

Location of the breakpoints of four reciprocal translocations involving linkage group V and their influence on recombination distances between neighboring markers

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Mapping the breakpoints of reciprocal translocations on a recombination map is an important part of genetic work with pea. Some of translocations have been known for several decades, but there is still a controversy in the map positions of their breakpoints, probably owing to different crosses being used to determine linkage with different loci. In this work we attempt to map the breakpoints of four translocations involving linkage group V by crossing them with either of two testerlines carrying appropriate markers. Three of these translocations are well known, namely, Hammarlund, Nilsson and Winge translocations, the fourth is the translocation carried by the line WL1393. The first and second translocations have their breakpoints near the loci *Cri* and *Gp*, the third and fourth near the loci *R* and *Tl*. As we dealt with genetic but not cytological analysis, we will use the linkage map nomenclature only. Thus "chromosome 5" will mean "chromosome associated with linkage group V". For a new version of the pea linkage map and correspondence of linkage group and chromosome nomenclature see ref. 15.

Materials

The following translocation stocks were used: i) Hammarlund translocation 2-5, with a breakpoint near the loci *Cri* and *Gp*. Our original stock HT-2 was used homozygous for this translocation and marked with the alleles *a* and *lf* (see ref. 2). It was derived from the hybrid Tau2, described in Gorel et al. (5) by fixing a cross-over event between the loci *A* and *His*(2-6) transferring the alleles *a* and *lf* on a translocated chromosome. ii) Nilsson translocation 3-5: line WL1718 (= Lamm's L83), with a breakpoint near the loci *Cri* and *Gp*. iii) Winge translocation 7-5: line WL1479 (= Lamm's L112), with a breakpoint near the locus *R*. iv) Line WL1393 shown to have a translocation with a breakpoint near the locus *R* (9). Among many other markers, WL1393 has *b* and *st* of linkage group III.

Line WL110 and all the translocated lines except WL1393 had the alleles *Cri*, *Gp*, *Det*, *R*, *His*¹, *Sca*, *Curl*, *B*, and *St*. The lines HT-2 and WL1718 were crossed with the line Yellow Crispa, possessing a normal karyotype and marked with alleles *cri*, and *gp*. Yellow Crispa was derived from the cross SGE182 x WL1238, where the former is a mutant *cri* induced in the stock SG with EMS. The lines WL1479 and WL1393 were crossed with the line DRC (see ref. 1) homozygous for the alleles *det*, *r*, *His*¹, *Sca*, and *curl*.

To evaluate changes of recombination distances between the markers involved we used as a control the line WL110, accepted as possessing the standard karyotype in the pea (3). This line was crossed with both Yellow Crispa and DRC, and F₂ populations were generated.

All the Weibulsholm lines, designated by WL, were generously provided by Dr. S. Blixt, Alnarp, Sweden.

Methods

Protein isolation and electrophoresis: The phenotype for protein markers *His*¹ and *Sca* were determined using electrophoresis as is described in (10) and (1), respectively. Phenotype for the gene *r* was scored by microscopic analysis of the starch grains in seeds.

Linkage estimation: In all the crosses the F₁ progenies had semisterile pollen manifesting heterozygosity for a translocation. Semisterility versus full fertility of pollen was used in F₂ or test cross progenies for scoring structural heterozygotes for a translocation versus structural homozygotes. Linkage of a breakpoint to markers

was evaluated either in a test cross, in the progeny of which the breakpoint behaved as a dominant marker producing pollen semisterility, or in F_2 progenies. In the latter case two phenotypic classes are observed with respect to pollen sterility: structural heterozygotes with semisterile pollen and both types of structural homozygotes, with fertile pollen, as a single class. The recombination fraction between the breakpoint and a marker was estimated here by the maximum likelihood method (see 3). Calculations were made with the aid of the original program TBREAK for IBM PC, written by S.M. Rozov. Calculations of recombination fraction between markers were made, also by maximum likelihood method, with the program CROS by the same author.

