Theor Appl Genet (1987) 73:343–349

Linkage studies of the *h* gene with plant weight in flax genotrophs

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Received December 12, 1985; Accepted July 5, 1986
Communicated by R. Hagemann

Summary. The association of the $H-h$ (hairy-hairless septa) character with plant weight was studied in the coupling and repulsion phases in F_2 of reciprocal crosses between large (L) and small (S) genotrophs of flax variety 'Stormont Cirrus'. F_2 plants of reciprocal crosses in coupling ($L^H \times S^h$) and in repulsion ($L^h \times S^H$) giving $H-h$ segregations were grown with their parents at two sowing times. Significant positive and negative associations between *h* and plant weight were obtained. A model is proposed based on the hypothesis that the *H* phenotype had changed to the *h* phenotype at the time of induction by a heterochromatic region extending over this locus. In the heterozygote, stable equilibria of the homozygotes are destroyed and transfer of heterochromatin, or number of reiterated sequences, or a decrease in one homologue and an increase in the other, occur in this region between homologous chromosomes. The amount and direction of the association is dependent upon the frequency of transfer: 0% transfer gives complete positive association; 50% transfer, no association; 100% transfer, complete negative association. This mechanism or heterochromatic transfer preserves the Mendelian ratio of 3 : 1 of $H : h$ in the F_2. It is also supposed that there must be other controlling elements present as well.

Key words: Linkage – Coupling – Repulsion – *h* gene – Plant weight – Flax genotrophs

Introduction

A number of studies have been made on the association of capsule character (*h*) with the induced changes in plant weight in the variety 'Stormont Cirrus' (Durrant and Nicholas 1970; McLellan and Durrant 1973; Durrant 1974). In the present study, an attempt was made to provide further information on the association of *h* with plant weight in the coupling and in the repulsion phases using large (hairless) and small (hairy) genotrophs, and vice versa.

Materials and methods

The following seed stocks were used:

1 Large genotroph with hairless septa induced with NPK in 1954: phenotype, Lh; genotype, L/L *h*/*h*.

2 Small genotroph with hairy septa induced with NK in 1954: phenotype, SH; genotype, S/S *H*/*H*.

3 Large genotroph with hairy septa induced with N in 1960: phenotype, LH; genotype, L/L *H*/*H*.

4 Small genotroph with hairless septa induced with P in 1960: phenotype, Sh; genotype, S/S *h*/*h*.

Several plants of two ancestral lines of each of the above four stocks maintained over the years were reared in the greenhouse and later transplanted into the field for crossing in 1971. Five F_1 plants from each of the two plants of different ancestral lines from each of the crosses and parents were grown in 1972 in 7 inch pots in a heated greenhouse. Parents and crosses with symbols are given below:

Parents

G_1 L^h P_1 G_3 S^h P_3
G_2 L^H P_2 G_4 S^H P_4

Crosses		Reciprocal sum	Reciprocal diff.	
G_5 $L^h \times L^H$ G_6 $L^H \times L^h$		C_1	RC_1	B_I
G_7 $S^h \times S^H$ G_8 $S^H \times S^h$		C_2	RC_2	
G_9 $L^h \times S^H$ G_{10} $S^H \times L^h$		C_3	RC_3	B_{II}
G_{11} $L^H \times S^h$ G_{12} $S^h \times L^H$		C_4	RC_4	

There are four parents and four crosses in the first batch (B_I) respectively between L and between S, and four crosses of the second batch (B_{II}) between L and S, one set (C_3) in repulsion, and the other (C_4) in coupling. The parents crossed in each case contained different hair alleles to give segregation in every F_2.

The F_2 was sown in small pots in the greenhouse in 1973 and transplanted into the field after about 6 weeks. There were ten replicates each containing 12 randomized plots with 12 genotrophs, G_1 to G_{12}, with 10 plants per plot. A large number of plants were damaged in the greenhouse so the tops of the central shoots were cut off all of them so that they all had the same 'treatment'. A subsequent sowing was made six weeks afterward in the greenhouse and about four weeks later they were transplanted into the field. These later crosses did not receive the same 'treatment', and there were 12 replicates, 8 plants in each of the 12 randomized plots per replicate. The total number of plants grown were 2292. The two sowings were analysed separately throughout.

