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Effect of a substitution of a short chromosome segment carrying a histone H1 locus on expression of the homeotic gene *Tl* in heterozygote in the garden pea *Pisum sativum* L.

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Summary

Mutation tl^x was induced in pea by gamma-irradiation in the homeotic gene *Tl*, responsible for transformation of the upper leaflets into tendrils. Heterozygotes tl^x/Tl^+ possess 'flat tendrils' having narrow rudimentary leaf blades. By 15 generations of selfing the line Delta was established, in which closely linked loci *Tl* and *His1* were maintained heterozygous, *His1* encoding the most abundant histone H1 subtype. The heterozygous chromosome region was flanked by several recessive markers. The genotype of line Delta with respect to loci *His1* and *Tl* was: tl^x $His1^2/Tl^+$ $His1^1$. There were derived two sublines, Delta-s and Delta-f, homozygous for each of the *His1* alleles but heterozygous for the *Tl* and flanking markers. The three lines were compared for a number of quantitative traits, including parameters of flat tendrils reflecting the expression of the gene *Tl* in heterozygote, and for recombination rate within the heterozygous segment. The line Delta-s exceeded Delta-f in terms of the level of the first inflorescence, length of the internode 8–9, number of pods on the main stem and mean seed mass, but was inferior regarding the mean number of seeds per pod. The flat tendrils were wider in the Delta-s as compared with the Delta-f line; the difference was highly significant and exceeded 15% at node 17. Recombination rate between the flanking markers also showed significant differences, being highest in the Delta-f and lowest in the Delta line. We do not exclude the possibility that the observed differences in the quantitative traits are caused by the substitution of alleles of the histone H1 gene. The effects of such substitution appeared to be especially strong for expression of the homeotic gene *Tl*.

1. Introduction

Knowledge of the nature of loci that determine continuous variation of quantitative traits is of great importance in both practical and theoretical studies. These loci (so-called QTL) are responsible for changes in the morphology of an organism subjected to natural or artificial selection. Genetic analysis of traits affected by selection shows that the major part of the effect of the selection is due to allelic substitutions in a relatively small number of loci (Mackay, 1995). A thorough analysis of this type was carried out in *Drosophila* lines obtained in the course of selection for the number of the sensory chaetae – abdominal and sternopleural bristles. It was found out that the QTLs responsible for the major part of the selection effect commonly fall into the loci well known for their qualitative effect on bristle development, all probably being alleles of these genes. At the same time, there is no doubt that there exist QTLs without such an evident relation to the development of a selected trait. A good example of this kind is the locus *bobbed*, which contains multiple copies of the rRNA cistrons (Frankham, 1988). Changes in the number of these cistrons affect the level of translation and thus influence development of many morphological structures including the bristles.

It is well established that morphogenesis is regulated to a large extent at the level of transcription, numerous

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protein factors being involved. Notable among these factors are histones, which are constituents of chromatin of any eukaryotic cell and, to a certain extent, determine a molecular environment for the functioning of the transcription machinery. From general considerations it is clear that changes in the structure or expression of histone genes could affect development of many quantitative traits, that is, the histone genes can act as QTLs. It is sufficient to remember the modification of the position effect variegation by histone gene dosage in *Drosophila* (Moore *et al.*, 1983). In this respect histone H1 is of special interest. This histone does not participate in formation of the nucleosome core and interacts with linker DNA. As a rule it is represented by multiple non-allelic subtypes, frequently encoded by single gene copies. Most probably H1 subtypes have functional specificities, since the spectra of subtypes change in the course of ontogenesis and cellular differentiation (Poccia, 1986).

Histone H1 is traditionally supposed to take part in organization of the higher-order chromatin structure and also to play the role of a non-specific gene repressor (Felsenfeld, 1992). However, in recent years the structural role of histone H1 in chromatin organization has been subjected to critical consideration. It was shown that complete elimination of expression of histone H1 genes in *Tetrachymena thermophyla* led only to some chromatin decondensation and did not reduce viability of the protist (Shen *et al.*, 1995). Loss of linker histone H1^{o}, accumulating normally in non-dividing cells, had no apparent effect in mice (Sirotkin *et al.*, 1995). Nevertheless, maintenance of the H1 genes in all the eukaryotes that have been studied, separated by at least a billion years of evolution, leaves no doubt that this protein does perform some important functions.

An attractive idea was to ascribe to histone H1 a regulatory role as a factor capable of competing with specific gene activators or repressors for DNA binding sites (Brown, 1984). This idea is supported by *in vitro* evidence (Schlissel & Brown, 1984). During the past few years data have accumulated from *in vivo* systems in favour of H1 histone as a factor regulating gene expression. Most impressive in this respect are the results of experimental depletion and overproduction of histone H1 subtypes in the course of early embryonic development of *Xenopus* (Steinbach *et al.*, 1997), which show that specific H1 subtypes differentially affect transcription of certain genes and the competence of cells to activin induction. Knockout of H1 histone in *Tetrachymena* did not exert a major effect on global transcription; however, in starved cells there was a change in gene expression, one gene studied being repressed and another gene activated (Shen & Gorovsky, 1996). Hence, H1 histone can act as either a positive or a negative gene-specific regulator of transcription *in vivo*.

If H1 histone is part of a system of gene regulation, its molecular evolution should be related to some extent to adaptive evolution. We carried out an analysis of electrophoretic mobility of H1 histone in more than 400 insect species belonging to seven orders (Berdnikov *et al.*, 1993*a*) and found a correlation between speciation rate in an order and the rate of increase of the variance of the electrophoretic mobility of H1 histone. This fact apparently indicates that, in the course of adaptive evolution of insects, fixation of new allelic variants in histone H1 loci occasionally accompanied speciation.

Having obtained these results we wanted to find microevolutionary evidence of natural selection acting upon alleles of H1 histone and to demonstrate a phenotypic effect of allelic substitutions in the histone H1 genes. As a convenient object for this purpose we chose the garden pea (*Pisum sativum* L.), which is rather well genetically studied, has a short generation time and, as we have shown (Kosterin *et al.*, 1994), possesses seven histone H1 subtypes encoded by the genes *His1–His7*. All these genes have several allelic variants including null alleles. Allelic variants of H1 histone in legumes differ as a rule by the length of the C-terminal positively charged domain of the molecule (Belyaev & Berdnikov, 1985), responsible for the binding to linker DNA.

We have undertaken a large-scale study of the geographic distribution of allelic frequencies of H1 histone among aboriginal pea forms (Berdnikov *et al.*, 1993*b*) and found a strong negative correlation of the frequency of the slow allele of subtype 5 with the accumulated temperature of a vegetation period (the sum of aerial temperatures above $+10\,^{\circ}C$). In addition, the subtype 1 fast allele was not found north of the 44th parallel. In a study of a local population of the wild legume *Vicia unijuga* A. Br. in the territory of Novosibirsk Academy Town the allelic frequencies of one histone H1 subtype were found to form a radial cline (Berdnikov *et al.*, 1992). Most probably, these findings are evidence for natural selection acting upon some allelic variants of histone H1.

Comparison of three pairs of pea isogenic lines differing only by alleles of histone H1 and their immediate neighbourhood (Bogdanova *et al.*, 1994) showed statistically significant differences of some quantitative traits.

All these results allowed us to consider histone H1 loci as QTLs, allelic substitutions in these loci resulting in a shift of some quantitative traits.

