*Genet. Res., Camb.* (1994), **64**, *pp.* 35–41 *With 5 text-figures* Copyright © 1994 Cambridge University Press 

# Phenotypic effect of substitutions of short chromosomal segments containing different alleles of histone H1 genes in garden pea (*Pisum sativum* L.)

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(*Received 3 December 1992 and in revised form 5 May 1994*)

## Summary

A hypothesis has been tested that alterations in the molecule of histone H1 are capable of influencing quantitative traits of an organism. Two pairs of isogenic lines were constructed by selfing of plants kept heterozygous either for allelic variants of histone H1 subtype 1 (*His1* gene) or for allelic combinations (haplotypes) of closely linked genes of H1 subtypes 3, 4, 5 and 6 (gene cluster *His*(*2-6*)). After 19 and 15 generations of selfing, respectively, expectation of the length of chromosome which remained heterozygous has comprised 2·6 cM near *His1* and 3·5 cM near *His*(*2-6*). The third pair of isogenic lines was obtained as a result of two successive intracluster cross-over events bordering the gene of the subtype 5 (*His5*). In each pair of isogenic lines there have been revealed some statistically significant differences between mean values of a number of quantitative traits.

## 1. Introduction

Histone H1 is known as a major factor of chromatin folding into a higher-order structure (Thoma *et al.* 1979; Allan *et al.* 1980). It was shown to be a non-specific repressor of gene expression (Weintraub, 1985; Zlatanova, 1990). Besides, histone H1 appears to compete with specific gene activators for regulatory sites of tissue-specific genes, thus determining their ability for further transcription (Brown, 1984; Zlatanova, 1990). Moreover, recently a hypothesis has been put forward (Oikarinen, 1991) that H1 is a member of the regulatory cascade involved in the action of nuclear receptors which leads to selective activation of chromatin. For this reason alterations of molecular structure of histone H1 affecting its affinity to regulatory sites of genes or ability to interact with protein factors should bring about changes of differential gene activity. This would result in certain phenotypic modifications. Most probably they would be of a quantitative nature, since the activity of many genes should be affected while the effect on any given gene may be quite subtle.

Earlier we obtained indirect evidence of the adaptive value of H1 variation. We found a correlation between the variance of H1 molecular size and the number of recent species in different insect orders (Berdnikov *et al.* 1993*a*), indicating that certain changes in the H1 molecule were more or less frequently involved in evolutionary processes of species transition, i.e. were of selective advantage. In a small population of *Vicia unijuga* A.Br. a radial cline of H1 allelic frequencies was found, corresponding well to the spatial and temporal patterns of urbanization of the natural habitat (Berdnikov *et al.* 1992). Analysis of a world-wide collection of garden pea local forms showed that the frequency of a slow allelic variant of one of the H1 subtypes was correlated with the accumulated temperature above 10 °C (Berdnikov *et al.* 1993*b*).

To obtain more immediate evidence of the phenotypic effect of H1 allelic substitutions we have chosen the garden pea (*Pisum sativum* L.) as being genetically well studied and possessing relatively short generation time.

Histone H1 of the garden pea is heterogeneous, being represented by a family of several proteins, or H1 subtypes, enumerated according to an increase of their electrophoretic mobility. Each subtype has a number of electrophoretic variants (Berdnikov *et al.* 1993*b*), exhibiting Mendelian segregation, that is encoded by alleles of a single gene, seven genes existing for seven H1 subtypes. They have been located on the pea genetic map. The gene *His1* of the slowest subtype 1, comprising approximately half of

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total histone H1, was mapped on chromosome 5 (chromosome 7 in earlier nomenclature) (Rozov *et al.* 1986; Smirnova *et al.* 1990). Genes of subtypes 2, 3, 4, 5 and 6 are very closely linked to each other and segregate as a single unit. This cluster of five genes, designated as *His(2-6)*, was located in chromosome 1 (Belyaev & Berdnikov, 1981; Kosterin, 1992). The gene of subtype 7 has been mapped 33 cM from *His(2-6)* (Kosterin, 1992).

We constructed three pairs of isogenic lines differing in either of two loci for H1 genes (*His1* or *His(2-6)*) and compared them for a number of quantitative characters. Some statistically significant differences were found between the lines compared, which we suggest to be the result of H1 allelic substitutions.

