111 of which succeeded, yielding 661 seeds which all gave full-grown plants. For 27a the numbers are: 116 back-crosses, 87 succeeded, 227 seeds, 212 full-grown plants. The relatively small number of seeds in 27a is brought about by the inherited abortion.

The detailed results of the back-crosses are given in the appendix to this paper on p. 517. The usual methods of calculation are used, as recently described in detail (6, pp. 8-9). In the data on the factor-relations (§§ 2-3) the two numbers at the right side of the vertical line represent the sums of outer and inner terms of the four class ratio at the left of the line. Expectations in case of independent inheritance are put in parentheses, while c indicates actual deviation from expectation divided by standard error of expectation. Unless otherwise stated, the two groups of 27a are taken together.

<center>EXPERIMENTAL RESULTS</center>

<center>§ 1. *Monofactorial segregations*</center>

The following segregations of the single characters were obtained.

	Back-cross 14a			*Back-cross 27a*	
Gp.	342	: 319		104	: 108
	(330.5)	: (330.5)		(106)	: (106)
	c=0.9			c=0.3	
N.	362	: 299		103	: 109
	(330.5)	: (330.5)		(106)	: (106)
	c=2.5			c=0.4	
S_s.	357	: 304		104	: 108
	(330.5)	: (330.5)		(106)	: (106)
	c=2.1			c=0.3	

	Back-cross 14a	Back-cross 27a
A.	345 : 316	
	(330.5) : (330.5)	
	c=1.1	
P.		104 : 108
		(106) : (106)
		c=0.3
Q.		137 : 75
		(106) : (106)
		c=4.3

The agreement in the case of Q is too bad. Therefore this segregation cannot be considered as a monofactorial back-cross ratio and the data on Q may not be used in studying factor-relations. However, when we consider the two groups of which back-cross 27a consists separately, we find the following segregations:

|	group β	group γ
	117 : 53	20 : 22
	(85) : (85)	(21) : (21)
	c=4.9	c=0.3

Consequently group γ gives a perfect 1:1 ratio, but group β does not. This makes it evident that line β has not been homozygous for Q or q, but that line γ has been qq (cp. the formulas on p. 510). In the following only data on the reliable group γ will be used in so far as relations to Q are concerned.

§ 2. The linkage-group [$G\underset{.}{p}$, N, S_s]

Back-cross 14a — $(G\underset{.}{p} \, n \, s_s \times g\underset{.}{p} \, N \, S_s) \times g\underset{.}{p} \, n \, s_s$ — has given the following results:

Gp—N.	187 : 155 : 175 : 144	331 : 330
	(187) : (155) : (175) : (144)	(331) : (330)
		c=0.0
Gp—S_s.	222 : 120 : 135 : 184	406 : 255
	(185) : (157) : (172) : (147)	(332) : (329)
		c=5.8
N—S_s.	307 : 55 : 50 : 249	556 : 105
	(196) : (166) : (161) : (138)	(334) : (327)
		c=17.3

Linkage is evident for Gp-S_s and for N-S_s, not for Gp-N however. If we surmise about 50 % crossing-over between Gp and N and calculate the exact percentages of crossing-over, we find 50.1 % for Gp-N, 15.9 % for N-S_s and 61.4 % for Gp-S_s. This would lead to the following localization:

Gp ←————————50.1 % ——————————→ N ←15.9% → S_s

——|————————————————————————————|—————————|————

←————————————— 61.4 % —————————————→
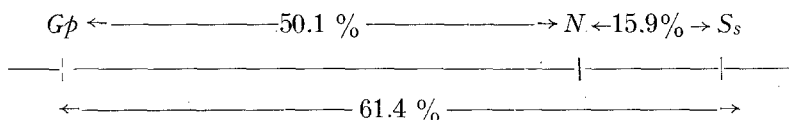
The three-factorial ratio $Gp\,N\,S_s$: $Gp\,N\,S_s$: . . . . : $gp\,n\,s$ equals 180 : 7 : 42 : 113 : 127 : 48 : 8 : 136. Since the second and the seventh term are double cross-overs, the percentage of double crossing-over is 2.3 %.