In the present work we have attempted to reveal an effect of allelic substitutions in a histone H1 locus on expression of a homeotic gene presumably coding for a transcription factor. We have chosen the gene *tl*, which participates in the control of the development of compound pea leaves. The null allele of this gene (*tl*) conditions development of the leaflets instead of

Fig. 1. Effect of the gene *Tl* on the compound leaf of pea. (*a*) General view of a node of the line Delta, showing the stipulae, leaflets and flat tendrils. (*b*) A normal pea leaf, genotype Tl/Tl. (*c*) A leaf of the line Delta, genotype Tl/tl^x. (*d*) A leaf of a homozygote tl^w/tl^w.

tendrils (Fig. 1 *d*). Heterozygous plants have tendrils with a very narrow rudimentary leaf blade (Fig. 1 *a*, *c*) the width of which varies depending on the genetic background, thus providing us with a tool for registering the expression level of this gene. To study the effect of histone H1 on expression of the *Tl* gene we have developed a convenient genetic system. It was synthesized on the base of a quasi-isogenic line Delta, obtained in our laboratory, with a genomic segment of 8 cM carrying the loci *Tl* and *His1* maintained in a heterozygous state. Two sublines were isolated from it, homozygous for *His1* (coding for histone H1 subtype 1) and heterozygous for *Tl*, which differ only in the immediate neighbourhood of the *His1* gene. We compared these lines for the degree of development of the rudimentary leaf blade of the tendrils and have registered significant differences (15–20 %) which we attribute to the effect of the allelic substitutions in the H1 histone gene on expression of the gene *Tl*.

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2. Materials and methods

(i) *Methods of phenotype testing*

(a) *Histone H1 isolation and electrophoresis*

Histone H1 was isolated with an express method that is a modification (Rozov *et al.*, 1986; Kosterin *et al.*, 1994) of Johns' (1964) method. Between 200 and 400 mg of pea leaves were rubbed with a rubber-headed pestle through a stainless steel grid (1 × 1 mm) into a vessel containing 12 ml of 0·15 M-NaCl, and the resulting homogenate 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 six 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*′-methylenbis-acrylamide gel containing 6·25 M urea and 0·9 M acetic acid following a modification (Berdnikov & Gorel, 1975) of Panyim & Chalkley's (1969) method. After electrophoresis, the gels were stained in 0·01% (w/v) Coomassie R-250 in 0·9 M acetic acid solution and destained by diffusion in 0·9 M acetic acid. An example of an electrophoregram is given in Fig. 2.

For a detailed assessment of the electrophoretic spectrum of histone H1 of the garden pea and its inheritance see Kosterin *et al.* (1994).

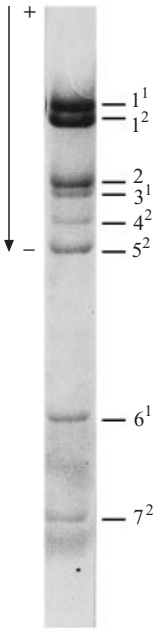

Fig. 2. An electrophoregram of histone H1 of the line Delta. Numerals represent H1 subtypes, superscripts their allelic variants.

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 displays easily detectable electrophoretic differences between allelic variants.

(b) *Isolation and electrophoresis of the SCA protein*

Electrophoretic analysis of the SCA protein was carried out by the method described in (Smirnova *et al.*, 1989, 1992), in its express modification. From a small area of the dry seed surface the testa was removed and 5 mg of flour was scratched from the cotyledons. The flour was dispersed in 12 μl of a solution containing 0·5 M acetic acid, 4 M urea and 7% sucrose. This suspension was loaded onto an 8 cm long gel, subjected to electrophoresis and stained as described above. Fast-migrating SCA protein is easily distinguished in electrophoregrams that contain many of the cotyledonary proteins.

(c) *Testing morphological traits*

Testing of the phenotype for the gene *r* (wrinkled seeds) was made by microscopic investigation of the form of starch grains of dry seeds.

The width of flat tendrils was measured with a binocular microscope with an ocular micrometer. The weight of leaflets, tendrils and seeds was determined with a Sartorius 2006 mp balance.

(ii) *Plant culture*

Seeds were planted at a depth of 6 cm 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.

(iii) *Mutagenesis*

Mutations were induced by irradiation of dry seeds with 7000 R gamma-rays or by submerging seeds in 0·15% ethylmethanesulphonate solution for 24 h.

(iv) *Material*

In this work we used the following pea lines (indicated in parentheses is the genotype for some genes of linkage group V):

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Standard lines
WL1018 (*r*, tl^w, $His1^2$, Sca^s);
WL1476 (*R*, *Tl*, $His1^2$, Sca^s);
WL1255 (*R*, *Tl*, Sca^s)
Cultivar 'Svoboda' (*Tl*, $His1^2$, Sca^s).

Lines obtained in our laboratory
Sprint-1 (*R*, *Tl*, $His1^3$, Sca^s), obtained as F23 of a cross of the accession VIR7034 (Nepal) × cultivar Avanti;
Sprint-Vologda (*R*, *Tl*, $His1^2$, Sca^s), obtained as F8 of a cross of the line Sprint-1 × accession VIR3262 (Vologda Region, Russia);
SG (*R*, *Tl*, $His1^1$, Sca^f), obtained on the basis of the line Sprint-1 and the accessions VIR6135 (Greece) and VIR320 (*Pisum sativum syriacum*, Palestine).

In the line SG recessive mutations were induced, allelic to the previously known loci *det*, *r* and *curl* of the linkage group V. The former two were induced by gamma-rays, the latter by ethylmethanesulphonate. By a series of crosses the three mutations were combined in a single line DRC, which was used in the present work.

3. Results

(i) tl^x *mutation*

A homeotic gene *Tl* is responsible for transformation of the upper leaflets of the compound leaf into tendrils (Blixt, 1972). In homozygotes for the normal allele *Tl* the rachis of the compound leaf in its proximal part carries 1–3 leaflet pairs and in its distal part 1–3 pairs of tendrils, and is completed with a non-paired terminal tendril (Fig. 1*b*). The tendrils are cylindrical structures homologous to the central vein of the leaflet. They are capable of secondary branching. The mutant allele tl^w results in development of normal leaflets instead of all tendrils, thus making the compound leaf imparipennate (Fig. 1*d*). In the heterozygotes tl^w/Tl the tendrils have a very narrow rudimentary leaf blade and in fact are extremely narrow leaflets. Later in the text we call these intermediate structures 'flat tendrils' (Fig. 1*a*, *c*). The degree of development of the leaf blade of the flat tendril of the heterozygotes tl^w/Tl varies substantially depending on the genetic background of the plant. Thus, parameters of the flat tendril can serve as a sensitive indicator of the action of modifiers of the *Tl* gene, possibly including H1 histone.

In 1987 we obtained F1 seeds from the cross Sprint-1 (*R*, *Tl*) × WL1018 (*r*, tl^w). The seeds were gamma-irradiated and planted in the field resulting in 1208 M1 plants. Their leaves were of a phenotype typical for the heterozygotes Tl/tl^w. However, 15 plants possessed branches of the phenotype tl^w, that is, were chimeric with respect to gene *Tl*. All these branches were sterile, except for one which produced seeds: 11 wrinkled (*r*) and 18 round (*R*). In subsequent generations plants grown from the round seeds gave round and wrinkled seeds in approximately the same proportion. An analysis of three crosses described in an earlier communication (Gorel *et al*., 1994), as well as an analysis given below of the line Delta, led us to the following conclusions:

(a) Gamma-irradiation had induced a loss-of-function mutation in the *Tl* locus which is a recessive sporophyte lethal, while in the heterozygote with alleles *Tl* or tl^w it behaves as the mutant allele tl^w. The allele obtained was designated tl^x.

(b) The mutation is associated with substantial repression of crossing-over in its vicinity, mostly in the segment *det–r–tl*.

(c) Mutation tl^x is detrimental at the gametophyte level, since it is transmitted to the following generation with a reduced probability: through the female gametophyte about 44%, and through the male gametophyte about 13% (Table 1). This is not related to the pollen sterility: less than 10% of pollen grains are abortive in the heterozygotes tl^x/Tl.