## 2. Materials and methods

### (i) *Materials*

Pea accessions VIR-7036 (*Pisum sativum asiaticum* Govorov; Nepal), VIR-5195 (*P.s. asiaticum* Gov.; India), VIR-4871 (*P.s. transcaucasicum* (Gov.) Makash.; Georgia), and two cultivars: Torsdag and Avanti were used as ancestors for the isogenic lines constructed.

### (ii) *Plant growing*

Plants were grown in a greenhouse in hydroponic vermiculite/ceramsite (2:1, v/v) beds fed by standard Knop nutrient solution. They were illuminated by 8 h daylight/16 h incandescent light of 10000–12000 lx intensity. Quantitative characters were measured in the winter–spring vegetation period.

### (iii) *Histone H1 isolation and electrophoresis*

Histone H1 was isolated according to Johns (1964) with further modifications (Rozov *et al.* 1986): 200–400 mg of pea leaves were homogenized in 12 ml of 0·15 M NaCl, the homogenate was filtered through two layers of gauze and centrifuged at 1500 ***g*** for 5 min. Histone H1 was extracted by resuspending the pellet in 1 ml of 5% $HClO_4$. After centrifugation the protein was recovered from the supernatant by adding sulphuric acid to a final concentration of 0·5 M and 6 volumes of cold acetone. The precipitated protein was centrifuged and then dissolved in 0·2 ml of a medium containing 0·9 M acetic acid, 8 M urea, and 15% (w/v) sucrose. The preparations were subjected to electrophoresis in long (10–30 cm) slabs of 15% polyacrylamide / 0·5% *N,N'*-methylenbisacrylamide gel containing 6·25 M urea and 0·9 M acetic acid following a modification (Berdnikov & Gorel, 1975) of Panyim & Chalkley (1969) method. After electrophoresis, the gels were stained in 0·01% (w/v) Coomassi R-250 in 0·9 M acetic acid solution and destained by diffusion in 0·9 M acetic acid. The resolution of slightly differing allelic variants of the slowest subtype 1 was facilitated by cleavage of the histone H1 molecule at tyrosine residues by treating preparations with *N*-bromosuccinimide added to a final concentration of 1 mg/ml for 15 min (Sherod *et al.* 1974). The H1 fragment containing the C-terminus of the molecule demonstrates easily detectable electrophoretic differences between allelic variants.

## 3. Results

To study the effect of allelic substitutions in the gene cluster *His(2-6)* two isogenic lines differing in the four histone H1 genes were produced. One parental line, VIR-4871, possessed the fast allelic variant of H1 subtypes 3, 5 and 6, and the slow variant of subtype 4, while another parental line, obtained from cultivar Torsdag, had, oppositely, the slow variants of subtypes 3, 5 and 6, and the fast one of subtype 4. According to the nomenclature of alleles and the designation of their combinations adopted by Berdnikov *et al.* (1993*b*), the former had H1 haplotype 2122 (an allelic combination of H1 subtypes 3-6), the latter had haplotype 1211 (as shown in Fig. 1).

The isogenic lines were obtained according to the

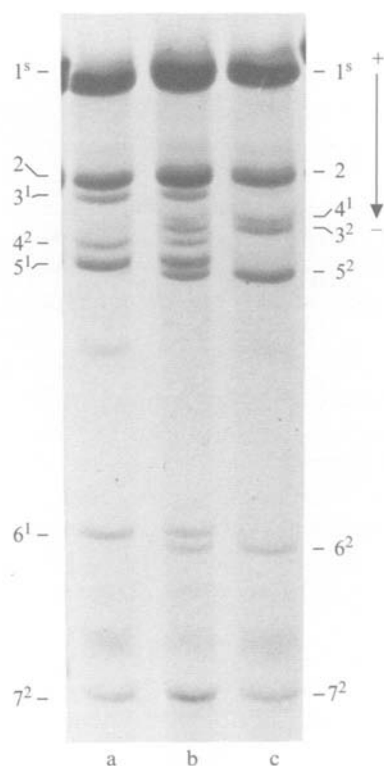


Fig. 1. Electrophoresis of histone H1 from individual plants segregating for allelic combinations of the locus *His(2-6)*. (*a*) haplotype 1211; (*b*) heterozygote 1211/2122; (*c*) haplotype 2122. Figures denote H1 subtypes, superscripts, their allelic variants.