Results

Crosses with the line Yellow Crispa concerning the *cri-gp* region

i) The cross WL110 x Yellow Crispa yielded the following segregation of phenotypes in 337 plants of the F_2 progeny: 226 Cri Gp, 18 Cri gp, 21 cri Gp, 72 cri gp, that suggests linkage of $11.9 \pm 1.9\%$ with the joint chi-square of 168.8 ($P < 0.0001$).

ii) A cross WL1718 x Yellow Crispa was performed and joint segregation of the breakpoint and the markers *cri* and *gp* was scored in the F_2 population, as presented in Table 1. No plant appeared that could be considered an obvious trisomic. The data of Table 1 suggest the map segment on the right.

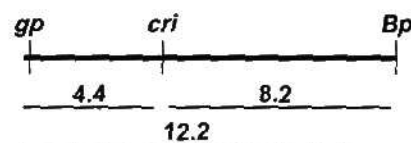


Table 1. Segregation of the F_2 progeny of the cross WL1718 x Yellow Crispa for the genes *cri*, *gp*, and the breakpoint*

	Phenotypes for Cri and Gp fertility					Cri	cri
	Cri	cri	Gp	gp	phenotype for gp		
ss	146	11	140	16	Gp	208	4
ft	77	52	80	49	gp	7	51
RF (%)	8.18 ± 2.43		12.18 ± 2.91			4.42 ± 1.28	
Joint seg. χ^2	45.7		30.8			207.9	

*Structural heterozygotes with semisterile pollen are designated as ss, structural homozygotes with fertile pollen as ft. Significance of all linkages, as estimated with joint chi-squares, far exceeds 0.1% level.

Hence, according to the most recent recombination map of *Pisum* (16), the position of Nilsson's translocation breakpoint is as follows:



iii) We performed a cross HT-2 x Yellow Crispa and analyzed joint segregation of the breakpoint and the markers *cri* and *gp* in an F_2 population and three F_8 populations (produced from heterozygotes for translocation and all the markers involved). The results shown in Table 2 reveal an absence of recombination between the

Table 2. Segregation of an F_2 and three F_8 progenies of the cross HT-2 (Hammarlund translocation, a, Cri, Gp) x Yellow Crispa (normal karyotype, A, cri, gp)

Family	A cri gp, homozygote N/N	A Cri Gp heterozygote N/K	a Cri Gp homozygote K/K	A Cri gp trisomic	Total
F_2	13	16	10	1	40
F_8 No. 1	9	25	12	0	46
F_8 No. 2	13	33	8	0	54
F_8 No. 3	12	22	12	2	48
Total	47	96	42	3	188

loci A, Cri, and Gp. An analysis of the hybrid Tau-2 presented in another communication in this issue (5) has shown that the cross-over rate between Cri and Gp is extremely low in heterozygotes for the Hammarlund translocation.

Crosses with the line DRC concerning the *r*-*Sca* region.

i) The distance between *R* and *Sca* in a normal karyotype was estimated in an F_2 population of the cross WL110 x DRC as 27.5 ± 3.1 % (Table 3).

Table 3. Segregation for the phenotypes for the genes *Sca* and *r* in the F_2 of the cross WL110 x DRC

Genes	<i>Sca</i> ⁺	<i>Sca</i> ⁺ + <i>Sca</i> ^f	<i>Sca</i> ^f	Total
<i>R</i>	68	105	26	199
<i>r</i>	6	31	34	71
Total	74	136	60	270
recombination fraction	27.5 ± 3.11 %			
joint segregation χ^2	42.15 $p < 0.0001$			

ii) Kosterin (9) previously showed that the breakpoint of the translocation of the line WL1393 is located about 10 cM from *Tl* (9). In a cross WL1393 x Yellow Crispa, linkage of the breakpoint was evaluated to the markers of the *R*-*Curl* segment of chromosome 5 as well as to some other markers, including *st* of chromosome 3. This cross yielded 306 F_2 seeds for which segregation of markers *r* and *Sca* was scored (Table 4) to provide an estimation of distance between them as 9.3 ± 1.7 cM. From these seeds we grew up 165 plants and scored the markers *st*, *His1*, *curl* and pollen fertility (Tables 5 and 6). No recombination was observed between *Sca* and *curl*, so the data for *curl* are not included in the tables. The results obtained suggest the map segments on the right. Above the bold line the marker-breakpoint distances are presented, while below the line there are distances between the markers other than the breakpoint. One can note a good additivity of marker-marker distances. A substantial difference is observed between the marker-marker distances and the marker-breakpoint distances (see Discussion). However, both sets of differences suggest the same gene order.

