

## The effects of heterozygosity at the *af*, *st* and *tl* loci in peas

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Accepted March 12, 1985

Communicated by R. Riley

**Summary.** Eight near-isogenic lines of pea representing all the homozygous combinations of three genes *af*, *st* and *tl*, which modify leaf shape and size, were crossed in all possible ways excepting reciprocals. An analysis of the resulting 36 families has shown that homozygous mutant alleles at the *tl* locus acting with homozygous mutant alleles at the *af* and *st* loci increase both seed weight and plant haulm weight. The mutant alleles at the *af* and *st* loci seem, when homozygous, to have little effect by themselves upon seed weight but they do increase or decrease haulm weight, respectively. There is clear evidence of heterotic effects resulting from heterozygosity at each one of the three loci which modify seed weight, haulm weight and basal branching. The implications of such heterotic effects in pea breeding programmes are discussed.

**Key words:** Peas – Genetics – Foliage – Heterozygosity – Heterosis

### Introduction

In a previous paper the effects of three recessive mutant genes *af*, *st* and *tl* which modify leaf and stipule form and size in peas were examined: *afaf* converts all leaflets to tendrils, *stst* reduces stipule size considerably and *tltl* converts all tendrils into leaflets (Snoad et al. 1985). The effects of the mutant alleles when homozygous at any one of the three loci was to reduce the productivity of the plants, with *stst* having the most pronounced effects. The characters measured tended to respond in a similar direction but not necessarily to the same extent in all families that were homozygous for the recessive alleles. There was a clear indication of

partial dominance associated with heterozygosity at the *tl* locus which was especially obvious in *afaf*·*Tltl* families.

This study of gene effects and gene dosage effects has been extended to additional characters recorded in the same experiment in order to learn more about the effects of heterozygosity of the *af*, *st* and *tl* alleles in peas.

### Materials and methods

Full details of the experiment have been presented in an earlier paper (Snoad et al. 1985) and only a brief summary is provided here.

Eight near-isogenic pea lines representing all the homozygous combinations of the three genes *af*, *st* and *tl* were crossed in all ways but excluding reciprocals. The crossing programme generated 36 families; eight reconstituted parental lines and 19 recombinant genotypes, some of which were represented more than once. The genotypes of the 36 families are summarised in Table 1 where they have been divided into their eight phenotypic groups.

The F<sub>1</sub> plants were grown against wire in a field plot at the John Innes Institute using three blocks and a single plant randomisation of 10 plants from each family in each block. The plants were spaced at 20 cm and were protected by guard rows and guard plants at the end of each row. The whole area was enclosed by netting in order to prevent damage by birds.

Four of the characters recorded are discussed in this paper.

1. The weight of individual seeds when sown (mg). It was decided that this character should be recorded so that correlations between it and other characters could be made if required. It has, at the same time, provided an opportunity to estimate the effects of the three genes upon seed weight prior to this experiment.

2. The number of fertile basal branches per plant.

3. The oven-dried weight of haulm developed at maturity – i.e. excluding seeds (g).

**Table 1.** The mean values and their standard errors for the four characters examined. The 36 families are arranged in their eight phenotypic groups together with an indication of their genotype which shows whether the alleles were homozygous dominant, homozygous recessive or heterozygous

