Further data on the genes controlling anthocyanin pigmentation in the pea

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The pattern of anthocyanin colouration of various organs, other than the corolla, of the pea plant is known to be controlled by a number of genes (1, 7). The gene *D* causes a single or double anthocyanin ring or several spots at the base of the stipules; the gene *And* determines irregular dots on the upper surface of the leaflets and stipules; the gene *Ans* brings about a red suffusion of the stem. Several symbolised genes control colouration of the pod: the dominant alleles of genes *Pur* and *Pu* together confer purple pods (3) and gene *Astr* causes the purple stripes on the pod wall in some accessions of *Pisum fulvum* (4). It was reported (6) that two recessive genes *rup* and *rups* are responsible for a row of purple, roundish spots on the pod wall corresponding to the seeds forming inside, while two genes *sru* and *srub* control the purple colouration of the upper seam of the pod; the same line, WL1293, was specified as the type line for all four genes *rup, rups, sru* and *srub*. No further data have been reported on the inheritance of the two latter characters and the reality of the four corresponding genes demands further thorough investigation. The genetic control of other patterns, such as the variable anthocyanin colouration of the pedicel and of the red spots at the leaflet bases, has not been investigated.

Identification of the genotype of a plant by phenotypic manifestation of the above genes is difficult due to an interaction of the genes and, in some cases, their probable identity to each other.

Four of the genes pertinent to anthocyanin distribution, namely, *D, Pur, And,* and *Ans,* are known to be located in the same region of the genome (1, 7). Although the data available demand certain caution in interpreting, one can infer the genes controlling the distribution of anthocyanin pigmentation form clusters on the linkage map.

We present here some observations on the inheritance of the pod colouration, the axillary anthocyanin ring, and the spots at the leaflet bases. We suggest that the funiculus colouration can aid in identification of the genes *Pur* and *Pu.* In addition, we propose that *Astr* and *Pur* may be the same gene and that the spots at the leaflet bases may be another effect of the gene *D.*

We have analysed segregation for the purple colouration of the pod in the cross WL-577 x VIR-5797. Line WL-577 has purple pods and is homozygous dominant for *Pur* and *Pu* (1). Line VIR-5797 has green pods. In the same cross we observed segregation for anthocyanin colouration of the funiculus. This kind of colouration was also observed by Lamprecht (2) in plants with purple pods but detailed analysis of inheritance of this character was not carried out.

Among 290 plants of the F_2 generation, we observed segregation for the colour of the pod wall and the colour of the funiculus as shown in Table 1 (sometimes the anthocyanin colouration of the funiculus was reduced only to its very base, but yet was distinctly observable).

Table 1. Segregation for the colour of the pod wall and the colour of the funiculus in the cross WL577 x VIR-5797.

	Purple pod	Green pod	Total
Coloured funiculus	126	39	165
Colourless funiculus	0	50	50
Not determined*	38	37	75
Total	164	126	290

* Funiculus colour was not detennined for 75 of the F_2 plants because they had not attained the relevant slage of development before the experiment was terminated due to restrictions on time and space. Pod colour was already apparent just after pod elongation ceased while funiculus colour was not examined until the seeds had undergone further development.

Table 2. Segregation for the presence/absence of the anthocyanin ring in the leaf axils and presence/absence of colour of the funiculus in the F_2 of cross WL-1081 x "Sprint-26".

	Ring present	Ring absent	Total
Coloured funiculus	66	1	67
Colourless funiculus	6	26	32
Total	72	27	99

Chi-square (3:1) is 2.83 ($P<0.05$) for the funiculus colour and 0.27 ($P>0.6$) for the ring. The joint segregation Chi-square is 69.5 ($P<0.0001$) and the recombination fraction 6.84 ± 2.84%.

Table 3. Segregation for the presence/absence of the dots at the leaflet bases and presence/absence of purple stripes on the pod wall in the F_2 of cross WL-1238 x F_3 [VIR-6070 (*P.fulvum*) x WL-1255]

	Dots present	Dots absent	Total
Stripes present	52	8	60
Stripes absent	6	20	26
Total	58	28	86

Chi-square (3:1) is 2.62 ($P>0.1$) for the dots and 1.25 ($P>0.2$) for the stripes. The joint segregation Chi-square is 33.4 ($P<0.0001$) and the recombination fraction 16.0 ± 4.4%.

The observed ratio of the number of purple-podded plants to that of green-podded plants (164:126) corresponds well to the 9:7 ratio (Chi-square = 0.01) expected in the case of two complementary dominant genes. Thus, we can confirm that in our case the purple colouration of the pod is determined by the simultaneous presence of two dominant genes, as is assumed for the line WL-577. At the same time, segregation for the anthocyanin colouration of the funiculus followed a monogenic mode of inheritance, the observed ratio 165:50 corresponding well to 3:1 (Chi-square = 0.26). If we consider separately the classes with coloured and colourless funiculus, we notice that in the former class segregation for the

colouration of the pod is of a monogenic character, as the ratio 126 purple : 39 green is in a good accordance with 3:1 (Chi-square = 0.17). However, segregation for the pod colouration was not observed among plants with a colourless funiculus. Thus, not one of the purple-podded plants had a colourless funiculus, while the green-podded plants segregated for colouration of the funiculus in the proportion 39 coloured: 50 colourless, corresponding well to 3:4 ratio (Chi-square = 0.03). The proportions of phenotypic classes: 126 purple pod, coloured funiculus : 39 green pod, coloured funiculus : 50 green pod, colourless funiculus are well described by the digenic ratio 9:3:4 (Chi-square = 0.69).

