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THE LOCALIZATION OF ECERIFERUM LOCI IN BARLEY V. THREE POINT TESTS OF GENES ON CHROMOSOME 1 AND 3 IN BARLEY

by

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Five three point tests are reported for chromosome 1 and four three point tests for chromosome 3. The tests were analysed in the F_3 generation.

Maps for two regions on chromosome 1 comprising five and four genes respectively, can be constructed from the data obtained. The map spanning the distance *cer-f* to *ert-m* confirms previous results for this interval. As observed earlier, coefficients of coincidence larger than 1 corresponding to negative interference are found in this region of chromosome 1. It is suggested that the consistent occurrence of negative interference in this interval is due to its location close to the centromere. In the other region studied on chromosome 1 normal positive interference is observed.

On chromosome 3 a linkage map of the four markers analysed is presented. Positive interference is found in the three point tests of this region.

1. INTRODUCTION

In a previous paper (24) a linkage map comprising five barley genes on chromosome 1, namely eceriferum *cer-f*, albina ac_2, erectoides *ert-d*, *ert-a* and *ert-m* has been presented. In all three point tests involving these genes more double crossovers were observed than expected from the frequencies of crossover in each of the two intervals covered by the three markers. This result was surprising and I have therefore repeated these three tests and can confirm the occurrence of negative interference in this region of chromosome 1.

In order to investigate whether negative interference is a common characteristic for chromosome 1 markers in barley a different region on chromosome 1 has been studied by three point tests. The region contains the four genes virescent *yc*, chlorina *fc*, ecriferum *cer-a* and brachytic *br*. Normal positive interference was obtained in two three point tests involving these markers.

One region on chromosome 3 has been mapped with four three point tests involving

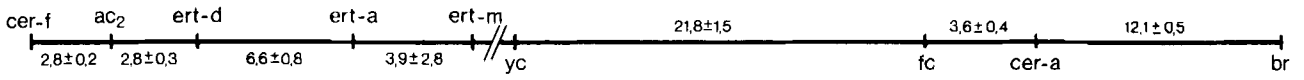

Figure 1. Sequence, distances and standard deviations based on the maximum likelihood method of calculation for the nine genes on chromosome 1.

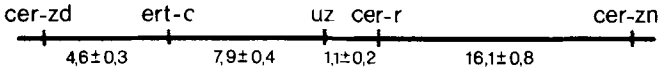

Figure 2. Sequence, distances and standard deviations based on the maximum likelihood methods of calculation for the five genes on chromosome 3.

five genes: eceriferum *cer-zd, cer-r* and *cer-zn,* erectoides *ert-c* and semibrachytic *uz*. Normal positive interference is found in this region. The maps derived are given in Figures 1 and 2.

2. MATERIALS AND METHODS

The mutants used as markers have been either induced in the cultivar Svalöf's Bonus or have been transferred by repeated back crossing into Bonus background by PERSON & HAGBERG (17). The following mutants have been employed:

albina	chromosome 1	ac_2	(19)
erectoides	chromosome 1	*ert-a*^{23}	(16, 8, 22)
erectoides	chromosome 1	*ert-d*^{33}	(16)
erectoides	chromosome 1	*ert-m*^{40}	(16)
eceriferum	chromosome 1	*cer-f*^{9}	(11, 21)
eceriferum	chromosome 1	*cer-a*^{1}	(11, 8)
brachytic	chromosome 1	*br*	(18)
chlorina	chromosome 1	*fc*	(19)
virescent	chromosome 1	*yc*	(19)
semibrachytic	chromosome 3	*uz*	(26)
erectoides	chromosome 3	*ert-c*^{39}	(16)
eceriferum	chromosome 3	*cer-r*^{19}	(11, 22)
eceriferum	chromosome 3	*cer-zd*^{67}	(11, 8)
eceriferum	chromosome 3	*cer-zn*^{244}	(12, 21, 22)

Form crosses involving these mutants (21,22,23) the following double mutant lines have been isolated: *ert-a*^{23} *cer-f*^{9}, *ert-d*^{33} *cer-f*^{9}, *ert-m*^{40} *cer-f*^{9}, *br cer-a*^{1}, *uz cer-r*^{19}, *ert-c*^{39} *cer-zn*^{244}, *uz cer-zn*^{244}, *ert-c*^{39} *cer-r*^{19}. Each of these double mutant lines was crossed with one or more markers. The combinations in the nine three point tests are given in Table I, where the linked markers in coupling phase from the double mutant lines are designated as m and c. The marker of the other parent which then is present in the F_1 in repulsion phase is designated as r. Crosses, growth of the material in the field or the phytotron (3) and analyses were carried out as described in the previous paper of this series (25).