	Family	<i>Af</i>	<i>St</i>	<i>Tl</i>	Wt. of individual seeds when sown	No. of fertile basal branches	Wt. of oven dried haulm	Protein content of harvested seed (N×6.25 as % dry matter)
					(mg)		(g)	
					Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Group 1	1	D	D	D	<u>256.47 ± 5.11</u>	<u>1.80 ± 0.19</u>	<u>28.89 ± 1.89</u>	<u>20.54 ± 0.70</u>
	9	H	D	D	314.27 ± 4.73	2.03 ± 0.16	36.48 ± 2.92	20.73 ± 0.63
	10	D	H	D	284.60 ± 8.33	2.29 ± 0.22	31.27 ± 2.58	19.93 ± 0.78
	11	D	D	H	320.87 ± 7.86	1.87 ± 0.22	37.48 ± 2.43	20.64 ± 0.70
	12	H	H	D	225.10 ± 2.53	2.29 ± 0.25	33.36 ± 3.39	21.42 ± 0.56
	16	H	H	D	261.20 ± 11.73	2.34 ± 0.22	33.07 ± 2.58	21.04 ± 0.68
	13	H	D	H	309.73 ± 3.62	1.88 ± 0.23	39.88 ± 3.18	20.27 ± 0.76
	17	H	D	H	324.30 ± 2.25	2.60 ± 0.26	35.69 ± 3.06	21.56 ± 0.93
	14	D	H	H	297.03 ± 1.97	1.51 ± 0.26	33.44 ± 2.26	19.99 ± 0.63
	22	D	H	H	301.70 ± 8.15	2.56 ± 0.22	32.68 ± 2.04	20.82 ± 1.22
	15	H	H	H	311.20 ± 1.32	2.57 ± 0.24	32.87 ± 2.41	19.96 ± 0.34
	20	H	H	H	284.93 ± 8.35	2.38 ± 0.18	31.32 ± 2.14	20.06 ± 0.36
	24	H	H	H	300.20 ± 6.62	2.76 ± 0.23	37.67 ± 2.36	19.79 ± 0.67
27	H	H	H	250.80 ± 8.92	1.76 ± 0.14	29.03 ± 2.55	20.12 ± 0.42	
Group 2	2	R	D	D	<u>250.23 ± 6.16</u>	<u>1.86 ± 0.19</u>	<u>30.38 ± 0.17</u>	<u>20.08 ± 0.95</u>
	18	R	H	D	276.03 ± 7.98	1.81 ± 0.16	21.91 ± 1.69	19.73 ± 0.30
	19	R	D	H	284.50 ± 5.34	1.65 ± 0.15	36.10 ± 1.96	19.49 ± 0.89
	21	R	H	H	283.97 ± 8.82	1.83 ± 0.18	28.32 ± 1.94	21.06 ± 1.07
	31	R	H	H	310.00 ± 4.99	2.14 ± 0.24	31.88 ± 2.21	22.32 ± 1.18
Group 3	3	D	R	D	<u>246.41 ± 3.09</u>	<u>2.14 ± 0.23</u>	<u>17.86 ± 2.04</u>	<u>21.13 ± 0.64</u>
	23	H	R	D	271.20 ± 8.06	2.46 ± 0.28	22.96 ± 2.88	23.72 ± 0.44
	25	D	R	H	297.67 ± 9.05	2.65 ± 0.28	19.89 ± 1.66	19.64 ± 1.07
	26	H	R	H	312.50 ± 8.34	2.57 ± 0.26	21.81 ± 1.53	20.41 ± 0.27
	32	H	R	H	270.23 ± 5.28	1.80 ± 0.16	16.80 ± 2.08	20.63 ± 0.72
Group 4	4	D	D	R	<u>252.23 ± 4.57</u>	<u>1.83 ± 0.20</u>	<u>28.59 ± 3.07</u>	<u>20.06 ± 1.01</u>
	28	H	D	R	302.40 ± 9.01	2.86 ± 0.22	38.73 ± 3.61	20.08 ± 0.48
	29	D	H	R	303.37 ± 8.63	2.46 ± 0.27	40.45 ± 3.13	20.74 ± 0.70
	30	H	H	R	341.23 ± 5.94	2.88 ± 0.27	37.59 ± 2.59	19.97 ± 0.47
	34	H	H	R	318.20 ± 5.63	2.46 ± 0.20	37.70 ± 2.83	20.83 ± 0.56
Group 5	5	R	R	D	<u>255.77 ± 4.90</u>	<u>2.06 ± 0.21</u>	<u>12.73 ± 1.60</u>	<u>20.59 ± 0.67</u>
	33	R	R	H	260.17 ± 7.24	1.61 ± 0.21	14.00 ± 1.24	19.62 ± 0.82
Group 6	6	R	D	R	<u>275.50 ± 5.32</u>	<u>1.56 ± 0.22</u>	<u>36.29 ± 2.15</u>	<u>20.67 ± 0.78</u>
	35	R	H	R	270.37 ± 8.64	1.79 ± 0.16	32.21 ± 2.76	20.92 ± 0.52
Group 7	7	D	R	R	<u>278.43 ± 5.60</u>	<u>2.47 ± 0.30</u>	<u>26.10 ± 2.46</u>	<u>20.63 ± 0.56</u>
	36	H	R	R	313.80 ± 7.81	2.21 ± 0.26	22.45 ± 1.76	20.90 ± 0.41
Group 8	8	R	R	R	<u>295.27 ± 5.41</u>	<u>1.16 ± 0.11</u>	<u>22.20 ± 1.64</u>	<u>22.47 ± 0.79</u>