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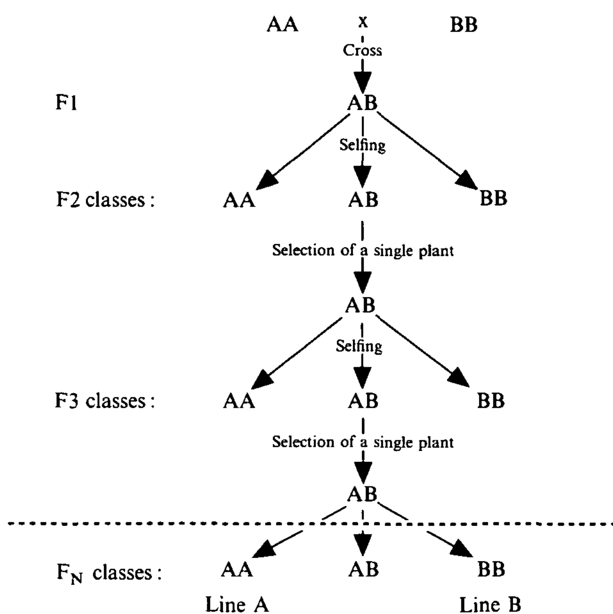


Fig. 2. The scheme employed to obtain isogenic lines of pea. A and B are the conventional designations of alleles of a single locus (or allelic combination of a gene cluster).

scheme shown in Fig. 2. The ancestral lines were crossed in 1980. Selfing of the resulting hybrid plants (which had the heterozygous H1 phenotype, Fig. 1 *b*, and fully fertile pollen and ovules) produced F2 progeny which segregated for *His*(*2-6*) genes. A single plant was chosen from the heterozygous class and allowed to self-pollinate. A single heterozygous plant was again chosen from its progeny, and so on. This procedure was repeated for 15 generations. In its course homozygosity was rapidly rising all over the genome except for the chromosome segment containing the *His*(*2-6*) locus. The average recombination length $y$ of the segment that retained heterozygosity is given by the formula (Serra, 1966):

$$y = 1/(2N - 0{\cdot}5 \ln N),$$

where $N$ is the number of generations of selfing. At $N = 15$ expectation of this length is 3·5 cM. (It should be noted that in our experiment the gene *a* located at 4 cM from *His*(*2-6*) and controlling anthocyanin production became homozygous after 6 generations of selfing.)

Two homozygous plants, one possessing the H1 allelic combination of cultivar Torsdag, the other – of line VIR-4871, were taken from the progeny of a heterozygote obtained after 15 generations of selfing (segregation for the allelic combinations of the locus *His*(*2-6*) is shown in figure 1). Both plants possessed the same (fast) allelic variant of the *His1* gene. Their progenies, 60 individuals of the former (designated as line A) and 62 individuals of the latter (line B) were planted in a hydroponic bed in a greenhouse in 1986. The plants of the two lines were arranged in alternate rows.

A number of quantitative traits of mature plants of lines A and B were measured. Their means and standard deviations are given in Table 1. The differences between their means range from 2 to 19%. For such traits as node of the first inflorescence, mean number of pods per node, the differences turned out to be significant.

We also measured the lengths of internodes from node 6 to 16 at the moment when they attained approximately half their final lengths, that is at the moment of their maximal growth rate. As the plants of lines A and B grew in alternate rows, the internode lengths were compared in pairs of plants of each line growing next to each other. The internode lengths of

