Genes *a* and *d* may not be in the same linkage group

Kosterin, O.E. Institute of Cytology and Genetics
Novosibirsk 630090, Russia

Recently, the gene *His7,* coding for the histone HI subtype with the highest electrophoretic mobility, was mapped in linkage group 1 of pea (4). The following arrangement of genes was proposed:

6.5-7 cM 8-10 cM 22-24 cM
*His(2-6)*_____*a*_____*lf*_____*His7*

Since that paper was written, the scheme was corroborated in several additional crosses. According to the hitherto constructed pea linkage maps (1, 6, 7), the gene *His7* should be located fairly close to gene *d (maculum).* In order to reveal the arrangement of genes *His7* and *d,* I carried out several crosses. Surprisingly, the two genes segregated independently in all populations examined.

First, a testcross (WL1393 x WL1688) x WL1393 was made. Line WL1393 has genotype *d,* allele 2 of gene *His7,* and haplotype 1323 of the cluster *His(2-6)* of the linked histone H1 genes (4). Line WL1688 has allele D^{co}, allele 3 of *His7,* and haplotype 1121 of *His(2-6).* The progeny of the testcross segregated as follows: 47 D^{co} $His7^{3/2}$: 41 D^{co} $His7^{2}$: 39 *d* $His7^{3/2}$: 49 *d* $His7^{2}$*;* these data give a recombination fraction of 45.4 ± 3.8% but the deviation from 50% is not significant (P > 0.2). However, line WL1393 turned out to carry a chromosome rearrangement and, because this work was done on seedlings, I had no opportunity to check this progeny for semisterility. To exclude the possibility that the rearrangement of WL1393 resulted in the decoupling of genes *His7* and *d,* three other testcrosses were made between parents known to have a normal karyotype. The descriptions of these crosses are given in Tables 1, 2, and 3. In all three crosses no linkage was found between *d* and either *His(2-6)* or *His7.*

Taking into account that *His7* is located about 26-27 cM from gene *a* in the direction of the putative position of *d,* (4) these results imply that genes *a* and *d* would also not be expected to exhibit linkage. However, the widely accepted assignment of *a* and *d* to the same linkage group appears to be based primarily on direct segregation data. Lamprecht (5) tested the joint segregations of *a* and *d* in numerous crosses. In some of the crosses the genes segregated independently, whereas in others a weak linkage was detected. Gene *d* is hypostatic to *a*, so, the phenotypic class *a* gave no information on gene *d.* In order to calculate the recombination fractions and their standard errors by the product ratio method, Lamprecht deliberately divided the *a* class into the classes *a D*

and *a d,* letting the former to be equal to the reciprocal class *A d.* For example, the segregation data 258 *A D* : 93 *A d* : 103 *a* (repulsion) became 258 *A D :* 93 *A d* : 93 *a D* : 10 *a d;* and 215 *A D* : 125 *A d* : 101 *a* (repulsion) became 215 *A D : 125 A d:* 101 *a D : 0 a d*! In the latter case the entire class *a* was less than *A d.* This procedure introduces artifactual information not resulting from the experiment, and hence reduces the standard errors. For this reason a "significant" linkage was detected in several cases where the correct calculation does not reveal it. Thus, for the former of the above examples the calculation by the method of maximum likelihood for the case of cryptomery provides a recombination value of 45.23 ± 7.92% instead of the reported 32.4 ± 3.98%. The maximum likelihood estimation of the recombination value for the second example is zero with an infinite standard error, implying that the segregation provides no information on linkage. The processed data, with one zero class, in this case could also not be treated by the product ratio method, and the reported value of 9.1 ± 4.71% was obtained by substituting 1 in place of 0.

When the data reported in (5) were recalculated by the method of maximum likelihood, all but one of the crosses gave recombination values which deviate from 50% by less than twice their standard errors. The exceptional cross 684, which gave a recombination fraction of 23.5 ± 3.7%, might involve some chromosome rearrangement. Nevertheless, the combined data for all crosses, including those published prior to (5), seem to provide evidence for a very weak but significant linkage. The total segregation data for all coupling crosses gives a recombination value of 40.55 ± 2.06%; that for repulsion phase crosses gives 37.8 ± 2.46%. However, the combined data should be considered with caution, since any aberrant cross can bias the whole sample.

In the same paper (5), Lamprecht reported data on gene *au (aurea)* suggesting its location between *a* and *d.* Two crosses were reported with the following segregations for classes *Au A, Au a, au A,* and *au a:* 194:85:86:0 (repulsion) and 179:15:15:36 (coupling). Since *au* plants probably could not be classified for *a* as they die before flowering, it appears likely that the *au* class was artificially divided into *au A* and *au a* classes, in the same way as in the case of cryptomeric genes *a* and *d.* The proper estimations of the recombination values are 28.03 ± 17.5% and 12.36 ± 3.37%, respectively. The latter case suggests a significant linkage between *a* and *au.* Moreover, for the latter cross (Number 880) the segregation 165 *Au* D^{co}, 69 *Au d,* 57 *au* D^{co}, 0 *au d* (repulsion) was also presented. It should be noted that the *au* class could not be classified for *d* because alleles *au* and *a* were in the coupling phase, therefore, these data should be treated by the cryptomery model. (Besides, the *Au* class is expected to have contained about 8% of *a* plants, which were probably added to the class *Au d).* The maximum likelihood estimation of the recombination fraction for the above segregation is 34.0 ± 12.2% (instead of the reported 14.1 ± 5.72%). This result does not provide evidence for the linkage of genes *au* and *d* and thus does not prove the presence of genes *a* and *d* in the same linkage group.