D = dominant alleles; H = heterozygous; R = recessive alleles  
Mean values for the eight parental lines are underlined

4. Protein content of seed harvested from the first podding node (N×6.25 as a percentage of the dry matter). It has been suggested that the standard factor of 6.25 may overestimate crude protein content and that a factor of 5.50 should be used for pea seed protein (Sosulski and Sarwar 1973). However, since this change has not been universally adopted and its use would not influence the interpretation of these data the factor of 6.25 has been retained.

The data were analysed by the analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMR) in order to estimate the effects of genes singly and in combination and to determine whether or not there were effects associated with heterozygosity.

## Results

### *The weight of individual seeds when sown (mg)*

*Parents.* The ANOVA shows clearly that it is the *tl* locus in particular which modifies pea seed weight (Table 2). At the same time the effects of the *af* and *st* loci, together with the interactions between them and the *tl* locus, are also highly significant (Table 2).

Examination of the mean values recorded for the eight parental families (1–8) suggests that it is the mutant allele when homozygous at the *tl* locus in consort with the mutant alleles at the *af* and/or *st* loci which is associated with an increase in seed weight (Table 1).

*All families.* The overall ANOVA confirms the major effect of the *tl* locus upon seed weight but it is noticeable that the significance of the effect of the *af* locus has increased and that heterozygous effects are highly significant at all three loci but especially so at the *af* and the *tl* locus (Table 3).

The mean values obtained for seed weight in heterozygous families suggest that the effects are not those of partial dominance but of a form of heterosis especially associated with the *af* and *tl* loci. In relation to the seed weight data this takes the general form,

$$Aa > aa > AA.$$

An analysis of the data for Groups 1, 2 and 3 using DMR confirms that all families which are heterozygous at the *tl* locus have significantly heavier seeds than families, 1, 2 and 3 which are homozygous for the wild type allele. There is one exception in Group 1, however,

in that family 27 does not differ from family 1 but this seems to be a peculiarity of family 27 which has been noted in an earlier paper (Snoad et al. 1985) and can be seen to affect the values obtained for other characters being investigated in this paper (Table 1). It may be a reflection of residual heterozygosity and/or genetic drift having modified the parental lines since families 15, 20, 24 and 27 are expected to be genetically and phenotypically identical as far as the three loci under consideration are concerned.

Similar analysis of Group 4 data confirms that heterozygosity at either the *af* and/or *st* locus is associated with heavier seeds than those in family 4 which are homozygous for the wild type allele.

### *The number of fertile basal branches per plant*

*Parents.* The genotypes differ significantly but the ANOVA does not suggest that basal branching is affected by any one of the three genes although there is some slight indication of an effect due to the interactions between *af* × *st* and *af* × *tl* (Table 2).

*All families.* The overall ANOVA demonstrates a highly significant effect of the *af* locus upon branching and this is probably due to the effects resulting from heterozygosity at the *af* locus (Table 3). The greatest amount of basal branching is frequently to be found in families in which there is heterozygosity at the *af* locus.