Table 1. *The comparison of quantitative characters in isogenic lines* $A^{+}$ *and* $B^{++}$, *differing in alleles of genes of histone H1 subtypes 3-6* (*locus His*(*2-6*); *line A has H1 hapolotype 1211*; *line B*, *2122*)

| Character | Means (M) ± standard errors | | Standard deviations | | | |
|---|---|---|---|---|---|---|
| | Line A | Line B | Line A | Line B | $M_B - M_A$ | $\frac{(M_B - M_A)}{M_A}$ (%) |
| Total number of nodes per plant | 91·49 ± 4·38 | 102·79 ± 4·39 | 33·62 | 34·56 | 11·30 | 12·4 |
| Number of nodes in main stem | 38·64 ± 0·73 | 40·08 ± 0·70 | 5·62 | 5·51 | 1·44 | 3·7 |
| Number of laterals | 5·78 ± 0·48 | 6·87 ± 0·48 | 3·74 | 3·84 | 1·09 | 18·8 |
| Number of fertile nodes | 22·33 ± 1·29 | 25·23 ± 1·28 | 10·03 | 10·07 | 2·90 | 13·0 |
| Node of the first inflorescence | 16·17 ± 0·05 | 17·06 ± 0·04 | 0·43 | 0·30 | 0·89*** | 0·05 |
| Day of flowering | 40·44 ± 0·17 | 40·75 ± 0·17 | 1·42 | 1·35 | 0·31 | 0·02 |
| Number of pods | 32·63 ± 1·89 | 35·34 ± 1·87 | 14·92 | 14·72 | 2·71 | 8·3 |
| Mean number of pods per inflorescence | 1·47 ± 0·02 | 1·39 ± 0·02 | 0·15 | 0·17 | −0·08** | −5·4 |
| Mean number of seeds per pod | 4·04 ± 0·07 | 4·14 ± 0·07 | 0·55 | 0·57 | 0·10 | 2·5 |
| Total seed number | 130·40 ± 7·83 | 142·79 ± 7·97 | 60·64 | 62·78 | 12·39 | 9·5 |
| Mean seed mass (mg) | 131·12 ± 1·22 | 128·60 ± 1·37 | 10·07 | 11·01 | −2·52 | −1·9 |

+, Number of plants of line A = 60.
++, Number of plants of line B = 62.
Here and further asterisks indicate the levels of significance $\alpha$ estimated by Student's $t$ test, as follows: *, $0{\cdot}05 \geqslant \alpha \geqslant 0{\cdot}01$; **, $0{\cdot}01 \geqslant \alpha \geqslant 0{\cdot}001$; ***, $\alpha \leqslant 0{\cdot}001$.

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Table 2. *The mean length (mm) of internodes 6–7 and 7–8 in lines A and B at different stages of their growth*

| | Length of internode 6–7 | | Length of internode 7–8 | |
|---|---|---|---|---|
| Day of growth | Line A | Line B | Line A | Line B |
| 19 | 31·04 ± 1·34 | 35·42 ± 1·38 | | |
| 22 | 83·42 ± 0·63 | 86·28 ± 0·65 | 49·34 ± 1·66 | 55·97 ± 1·81 |
| 25 | | | 89·09 ± 0·80 | 89·85 ± 0·67 |
| Elongation for 3 days | 52·19 ± 1·20 | 50·88 ± 1·46 | 38·38 ± 2·17 | 34·83 ± 2·17 |

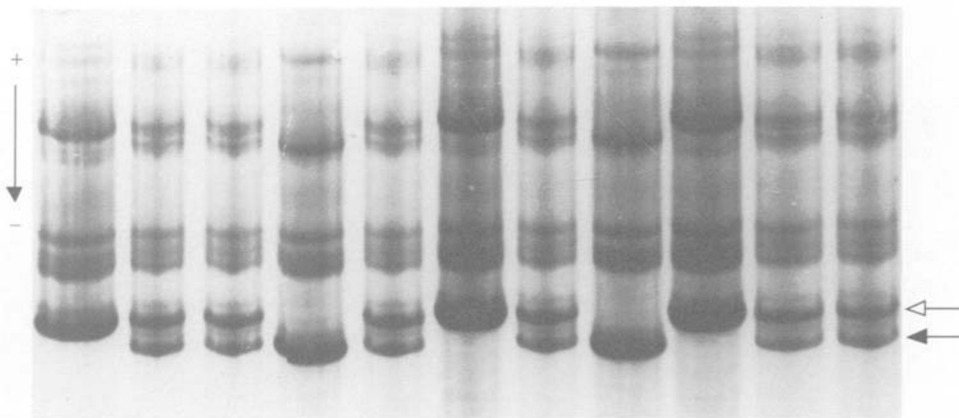


Fig. 3. Electrophoresis of histone H1 from individual plants segregating for *His1* alleles and treated with *N*-bromosuccinimide. Indicated are the *C*-terminus-containing fragments of the slowest *H*1 subtype (open arrow, variant *His*1$^s$; closed arrow, variant *His*1$^f$).