Plants homozygous for the chlorina marker *fc* are of poor viability in mixed stands with phenotypically normal plants. Many chlorina plants die before heading and they were therefore in the F_2 rows tagged and counted at the three leaf stage as well as at harvest time. The surviving chlorina plants were sown separately in the F_3 generation to avoid competition with normal plants.

3. RESULTS AND DISCUSSION

3.1. Chromosome 1

The observed number of plants in the 18 viable genotype classes of the three point tests number 1 to 4 and in the 27 viable genotype classes in test number 5 are listed in Table II. In the latter

Table I

The gene combinations analysed in nine three point tests. The letters m and c designate the markers which are in coupling; and r designates the marker which is in repulsion with respect to m and c.

Chromosome	Test number	m	c	r
1	1	*ert-d*[33]	*cer-f*[9]	ac_2
1	2	*ert-m*[40]	*cer-f*[9]	ac_2
1	3	*ert-a*[23]	*cer-f*[9]	ac_2
1	4	*br*	*cer-a*[1]	*yc*
1	5	*br*	*cer-a*[1]	*fc*
3	6	*uz*	*cer-r*[19]	*cer-zd*[67]
3	7	*cer-zn*[244]	*ert-c*[39]	*cer-zd*[67]
3	8	*uz*	*cer-zn*[244]	*cer-zd*[67]
3	9	*cer-r*[19]	*ert-c*[39]	*cer-zd*[67]

Table II

Observed number of F_2 plants in the 27 genotype classes from nine three point tests.
M = wildtype allele; m = the mutant allele which is in coupling with c
C = wildtype allele; c = the mutant allele which is in coupling with m
R = wildtype allele; r = the mutant allele in repulsion to c and m

Figures in parenthesis under test 5 refer to the number of plants after correction for sublethality of the genotype fcfc.

Genotype	Test number 1	2	3	4	5	6	7	8	9
1 MmCcRr	824	547	504	309	415	620	279	399	447
2 mmccRR	409	270	248	127	197	297	118	189	217
3 MMCCrr	–	–	–	–	153(205)	300	125	185	222
4 MmCCrr	–	–	–	–	34 (52)	0	85	3	48
5 MmccRR	17	83	48	45	58	0	81	1	44
6 MMCcRr	18	83	46	48	59	2	86	7	46
7 mmCcRr	23	81	48	40	56	3	88	6	43
8 mmccRr	4	5	7	93	15	75	16	43	22
9 MMCCRr	7	4	2	97	18	71	13	46	24
10 MmCcRR	5	6	4	102	19	79	15	47	26
11 MmCcrr	–	–	–	–	9 (14)	72	13	43	24
12 MMCcrr	–	–	–	–	1 (1)	5	2	61	2
13 mmCcRR	20	11	12	10	2	8	2	54	1
14 MmCCRr	19	15	16	27	4	8	4	57	5
15 MmccRr	22	13	14	26	4	7	6	55	3
16 MMCcRR	0	0	0	16	2	0	3	0	2
17 mmCcrr	–	–	–	–	1 (1)	0	4	0	0
18 mmCCrr	–	–	–	–	1 (2)	0	14	0	0
19 MMccRR	0	5	6	2	6 (0)	0	17	0	3
20 mmccrr	–	–	–	–	0	7	0	2	1
21 MMCCRR	0	0	0	18	0	5	1	3	0
22 mmCCRr	0	0	0	0	0	0	0	1	0
23 MMccRr	0	1	2	2	0	0	2	0	1
24 MmCCRR	0	0	0	3	0	0	0	5	0
25 Mmccrr	–	–	–	–	0 (0)	1	0	6	0
26 mmCCRR	0	0	0	1	0	0	0	3	0
27 MMccrr	–	–	–	–	0 (0)	0	0	2	0

Table III

Origin of the genetypes listed in Table II. P = parental non-crossover gamete; I = gamete with crossover in region I. II = gamete with crossover in region II; I $ II ∅ double crossover gamete. In test 1-3, region I corresponds to c-r, and region II to r-m. In tests 4-5, 7, 9 region I corresponds to c-r, and region II to c-m. In test 6, 8 region I corresponds to r-m and region II to m-c.