Due to a combination of sporophytic lethality of the homozygotes tl^x/tl^x and reduced transmission of the tl^x allele through the male gametophyte self-pollination of the tl^x/Tl heterozygotes results in approximately equal proportions of heterozygotes (of the same type) and homozygotes Tl/Tl.

We suppose that the tl^x mutation is a small deletion covering, in addition to *Tl*, at least one essential gene. It might also be a small inversion or duplication with

Table 1. *Transmission of the* tl^x *allele via the male and female gametophyte in the off-spring of the test-crosses Sprint-Vologda × Delta and Delta × Sprint-Vologda*

Cross	No. of plants			Percentage
	Tl/Tl	Tl/tl^x	Total	Tl/tl^x
Sprint-Vologda × Delta	479	71	550	12·91 ± 1·43
Delta × Sprint-Vologda	786	615	1401	43·90 ± 1·33

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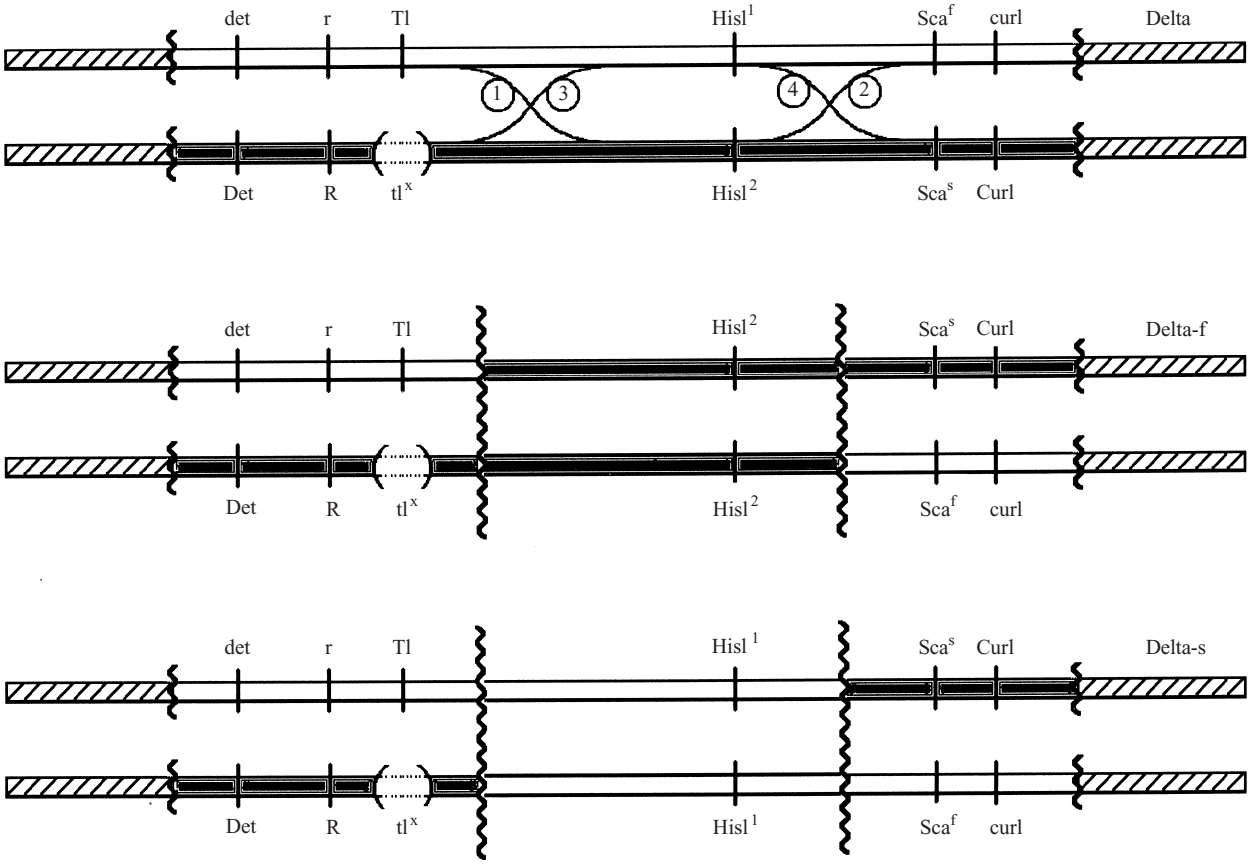

Fig. 3. Diagram of a chromosome region maintained heterozygous in the lines Delta, Delta-s and Delta-f. Filled and empty bars designate the homologues in the heterozygous segment of the line Delta. Hatched bars show the regions isogenic in all three lines. Circled numerals indicate arbitrary points of cross-over events in the line Delta used for construction of its derivatives Delta-s and Delta-f.

breakpoints in *Tl* and some vital gene. The described properties of the tl^x mutation were thoroughly investigated quantitatively in the line Delta; the results are given below.

(ii) *Line Delta*

Line Delta is a quasi-isogenic line of complicated genealogy. It was synthesized on the basis of the above-described mutation tl^x, cultivar 'Svoboda', lines WL1476 and DRC. It was isogenized for 15 generations as follows. Each subsequent generation was obtained by selfing of a single, randomly chosen plant heterozygous for the linked loci *det*, *r*, *tl*, *Sca* and *curl*. As a result we obtained a line isogenic for all the genome except for a small segment of about 10 cM referring to the linkage group V which was being maintained heterozygous (Fig. 3).

The left end of the heterozygous segment is marked by a group of the three closely linked genes *det*, *r* and *tl*, and the right end by tightly linked genes *Sca* and *curl*. The plants *det*/*det* have determinate growth, homozygotes *r*/*r* have wrinkled seeds with compound starch grains. The *Sca* gene codes for a perchloric-acid-soluble cotyledon albumin (Smirnova *et al.*, 1989). The genotype *curl*/*curl* determines a curled structure of the leaflets and stipules and is easily identified in the seedling stage. The alleles $His1^1$ and $His1^2$ encode, respectively, slow and fast variants of the subtype 1 of H1 histone, subtype 1 being of the lowest electrophoretic mobility and comprising about one-half of total H1 histone (Fig. 2). The alleles $His1^1$ and Sca^f in the line Delta come from the wild pea *Pisum sativum syriacum*, accession VIR320 collected by N. I. Vavilov in Palestine. The alleles $His1^2$ and Sca^s are common to cultivated peas.

According to the recombination map of the region resulting from the generalized data of more than 20 crosses including plants of the normal karyotype, constructed with the aid of the program JOINMAP (Stam, 1993), the markers utilized are arranged with the following recombination distances (Rozov *et al.*, 1993 and unpublished data):

```
det-----r---tl----------His1-----
     3·4     1·8          9·7          4·2

Sca-curl                          (% crossing-over)
  0·7
```

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Table 2. *Rates of crossing-over between the genes* det, r, Sca, His1 *and* curl *in the line Delta as determined on the basis of the* r *genotype*

Gene pairs	No. of plants[a]	No. of crossovers	Recombination frequency (%)	Standard error (%)
det–r	812	4	0·25	0·12
det–tl	812	5	0·31	0·14
r–tl	1018	2	0·10	0·07
r–His1	643	67	5·21	0·62
r–Sca	1928	271	7·03	0·41
His1–Sca	643	23	1·79	0·37
Sca–curl	1154	17	0·74	0·18

[a] Cross-over rates were estimated in different experiments, not all the gene pairs being tested in each.