Results

A summary of mean plant weights is given in Table 1, and the variance analysis in Table 2. ANOVA displayed highly significant differences in both sowings between mean plant weights of the genotrophs. Table 3 gives dominance (i.e., potency) tests for each of the four sets of crosses. There is no significant dominance for C_3 or C_4 (the potency ratio here is about 0.1 on average) but there is significance for the C_1 and C_2 crosses, L × L and S × S, in a positive direction. Here the average potency ratio is about unity.

H − h segregation

The numbers of *H* and *h* plants in the F_2 of the eight crosses, their ratios and χ^2's testing for 3 : 1 ratio and heterogeneity are given in Table 4. Data agree in giving a 3 : 1 ratio in both sowings. Therefore, this factor is apparently segregating uniformly as a normal Mendelian gene.

The association of H − h with plant weight

The mean plant weights for *H* and *h*, for each of the eight types of crosses are given in Table 1, and four parents grown with the F_2 are given in Table 5 for comparison. The difference, $\bar{d}$, between $\bar{H}$ and $\bar{h}$ is denoted as positive and negative depending upon

Table 1. Mean plant weights of *H* and *h*, $\bar{d}$ values and $\bar{d}/(Pr - Ps)$ ratios in SO_1 and SO_2 compared with expected values and ratios calculated on the assumption given in the text

	Observed values in F_2				Expected values in F_2 assuming complete linkage							
					No dominance				Complete dominance			
	H	*h*	$\bar{d}$	$\frac{\bar{d}}{Pr-Ps}$	*H*	*h*	$\bar{d}$	$\frac{\bar{d}}{Pr-Ps}$	*H*	*h*	$\bar{d}$	$\frac{\bar{d}}{Pr-Ps}$
First sowing												
$L^h \times L^H$	10.74	10.81	−0.07	0.02	11.71	9.70	+2.01	0.67	12.72	9.70	+3.02	1.00
$L^H \times L^h$	10.83	14.14	−3.31	−1.10								
$S^h \times S^H$	4.59	6.13	+1.54	1.08	4.23	5.18	+0.95	0.67	4.71	5.18	+0.47	0.33
$S^H \times S^h$	6.03	7.57	+1.54	1.08								
$L^h \times S^H$	5.82	7.35	+1.53	0.26	5.72	9.70	+3.98	0.67	7.72	9.70	+1.98	0.33
$S^H \times L^h$	7.07	6.51	−0.56	−0.09								
$L^H \times S^h$	7.46	10.74	−3.28	0.44	10.21	5.18	+5.03	0.67	12.72	5.18	+7.54	1.00
$S^h \times L^H$	6.84	8.56	−1.72	−0.23								
Second sowing												
$L^h \times L^H$	25.73	29.67	−3.94	−0.75	21.54	18.02	+3.52	0.67	23.29	18.02	+5.27	1.00
$L^H \times L^h$	25.51	25.74	−0.23	−0.04								
$S^h \times S^H$	8.96	10.05	+1.09	+0.25	6.22	9.10	+2.88	0.67	7.66	9.10	+1.44	0.33
$S^H \times S^h$	8.00	9.55	+1.55	+0.36								
$L^h \times S^H$	10.36	16.75	+6.39	+0.48	9.19	18.02	+8.83	0.67	13.61	18.02	+4.41	0.33
$S^H \times L^h$	10.31	14.88	+4.57	+0.36								
$L^H \times S^h$	18.90	15.42	+3.48	+0.25	18.56	9.10	+9.46	0.67	23.29	9.10	14.19	1.00
$S^h \times L^H$	16.42	13.13	+3.29	+0.23								

Table 2. Analysis of variance of mean plant weight in the F_2 generation for SO_1 and SO_2