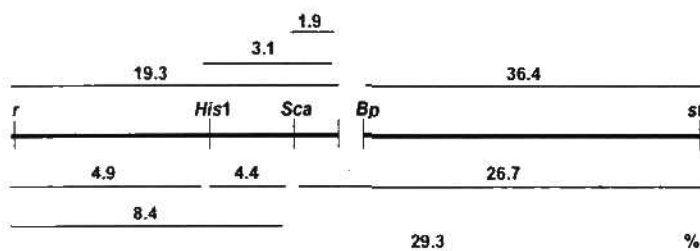


Table 4. Segregation for the phenotypes of the genes *r* and *Sca* as observed in the F_2 seeds resulted from the cross WL1393 x DRC

Genes	<i>Sca</i> ⁺	<i>Sca</i> ⁺ + <i>Sca</i> ^f	<i>Sca</i> ^f	Total
<i>R</i>	83	152	17	252
<i>r</i>	0	8	46	54
Total	83	160	63	306
recombination fraction	9.26 ± 1.72 %			
joint segregation χ^2	168.3 $p < 0.0001$			

iii) We made a testcross WL1479 x DRC x DRC (cross 1 of Tables 7 and 8) which produced 428 seeds scored for *r* and *Sca*. The segregation observed is shown in Table 7 (cross 1). One can see a drastic reduction of the distance *r*-*Sca* as compared with that for the normal karyotype. All seeds were planted in the greenhouse but only 129 produced healthy plants, probably due to damage while testing the phenotype for *r* and *Sca*.

Because the number of cross-overs in the region studied was greatly suppressed (Table 8, cross 1), that sample was insufficient to determine linkage relationships. Therefore, we performed testcrosses analogous to those in cross 1 by choosing from cross 1 plants heterozygotes for all the loci considered and pollinating them

Table 5. Segregation for the phenotypes for the genes *r*, *His1*, *Sca* and *st* as observed in the F₂ plants resulted from the cross WL1393 x DRC

Gene A	Gene B	Phase	Number of plants w/phenotypes designated									Joint	Rec. Fract.	St. Error
			A/B	A/h	A/b	h/B	h/h	h/b	a/B	a/h	a/b			
<i>r</i>	<i>His1</i>	-	50	83	1	-	-	-	0	6	25	122.03***	4.9	1.7
<i>r</i>	<i>Sca</i>	-	49	81	4	-	-	-	0	8	23	94.95***	8.4	2.2
<i>r</i>	<i>st</i>	R	99	—	35	-	-	-	25	-	6	0.62	44.7	6.1
<i>His1</i>	<i>Sca</i>	-	23	75	26	-	-	-	3	14	24	20.78***	29.3	4.1
<i>st</i>	<i>Sca</i>	-	23	78	23	-	-	-	4	11	26	29.77***	26.6	3.9

N=165.

Capital letters A and B stand for dominant phenotypes, lower case ones a and b for recessive phenotypes. In the case of genes *His1* and *Sca* with co-dominant alleles, h stands for heterozygotes, upper case letters stand for *His1*² and *Sca*², coming from WL1393, lower case letters stand for *His1*¹ and *Sca*¹, coming from DRC.

*, **, *** = $P < 0.01$, 0.001 and 0.0001, respectively.

Table 6. Segregation of the F₂ progeny of the cross WL1393 x DRC for the genes *r*, *Sca*, *st* and the translocation breakpoint. Structural heterozygotes with semisterile pollen are designated as *ss*, structural homozygotes with fertile pollen as *ft*.

fertility	phenotype for the four loci									
	st		r		His1			Sca		
	St	st	R	r	2	2+1	1	s	s+f	f
ss	79	45	80	11	1	85	5	1	87	3
ft	12	29	54	20	49	4	21	48	2	24
RF (%)	36.39 ± 10.65		19.32 ± 5.66		3.13 ± 3.32			1.85 ± 3.26		
J.s. χ^2	14.78		5.97		129.27			142.35		
P <	0.0005		0.025		0.0001			0.0001		