Table 1. Segregation for genes *d, His(2-6)* and *His7* in the progeny of testcross A[1].

His(2-6)	1123/1221			1221/1221	
His7	2/2	2/3		2/2	2/3
D^{co}	24	11		10	37
d	23	10		14	29
	Recomb. fract.		Joint χ^2	Probability	
His7 – d	53.8 ± 4.0%		0.83	P > 0.3	
His(2-6) – d	49.4 ± 4.0%		0.01	P > 0.9	
His(2-6) – His7	28.5 ± 3.6%		28.21	P << 0.0001	

[1] Line RT-1 (D^{co}, $His(2\text{-}6)^{1123}$ – $His7^{2}$) was pollinated by WL1238 (*d*, $His(2\text{-}6)^{1221}$ $His7^{3}$) yielding four fully fertile F_1 plants. An extra rapid line 6-14 (*d*, $His(2\text{-}6)^{1221}$, $His7^{2}$) was fertilised by the pollen of the F_1 plants. Line RT-1 was obtained by S.M. Rozov as F_6 of cross 6-14 x 5-11 (which is F_{11} of VIR7036 x WL1018). Line 6-14 was obtained by S.M. Rozov as F_{14} of cross VIR320 (Palestine) x Sprint-1 (which is F_{25} of cross VIR7036 (Nepal) x cv. Avanti). The D^{co} allele in RT-1 causes not only the single anthocyanin ring in the axils but also red spots at the leaflet bases.

Table 2. Segregation for genes *d, His(2-6)* and *His7* in the progeny of testcross B[1].

His(2-6)	1323/1221			1221/1221	
His7	2/2	2/3		2/2	2/3
D^{co}	32	15		13	49
d	20	13		14	43
	Recomb. fract.		Joint χ^2	Probability	
His7 – d	49.2 ± 3.5%		0.25	P > 0.5	
His(2-6) – d	52.3 ± 3.5%		0.85	P > 0.3	
His(2-6) – His7	27.6 ± 3.2%		35.78	P << 0.0001	

[1] Analogously to testcross A, line 6-14 plants were pollinated by fully fertile pollen of F_1 hybrids between WL1238 and an individual plant (D^{co}, $His(2\text{-}6)^{1323}$, $His7^{2}$) which resulted from the complex testcross [(a single plant in the F_2 of WL1292 x WL1688) x WL102] x WL102, i.e. Cross 1 in (4).

Table 3. Segregation for genes *d*, *His(2-6)* and *His7* in the progeny of testcross C[1].

His(2-6)	1221/1121			1121/1323	
His7	2/2	2/3		2/2	2/3
D^w	12	3		0	9
D^{co}	10	3		5	11
	Recomb. fract.		Joint χ^2	Probability	
His7 – d	50.9 ± 6.9%		0.05	P = 0.9	
His(2-6) – d	40.5 ± 6.8%		1.61	P = 0.2	
His(2-6) – His7	28.8 ± 3.3%		18.13	P < 0.0001	

[1] Two fully fertile F_1 hybrids of cross SGE *(D^w, $His(2\text{-}6)^{1221}$, $His7^2$)* x individual plant *(D^{co}, $His(2\text{-}6)^{1323}$, $His7^3$)* were pollinated by WL102 *(D^{co}, $His(2\text{-}6)^{1121}$, $His7^2$).* Line SGE was derived by V.A. Berdnikov from VIR6135 (Greece), VIR320 (Palestine), and Sprint-l (see Table 1). The individual plant came from Cross 1 of (4) (see Table 2).

Surprisingly, I failed to find information on the existence of any other markers linked both to genes *a* and *d.* The accumulated results provide evidence for two groups of subsequently linked genes traditionally attributed to linkage group I, one containing gene *a* and the other genes *d* and *i.* The assignment of these two groups to the same chromosome seems to be problematic. Thus, it cannot be excluded that the pea linkage map may require more major reconstruction even after the recent alterations (2, 3, 7).

1. Blixt, S. 1972. Agri Hort. Genet. 30:1-293.
2. Folkeson, D. 1990. Hereditas 112:249-256.
3. Folkeson, D. 1990. Hereditas 112:257-264.
4. Kosterin, O.E. 1992. Pisum Genet. 24:56-59.
5. Lamprecht, H. 1956. Agri Hort. Genet. 14:66-106.
6. Weeden, N.F., Ambrose, M. and Swiecicki, W.K. 1991. Pisum Genet. 23: cover.
7. Weeden, N.F. and Wolko, B. 1990. *In* Genetic Maps, Ed. S.J. O'Brien, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York, pp. 6106-6112.