### *The oven-dry weight of haulm at maturity – i.e. excluding seeds (g)*

*Parents.* The ANOVA indicates that the amount of haulm developed is mostly influenced by the *st* and *tl*

**Table 2.** An analysis of variance of the eight parents to estimate the effects of the genes *af*, *st* and *tl* upon the four characters examined

	d.f.	Wt. of individual seeds when sown	d.f.	No. of fertile basal branches	d.f.	Wt. of oven dried haulm	d.f. <sup>a</sup>	Protein content of harvested seed (N × 6.25 as % dry matter)
Total	238		154		189		71	
Block	2	625.8		1.687		506.9		2.313
Genotype	7	8,871.4***		2.900***		1,399.4***		6.224
Bl × Geno	14	438.9		1.206		137.0		7.147
<i>Af</i>	1	6,850.0***		6.240		54.3		13.261
<i>St</i>	1	6,591.6***		1.113		6,664.9***		2.457
<i>Tl</i>	1	31,865.5***		1.579		1,455.1***		0.307
<i>Af</i> × <i>St</i>	1	303.6		3.636*		872.5**		0.823
<i>Af</i> × <i>Tl</i>	1	5,188.3**		5.491*		218.2		2.383
<i>St</i> × <i>Tl</i>	1	9,487.4***		0.137		464.0*		12.087
<i>Af</i> × <i>St</i> × <i>Tl</i>	1	1,813.1		2.107		66.7		12.251
Residual	215	801.8	131	0.821	166	114.0	48	5.045

\*  $P = 5.0-1.0\%$ ; \*\*  $P = 1.0-0.5\%$ ; \*\*\*  $P = < 0.5\%$

<sup>a</sup> For protein content, seeds from only three plants were assayed from each family in each block, hence only 71 d.f.

**Table 3.** An analysis of variance of the 36 families which estimates gene effects, interactions and the effects of heterozygosity

	d.f.	Wt. of seeds when sown	d.f.	No. of fertile fertile basal branches	d.f.	Wt. of oven dried haulm	d.f.*	Protein content of harvested seed (N × 6.25 as % dry matter)
Total	1,077		833		938		323	
Block	2	1,621		12.681***		5,642.3***		3.951
Family	35	21,410***		3.643***		1,443.7***		4.799
Genotype	26	23,838***		3.655***		1,879.4***		5.590
Replicated Genotypes	9	14,427***		3.606***		185.0		2.515
Bl × Geno	52	1,638		1.075		170.2		5.410
<i>Af</i>	2	32,631***		23.697***		1,515.2***		5.798
<i>St</i>	2	6,834*		4.745*		17,772.3***		0.396
<i>Tl</i>	2	95,460***		0.549		1,918.1***		4.841
<i>Af</i> × <i>St</i>	4	20,035***		2.701		538.3**		0.940
<i>Af</i> × <i>Tl</i>	4	19,540***		3.221		290.0		2.478
<i>St</i> × <i>Tl</i>	4	16,695***		1.295		449.4*		6.784
<i>Af</i> × <i>St</i> × <i>Tl</i>	8	15,574***		1.023		167.7		10.308*
<i>AfAf</i> × <i>Afaf</i>	1	44,574***		48.114***		2,690.1***		10.849
<i>StSt</i> × <i>Stst</i>	1	14,672***		0.181		34,601.0***		0.562
<i>TlTl</i> × <i>Tltl</i>	1	35,120***		0.091		165.2		9.699
Residual	988	1,485	744	1.167	849	145.1	234	4.603

\*. \*\*. \*\*\* see Table 2

\* For protein contents, seeds from only three plants were assayed from each family in each block, hence only 323 d.f.

loci with the interaction between the *af* and *st* loci also being of some significance (Table 2). There is little doubt that homozygosity of the mutant alleles at the *st* locus is responsible for the development of less haulm, as in Groups 3 and 5, but when the mutant alleles at the *af* and/or *tl* loci are also homozygous there is compensation for this *stst* effect as in Groups 7 and 8 (Table 1). The genetic combination *afaf* · *StSt* · *tltl* leads to the development of the most haulm.