Source of variation	First sowing (SO_1)			Second sowing (SO_2)		
	d.f.	M.S.	V.R.	d.f.	M.S.	V.R.
Replicates	9	0.078	–	11	0.014	–
Genotrophs (G)	11	82.45	12.18*	11	594.09	15.15*
Error	99	6.77		121	39.22	
Total	119			143		

* $P \leqq 0.001$

Table 3. Mean squares for individual tests of dominance for plant weights

	d.f.	M.S.	V.R.
First sowing			
C_1 dominance	1	5.01	0.74
C_2 dominance	1	5.96	0.88
C_3 dominance	1	0.76	0.11
C_4 dominance	1	8.93	1.32
Error	99	6.77	
Second sowing			
C_1 dominance	1	262.21	6.69*
C_2 dominance	1	46.81	1.19
C_3 dominance	1	5.83	–
C_4 dominance	1	127.69	3.26
Error	121	39.22	

Test of dominance

C_1 dominance $= 1/4\,[L^h + L^H - L^h \times L^H - L^H \times L^h]^2$
C_2 dominance $= 1/4\,[S^h + S^H - S^h \times S^H - S^H \times S^h]^2$
C_3 dominance $= 1/4\,[L^h + S^H - L^h \times S^H - S^H \times L^h]^2$
C_4 dominance $= 1/4\,[L^H + S^h - L^H \times S^h - S^h \times L^H]^2$

* $P \leqq 0.05$

whether the association is in the direction of linkage ("positive linkage") or in the opposite direction to linkage ("negative linkage"). Two parents occurring in each cross will be referred as Pr and Ps, where Pr is the larger of the two parents. For example, L^H is the larger of the two parents in the $L^H \times S^h$ cross, therefore, $\bar{d} = \bar{H} - \bar{h}$; in the $L^h \times S^H$ cross $\bar{d} = \bar{h} - \bar{H}$ because L^h is the larger.

The $\bar{d}$ values expressed as ratios (Pr – Ps) in Table 1 can be compared with similar values calculated on the assumption that plant weight is determined by a single genetic factor which is completely linked to $H - h$, and either that plant weight shows no dominance, or that it is completely dominant in a plus direction. In the crosses between L and S there is a very little dominance as judged by the F_2 but they have a ratio about 0.3 to 0.4, well below the 0.67 expectation (Table 1), showing that one or more of the above assumptions are incorrect. All the associations given by the crosses between L^H and L^h are negative and none are significant (Table 5).

The variances of the means, $V_{\bar{H}}$ and $V_{\bar{h}}$, and the standard deviation of the mean difference, $S\bar{d}$, were obtained from sums of squares calculated separately for each replicate and summed over replicates for each of the four crosses (Table 5). The repulsion cross, between L^h and S^H, has a significant positive association in SO_2, and the coupling cross, between L^H and S^h, has a significant negative association in SO_1. There is substantial dominance in the F_2 of crosses between S^H and S^h (Table 3), and the highly significant positive association in SO_1 (Table 5) gives a $\bar{d}/(Pr - Ps)$ ratio of 1.08 (Table 1), which is greatly in excess of the expected 0.33 value. This indicates that there are substantial genetic differences between these two genotrophs. The main features are, however, that although the segregation of $H : h$ is normal in the F_2, there are apparently strong positive and negative linkages in the coupling and repulsion crosses which are altered by SO_1 and SO_2 environments.

The transfer frequency is calculated from the ratio of the $\bar{d}$ obtained (difference between H and h plants) to the estimated $\bar{d}$ value assuming complete "positive linkage" i.e.,

$$\frac{\bar{d}\text{ obtained}}{\bar{d}\text{ estimated}} = \text{association ratio (A.R.)}.$$

The estimated $\bar{d}$ value is obtained by multiplying (Pr – Ps) by a factor in Table 1 for the appropriate phase and dominance. In the absence of dominance the association ratio is 0 for 50% transfer, 1.0 for no transfer and –1.0 for 100% transfer. Hence the transfer frequency may be calculated from the association ratio $1 - A.R./2$. In the presence of complete dominance in the coupling phase the ratios respectively are 1.0, 0.33 and –0.33, and the transfer frequency may be calculat-