Table 7. Segregation for phenotypes for the genes *r* and *Sca* in the seed samples produced from two test-crosses^a

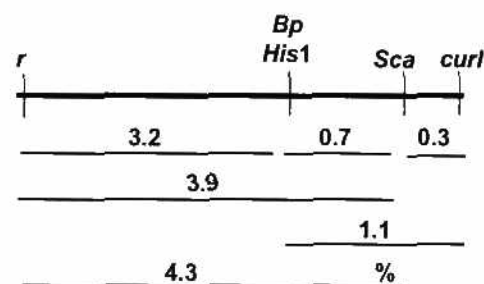
	Phenotypes ^b						N	Recomb. Fraction (%)
	R <i>Sca</i> ^{s+ft}	R <i>Sca</i> ^t	r <i>Sca</i> ^{s+ft}	r <i>Sca</i> ^t	R <i>Sca</i> ^{s+ft}	r <i>Sca</i> ^{s+ft}		
Cross 1	216	8	7	188	1	8	428	5.4 ± 1.1%
Cross 2	114	14	9	106	2	2	247	10.1 ± 1.9%
Combined	330	22	16	294	3	10	675	7.6 ± 0.1%

^aCross 1, WL1479 x DRC x DRC; and Cross 2, full heterozygotes of Cross 1 x full homozygotes of Cross 1.

^b+ indicates heterozygote or dominant phenotypes, - for homozygote or recessive phenotypes. *Sca*^{s+ft} indicates a heterozygote phenotype with equally expressed allelic variants *Sca*^s and *Sca*^t; *Sca*^{s+ft} indicates a heterozygote phenotype with the variant *Sca*^s about twice as abundant as *Sca*^t.

with homozygotes for all the markers derived from the line DRC. The progeny from these crosses are referred to as cross 2 in Tables 7 and 8. In cross 2 the distance r - Sca obtained appears to be about twice as large as that for the initial cross WL1479 x DRC x DRC (Table 7). Comparison of the values with the aid of Fisher j -transformation and Student criterion suggests a weak significance of differences at the 5% level, but the values are too small for even j -transformation to make an approach to normality enough for a correct use of the Student criterion.

We obtained 150 plants from the seeds of cross 2. The data on segregation of markers and the breakpoint for both crosses are given in Tables 7 and 8. A shortage of the markers derived from the line DRC can be noted. The sample of plants generates a somewhat smaller r - Sca distance than was observed in the full sample of seeds, but for the sake of mutual correspondence, we use the former value in the diagram on the right of linkage relationships obtained from the combined sample of plants of the two crosses. No cross-over event was observed between the breakpoint and the gene $His1$.



In this cross we faced the following phenomenon: Nine of 428 seeds obtained from the cross 1, and 4 of 247 seeds from the cross 2 (Table 8), had a strange electrophoretic pattern of the SCA protein: both allelic variants, SCA^s and SCA^f , were present but the former looked twice as abundant as the latter while in normal heterozygotes the variants are equally abundant. None of these seeds germinated in a greenhouse. This phenotype cannot be ascribed to tertiary trisomics, because one dose of Sca^f always comes from the testerline while an unequal resolution of a chromosomal cross in a structural heterozygote, being a female parent, cannot provide a gamete with a double dose of the same Sca^s allele. Such a phenotype could be exhibited by a trisomic resulting from self-pollination. But self-pollination was almost completely prevented in this testcross. Only one clear example of a seed derived from selfing (phenotype $R Sca^s$) was found among the 429 seeds considered, and it was excluded from our analyses. So, we putatively ascribe these problematic phenotypes to some rare alteration of a relative expression of the Sca alleles and consider these individuals as heterozygotes.

Table 8. Segregation of plant populations resulting from the two test-crosses presented in Table 7

Phenotypes ¹				Cross 1		Cross 2		Combined	
r	$His1$	Sca	$Curl$	Ss	F	Ss	F	Ss	F
Non-cross-over phenotypes									
+	+	+	+	84	0	80	0	164	0
-	-	-	-	0	42	0	61	0	103
Cross-over phenotypes									
+	+	+	-	0	0	1	0	1	0
+	-	-	-	0	1	0	1	0	2
-	+	+	+	2	0	5	0	7	0
-	-	+	+	0	0	0	2	0	2
Totals				129		150		279	

¹+ stands for heterozygote or dominant phenotypes, - for homozygote or recessive phenotypes.