In the line Delta these distances are substantially distorted in the vicinity of the gene *Tl* (Table 2):

```
det--r-tl----------His1----Sca-curl
   0·3   0·1          5·1          1·8      0·7
```

(% crossing-over)

We observed practically complete suppression of crossing-over between the genes *r* and *tl*, as well as a great decrease in its intensity between the genes *det* and *r*, *tl* and *His1*. It should be noted that in the line Delta lethality of the tl^x gene results in a substantial deviation from Mendelian segregation. This prevents the use of standard methods for F2 segregation to determine cross-over distances. For this reason we estimated recombination distances in the offspring of line Delta on the basis of the proportion of recombinants among the homozygotes Tl/Tl or, which is virtually the same, among the homozygotes r/r, since recombination between the genes *r* and *Tl* is practically suppressed – in the sample of 1018 r/r plants only two cases of crossing-over between *r* and *tl* were found (Table 2). Due to the co-dominance of the genes *Tl*, *His1* and *Sca*, coupling of the recessive alleles *r* and *curl*, as well as the small recombination length of the segment under investigation, it is possible unambiguously to identify all the genotypes formed in the *Tl* class. Therefore, it is possible to register all recombination events, and to estimate recombination distances by dividing their numbers by the total number of gametes.

In the course of isogenization of the line Delta the total length of the genome fraction retaining heterozygosity beyond the segment *det–curl* should have decreased steadily. The expectation of the length *y* (in cM) around the segment maintained heterozygous can be calculated according to the formula (Serra, 1966)

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

where *N* represents the number of generations of self-pollination. For 15 generations of selfing *y* equals 3·5%.

To estimate the probability of transmission of the allelle tl^x to the next generation we sowed the Delta plants in the field and obtained 5739 round seeds (R/r) and 6471 wrinkled (r/r), the proportion of the former being equal to $47{\cdot}00 \pm 0{\cdot}45$%. This proportion is practically identical to the proportion of the genotypes Tl/tl^x and Tl/Tl.

To determine the percentage transmission of the tl^x allele to the next generation via the male and female pathway we performed two reciprocal test-crosses. In the first cross, plants of the Sprint-Vologda line (*Tl* homozygous) were pollinated by the line Delta; in the second cross the Delta line was used a female parent. The results are given in Table 1. The data show that the tl^x allele is transmitted via male gametophytes at a rate of 12·9%, and via female gametophytes at a rate of 43·9%. The calculated probability of transmission of this allele by self-pollination is 48·2%, which corresponds well with the observed 47·0%.

(iii) *Synthesis of the lines Delta-s and Delta-f*

To study the effect of a substitution of allelic variants of H1 histone on a number of traits including expression of the *Tl* gene in heterozygote and recombination rate in the heterozygous segment we isolated in 1996 two sublines from the line Delta where the *His1* gene was fixed as homozygous. The other markers, however, were kept heterozygous. A subline carrying the allele coding for the 'fast' allelic variant of histone H1 subtype 1, $His1^2$, was designated as Delta-f, while a subline with the allele $His1^1$ was designated Delta-s. To obtain each of these sublines we fixed two cross-over events giving rise to four recombinant chromosomes:

$r\ \ Tl\ \ His1^2\ \ Sca^s\ \ Curl$

$R\ \ tl^x\ \ His1^2\ \ Sca^f\ \ curl$

$r\ \ Tl\ \ His1^1\ \ Sca^s\ \ Curl$

$R\ \ tl^x\ \ His1^1\ \ Sca^f\ \ curl$

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Table 3. *The values of quantitative traits in the lines Delta, Delta-s and Delta-f*

Character	M_h	M_s	M_f	S_h	S_s	S_f	N_h	N_s	N_f	$M_h - M_s$	$M_h - M_f$	$M_s - M_f$	T_{hs}	T_{hf}	T_{sf}
Day of seedling appearing	8·12 ± 0·08	8·36 ± 0·08	8·56 ± 0·08	0·79	0·80	0·82	107	102	105	−0·24	−0·44	−0·20	2·18*	3·98***	1·78
Day of flowering	31·35 ± 0·15	31·35 ± 0·14	30·94 ± 0·18	1·50	1·37	1·87	104	101	103	0·00	0·41	0·41	0·00	1·74	1·74
Node of first flower	11·61 ± 0·10	12·11 ± 0·09	11·54 ± 0·12	0·99	0·87	1·17	104	101	104	−0·50	0·07	0·57	3·84***	0·47	3·95***
Length of internode 8–9 (mm)	109·70 ± 0·76	112·91 ± 0·77	110·31 ± 0·86	7·81	7·76	8·79	105	102	104	−3·21	−0·61	2·60	2·97**	0·53	2·25*
Length of internode 9–10 (mm)	104·30 ± 1·03	105·18 ± 1·07	102·87 ± 8·57	10·57	10·80	10·99	105	102	104	−0·88	1·43	2·31	0·59	0·96	1·52
No. of pods on main stem	5·45 ± 0·12	6·02 ± 0·15	5·43 ± 0·13	1·20	1·43	1·26	96	94	95	−0·57	0·02	0·59	2·98**	0·11	3·01**
Mean no. of seeds in a pod	3·12 ± 0·06	2·89 ± 0·07	3·30 ± 0·05	0·55	0·71	0·49	96	94	95	0·23	−0·18	−0·41	2·50*	2·39*	4·62***
Total no. of seeds on main stem	16·90 ± 0·45	17·22 ± 0·55	17·86 ± 0·50	4·44	5·32	4·87	96	94	95	−0·32	−0·96	−0·64	0·45	1·47	0·86
Proportion of round seeds	0·438 ± 0·012	0·466 ± 0·012	0·452 ± 0·012	0·496	0·499	0·498	1651	1644	1721	−0·028	−0·004	0·014	1·61	0·82	0·81
Mean mass of round seeds (mg)	225·37 ± 2·43	232·09 ± 3·03	222·19 ± 2·47	24·19	29·65	24·61	99	96	99	−6·72	3·18	9·90	1·74	0·92	2·54*
Mean mass of wrinkled seeds (mg)	210·29 ± 1·86	216·00 ± 2·81	208·01 ± 2·63	18·51	27·53	26·20	99	96	99	−5·71	2·28	7·99	1·70	0·71	2·08*
Mean mass of all seeds (mg)	217·22 ± 2·06	223·70 ± 2·90	214·38 ± 2·48	20·50	28·42	24·72	99	96	99	−6·48	2·84	9·32	1·83	0·88	2·45*

M, means; S, standard errors; T, Student criterion values; N, sample size. The subscript h (heterozygous) refers to the line Delta, s to the Delta-s, f to the Delta-f.
Total numbers of plants are not equal for different characters since some plants were damaged before such characters were formed. For the character 'proportion of round seeds' the total number of seeds is given.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Table 4. *Values of the parameters maximum width* (*w*), *length* (*l*), *fresh mass* (*m*), *and ratios w/l and m/l, of the flat tendrils on node 11 in the lines Delta, Delta-s, and Delta-f*