Table 4. Number of *H* and *h* plants in the F_2 segregating crosses, *Hh* ratios, χ^2's testing for 3 : 1 ratios and heterogeneity χ^2's for first and second sowing

Batches	Genotrophs	First sowing					Second sowing				
		H	*h*	*H* : *h* ratio	χ^2's	d.f.	*H*	*h*	*H* : *h* ratio	χ^2's	d.f.
I	$L^h \times L^H$	62	17	3.65 : 1	0.51	1	63	22	2.86 : 1	0.04	1
	$L^H \times L^h$	61	19	3.21 : 1	0.07	1	64	17	3.76 : 1	0.70	1
	$S^h \times S^H$	57	20	2.85 : 1	0.04	1	61	17	3.59 : 1	0.43	1
	$S^H \times S^h$	55	15	3.67 : 1	0.48	1	55	21	2.62 : 1	0.28	1
II	$L^h \times S^H$	63	22	2.86 : 1	0.04	1	59	16	3.69 : 1	0.54	1
	$S^H \times L^h$	52	17	3.06 : 1	0.00	1	59	20	2.95 : 1	0.00	1
	$L^H \times S^h$	59	15	3.93 : 1	0.88	1	62	22	2.82 : 1	0.06	1
	$S^h \times L^H$	55	12	4.58 : 1	1.80	1	63	19	3.32 : 1	0.15	1
	Total	464	137	3.39 : 1	1.56	1	486	154	3.16 : 1	1.89	1

	Heterogeneity χ^2			
	First sowing		Second sowing	
Item	d.f.	χ^2	d.f.	χ^2
Heterogeneity of crosses	3	2.51	3	0.51
between batches	1	0.04	1	0.30
within batches	2	0.47	2	0.21
Heterogeneity of reciprocals	4	1.30	4	1.68

Table 5. Mean plant weights (g) for SO_1 and SO_2 of *H* and *h* ($\bar{H}$ and $\bar{h}$), $\bar{d}$, the difference between $\bar{H}$ and $\bar{h}$, their error variance, standard deviation of their mean difference, t-values and probability after summation over reciprocals

	First sowing				Second sowing			
Genotrophs	$L^h \times L^H$ $L^H \times L^h$	$S^h \times S^H$ $S^H \times S^h$	$L^h \times S^H$ $S^H \times L^h$	$L^H \times S^h$ $S^h \times L^H$	$L^h \times L^H$ $L^H \times L^h$	$S^h \times S^H$ $S^H \times S^h$	$L^h \times S^H$ $S^H \times L^h$	$L^H \times S^h$ $S^h \times L^H$
$\bar{H}$	10.78	5.30	6.39	7.16	25.62	8.51	10.33	17.65
No. of *H* plants	123	112	115	114	127	116	118	125
$\bar{h}$	12.57	6.85	6.98	9.77	27.96	9.77	15.71	14.33
No. of *h* plants	36	37	39	27	39	37	36	42
$\bar{d}$	−1.79	+1.55	+0.59	−2.61	−2.34	+1.26	+5.38	+3.32
$V_{\bar{H}}$	0.41	0.08	0.09	0.13	1.62	0.14	0.25	0.97
$V_{\bar{h}}$	1.55	0.23	0.33	0.87	4.95	1.12	1.39	1.00
$S_{\bar{d}}$	1.40	0.56	0.65	1.00	2.60	1.12	1.28	2.37
t-value	1.28	2.77	0.91	2.61	0.90	1.13	4.20	2.37
P	0.20	0.01	0.4−0.3	0.01	0.4−0.3	0.3−0.2	0.001	0.02

Mean plant weights (g) of parents grown with the F_2 plants

	First sowing				Second sowing			
Parents	L^h	L^H	S^h	S^H	L^h	L^H	S^h	S^H
Mean	9.70	12.72	5.18	3.76	18.02	23.29	9.10	4.10

ed from 1 − A.R./1.33. In the presence of complete dominance in the repulsion phase the ratios, respectively, are 1.0, −1.0 and −3.0, and the transfer frequency is calculated from 1 − A.R./4.