Discussion

Effect of breakpoints on cross-over rate between neighboring markers.

Both series of crosses demonstrate the same trend of a progressing shrinkage of recombination distances between markers in a following order: normal karyotype; translocation breakpoint on one side of the markers; and breakpoint between the markers. Thus, one can see a reduction of the distance cri - gp from 11.9% to 4.4%,

to almost zero in a sequence of three crosses between the same testerline Yellow Crispa and WL110 (no translocation), WL1718 (Nilsson translocation, with a breakpoint on one side of these markers), and HT-2 (Hammarlund translocation, with a breakpoint between these markers), respectively. Similarly, in the series of crosses involving the line DRC the recombination length of the *r-Sca* segment decreases from 27.5% (cross with WL110), to 8.4% (cross with WL1393—breakpoint outside the segment), to 3.9% (cross with WL1479—Winge translocation with a breakpoint within the segment). This regularity suggests that cross-over rates are suppressed near translocation breakpoints. It would be of interest to learn to what an extent this effect varies in different translocations, but this is a matter of special studies involving a chain of closely set markers in relevant chromosomal regions, which are still missing in pea.

Linkage of translocation breakpoints to histone H1 loci.

An interesting point is that we have two translocations with breakpoints that cosegregate with loci coding for histone H1. Here we find a perfect association between *His1* and the Winge translocation, and Gorel et al. (5) report cosegregation of *His(2-6)* and the Hammarlund translocation breakpoint was observed in a sample of 800 families. One of such loci, *His(2-6)*, is a cluster of five tightly linked genes (10). The large sample allowed us to identify a case of cross-over within the *His(2-6)* cluster, known to be about 1 cM long (10). This recombination event provided an exchange H1 haplotype 2123 on a normal karyotype, suggesting that the breakpoint is very tightly linked to the particular histone gene *His6* within the cluster. The cluster *His(2-6)* may contain repetitive DNA sequences which could somehow facilitate chromosome breakage. In addition, this cluster is located close to the centromere. However, the locus *His1* seems to contain a single histone H1 gene and is located far from the centromere, although there may be some structural similarity of its chromosomal surroundings to those of *His(2-6)* which could have facilitated the origin of Winge translocation.

Evaluating recombination distances between a breakpoint and markers by the maximum likelihood method.

In two crosses, WL1718 x Yellow Crispa and WL1393 x DRC, we applied a maximum likelihood method for evaluation of recombination distances between markers and a translocation breakpoint in F_2 populations. The breakpoints were followed by pollen fertility (reflecting structural homozygosity) versus semisterility (structural heterozygote). Equations developed for the two cases of co-dominant and dominant markers are presented in Kosterin et al. (10). The former is simple, the latter can be easily solved numerically by a computer using the program T-BREAK.

In the cross WL1718 x Yellow Crispa we have obtained a map segment where the three distances (*cri-gp*, *cri-Bp*, *gp-Bp*) correspond well to each other. In the cross WL1393 x DRC the distances between the breakpoint, on the one hand, and the markers, on the other hand, (above the bold line) suggest the same gene order, but appeared quite different: the long distances were much longer and the short ones were shorter. This discrepancy may be ascribed to the fact that the maximum likelihood method is sensitive to deviation from the implied models of single gene segregation (8). In the F_2 seeds resulting from our cross, there is a substantial shortage of the alleles *Sca^f* and, especially, *r*, which came from the line DRC (Table 4). This shortage could be explained by the presence in the vicinity of the allele *r* of some factor lowering the competitive ability of gametophytes. This imbalance is even stronger in the population of plants grown from them (Tables 5 and 6) and may affect differently the results obtained with the maximum likelihood method for different models of inheritance, marker-marker and marker-breakpoint joint segregations. Nevertheless, in spite of the discrepancies between breakpoint-marker and marker-marker distances, they suggest the same order of the genes and breakpoint.

Position of the Nilsson translocation breakpoint.