the plants of line B were steadily greater than those of their counterparts in line A. The lengths of internodes 6–7 and 7–8 were measured twice – when the internodes were undergoing intensive elongation and when they had attained their final length. Table 2 shows that at the time of intensive growth the internode lengths in line B were on average greater than in line A. But when growth finished the differences were much less (internode 6–7) or had disappeared (internode 7–8). This means that elongation rate at the earlier period of internode growth is higher in line B, and, oppositely, in the later period – in line A. Thus, the lines differed in the dynamics of internode growth but not in the final internode length.

The effects of allelic substitutions in gene *His1* were studied in an analogous experiment. The ancestral line with the fast allelic variant of H1 subtype 1 was isolated from accession VIR-7036, with the slow variant – from the cultivar Avanti (Electrophoresis of *N*-bromosuccinimide cleaved H1 subtype 1 variants is presented in Fig. 3). They were crossed in 1983. No semisterility was observed in F1 and the descendant hybrids. The isogenic lines C and D homozygous for the variants His1$^s$ (slow) and His1$^f$ (fast), respectively, were obtained by the scheme described above of isogenization for 19 generations of selfing. The expected length of the chromosome segment retaining heterozygosity has comprised 2·7 cM. The obtained lines C and D were characterized by a miniature habitus and a very short life cycle – about 32–40 days under permanent illumination. As these peas were of relatively low seed productivity, a generation of reproduction was added before the lines were tested. 86 plants of line C and 83 plants of line D were planted in chess-board order in hydroponic beds in 1986. Both lines possessed the same 1221 haplotype of the subtypes 3–6.

Significant differences between lines C and D were observed for 7 of the 11 quantitative traits measured, namely, mean seed mass, total number of nodes per plant, number of lateral branches per plant, node of the first inflorescence, the day of flowering, number of seeds per pod. The distributions of the two former traits are given in Fig. 4.

It should be noted that the observed effects were reproducible at different generations of selfing. A preliminary comparison of plants of both homozygous and heterozygous classes was made after 10 selfing generations. The mean values of traits characterizing general size and productivity of a plant of His1$^f$ homozygous class exceeded that of His1$^s$ class (not shown). The most pronounced effect was observed in the number of nodes per plant and in the number of lateral branches. The heterozygous class exhibited intermediate values of all the traits studied.

The third pair of isogenic lines was obtained by a somewhat different way. This pair was to elucidate the effects of allelic substitution in only one gene of the

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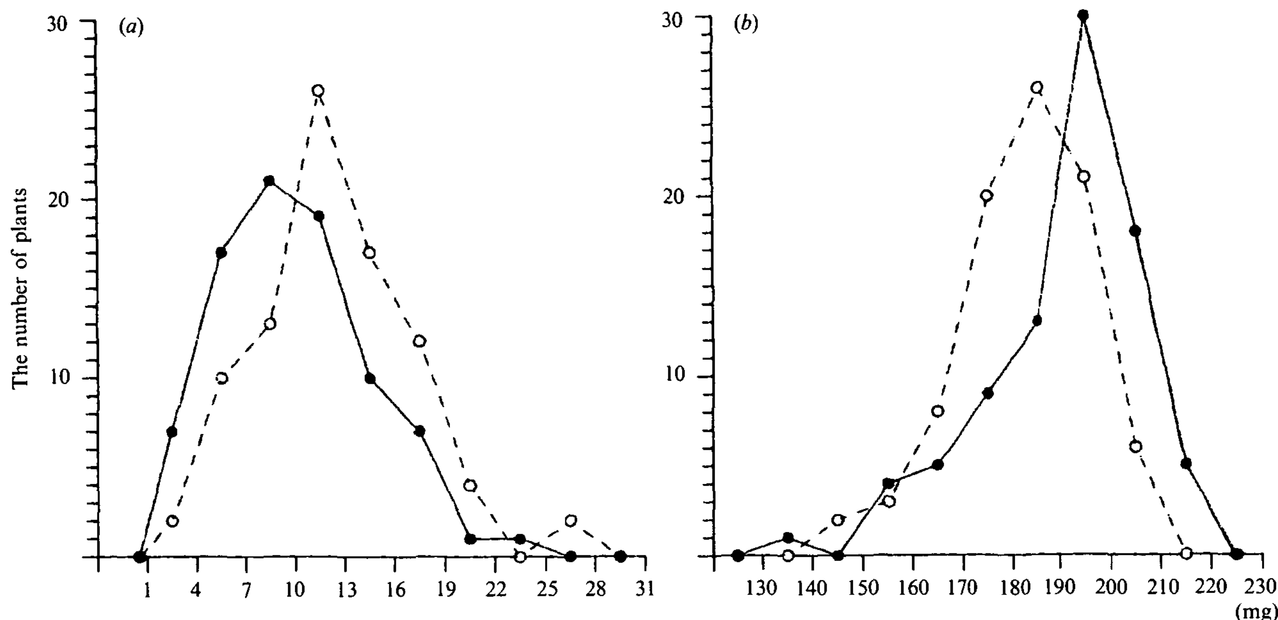