Discussion

The $\bar{d}$ values for plant weight are summarized in Table 6. In repulsion the association drops from a positive association in SO_2 to a non significant association in SO_1. In coupling the association is positive in SO_2 though less strong; in SO_1 it drops to highly significant negative value. The change in association for this trait is about the same in amount and direction for both coupling and repulsion. Genetic instability occurs at the *h* locus as well as in the F_1 crosses between L and S (Joarder et al. 1975), and continues to occur in *Hh* heterozygotes of the F_2 and later generations. Heritable changes $H \rightarrow h$ and $h \rightarrow H$ occur, and appear to be paramutation-like changes where one allele alters its homologue on the other chromosome, either directly or via associated elements.

A model is suggested to explain the F_2 association between *H*−*h* and plant weight (Fig. 1). A heterochromatic region has been incorporated into the model. In repulsion, in the L genotroph, it is shown extending over the *h* locus thereby switching it off, i.e., a $H \rightarrow h$ change has occurred. In the S genotroph, which has less DNA, it is assumed that the heterochromatin does not cover the *h* locus, and so it is a fully active *H*. In the heterozygotes of the repulsion cross, the heterochromatic region may, or may not, transfer from the L chromosome to the S chromosome. If it does, $L^h \rightarrow L^H$ and $S^H \rightarrow S^h$ changes occur, giving reassociation. If there is a heterochromatic transfer in every meiotic cell, complete (100%) "negative linkage" will occur. If there is no transfer there will be complete (100%) "positive linkage". If heterochromatic transfer occurs in 50% of the meiotic cells, there will be no apparent linkage. In crosses, during transfer, one allele is switched on and the other is switched off, thus preserving the frequencies of the two alleles, *H* and *h*, with the consequence that 3 : 1 ratio occur (Table 4).

The transfer frequencies (Table 7) for plant weight, and in the crosses between L and S, vary from 20% to 76%. They are consistently higher in coupling than in repulsion, and consistently higher in SO_1 than SO_2. The model supposes that the transfer of heterochromatin occurs in the F_1 and consequently one might therefore suppose that the coupling phase, or the genotrophic background of L^H and S^h genotrophs, increased the frequency of transfer. It might be further conjectured that the L^h and S^H chromosomal associations are favoured more than L^H and S^h. On the contrary, since the two sowing times were applied to the F_2 generation the difference in the transfer frequency between SO_1 and SO_2 cannot be directly due to the effect of sowing time on the transfer frequency itself. Sowing time must either change the association between *h* and plant weight in the F_2 by some other, entirely different, mechanism, or it has a modifying effect in the distribution of heterochromatin. In the present investigation, changes, perhaps heterochromatic transfer, occurring in the F_1 may be incomplete and require further stimulus of the appropriate F_2 environment before they can appear as changes in amount or direction of association.

Table 6. Summary of $\bar{d}$ values for plant weight

			Repulsion	Coupling
	$L^h \times L^H$ + $L^H \times L^h$	$S^h \times S^H$ + $S^H \times S^h$	$L^h \times S^H$ + $S^H \times L^h$	$L^H \times S^h$ + $S^h \times L^H$
SO_1	−1.79	1.55**	0.59	−2.61**
SO_2	−2.34	1.26	5.38**	3.32*
$SO_1 - SO_2$	−0.55	−0.29	4.79*	5.93***