Our results on the Nilsson translocation contradict to some extent the data of previous researchers. Our distance *cri-Bp* of $8.18 \pm 2.34\%$ is practically identical to $8.6 \pm 2.5\%$ reported by Lamm and Miravalle (14) and $8.0 \pm 2.7\%$ by reported by Snoad (15). But our value of the distance *gp-Bp* of $12.2 \pm 2.9\%$ is greater than the

6.5% reported by Lamm (12) and less than $5 \pm 3.3\%$ reported by Snoad (15), although it corresponds well to the $13.8 \pm 2.8\%$ reported by Folkesson (4). All the researchers worked with the same line (L83), but both Lamm and Snoad estimated the linkages of each of the two markers to the breakpoint in different experiments. Our figures were obtained in the same experiment and correspond well to each other and both markers.

Our results contradict those by Lamm and Miravalle (14) and Snoad (15), which suggest that the breakpoint lies between *Gp* and *Cri*. The results of Lamm and Snoad are also surprising because a breakpoint between two markers (such as Hammarlund's and Winge's translocation in our study) dramatically reduces the recombination distance between the markers. However, combining the data for Nilsson's translocation by Lamm and Miravalle (14) and Snoad (15), we obtain a *Cri* - *Gp* distance of 13-16 %, nearly the same as in the normal karyotype. Our data reveal a moderate decrease in the recombination distance *Cri* - *Gp* to 12 %. If the breakpoint were indeed between *Cri* and *Gp* we would expect to observe a suppression of crossing over comparable to that exerted by Hammarlund translocation.

Finally, in a paper by Hall et al. (6) the position of the breakpoint of a translocation in the line JI61 (which is considered to be equivalent to L83) is as follows:

—*Fs*—*5S1*—*Vc2*—*Bp*—

This result, in combination with the data from a second communication of the same authors (7), suggests the order:

—*Fs*—*5S1*—*Gp*—*Vc2*—*Tl*—*R*—

or:

—*R*—*Tl*—*Bp*—*Gp*—

which is a different order of loci than either ours or that of Lamm and Snoad. The key to the problem may be in a non-identity of the 3-5 translocations involved. Hall et al. (6) wrote: '... JI61 (WBH 761) carries a 3S-5L translocation. It is probably equivalent to L83 (JI145) and WL379 (3), as all these lines were derived from "Extra Rapid", which is the source of the translocation.' In fact, Lamm (13) wrote that 'L379 seems to be identical with L83'. His distances between the breakpoint and *Gp*, reported in the cited communication for these two lines, differed three fold: 18.2 ± 1.64 for L83 and 6.5 ± 3.32 for L83 (note Folkesson's estimation of this distance (4) for L83 and ours for WL1718 = L83 are about intermediate between them).

In the Weibullsholm's germplasm listing, kindly provided to us by Dr. S. Blixt, the following can be found about WL1718 we used and WBH 761 used by Hall (WBH and WL both mean Weibullsholm lines):

1718: 'Selection, L5056 = Lamm L83; Year obtained: 1957; T(3-5)

761: Cross Derivative, Origin: k 199, F9 9165/11 1943 118 x 206; Year included: 1943; T() + NK.

To follow the pedigree of the latter, let us see the lines 118 and 206.

206: Cultivar, Name: Olympia; no other interesting information

118: Cultivar, Name: Extra Rapid, Year obtained: 1927; T(1-4-6) + NK

Finally, at WL379 we read:

379: Mutant; Origin: W Ambrosia I Extra Rapid; Year included: 1936; T(3-5)

Hence, WBH 761 was derived from the cross of two varieties, one of which was named Extra Rapid. At WL379 the word 'Extra Rapid' is given in the Origin. We could not find the label 'Extra Rapid' in the description for WL1718, but it was called so by Lamm and Miravalle (14). Hence, it is possible that some of these Extra Rapids are unrelated. It is also possible that our WL1718 was indeed the same as L83 of Lamm and Snoad, while Hall et al. dealt with another translocation. The discrepancy in the breakpoint position with respect to *cri* and *gp* may exist only in the data, as neither Lamm and Miravalle (14) nor Snoad (15) analyzed both markers in the same cross.

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