Fig. 4. Distributions of plants of lines C (solid line) and D (dashed line) for the number of lateral branches (*a*), and the mean seed mass (*b*). Numbers of plants are summed over the intervals of a character value indicated on the horizontal axis.

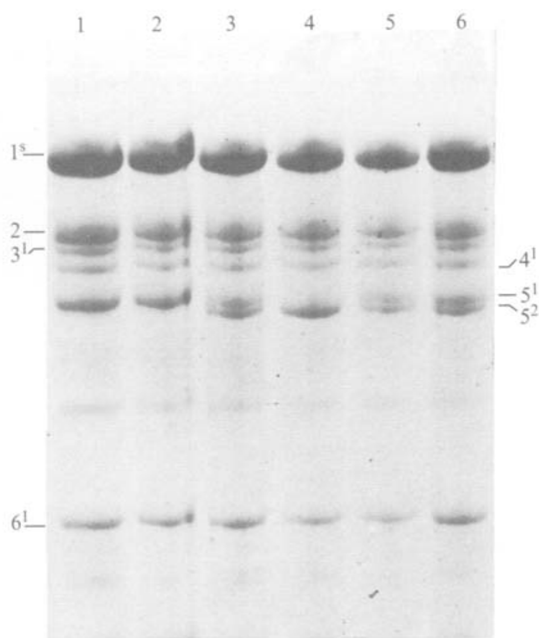
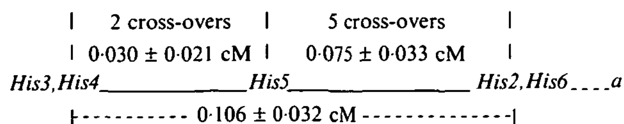



Fig. 5. Electrophoresis of histone H1 from individual plants segregating for the alleles of the *His5* gene. Designations as in Fig. 1. Lanes 1 and 2, homozygotes for the variant $5^1$ (haplotype 1111), lane 4, homozygotes for the variant $5^2$ (haplotype 1121), lanes 3, 5, and 6, heterozygotes.

cluster (*His2-6*). The line D described above, with the haplotype 1221 of the locus *His(2-6)* and the fast allele of the subtype 1, became a recurrent parent in two series of back-crosses. As the second parent there was used either VIR-5195 (India) line with the haplotype 1123 or the above mentioned line B with the haplotype 1211. In the course of isogenization the heterozygote 1221/1123 or 1221/1211 was back-crossed on the homozygote 1221 (line D). After 12 generations of back-crossing in the result of segregation we obtained two nearly isogenic plants with haplotypes 1123 and 1211, respectively, which were crossed in 1991 to produce heterozygote 1123/1211. Among the F2 progeny we found a cross-over within the *His(2-6)* cluster between the genes of the subtypes 4 and 5 possessing 1123/1111 genotype. Quite unexpectedly, in the following generation this heterozygote gave rise to another cross-over within the cluster, namely between the genes of the subtypes 5 and 6, which had a 1121/1111 constitution of the *His(2-6)* locus. Segregation for the alleles of the *His5* gene in the progeny of this heterozygote is shown in figure 5.