The observed segregation for the two characters allows us to suppose that one of genes of the pair *Pur* and *Pu* determines anthocyanin colouration of the funiculus, while addition of the other gene extends this colouration to the entire pod.

In order to elucidate which of the genes *Pu* and *Pur* is responsible for each effect we tried to estimate genetic linkage between anthocyanin colouration of the funiculus and other traits of the plant We performed a cross WL-1081 x "Sprint-26"; the former line has a red funiculus and markers *D, wsp, n* and *i,* while the latter line has a colourless funiculus and markers *d, Wsp, N* and *I*. In addition, these lines also differed in allelic composition of the histone H1 loci *His(2-6)* and *His7.* An analysis of 99 plants in the F_2 generation (Table 2) showed significant linkage between colourless funiculus and gene *d,* with a recombination fraction of 6.84±2.84% (estimated by the maximum likelihood method) and a joint segregation Chi-square of 69.5 ($P<0.0001$). Thus we conclude the gene determining anthocyanin colouration of the funiculus is *Pur* since this gene is known to be linked to *D* (1).

Purple colouration of the pod has rather a variable expression and the gene *Pur* is supposed to be highly mutable and to be associated with a series of weaker alleles determining variation in the extent of the coloured area of the pod surface (2). However, an alternative explanation can be proposed for this variability - the existence of a series of genes modifying expression *of Pur,* and *Pu* may be one of the strongest of these modifiers.

Another character associated with the distribution of anthocyanin colouration is the presence of anthocyanin dots at the base of leaflets. The character was involved in the testcross (RT1 x WL-1238) x 6-14. 158 plants resulting from this testcross segregated for presence/absence of the dots at the base of leaflets. At the same time, they also segregated for presence/absence of the anthocyanin ring at the bases of the stipules caused by alleles D^{co} and *d,* respectively. Since one of the parents, WL-1238 (as well as the line 6-14 used for pollination of the F_1, hybrids), was void of both dots and axillary ring, these characters evidently came from RT1 where they were not observable because of the repressed anthocyanin production (this line carries allele *a).* (Note that all the plants of the testcross progeny received the allele A from line 6-14 and so did not segregate for anthocyanin production). We found that the dots at the leaflet bases were absolutely coupled with the anthocyanin ring caused by D^{co}: 82 plants had both dots and rings while 76 plants lacked both characters; those numbers are in good accordance with a 1:1 ratio (Chi-square = 0.23, $P>0.6$). Taking into account the full fertility of the F_1 plants, suggesting that no noticeable chromosomal rearrangements were involved in the cross, one can suppose that these two evidently homologous characters are coded by two extremely closely linked genes or, more probably, are manifestations of the same allele D^{co} of the gene *D.*

We made another attempt at mapping the gene determining the dots at the leaflet bases in the cross WL-1238 x F_3 [VIR-6070 (*P. fulvum*) x WL-1255]. The latter parent was

represented by a single F_3 plant originating from the mentioned cross. This F_3 plant possessed red dots at the base of leaflets (but no axillary anthocyanin ring), anthocyanin stripes on the pod surface, yellowish colouration of the flower (evidently coming from *P. fulvum*), as well as genes *wlo* and *p* (evidently from WL-1255). The WL-1238 parent is void of anthocyanin colouration except for the flowers, lacks yellowish colouration of the corolla, and has a number of recessive markers - *r, tl*^{w}*, gp, le, wb, b* and *k.* In addition, the parent plants differed in allelic composition of histone H1 loci *His(2-6)* and *His7.* All descendants of the cross were fully fertile, thus indicating absence of chromosomal rearrangements, although there occurred in the F_2 a number of plants with the "chlorotica" phenotype which eventually died.

An analysis of 86 F_2 plants (Table 3) showed significant linkage (recombination fraction 16.0 ± 4.4%; joint seg. Chi-square = 33.4, P<0.0001) between ihe gene determining red dots at the bases of leaflets and the gene conditioning purple stripes on the pod (*Astr*). Both genes came from *P. fulvum* and, as discussed earlier, the former of them might be the gene *D* represented in this species by an allele which is expressed in such a way. The distance between the gene determining the red dots at the bases of the leaflets and *Astr* is quite similar to that between *D* and *Pur,* thus allowing us to speculate that the *Astr* phenotype is a manifestation of *Pur* specific to *P. fulvum.*

Thus, our data suggest that colouration of the funiculus is the character which reliably indicates the presence of the dominant allele of the gene *Pur* and hence, allows the phenotypic classes *Pur Pu* and *Pur pu* to be distinguished. In addition, our data indicate that the two traits purple stripes on the pod (*Astr*) and purple pods (*Pur*) may be manifestations of the same gene. Likewise, the two traits reds spots at the leaflet bases and anthocyanin ring in the leaf axils (*D*) may be determined by the same gene.

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