Characters	Position	M_h	M_s	M_f	S_h	S_s	S_f	N_h	N_s	N_f	$M_h - M_f$	$M_h - M_s$	$M_s - M_f$	T_{hs}	T_{hf}	T_{sf}
Maximum width (w, mm)	0	1·93 ± 0·07	2·09 ± 0·08	1·75 ± 0·08	0·72	0·81	0·81	102	101	104	−0·16	0·18	0·34	1·49	1·68	3·00**
	1	1·28 ± 0·03	1·40 ± 0·04	1·30 ± 0·04	0·27	0·37	0·36	101	99	102	−0·12	−0·02	0·10	2·62**	0·45	1·94
	2	1·26 ± 0·03	1·45 ± 0·06	1·31 ± 0·03	0·31	0·57	0·30	93	93	97	−0·19	−0·05	0·14	2·82**	1·13	2·13*
Length (l, mm)	0	49·75 ± 1·10	48·17 ± 1·1	52·20 ± 1·05	14·16	11·6	10·1	102	101	104	1·58	−2·45	−4·03	0·85	1·40	2·64**
	1	33·06 ± 0·51	32·26 ± 0·9	33·62 ± 0·61	5·16	5·88	6·17	101	99	102	0·80	−0·56	−1·36	1·02	0·70	1·60
	2	43·08 ± 0·64	41·82 ± 0·9	44·87 ± 0·58	6·13	5·73	5·71	93	93	97	2·16	−0·89	−3·05	2·48*	1·04	3·67***
Mass (m, mg)	0	9·66 ± 0·29	9·93 ± 0·27	9·66 ± 0·30	2·89	2·76	3·06	102	101	104	−0·27	0·00	0·27	0·68	0·00	0·68
	1	4·89 ± 0·17	5·12 ± 0·18	4·90 ± 0·19	1·68	1·80	1·93	101	99	102	−0·23	−0·01	0·22	0·93	0·04	0·84
	2	7·23 ± 0·25	8·06 ± 0·26	7·83 ± 0·30	2·41	2·48	2·93	93	93	97	−0·83	−0·60	0·23	2·31*	1·54	0·58
w/l × 100	0	4·46 ± 0·37	4·90 ± 0·38	3·66 ± 0·29	3·73	3·82	2·94	102	101	104	−0·44	0·80	1·24	0·83	1·71	2·61*
	1	4·01 ± 0·13	4·72 ± 0·32	4·21 ± 0·27	1·29	3·15	2·78	101	99	102	−0·71	−0·20	0·51	2·09*	0·66	1·22
	2	2·93 ± 0·08	3·57 ± 0·18	2·94 ± 0·07	0·81	1·74	0·68	93	93	97	−0·64	−0·01	0·63	3·22***	0·09	3·31***
m/l × 10	0	2·13 ± 0·19	2·11 ± 0·06	1·87 ± 0·06	1·90	0·61	0·59	102	101	104	0·02	0·26	0·24	0·10	1·33	2·86**
	1	1·48 ± 0·04	1·59 ± 0·05	1·45 ± 0·05	0·45	0·46	0·47	101	99	102	−0·11	0·03	0·14	1·71	0·46	2·14*
	2	1·64 ± 0·05	1·92 ± 0·06	1·72 ± 0·05	0·47	0·55	0·54	93	93	97	−0·28	−0·08	0·20	3·73***	1·09	2·53*

Positions 0, 1 and 2 designate, respectively, the terminal tendril, last pair of tendrils, penultimate pair of tendrils. The sample size can differ for different positions if some leaves lacked flat tendrils in some positions. Designations as in Table 3.
* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}0001$.

We then combined them in a pairwise fashion by crosses as shown in Fig. 3. The sublines obtained differ from the initial line Delta by the homozygosity of a chromosome segment surrounding the *His1* gene and limited by the points of the crossover events. The line Delta-s is homozygous for the chromosome segment marked by the $His1^1$ allele, and the Delta-f for the segment with $His1^2$. In these two Delta derivatives an additional homozygous zone in the vicinity of the *His1* locus is determined by the points of two random cross-over events, and for this reason the lengths of these zones most probably are not equal. However, they do not exceed the distance between the nearest flanking markers, which were maintained heterozygous. Thus, the lines Delta-s and Delta-f are identical along all the genome, including its heterozygous region at genes *det–r* and *Sca–curl*, except for a region of no more than 7 cM around the *His1* gene. We suppose that this region of difference between the lines Delta-s and Delta-f do not contain elements of the *Tl* locus (which, as a homeotic gene, could be complex and large) since crossing-over is suppressed in the vicinity of the tl^x mutation. It is very unlikely that any of two cross-over events between *Tl* and *His1* used for obtaining the lines took place close to *Tl* or even within it.

(iv) *Comparison of the lines Delta, Delta-s and Delta-f with respect to quantitative traits*

In an autumn generation 1997 in two hydroponic beds of a greenhouse 110 heterozygous seeds $R/r\ Sca^s/Sca^f$ were planted of each of the three lines – Delta, Delta-s and Delta-f – in alternating rows. There emerged 110 plants of the line Delta, 107 plants of Delta-s and 109 plants of Delta-f. All had flat tendrils, that is, were heterozygous Tl/tl^x. Four of them (2 among Delta-s and 2 among Delta-f) were of the phenotype *curl*, that is, were cross-overs between the genes *Sca* and *curl*. Six plants (3 among Delta and 3 among Delta-s) were of the phenotype *det*, that is, were cross-overs between the genes *det* and *r*. The ten cross-over plants were excluded from the subsequent analysis. Several plants were lost during growth.

The plants of the three lines were compared with respect to a number of quantitative traits; the results are given in the Table 3. First the close similarity of the mean values of the traits in all the three lines should be noted, which corresponds well to their close genetic similarity. Such traits as the day of flowering, length of internode 9–10, number of seeds of the main stem and proportion of round seeds did not demonstrate statistically significant differences. However, for a number of traits there were registered significant differences. The difference in the mean seed mass attained the 5% level of significance, the phenotypic classes round and wrinkled both showing a similar result. The differences in the length of internode 8–9 and the number of pods on the main stem achieved the 1% level of significance, and those in the day of seedling emergence, node of the first inflorescence, and mean number of seeds per pod achieved the 0·1% level. Plants of the line Delta-s exceeded those of Delta-f in terms of the length of internode 8–9, the number of pods on the main stem and mean seed mass, but were inferior with regard to the mean number of seeds per pod. In addition the first flower in the Delta-s line appeared on average at a higher node.

It was found that in the line Delta heterozygous for the chromosome segment carrying the *His1* gene the level of the first inflorescence, length of internode 8–9 and number of pods on the main stem were practically indistinguishable from those in the line Delta-f; the mean number of seeds per pod and mean seed mass were intermediate between Delta-s and Delta-f; while the seedlings of this line emerged on average some 12 h earlier than in either of the lines homozygous for *His1*.

The mean seed mass was determined separately for the round (R/r) and wrinkled (r/r) seeds. Note that the round seeds on average were approximately 7% heavier than the wrinkled ones. A difference between lines Delta-s and Delta-f was seen in both seed classes, its magnitude being comparable with that of the class difference (4%).

(v) *Comparison of the lines Delta, Delta-s and Delta-f with respect to the degree of expression of the gene Tl*

It is known that the first two nodes of the pea shoot carry leaf scales (reduced stipules) instead of leaves, while each node above this level carries a compound leaf consisting of the central rachis with leaflets and tendrils arranged in pairwise fashion and terminating with a non-paired tendril. One or two lowest leaves carry one leaflet pair and an abortive central tendril. At higher nodes the number of leaflet and tendril pairs increases up to three for both leaflets and tendrils. At the border of the compound leaf zones carrying leaflets and tendrils, mixed leaflet/tendril pairs are frequent, both organs having proper identity.

The plants homozygous for tl^w develop normal leaflets in place of tendrils (Fig. 1*d*). In the heterozygotes Tl/tl^w and Tl/tl^x (Fig. 1*a*, *c*) all the tendrils, as indicated above, have a rudimentary leaf blade. Our measurements show (Tables 4 and 5; Fig. 4) that the width of the flat tendrils, as a rule, does not exceed 2 mm, although sometimes it can attain 6 mm.