We performed a special study of the fine structure of the *His(2-6)* gene cluster. Totally 3313 plants of the F2 progenies of four crosses were analysed, seven cross-over plants being detected. The results suggest the following arrangement of genes:




Thus, the haplotype 1121 resulted from two cross-over events flanking both sides of *His5* within the gene cluster and, hence, the chromosomal segment retaining heterozygosity cannot exceed 0·1 cM.

After a cycle of reproduction we obtained the third pair of isogenic lines E and F with the haplotypes 1121 and 1111, respectively, which differed only by the alleles of the *His5* gene. The analysis of this pair is

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Table 3. *The comparison of quantitative characters in isogenic lines $C^+$ and $D^{++}$, which differ in alleles of locus His1 encoding H1 subtype 1 (line C possesses variant s; line D, variant f)*

| Character | Means (M) ± standard errors | | Standard deviations | | | $\frac{(M_D - M_C)}{M_C}$ |
|---|---|---|---|---|---|---|
| | Line C | Line D | Line C | Line D | $M_D - M_C$ | (%) |
| Total number of nodes per plant | 68·91 ± 3·73 | 87·08 ± 4·06 | 33·94 | 37·65 | 18·17*** | 26·4 |
| Number of nodes in main stem | 17·30 ± 0·30 | 17·93 ± 0·28 | 2·73 | 2·63 | 0·63 | 3·6 |
| Number of laterals | 9·49 ± 0·49 | 11·89 ± 0·51 | 4·51 | 4·73 | 2·40*** | 25·3 |
| Node of the first inflorescence | 7·78 ± 0·05 | 7·57 ± 0·05 | 0·47 | 0·50 | −0·21** | −0·03 |
| Day of flowering | 24·65 ± 0·13 | 24·15 ± 0·13 | 1·19 | 1·23 | −0·50** | −0·02 |
| Number of pods | 6·94 ± 0·25 | 6·88 ± 0·17 | 2·25 | 1·59 | −0·06 | −0·9 |
| Mean number of seeds per pod | 2·61 ± 0·07 | 2·84 ± 0·06 | 0·65 | 0·57 | 0·23* | 8·8 |
| Total seed number | 17·52 ± 0·63 | 19·21 ± 0·53 | 5·75 | 4·94 | 1·69* | 9·6 |
| Mean seed mass (mg) | 189·99 ± 1·67 | 182·17 ± 1·40 | 15·23 | 12·96 | −7·82*** | −4·1 |
| Length of plant (cm) | 108·50 ± 1·68 | 110·335 ± 1·60 | 15·34 | 14·83 | 1·83 | 1·7 |
| Dry plant mass (g) | 7·14 ± 0·27 | 7·47 ± 0·25 | 2·47 | 2·33 | 0·33 | 4·6 |

+, Number of plants of line C = 83.
+ +, Number of plants of line D = 86.

Table 4. *The comparison of quantitative characters in isogenic lines $E^+$ and $F^{++}$, which differ in alleles of gene His5 encoding H1 subtype 5 (line E possesses haplotype 1121; line F, haplotype 1111)*