Genotypes in tests			
1, 2, 3	4, 5, 6, 7	6, 8	
1	1	1	P × P or I × I or II × II or I + II × I + II
2	2	2	P × P
3	3	3	P × P
12	8	8	P × I
13	9	9	P × I
14	10	10	P × I or I + II × II
15	11	11	P × I or I + II × II
4	4	12	P × II
5	5	13	P × II
6	6	14	P × II or I + II × I
7	7	15	P × II or I + II × I
8	12	4	P × I + II
9	13	5	P × I + II
10	14	6	P × I + II or I × II
11	15	7	P × I + II or I × II
22	16	24	I × II
23	17	25	I × II
26	20	20	I × I
27	21	21	I × I
18	18	26	II × II
19	19	27	II × II
24	24	16	I + II × I
25	25	17	I + II × I
16	22	22	I + II × I
17	23	23	I + II × I
20	26	18	I + II × I + II
21	27	19	I + II × I + II

test plants homozygous for *fc* are sublethal as mentioned under Materials and Methods. Only 205 of the 275 chlorina plants in the F_2 reached maturity and could be tested in F_3. Assuming equal lethality in all nine *fcfc* genotypes the distribution of the 275 chlorina plants can be calculated and is given in parenthesis.

The genotypes listed in Table II result from various combinations of non-crossover gametes, gametes carrying one crossover and gametes carrying two crossovers as tabulated in Table III. As genotypes resulting from non-crossover gametes with gametes carrying a double crossover (P x I + II) are less frequent than genotypes resulting from a fertilization between non-crossover gametes and single crossover gametes (P x I, P x II) the order of the three markers involved in each three point test can be derived from Tables II and III. The result is graphically presented in Figure 3, which also contains the recombination frequencies determined by the approximate method. Good agreement is obtained with the distances reported earlier (24). The interval between *cer-f* and ac_2 averages here 2,8 centimorgan versus 2,3 in (24). The distance ac_2 to *ert-d* with 2.8 compares to 2.5, that of ac_2 to *ert-m* with 13.3 to 12.8 and the interval ac_2 to *ert-a* with 9.4 to 8.5 centimorgan. In these three tests the frequency of observed double crossovers is consistently higher than the expectation (Table IV) leading to coefficients of

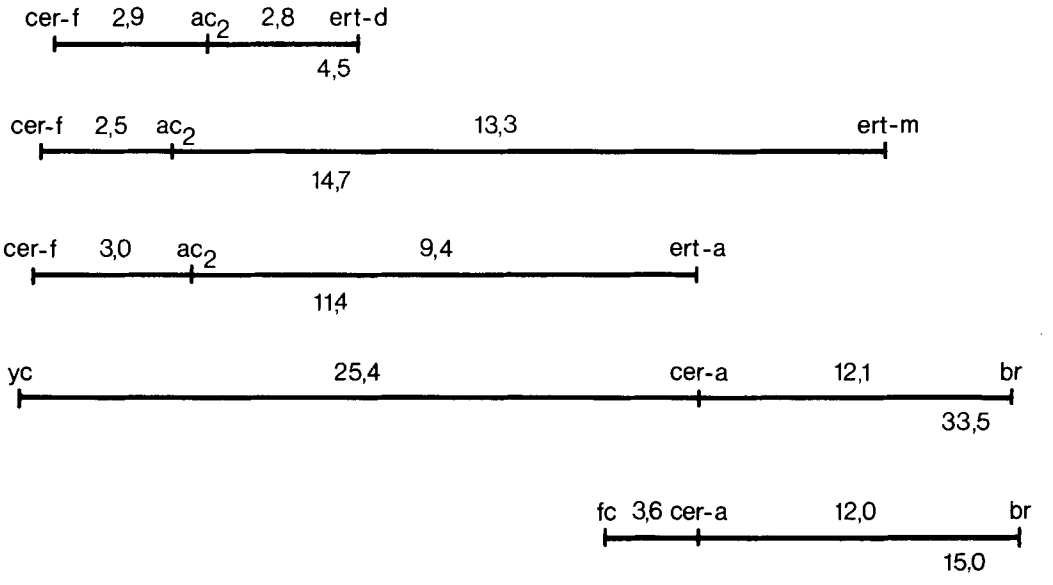

Figure 3. Distances on chromosome 1 based on the approximate method.

Table IV

Distances obtained from the estimated frequencies for the various genotypes as derived from the first principle according to the approximate method (14).

