Fs and *U* appear to be alleles of a locus near the end of linkage group V

Weeden, N.F. Montana State Univ., Bozeman, MT, USA

Both *Fs* (*violaceopunctata*) and *U* (violet testa) are described loci that have been placed on the lower half of linkage group (LG) V as that linkage group is traditionally depicted (1, 8). Phenotypes produced by alleles at *Fs* and *U* have an interesting interrelationship because both produce anthocyanin pigmentation in the testa. Dominant alleles at *Fs* generally produce more or less intense spots of anthocyanin (usually 20 to 100) on the testa, whereas the *U* allele causes anthocyanin to be produced uniformly throughout the testa, resulting in a dark violet or 'black' seed. However, occasionally in an *Fs* line seeds will be formed in which the spotted phenotype is replaced by a solid pigmentation that covers much or all the testa. This phenotype is referred to as 'obscura' (Fig. 1). The cause of the obscura phenotype is unclear because the next generation nearly always displays the spotted testa phenotype (1). Furthermore, a second allele of *U*, designated U^{st}, produces stripes or streaks of anthocyanin pigmentation on the testa, differing from a typical *Fs* pattern primarily by the streaks being larger and more irregular than the spots produced by *Fs* alleles, as well as by their fewer number (0-5) on the testa.

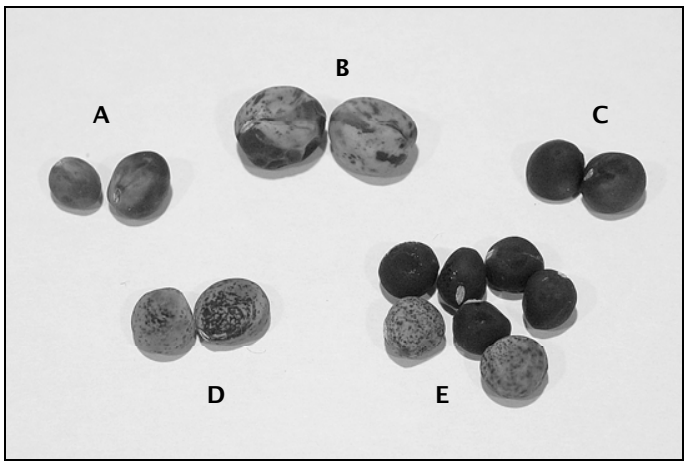

Fig. 1 Different expressions of the 'obscura' phenotype (A, B, D) compared with a U *phenotype (C). The seeds shown in E are from a single plant grown from a seed with an* Fs *testa phenotype. The obscura seeds in E are virtually indistinguishable from true-breeding* 'U' *seeds.*

Lamprecht (6) reported a recombination value between *Fs* and *U* of 23%, and this arrangement is reflected in Blixt's classical linkage map (1). However, in two other experiments he reported less than 1% recombination (4, 5) between the two phenotypes. Linkage analysis between *Fs* and *U* is complicated because the *Fs* phenotype is hypostatic to *U* and thus cannot be observed in seeds with the typical *U* phenotype. Furthermore, in lines homozygous U^{st}, not every seed displays the streaks characteristic of this allele. Thus, if relatively few seeds are collected from a plant possessing the U^{st} allele, it is possible to incorrectly score the plant as *u/u*. Finally, the *obscura* phenotype can complicate the scoring of phenotypes when both *Fs* and *U* are segregating in a population.

In order to determine the actual linkage intensity between *Fs* and *U*, I mapped both *Fs* and *U* relative to the isozyme locus *Pgdc*. Upon finding that both genes mapped to the same location within the error of the analysis, I attempted to reject the hypothesis that *Fs*, *U* and U^{st} are all alleles at the same locus, both by reviewing the literature and by performing the analyses described below. I am unable to reject the hypothesis and, indeed, developed evidence that neither *U* nor U^{st} are 100% penetrant when involved in crosses. Thus, the "recombination" observed between *Fs* and U^{st} by myself and other pea geneticists could merely reflect incomplete expression of the *U* or U^{st} phenotype. Although the history of the nomenclature for *U* and *Fs* is complicated, both genes being initially given different symbols [*Ast* for *U* (9) and *P* for *Fs* (3)], it appears *U* has precedence over *Fs*, and at this point it seems appropriate to retire the symbol *Fs* and identify the *violaceopunctata* phenotype in JI 261, A578-238 and many other lines as being produced by an allele of *U*, namely U^{fs}. This allele is dominant relative to absence of spotting (*u*) and codominant with U^{st}. A second locus (*F*)

ostensibly able to produce the *violaceopunctata* phenotype, has been described previously (7,9) and has been assigned to LG III (6). The elimination of *Fs* should have no effect on the validity of *F*, although I have not been able to verify the existence of this latter gene.