Back-cross 27a — ($gp\,N\,S_s$ × $Gp\,n\,s_s$) × $gp\,n\,s_s$ — yielded next results:

Gp-N.   56 : 48 : 47 : 61   117 : 95
            (51) : (53) : (52) : (56)   (107) : (105)
                                               c=1.4

Gp-S_s.   61 : 43 : 43 : 65   126 : 86
            (51) : (53) : (53) : (55)   (106) : (106)
                                               c=2.8

N-S_s.   94 : 9 : 10 : 99   193 : 19
            (51) : (52) : (53) : (56)   (107) : (105)
                                               c=11.9

Clear linkage only exists for N-S_s, although the value for c in the case of Gp-S_s is very high. In case of linkage the percentages of crossing-over would be 55.2 %, resp. 59.4 % and 8.9 % which results in the following map:

Gp ←———————— 55.2 % ——————————→ N ← 8.9 % → S_s

——|———————————————————————————————|—————————|——

←————————————— 59.4 % —————————————→
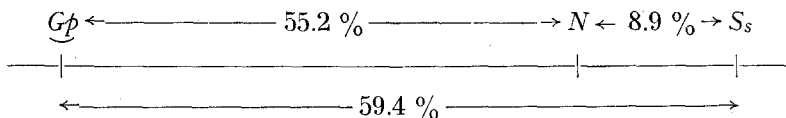
The three-factorial ratio is 51 : 5 : 10 : 38 : 43 : 4 : 0 : 61 and the percentage of double crossing-over is 2.4 %.

Comparing the results of back-cross 14a with those of 27a it follows

that the former positively point to the existence of a linkage-group
$[Gp, N, S_s]$, while the latter do not necessarily do so. The hypothesis
of linkage is materially supported by calculating the interference
relations from the trifactorial ratios. We then find:

					14a	*27a*
Crossing-over Gp-N among	non cross-overs	N-S_s			56.8 %	58.0%
,,	,,	,,	,,	cross-overs ,,	14.3 %	26.3 %
,,	,,	N-S_s	,,	non cross-overs Gp-N	27.3 %	14.7 %
,,	,,	,,	,,	cross-overs ,,	4.5 %	4.3 %
,,	,,	Gp-S_s	,,	non cross-overs Gp-N	27.3 %	14.7 %
,,	,,	,,	,,	cross-overs ,,	95.5 %	95.7 %
,,	,,	,,	,,	non cross-overs N-S_s	56.8 %	58.0 %
,,	,,	,,	,,	cross-overs ,,	85.7 %	73.7 %

There is no reason for the variations in the percentages of crossing-
over, as indicated in the above table, if not the three genes in question
are localized in the same chromosome. The parallelism between the
numbers for 14a and for 27a makes it evident that in both back-
crosses a linkage-group $[Gp, N, S_s]$ is involved, in which the per-
centages of crossing-over for Gp-S_s are more than 50 % which also
holds true for Gp-N in 27a.

§ 3. *The relations of Q to* $[Gp, N, S_s]$

As discussed at the close of § 1, only group γ of back-cross 27a has
given reliable data on the relations in which Q is involved. The num-
bers in this group are small, namely 42, so that no definite results can
be expected. The ratios are rather suggestive, however, and therefore
will be discussed. They are:

Q-Gp.   9 : 11 : 11 : 11   20 : 22
      (10) : (10) : (10) : (12)   (22) : (20)
                           c=0.6

Q-N.   17 : 3 : 0 : 22   39 : 3
      (8) : (9) : (12) : (13)   (21) : (21)
                           c=5.6

Q-S_s.   15 : 5 : 1 : 21   36 : 6
      (8) : (12) : (8) : (14)   (22) : (20)
                           c=4.4

Linkage between Q-N and Q-S_s is clear, so that Q, N and S_s anyhow belong to one linkage group. The crossing-over percentage for Q-N is 7.1 %, for Q-S_s it is 14.2 % and for N-S_s (in group γ of 27a) it is 7.1 %. This would point to a linear order Q-N-S_s. Gene Gp may then be so far apart from Q and N that there is about 50 % crossing-over for both Gp-Q and Gp-N. The material at hand is not large enough for definitely deciding this question. It is evident that Q belongs to the group $[Gp, N, S_s]$, but more extensive material must be studied for determining its locus.

§ 4. *The relations with A and P*

In 14a the relations of A to $[Gp, N, S_s]$ were studied and in 27a those of P to $[Gp, Q, N, S_s]$. All these relations were found to be independent. Since this is a confirmation of former results (5, 7), no particulars will be mentioned. The segregation-numbers follow from the summarizing tables in the appendix.