*All families.* According to the overall ANOVA the *st* locus still has a very significant effect upon haulm development with some implication of the *af* and *tl* loci (Table 3). It is, however, the effects of heterozygosity at the *st* locus which is the most outstanding feature of the ANOVA although a similar but smaller effect is associated with the *af* locus (Table 3). The effect of heterozygosity at the *st* locus is of a heterotic nature in that haulm weight tends to be higher in *Stst* than in *StSt* plants with the values for both exceeding those recorded for *stst* plants (Table 1). There is a similar indication of a heterotic effect associated with the *af* locus.

*Protein content of seed harvested from the first podding node (N × 6.25 as a percentage of the dry matter)*

*Parents.* The ANOVA gives no indication that there are significant differences between the parents for seed protein content (Table 2).

*All families.* There is only a hint of an interaction between the *af*, *st* and *tl* loci in the overall ANOVA (Table 3) and therefore no genetical variation for protein content has been detected.

## Discussion

Since the genotypes do not differ in protein content the effects of the three genes *af*, *st* and *tl* are only of significance in three of the four characters examined and of these three characters one, basal branching, was different from the others in that gene effects were only recorded when there was heterozygosity at the *af* locus.

Examination of the raw data for protein content exposes the large amount of variation within families, as shown by the high standard errors, and this reduces the possibility of demonstrating any gene effects upon this character. There are, however, data elsewhere (Wright and Hedley, personal communication) to suggest that protein content is lower in *stst* than in *StSt* seeds. This genetic effect has been more readily exposed because protein content was expressed as protein per seed rather than by the use of bulked samples which are never of uniform seed size.

The major effect of the homozygous mutant allele at the *tl* locus is to increase seed weight and haulm weight, especially in consort with homozygosity of the mutant alleles at the *af* and *st* loci. Homozygosity of the mutant alleles at the *af* and *st* loci alone seems to have

little effect upon seed weight but respectively to increase or decrease haulm weight significantly. It would be interesting to examine the larger seeds of *tttl* families and determine their protein, starch, sugar and lipid content.

It is, however, when the effects of heterozygosity at the three loci are examined that the results become of interest to the pea breeder. There is clear evidence of a heterotic effect associated with heterozygosity at all three of the loci examined in this paper and these effects modify seed weight, basal branching and haulm weight. In the case of seed weight and haulm weight there is evidence of gene interaction, albeit between genes on different chromosomes, being associated with the heterotic effect but there is no evidence of similar interaction being associated with the effect upon basal branching. Since only three loci have been under investigation other such interactions cannot be ruled out.

Heterosis in peas was reported as long ago as 1910 when Keeble and Pellew indicated that a hybrid between two forms of pea was taller than either parent and interpreted this effect as being due to the complementary effects of dominant alleles at two loci. Unfortunately the fact that the material used in the present experiment is not truly isogenic inhibits any attempt to interpret the heterotic effects that have been noted.

Partial dominance associated with heterozygosity at the *tl* locus affecting leaf weight and seed yield, both at a single node and per plant, have been reported in an earlier paper (Snoad et al. 1985). There is evidence now that heterozygosity at any one of three loci can also affect plant development in such a way that some measurements in plants heterozygous for alleles at a locus exceed those in plants homozygous for either the wild type or the mutant allele.

The implications of such findings are of importance to pea breeders who are often under such pressure to develop new cultivars that the amount of genetical work undertaken is limited. The dominance relationships between the various mutant and wild type alleles with which they are working are probably known but they could be unaware of the effects of heterozygosity so that selection in very early generations, as in pedigree breeding systems, might be misleading for some characters. The use of one of the many bulk selection methods in which selection is delayed until the F5 or even later would seem even more desirable. If glasshouse space is readily available then the single seed descent system has a special appeal because it combines delay in selection with speed in the production of generations.

*Acknowledgements.* We wish to express our gratitude to Mr. Jeremy Franklin of the Food Research Institute, Norwich for help in using GENSTAT to analyse the data.

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