At the first few nodes leaves of all Delta sublines often display an irregular arrangement: mixed leaflet/tendril pairs are frequent as well as organs intermediate between leaflets and flat tendrils. At higher nodes the pattern of arrangement of leaf parts becomes more

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Table 5. *Values of the parameters of flat tendrils on node 17 of the lines Delta, Delta-s and Delta-f*

Characters	Position	M_h	M_s	M_f	S_h	S_s	S_f	N_h	N_s	N_f	$M_h - M_f$	$M_h - M_s$	$M_s - M_f$	T_{hs}	T_{hf}	T_{sf}
Maximum width (w, mm)	0	1·69 ± 0·03	2·09 ± 0·06	1·66 ± 0·04	0·34	0·57	0·35	94	93	97	0·40	0·03	0·43	5·84***	0·60	6·29***
	1	1·42 ± 0·02	1·65 ± 0·03	1·40 ± 0·03	0·23	0·27	0·26	96	95	97	−0·23	0·02	0·25	6·34***	0·57	6·54***
	2	1·45 ± 0·05	1·73 ± 0·03	1·42 ± 0·04	0·48	0·31	0·35	96	93	96	−0·28	0·03	0·31	4·75***	0·49	6·44***
Length (l, mm)	0	50·63 ± 1·07	47·43 ± 1·01	48·32 ± 1·03	10·33	9·73	10·4	94	93	97	3·20	2·31	−0·89	2·18*	1·56	0·62
	1	32·18 ± 0·58	30·74 ± 0·50	31·04 ± 0·57	5·64	4·85	5·58	96	95	97	1·44	1·14	−0·30	1·89	1·41	0·40
	2	44·09 ± 0·72	41·77 ± 0·65	43·28 ± 0·71	7·02	6·23	6·96	96	93	96	2·32	0·81	−1·51	2·40*	0·80	1·57
Mass (m, mg)	0	10·04 ± 0·29	11·14 ± 0·34	9·36 ± 0·27	2·77	3·26	2·63	94	93	97	−1·10	0.68	1·78	2·49*	1·74	4·15***
	1	6·63 ± 0·21	7·31 ± 0·23	6·07 ± 0·16	2·03	2·23	1·61	96	95	97	−0·68	0·56	1·24	2·20*	2·12*	4·31***
	2	9·77 ± 0·27	10·98 ± 0·29	9·16 ± 0·27	2·61	2·78	2·63	96	93	96	−1·21	0·56	1·82	3·09**	1·61	4·62***
w/l	0	3·52 ± 0·12	4·71 ± 0·23	3·63 ± 0·12	1·15	2·23	1·19	94	93	97	−1·19	−0·11	1·08	4·59***	0·65	4·19***
	1	4·57 ± 0·12	5·51 ± 0·13	4·72 ± 0·15	1·18	1·23	1·48	96	95	97	−0·94	−0·15	0·79	5·39***	0·78	4·02***
	2	3·44 ± 0·16	4·29 ± 0·11	3·41 ± 0·11	1·57	1·10	1·11	96	93	96	−0·85	0·03	0·88	4·30***	0·15	5·47***
m/l	0	1·97 ± 0·04	2·37 ± 0·06	1·95 ± 0·04	0·34	0·62	0·44	94	93	97	−0·40	0·02	0·42	5·48***	0·35	5·40***
	1	2·03 ± 0·04	2·35 ± 0·05	1·96 ± 0·04	0·42	0·48	0·37	96	95	97	−0·32	0·07	0·39	4·90***	1·23	6·31***
	2	2·25 ± 0·07	2·63 ± 0·06	2·12 ± 0·05	0·66	0·54	0·51	96	93	96	−0·38	0·13	0·51	4·32***	1·53	6·68***

Designations as in Table 3.
* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

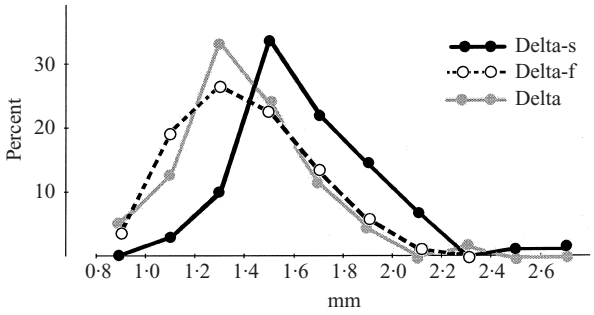

Fig. 4. Distributions of the maximum width of the flat tendril of the last pair (position 1) at the node 17 in the lines Delta, Delta-s and Delta-f.

standard. Starting from node 7 to node 9 (counting from the first leaf scale) the leaves normally have two leaflet pairs, two pairs of flat tendrils and a terminal flat tendril (Fig. 1 *a*, *c*). However, at higher nodes the leaflets of the second pair are often not well developed, being only few millimetres in length. We measured the parameters of flat tendrils at nodes 11 and 17. The leaf arrangement at node 11 was not stable, the second leaflet pair frequently being replaced by a mixed leaflet/tendril pair or a tendril pair, or the whole pair being omitted. The leaf at node 17 had a practically constant arrangement: the first pair of leaflets was followed by a pair of poorly developed leaflets, two pairs of flat tendrils and an unpaired terminal flat tendril.

We determined the following parameters of the flat tendril: length, maximum width, mass, and ratios of the maximum width to length and mass to length. The results are presented in Tables 4 and 5. The data were calculated separately for the terminal tendril (designated in the tables as position 0), the last tendril pair (position 1) and the penultimate tendril pair (position 2). Since in each pair parameters of the left and right tendril are correlated, that is, are not statistically independent, we averaged the parameter values of the right and left tendrils in each position of the leaf, and these mean values represented a plant in the samples.

Let us compare the data of Tables 4 and 5 referring to lines Delta-s and Delta-f, homozygous for the *His1* gene. Note that for all positions the mean values of the maximum width in Delta-s are higher than in Delta-f, but for the mean length the relation is reversed. In other words, the flat tendrils of the Delta-s line are shorter and wider than those of the Delta-f. The same tendency is reflected by the ratio of mass to length, linked to the mean width of the tendril, which is steadily higher in the line Delta-s. Note, however, that for position 1 of node 11 and all positions of node 17 the difference in tendril length between the lines in question is insignificant, while the differences in the maximum width and ratio of mass to length are consistently significant. This indicates that the substitution of the chromosome segment marked by the *His1* alleles affects the width of the flat tendril. This is also evidenced by the fact that the line Delta-s always has a tendril mass greater than that in line Delta-f, the difference being statistically insignificant for all the positions of node 11 but far exceeding the 0·1 % level of significance for node 17.

The magnitude of the effect on tendril width is greater at node 17 compared with node 11. The relative shift in the mean value of the maximum width increases from 16 % to 20 % for the terminal tendril, from 8 % to 15 % for the tendrils of the last pair, and from 11 % to 18 % for the penultimate pair. While at node 11 the difference in maximum width only of the terminal tendril attained the 1 % level of significance, at node 17 all the parameters reflecting the tendril width exceeded the 0·1 % level of significance.

Now let us turn our attention to the parameter values in the line Delta heterozygous for the *His1* alleles. It is reasonable to expect here that values will be intermediate between those of the lines Delta-s and Delta-f. In fact, few mean parameter values in Tables 4 and 5 for the line Delta fell beyond the interval between the means for lines Delta-s and Delta-f. On the other hand, however, in no case, except for the mass of the final tendril pair on node 17, did a mean value in the line Delta differ significantly from the corresponding value for line Delta-f. Thus, it can be stated that for all the parameters studied, the segment marked with the $His1^2$ allele dominates over its homologue with the allele $His1^1$ and conditions narrower flat tendrils.