DISCUSSION OF RESULTS

The main purpose of the present study has been to find out whether back-crosses would give the same results as F_2-material had given in so far as the occurrence of more than 50 % crossing-over is concerned. As indicated in the former pages, the back-crosses indeed gave similar results as F_2's studied before (5, see especially p. 238 for summarized results). There is another rather striking similarity between the new results and the former ones, namely in the variation of crossing-over percentages. Both in F_2's and in back-crosses involving Gp, N and S_s there was:

(1) little variation in the crossing-over between Gp and N.
(2) considerable variation in the crossing-over between N and S_s, namely relatively weak linkage in cross 14 and back-cross 14a, relatively strong linkage in cross 27 and back-cross 27a.
(3) weaker linkage between Gp and S_s in 14 and 14a than in 27 and 27a, although these differences are not very much pronounced in 14a and 27a. In as far as they are evident, they probably result from variations in the crossing-over between N and S_s.

From these three facts can be concluded that the genotype of the parents of the cross may influence the rate of crossing-over in only part of a chromosome (cf. 5, p. 23 8).

WELLENSIEK and KEYSER (7) already found linkage between Q, N

and S_s. In the present material this result is confirmed, while the possibility is shown that Gp also belongs to this linkage-group. WELLENSIEK and KEYSER (7) considered Gp as independent from $[Q, N, S_s]$, but their material was small and their conclusion therefore preliminarily. The study of more extensive material is still wanted to localize the four genes of the group, especially to localize Q with regard to Gp, N and S_s.

The finding of more than 50 % crossing-over between Gp and S_s is of double interest. Besides the interest from a general theoretical standpoint, the occurrence of very high frequencies of crossing-over is important in understanding the linkage situation in *Pisum*. I will not enter into a detailed discussion of this point now, but wait until the relations of a number of genes to Gp, N and S_s which are being studied, are known.

SUMMARY

1. In back-crosses the existence of more than 50 % crossing-over between Gp and S_s in the group $[Gp, N, S_s]$ was demonstrated.
2. Q also belongs to the above group, although its relative position is not definitely determined yet.
3. A is independent from $[Gp, N, S_s]$ and P is independent from $[Gp, Q, N, S_s]$ (confirmation of former results).

Wageningen, Sept. 19, 1929

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APPENDIX

In the two following tables, giving the detailed total numerical results of the two back-crosses, the symbols stand for the phenotypic characters, as observed in the field, unless in the heads of the table, where they represent genotypes.

BACK-CROSS 14a: $(a\ Gp\ n\ s_s \times A\ gp\ N\ S_s) \times a\ gp\ n\ s_s$

Phenotype	class	number
A Gp N S_s	1	96
———— N s_s	2	6
———— n S_s	3	17
———— n s_s	4	61
A gp N S_s	5	65
———— N s_s	6	22
———— n S_s	7	6
———— n s_s	8	72
a Gp N S_s	9	84
———— N s_s	10	1
———— n S_s	11	25
———— n s_s	12	52
a gp N S_s	13	62
———— N s_s	14	26
———— n S_s	15	2
———— n s_s	16	64
	total	661

BACK-CROSS 27a: $(gp\ p\ N\ S_s\ Q\ \times\ Gp\ P\ n\ s_s\ q)\ \times\ gp\ p\ n\ s_s\ \overset{?}{q}$

Phenotype	class	total	group β	group γ
Gp P N S_s Q	1	24	21	3
——— S_s q	2	0	0	0
——— s_s Q	3	5	3	2
——— s_s q	4	0	0	0
Gp P n S_s Q	5	0	0	0
——— S_s q	6	5	5	0
——— s_s Q	7	7	7	0
——— s_s q	8	15	8	7
Gp p N S_s Q	9	27	23	4
——— S_s q	10	0	0	0
——— s_s Q	11	0	0	0
——— s_s q	12	0	0	0
Gp p n S_s Q	13	3	3	0
——— S_s q	14	2	1	1
——— s_s Q	15	4	4	0
——— s_s q	16	12	9	3
gp P N S_s Q	17	19	15	4
——— S_s q	18	0	0	0
——— s_s Q	19	1	1	0
——— s^s q	20	0	0	0
gp P n S_s Q	21	0	0	0
——— S_s q	22	0	0	0
——— s_s Q	23	4	3	1
——— s_s q	24	24	14	10
gp p N S_s Q	25	24	20	4
——— S_s q	26	0	0	0
——— s_s Q	27	3	3	0
——— s_s q	28	0	0	0
gp p n S_s Q	29	0	0	0
——— S_s q	30	0	0	0
——— s_s Q	31	16	14	2
——— s_s q	32	17	16	1
total	212	170	42	