Test number	1	2	3	4	5	6	7	8	9
Total number of gametes	2736	2248	1914	1932	2260	3120	1948	2436	2362
Crossovers in region I	77	55	57	461	80	351	84	228	114
Crossovers in region II	74	283	181	235	271	34	493	279	214
Crossovers in I + II (double)	22	18	18	24	6	0	12	10	8
Crossover frequency in region I in %	2.8	2.6	3.0	23.9	3.5	11.3	4.3	9.4	4.8
Crossover frequency in region II in %	2.7	12.6	9.5	12.2	12.0	1.1	25.3	11.5	9.1
Double crossovers, expected	0.0008	0.0031	0.0028	0.0290	0.0042	0.0012	0.0109	0.0107	0.0044
Double crossovers, observed	0.0080	0.0080	0.0094	0.0124	0.0027	0.0000	0.0062	0.0041	0.0034

coincidence between 2.6 amd 10.5 (Table V) confirming the strong negative interference for this region.

The first case of negative interference has been reported for the centromere region of chromosome 3 in Drosophila melanogaster by MORGAN, STURTEVANT & BRIDGES (2,13). These authors also noted that coincidence values change with the chromosomal region studied and noted that in the second and third chromosomes the coincidence values are highest near the mid-points (centromeres) and

Table V

The coefficient of coincidence found in the nine three point tests.

Test number	Coefficient of coincidence and standard deviation found by maximum likelihood method	Coefficient of coincidence found by estimation method from the first principle
1	7.3 ± 1.6	10.5
2	1.7 ± 0.5	2.6
3	1.9 ± 0.6	3.4
4	0.7 ± 0.1	0.4
5	0.7 ± 0.3	0.6
6	0.3 ± 0.4	0.0
7	0.5 ± 0.2	0.6
8	0.3 ± 0.1	0.4
9	0.9 ± 0.3	0.8

fall off rapidly and symmetrically on each side. In the acrocentric X chromosome on the other hand the highest conincidence values were not found close to the contromere but again in the middle portion of the chromosome. M. M. GREEN (9) has reinvestigated and confirmed the occurrence of negative interference in the centromere region of chromosome 3 in Drosophila with coincidence values ranging from 1.5 to 2.8. Considerable higher negative interference (10.5) was obtained for the *cer-f, ac_2, ert-d* three point test in barley. As noted earlier (24) the coincidence value drops, but remains above 1 when the crossover distance in region II increases from 2.8 to 13.3 centimorgan (ac_2 to *ert-a* and ac_2 to *ert-m*).

The gene ac_2 is placed on the short arm of chromosome 1 by TSUCHIYA & SINGH (28) by means of telotrisomics analyses and considered to be close to the contromere because of absent crossover with translocation break points in the short arm (5). Gene *ert-m* has been placed by PERSSON (15) to the short arm of chromosome 1 with the aid of translocations. There is thus some evidence that the region *cer-f–ac_2–ert-d* is close to the contromere which suggest that the negative interference in this region in barley is comparable to the phenomenon in Drosophila.

The other two three point tests on chromosome 1 in barley (Fig. 3, Tables III, IV, V) permit the ordering of the four markers *yc, fc, cer-a* and *br* in agreement with the map derived from two-point tests. Positive interference is found in this chromosome region. The maps of the two regions with distances calculated by the maximum likelihood method (1,4) and weighted by the procedure of JENSEN & HELMS JØRGENSEN (10) are presented in Figure 1. The region *yc* to *br* is in the short arm of chromosome 1. The markers *fc, cer-a* and *br* have been placed in the short arm by means of telotrisomics (27) with *br* most distal (7). Definitive information on the location of *yc* relative to *ert-m* is not available but the distances obtained by two point tests, which are collected in Table VI all support a location of *yc* between *ert-m* and *fc*. The recombination frequencies of the intervals *ert-a* to *yc, ert-a* to *cer-a, ert-m* to *cer-a, ert-a* to *yc* and ac_2 to *cer-a* give roughly a range of 1 to 16 per cent crossover between *ert-m* and *yc*.