* $P \leqq 0.05$; ** $P \leqq 0.01$; *** $P \leqq 0.001$

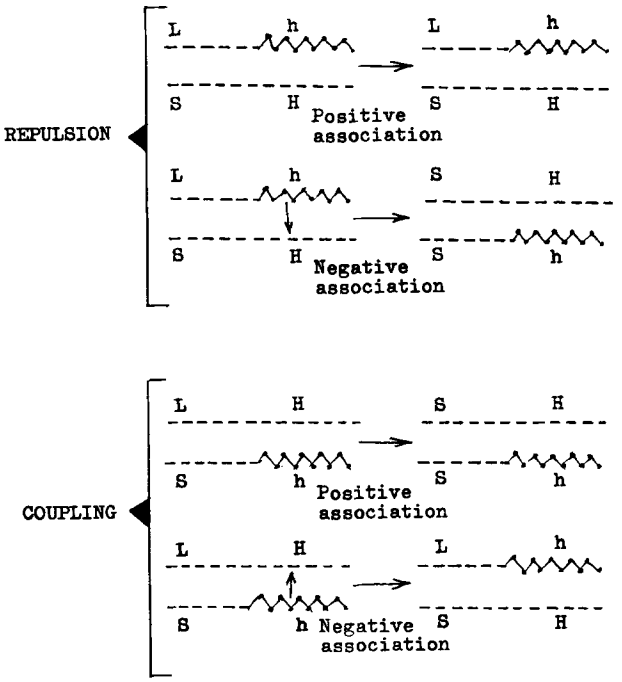

Fig. 1. Model to explain positive and negative association between *h* and plant weight. The wavy line indicates the heterochromatic region and the perpendicular arrows indicate the direction of heterochromatic transfer

Table 7. Calculation of heterochromatic transfer frequencies for plant weight (see text)

	$\bar{d}$ obtained	(Pr − Ps)	Multiplier from Table 1	$\bar{d}$ expected	Association ratio	Transfer frequency
SO$_1$						
Coupling $L^h \times L^H$ $L^H \times L^h$	−1.79	3.02	1.00	+3.02	−0.59	1.20
Repulsion $S^h \times S^H$ $S^H \times S^h$	+1.55	1.42	0.33	+0.47	+3.30	0.58
Repulsion $L^h \times S^H$ $S^H \times L^h$	+0.59	5.94	0.67	+3.98	+0.15	0.43
Coupling $L^H \times S^h$ $S^h \times L^H$	−2.61	7.54	0.67	+5.03	−0.52	0.76
SO$_2$						
Coupling $L^h \times L^H$ $L^H \times L^h$	−2.34	5.27	1.00	+5.27	−0.44	1.08
Repulsion $S^h \times S^H$ $S^H \times S^h$	+1.26	5.00	0.33	+1.44	+0.88	0.03
Repulsion $L^h \times S^H$ $S^H \times L^h$	+5.38	14.19	0.67	+8.83	+0.61	0.20
Coupling $L^H \times S^h$ $S^h \times L^H$	+3.32	13.92	0.67	+9.46	+0.35	0.33

Although the model has been considered with regards to the transference of heterochromatin from one chromosome to its homologue with a certain frequency, it does not necessarily imply that the heterochromatic material on one chromosome is transferred to the other. Although this is possible, in which case it would have characteristics in common with episomes, thought by several workers (McClintock 1968) to occur in maize, it is perhaps more likely that over a period of many cell divisions the number of reiterated sequences in one chromosome diminish while in the other they increase. Other instances are also known of chromosomal interactions resulting in heritable changes (Durrant 1974). Paramutation at the *R* locus in maize (Brink 1960) and at the *sulf* locus in *Lycopersicum esculentum* is due to the heterochromatisation of the locus (Hagemann and Snoad 1971). These are interactions between homologous chromosomes, parts of chromosomes or between these and environments inducing at least semi-permanent changes in the chromosomes and it is credible that chromosomal regions in flax in which changes are induced by the environment can also be altered by homologous chromosomes differing from them, or by changes in nearby segments. The suggested model, even if substantially correct, is by no means the complete explanation of these events. A primary question of course is why should they occur at all. There must be other factors which play a part and evidence for these have been obtained in genetic studies on the genotypes which differ in their ability to change in amount of DNA in response to different environments. These studies have shown that a cytoplasmic and a nuclear factor is involved (Al-Saheal and Larik 1985).

Acknowledgements. We wish to thank Professor A. Durrant for his help and erudite suggestions.

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