| Character | Means (M) ± standard errors | | Standard deviations | | | $\frac{(M_F - M_E)}{M_E}$ |
|---|---|---|---|---|---|---|
| | Line E | Line F | Line E | Line F | $M_F - M_E$ | (%) |
| Total number of nodes per plant | 28·19 ± 1·24 | 30·50 ± 1·54 | 13·53 | 18·01 | 2·31 | 8·2 |
| Number of nodes in main stem | 13·23 ± 0·15 | 13·10 ± 0·16 | 1·78 | 1·91 | −0·13 | −1·0 |
| Number of laterals | 3·72 ± 0·21 | 3·98 ± 0·23 | 2·47 | 2·70 | 0·26 | 7·0 |
| Node of the first inflorecence | 6·73 ± 0·04 | 6·73 ± 0·04 | 0·48 | 0·48 | 0·00 | 0·0 |
| Day of flowering | 20·89 ± 0·07 | 20·86 ± 0·07 | 0·81 | 0·88 | −0·03 | 0·1 |
| Number of pods | 4·29 ± 0·07 | 4·36 ± 0·07 | 0·81 | 0·77 | 0·07 | 1·6 |
| Mean number of seeds per pod | 4·17 ± 0·06 | 4·35 ± 0·05 | 0·71 | 0·63 | 0.18* | 4·3 |
| Total seed number | 17·78 ± 0·34 | 18·86 ± 0·32 | 3·99 | 3·80 | 1·08* | 6·1 |
| Mean seed mass (mg) | 145·07 ± 1·65 | 142·87 ± 1·55 | 19·33 | 18·37 | −2·20 | −1·5 |
| Length of plant (cm) | 82·21 ± 0·77 | 82·16 ± 0·74 | 9·03 | 8·78 | −0·05 | −0·06 |
| Length of main stem below node 5 (cm) | 25·23 ± 0·15 | 24·74 ± 0·16 | 1·75 | 1·86 | −0.49* | −1·9 |

+, Number of plants of line E = 137.
+ +, Number of plants of line F = 140.

given in Table 4. Significant differences between the two lines were found in 3 of the 11 quantitative traits measured: total number of seeds per plant, mean number of seeds per pod, and the length of the lower part of the stem (up to node 5).

## 4. Discussion

As stated above, the protein family of histone H1 plays an important role in chromatin folding into a higher-order structure. The protein probably acts as a non-specific repressor of gene expression and, besides, as a competitor for the regulatory sites of genes (Brown, 1984; Zlatanova, 1990; Oikarinen, 1991). Several kinds of indirect evidence suggest that molecular variations in these proteins are likely to have pleiotropic effects, including effects on quantitative traits. To attempt to obtain direct evidence on this question, we created three pairs of isogenic lines of *Pisum sativum*, differing only in electrophoretically detectable alleles of particular histone H1 genes and a small length of adjacent DNA still heterozygous. Eleven quantitative traits were compared in the pairs of lines.

Seven loci code for the H1 proteins: *His1*, the cluster *His(2-6)*, and *His7*. The isogenic lines differed in alleles of *His(2-6)* (lines A and B, Tables 1 and 2), alleles of *His1* (lines C and D, Table 3) and alleles of *His5* within the *His(2-6)* cluster (lines E and F, Table 4). The lengths of chromosomal segments, surround-

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ing the H1 alleles, which remained heterozygous were estimated from the numbers of selfing generations used to create the isogenic line pairs A/B and C/D, and from the known recombination length of the *His*(*2-6*) gene cluster for the pair E/F. These lengths are expressed in centimorgans (cM).

The estimated lengths of heterozygous DNA, together with the number of statistically significant differences between the two lines of each pair were as follows: for *His*(*2-6*), 3·5 cM and 3 traits different; for *His1*, 2·7 cM and 7 traits different; and for *His5* within the *His*(*2-6*) cluster, 0·1 cM and 3 traits different. Note that the cluster *His*(*2-6*) and the *His1* gene are located in different chromosomes (Rozov *et al.* 1986), and there are three traits significantly affected by allelic substitutions in different histone H1 loci (in two experiments of the three conducted), namely, node of the first inflorescence, mean number of seeds per pod, and total number of seeds per plant.

We cannot rule out the possibility that all these trait differences are the result of gene differences outside the histone H1 loci tested, but we consider it most likely that some or all of them are direct effects of the H1 allele differences, since otherwise one has to assume that several quantitative trait genes are located very close to each of the H1 gene family tested. A further period of inbreeding could restrict the heterozygous regions to the size of histone genes themselves and allow estimation of the extent of the chromosome segment required for retaining differences in the mean values of quantitative traits. Alternatively, isolation of a point mutation in the H1 gene in a pure line might solve this problem unambiguously.

The results reported here could be regarded as supporting the view that substitutions of alleles of histone H1 genes may cause slight effects on a number of quantitative traits of an organism.

This study was supported by a grant from the Soros Foundation for Novosibirsk Scientific Centre and by a grant from the Russian Foundation for Fundamental Research 93-04-20647.

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https://doi.org/10.1017/S0016672300032523 Published online by Cambridge University Press