Table 6. *Values of the leaflet parameters*: *maximum width* (*w*), *length* (*l*), *mass* (*m*), *ratios w/l and m/l, of the first pair leaflets on node 17 in the lines Delta-s and Delta-f*

Character	M_s	M_f	S_s	S_f	N_s	N_f	$M_s - M_f$	T
Maximum width (w, mm)	22·61 ± 0·21	22·62 ± 0·23	2·05	2·22	95	96	−0·01	0·03
Length (l, mm)	34·76 ± 0·32	34·32 ± 0·30	3·16	2·97	95	96	0·44	0·99
Mass (m, mg)	84·42 ± 1·86	82·31 ± 1·77	18·12	17·32	95	96	2·11	0·82
w/l × 10	6·51 ± 0·02	6·59 ± 0·02	0·21	0·22	95	96	−0·08	2·57*
m/l	2·40 ± 0·03	2·38 ± 0·03	0·31	0·33	95	96	0·02	0·43

Designations as in Table 3.
* $P < 0·05$.

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Table 7. *Recombination fractions (RF) between the genes* r *and* SCA *as calculated for the lines Delta, Delta-s and Delta-f separately for seeds of the phenotypes round and wrinkled*

Seed class	RF_s	RF_f	RF_h	N_s	N_f	N_h	$RF_s - RF_f$	$RF_s - RF_h$	$RF_f - RF_h$	T_{sf}	T_{sh}	T_{fh}
Round	10·57 ± 1·00	17·61 ± 1·33	7·68 ± 0·59	1076	1050	2254	− 7·04	2·89	9·93	−4·24***	2·63**	7·92***
Wrinkled	9·91 ± 0·92	15·44 ± 1·11	7·02 ± 0·42	1056	1060	3856	− 5·53	2·89	8·42	−3·83***	3·09**	8·50***

Designations as in Table 3.
** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Fig. 4 shows the distributions of the maximum width of the flat tendril in position 1 of node 17 for the three lines. The shape of the distribution for the line Delta-s is close to that of line Delta-f but the curve is substantially shifted to the right. The distribution for line Delta is somewhat wider and its mean value coincides with that of line Delta-f.

There arises a question whether the effect of the substitution of the chromosome segment studied is specific for *Tl* gene expression in the heterozygote or is extended to the leaflets as well. To clarify this we measured the same parameters for the first leaflet pair at node 17 for lines Delta-s and Delta-f (Table 6). The 5% level of significance was attained only by the shift of the parameter 'ratio of maximum width to length', the leaflets of line Delta-s being narrower. Thus, the effect on leaflet width is fairly weak and has an opposite sign to that on the flat tendrils.

The data obtained allowed us to conclude that substitutions of a short chromosome segment carrying the locus *His1* exert an appreciable (up to 20%) and specific effect on the width of the flat tendril – a peculiar structure observed only in pea plants heterozygous for the gene *Tl* – the magnitude of the effect being comparable to the standard deviation in the lines, that is, to the influence of environmental factors.

(vi) *Comparison of lines Delta, Delta-s and Delta-f with respect to the rate of meiotic recombination in the region* r-Sca

Our experimental model allows us to determine the rate of crossing-over in the investigated region of the chromosome 5 at the seed stage. Both flanking markers *r* and *Sca* are scored in the cotyledons, which are sporophyte organs. The genotype *r*/*r* determines wrinkled seeds and compound starch grains, rapidly and easily scored with a microscope. The genotype *R*/*r* gives round seeds and large simple starch grains. Seeds of the *R*/*R* genotype hardly ever form in the progeny of line Delta and its derivatives, since the allele *R* is extremely closely linked to the mutation tl^x. To determine the *Sca* genotype a few milligrams of cotyledon flour are sufficient. This makes it possible to support an accurate estimate of recombination rate by analysis of thousands of individuals.

Since the tl^x allele is a sporophyte lethal and reduces the probability of male gametophyte participation in fertilization, we can not use the standard methods of evaluating the recombination distance in the F2. For this reason we determined cross-over distances separately for the wrinkled *r*/*r* and round *R*/*r* seeds. As noted above, we can register all cross-over events among *r*/*r* seeds. To determine the percentage of recombination between the genes *r* and *Sca* among the round seeds *R*/*r* we used an equation obtained by the maximum likelihood method for the

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distance p between a co-dominant marker (in our case *Sca*) and a gene known to be heterozygous (in our case tl^x and r):

$$2ap^3+2(2a-b)p^2-2(a+b)p+b=0,$$

where a is the number of heterozygotes for the co-dominant marker and b is the total number of homozygotes of both types.

The data obtained are presented in Table 7. The line Delta heterozygous for gene *His1* had the lowest level of crossing-over between *r* and *Sca*. The line Delta-f has the highest recombination rate, and when compared with the other two lines the differences attain the 0·1 % level of significance. The rate of crossing-over in the investigated region *r–Sca* in the Delta-f line was 1·7 times higher than in the Delta-s line and 2·3 times higher than in the Delta line.

The rate of crossing-over estimated in the *R* class systematically exceeds that of the *r* class in all three lines, but the difference in no case attains the 5 % level of significance. Most probably, this bias is due to the different methods of estimating the recombination rate in the two phenotypic classes.

4. Discussion

The comparison of two pea lines differing by a segment of linkage group V revealed small but significant differences for a number of quantitative traits. The difference is especially pronounced for parameters of the flat tendril – a peculiar structure that is formed by reducing the dose of the normal allele of gene *Tl*. A normal (double) dose of the *Tl* gene provides the development of normal tendrils – cylindrical organs found only in representatives of the tribe Vicieae and thus, most probably, acquired in a relatively recent period of legume evolution. An ancestor compound leaf of the Vicieae tribe was of an imparipennate type (Yakovlev, 1991) possessing a structure close to the leaf of a pea plant homozygous for the null allele tl^w. The mutation tl^w does not affect the development of other plant organs, plant viability or fertility, and is even used in some commercial pea cultivars. All this indicates that the *Tl* gene participates in rather late stages of development of the compound leaf, which is usually an indication of a relatively late origin in evolution. Incomplete dominance of the *Tl* over its null allele is possible due to the same circumstance, which has not allowed complete stabilization of a set of modifiers.

In this work we used the null mutation tl^x obtained after gamma-irradiation of seeds of the heterozygotes $R\ Tl/r\ tl^w$. The tl^x mutation differs from the tl^w in that it not only affects the tendrils but is also a sporophyte recessive lethal and sharply reduces the probability of gamete transmission via the male gametophyte. Appearance of a mutation affecting at minimum two functions after gamma-ray treatment raises the possibility that this mutation is a short deficiency covering the *Tl* gene and at least one vital gene or, less probably, a small rearrangement with breakpoints in *Tl* and another vital gene.

Due to incomplete dominance of the wild-type allele the flat tendrils of the heterozygotes Tl/tl^x prove to be a sensitive system responding to the presence of modifiers. The data obtained in this study (Tables 4, 5) indicate that factors modifying *Tl* gene expression are present inside the segment *Tl–Sca*; in any case they are separable from gene *Tl* by recombination. Substitution of this segment containing the $His1^2$ allele by the homologous segment with the $His1^1$ allele results in an increase in mass and maximum width of the flat tendril by about 20 % (that approximately equals the value of the standard deviation of these parameters influenced by environmental factors).

The width of the flat tendrils can be considered as a typical quantitative trait and its variability should be associated with allelic differences in a relatively small number of loci. For example, *Drosophila* has fewer than 100 loci affecting the number of all types of sensory bristles (Mackay, 1995), while in rice the number of QTLs for a given trait varies from 1 to 19 (Yano & Sasaki, 1997). The most recent estimations of the total map length for the pea is between 1500 and 2400 cM (Hall *et al.*, 1997); therefore, the segment for which our lines differ (for its maximum length see Table 7) comprises no more than 1 % of the genome. It follows from the above estimates that a segment of such a size bears on average less than one QTL for a given trait. We can not exclude the possibility that the effects observed are due to some locus (or loci) other than *His1*. However, we have good reason to expect that it is histone H1 which serves as QTL for the studied character, as discussed below.