3.2 Chromosome 3

The results of the three point tests are presented in Figure 4 with the crossover values obtained by the approximate method. The sequence of the genes has to be as depicted in Figure 2, but the distance between *cer-r* and *cer-zn* will have to be redetermined as the distances between *cer-zd* and *cer-zn* in three point tests number 7 and 8 cannot be satisfactorily reconciled. Coincidence values are less than one indicating positive interference (Table V). Comparison of the distances determined here

Table VI

Recombination frequencies and standard deviations (S.D.) from the present three point tests compared with earlier results from two point tests. Data in parenthesis are from materials with abnormal segregation ratios. Distances in italics were obtained by summation of distances determined with intermediate marker.

	This work	Earlier work			
	Recombination frequency and S.D.	Recombination frequency and S.D.	Generation	n	References
Chromosome 1:					
cer-f - ac$_2$	2.8 ± 0.2	(4.7 ± 1.0)	F_3	224	(26)
cer-f - ert-d	*5.5 ± 0.4*	(2.4 ± 0.7)	F_3	229	(26)
cer-f - ert-a	*12.5 ± 0.8*	12 ± 3	F_2	793	(26)
cer-f - ert-m	*15.2 ± 2.8*	13.3 ± 1.8	F_3	208	(26)
cer-f - yc		17.0 ± 1.5	F_3	315	(26)
cer-a - br	12.1 ± 0.5	12.9 ± 2.1	F_3	124	(26)
cer-a - fc	3.6 ± 0.4	3.5 ± 0.9	F_3	187	(26)
cer-a - yc	25.4 ± 1.4	24.4 ± 1.6	F_3	346	(26)
cer-a - ac$_2$		39.9 ± 2.0	F_3	339	(26)
cer-a - ert-a		36 ± 3	F_2	817	(26)
cer-a - ert-m		36.1 ± 2.4	F_3	201	(26)
cer-a - ert-d		45 ± 3	F_2	644	(26)
ert-a - yc		20.4 ± 1.8	F_2	647	(14)
ert-a - ert-m	*3.9 ± 2.8*	17.2 ± 4.4	F_2	476	(15)
ert-d - yc		0	F_2	438	(15)
ert-d - fc		free	F_2	414	(15)
ert-d - ac$_2$	2.7 ± 0.3	1.2 ± 0.7	F_2	145	(15)
ert-d - br		free	F_2	521	(15)
ert-d - yc		24.6 ± 2.8	F_2	302	(15)
br - yc	*37.5 ± 1.5*	35.5 ± 4.2	F_2	228	(15)
Chromosome 3:					
cer-zd - ert-c	4.6 ± 0.3	(0)	F_2	418	(25)
cer-zd - uz	10.8 ± 0.4	(4.6 ± 0.6)	F_3	87	(25)
cer-r - cer-zd	*13.9 ± 0.4*	13.5 ± 1.7	F_3	316	(25)
cer-r - ert-c	9.0 ± 0.4	9.1 ± 1.9	F_3	126	(25)
cer-r - uz	1.1 ± 0.2	0.9 ± 0.7	F_3	112	(25)
cer-zn - ert-c	*19.1 ± 0.9*	24 ± 3	F_2	1036	(25)
cer-zn - uz	11.9 ± 0.7	12 ± 3	F_2	914	(25)

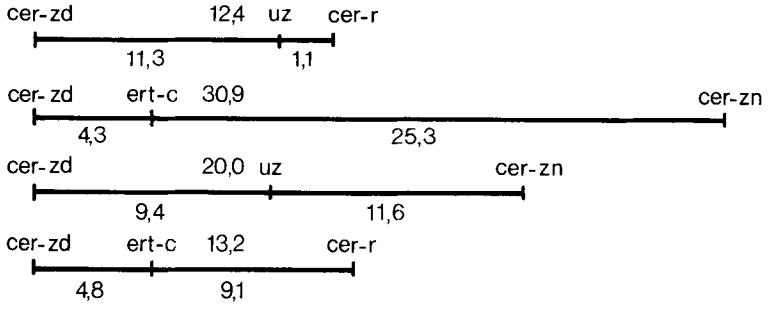

Figure 4. Distances on chromosome 3 based on the approximate method.

with earlier results (Table VI) reveal acceptable agreement.

There has been conciderable uncertainty about the assignment of genes on chromosome 3 to its short or long arm. Thus, *uz* has been transferred from the short arm to the long arm (6,15) and back again to the short arm (20). The distance *uz* and *ert-c* can roughly be calculated from the data given by ESLICK & MCPROUND (6) to 10% and is in agreement with the data in Figure 2. The genes *uz* and *cer-zn* are considered to be located in the short arm of the chromosome with *cer-zn* as the most distal marker (20).

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