Earlier we compared two isogenic lines, C and D, that differ in a region surrounding the *His1* locus (Bogdanova *et al.*, 1994). This region in line C originated from the pea accession VIR7036 (Nepal) and carried the allele $His1^3$; in line D it originated from the cultivar Avanti with the allele $His1^2$. Note, that line Delta also carries one of those electromorphs, while the other, $His1^1$, traces its origin to a wild-growing pea *Pisum sativum syriacum* (accession VIR320 from Palestine) and codes for a variant with a lower mobility than the allele $His1^2$. In the synthesis of lines C and D we maintained heterozygosity only for the locus *His1*, so the length of the heterozygous zone in the vicinity of the histone locus should have comprised 2·6 cM (Serra, 1966). Line C with the fast variant of H1 histone had a lower seed mass but more rapid development than line D with its less mobile variant. The comparison of the Delta-s and Delta-f lines presented here showed (Table 3) that line

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Delta-f with the faster histone H1 variant had slightly more rapid development and possessed smaller seeds than line Delta-s with the slower histone variant. The difference between the lines is of the same direction and order of magnitude as that observed in lines C and D. Note that the histone H1 variants encoded by the three mentioned *His1* alleles differ one from another by an altered length of the positively charged C-terminal domain.

Thus, comparison of both pairs of isogenic lines shows that the segment of linkage group V surrounding the gene *His1*, in spite of its different origin, possesses modifiers affecting the rate of development, seed mass and seed number per pod. Taking into account a small size of the segment, we suppose that both quantitative traits of the plant and expression of the *Tl* gene are affected by the same factor with a strong pleiotropic effect.

In our earlier experiment (Bogdanova *et al*., 1994) we compared two isogenic lines obtained by two cross-over events within the gene cluster *His*(2–6) so that they differed in a segment of the chromosome 1 no longer than 0·1 cM, containing a histone H1 gene *His5*. We found significant differences in three quantitative traits, among them the mean number of seeds per pod which was shown to differ in the present experiment. One can see that effects are recognizable even if the contrasting segment is about 100-fold shorter than in the case of Delta-s and Delta-f.

If substitution of a short chromosome segment affects the width of the flat tendrils, one can expect that the Delta line, heterozygous for that segment, should possess tendrils with parameters intermediate between the two homozygous classes. This is found to be the case for tendril mass on node 17, but for maximum width the value is displaced to that of Delta-f (Tables 4, 5). However, gene expression at the transcription level and leaflet width may not be linearly related. For example, the width of the leaf blade of the homozygote tl^w/tl^w is about 10 times larger than that of the heterozygote tl^w/Tl. If this relation between gene activity at the molecular and morphological level is accepted, in reality the width of the leaf blade of the flat tendril in the heterozygote should be much closer to that of the least wide of the homozygotes, that is, $His1^2$.

It is known that an overwhelming majority of homeotic genes in *Drosophila* (Morata, 1993), as well as in plants (Weigel & Meyerowitz, 1994), code for transcription factors. Therefore, we can say that the *Tl* gene, responsible for the transformation of the upper leaflet pairs into tendrils, most probably exerts its effect at the level of transcription. It is the histone H1 gene that is the best candidate for such a focus of action.

The most convincing evidence of the participation of histone H1 in transcriptional regulation was obtained in the study of molecular mechanisms of early embryonic development in *Xenopus*. Between the mid-blastula transition and early neurula a substitution occurs of the oocyte H1-like protein B4 for three somatic histone H1 variants. This is paralleled by substantial changes in gene activity; transcription of the oocyte 5S RNA genes is repressed and transcription of their somatic counterparts is switched on, and in cells of the animal pole a battery of genes providing mesoderm induction is activated (Dimitrov *et al*., 1993). It was shown that there exists competitive interaction between transcription factor TFIIIA, which activates transcription of oocyte 5S RNA, and histone H1 (Schlissel & Brown, 1984). Experimental overproduction of somatic histone H1 exerts its repressive effect even in the presence of excess TFIIIA. Depletion of the H1a subtype, comprising about 95 % somatic histone H1, prolonged the duration of expression of the oocyte genes. In contrast, overproduction of the minor H1c variant shortened this period (Bouvet *et al*., 1994).

Steinbach *et al*. (1997) injected into blastomeres of *Xenopus* a ribozyme selectively removing mRNA of a certain histone H1 subtype, or additional mRNA of this subtype. Embryonic explants with a reduced content of H1a had a prolonged period of competence to a mesodermal inductor activin, while overproduction of the H1c subtype shortened this period. None of the target genes of activin induction that were studied responded to manipulation with the oocyte-specific B4, while the response to changes in the level of somatic histone was of a similar although gene-specific manner. The molecular mechanism underlying this phenomenon is not clear, although it can be noted that a protein complex serving activin-dependent mesodermal induction includes a transcription factor containing a DNA-binding winged-helix domain (Chen *et al*., 1996). The central domain of histone H5, which is related to H1, has a tertiary structure extremely similar to that of the winged-helix domain of transcription factors (Clark *et al*., 1993; Ramakrishnan *et al*., 1993). It was shown (Cirillo *et al*., 1998) that a globular domain of histone H5 is capable of competing for DNA binding sites with the winged-helix transcription factor HNF3. It follows that the two types of proteins most probably interact inside chromatin, competing for regulatory *cis*-elements of target genes of activin induction.

Thus, we can consider the gene *His1* as a candidate locus for a QTL with a strong pleiotropic effect. Although the experiment presented here can not exclude the possibility that the effects observed are due to some other locus tightly linked to *His1*, the results of similar experiments with substitutions of much shorter chromosome segments containing alleles of other histone H1 genes (Bogdanova *et al*., 1994) and the most recent information on histone H1

function, taken together, make quite plausible the supposition that histone H1 genes can affect quantitative traits of an organism.

There are seven genes in the pea genome that code for different subtypes of histone H1 (Kosterin *et al.*, 1994). Each gene is represented by several alleles, null alleles also being found for some of them (*His3*, *His4*, *His5* and *His6*). Since allelic variants of histone H1 genes are easily registered by means of electrophoresis, these putative QTLs are in fact markers of themselves, which opens a way to construction of genotypes differing in the dose and allelic composition of all seven genes of the pea H1 histone.

The results of the comparison of our three lines for the rate of crossing-over between the genes *r* and *Sca* seem intriguing. There can be proposed three explanations for the phenomenon. First, histone H1, as an essential structural element of chromatin, could affect homologue pairing in meiotic prophase. However, it is difficult to imagine that a substitution of naturally occurring histone H1 variants, rather similar in their structure, could bring about almost a 2-fold effect on the rate of crossing-over.

The second explanation has only an indirect relation to the *His1* gene. There is evidence that in *Drosophila* males the histone gene cluster serves as a centre of pairing initiation of chromosome 2 due to peculiar repeats present in the same chromosome region (McKee, 1996). Naturally great care needs to be taken in referring to such a specific case as the male meiosis in *Drosophila*, which is unusual in many respects. Nevertheless, it can not be excluded that around gene *His1* are situated some sites somehow related to the initiation of chromosome pairing and, therefore, affecting recombination frequency in this region.

Finally, the lines Delta-s and Delta-f differ by a small homozygous chromosome segment. In the Delta line this segment is maintained in the heterozygous state, and it is this line that shows the minimum crossover rate. One can suppose that the regions of difference harbour some structural dissimilarities (duplications, inversions, deletions) that can result in a distortion of pairing when heterozygous and cause a reduction of the recombination rate. This explanation seems to be the simplest and most plausible.

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