Material and Methods

Several crosses, involving different sources of the alleles *U*, U^{st} or *Fs* were used in this analysis (Table 1). Each of the parents had been inbred several generations and consistently displayed the same testa phenotype in each generation. In two cases (WL1414 and WL1018), the line is homozygous *a*, and thus does not express *Fs* or *U* alleles. However, when crossed to a line that is homozygous *A*, the testa pattern can be observed in seed from the hybrid plant and in subsequent generations in seed of plants expressing the *A* allele.

Table 1. Populations used to examine the joint segregation of *U*, *Fs* and *Pgdc*

Cross designation	Parents of cross	Genotype of parents	Source of *U*
1	B77-257 x A578-238	*fs, u* x *Fs, u*	*U* not present
2	WL1238 x 'Sparkle'	*fs,* U^{st} x [*a*] (*fs, u*)[1]	S. Blixt
3	WL1414 x WL808	[*a*] (*Fs, u*) x *fs, U*	*P. s.* ssp. *abyssinicum*
4	WL1018 x 87-19i-a	[*a*] (*U*) x *Fs, u*	S. Blixt
5	87-19I-a x WL1018	*Fs, u* x [*a*] (*U*)	S. Blixt
6	C98-54 x C98-1-12	*U* x *Fs*	N.F. Weeden
7	WL1238 x JI 261	*fs,* U^{st} x *Fs,* U^{st}	S. Blixt and *P. s.* ssp *humile*
8	Marx 15241 x Marx 15098	*fs, oh* x (*Fs*), *U*	G.A. Marx
9	C01-1b x A03-123	(*Fs*?), *u/U* x *Fs, u*	N.F. Weeden

[1]**Parentheses indicate hidden phenotype of *Fs*, *U* or both that is not directly observable due to hypostatic interactions with other genes.**

Plants were grown in the glasshouse under 16 hr daylength or for a relatively few populations, in the field at Bozeman, Montana, USA. Pods were allowed to dry on the plant to allow full development of testa pattern.

Results and Discussion

As testa pattern is determined by the maternal genotype, the seed produced from a cross should display the same phenotype as the maternal parent. Furthermore, seed produced on all F_1 plants from either *U* x *Fs* or *Fs* x *U* crosses, should have the *U* phenotype. The former prediction was confirmed in all crosses. The phenotypes observed on seeds from the hybrid in each cross are presented in Table 2.

Table 2. Phenotype of seeds obtained from the hybrid plants in each of the crosses studied

Cross	Phenotype of Parents	Phenotype on seeds from hybrid	Phenotypes observed on seeds from F_2
1	*fs, u* x *Fs, u*	all seeds *Fs*	29 *Fs*, 10 *fs*
2	*fs,* U^{st} x *a*	all seeds U^{st}	53 U^{st}, 18 *u*, 29 *a*
3	*a* x *fs, U*	7 *U*, 3 U^{st}, 25 u, (*Fs* or *fs*)	0 *U*, 0 U^{st}, 9 *Fs*, 6 *fs*
4	*a* x *Fs, u*	all seeds *U*	4 *U*, 4 *Fs*, 7 *a*
5	*Fs, u* x *a*	*U* or *U**[1]	37 *U* or *U**, 10 *Fs*, 13 *a*
6	*U* x *Fs*	20 *U* and 14 *Fs*	4 *U*, 9 *U**, 11 *Fs*
7	*fs,* U^{st} x *Fs,* U^{st}	all seeds *Fs* and U^{st}	Not tested
8	*fs* x *U*	3 *U*, 2 *Fs*	1 U, 1 U*, 2 fs, v. faint U
9	*u/U* x *Fs, u*	both seeds *U**	*U* and *Fs* (backcross)

[1] **Asterisk indicates that the *U* phenotype only partially covered testa**

Cross 1 (B77-257 x B578-238)

This cross was used primarily to confirm normal segregation of *Fs* and to determine recombination distance between *Pgdc* and *Fs*. The original two seeds produced from the cross both were *fs, u*, the same phenotype as the maternal parent. The hybrid plants generated from these two seeds produced only *Fs* seed (24 seed from one plant and 15 from the other). Of the 39 F_2 plants grown, 29 produced only *Fs* seeds and 10 produced only *fs* seed. Joint segregation analysis of *Fs* and *Pgdc* gave a recombination distance between the two loci of 6 cM. Thus, this cross and F_1 and F_2 generations indicated that *Fs* was behaving as a single Mendelian factor, closely linked to *Pgdc*. Other crosses placed *Fs* distal to *Pgdc* relative to *Acp*1 (Weeden, unpublished).

Cross 2 (WL 1238 x 'Sparkle')

This cross was used primarily to confirm normal segregation of U^{st} and to determine recombination distance between *Pgdc* and U^{st}. Four seeds were produced from the original cross, and each had the maternal *fs*, U^{st} testa phenotype. The seed from the hybrid plant also displayed this same phenotype. If the white-flowered plants are excluded from consideration, the F_2 population segregated approximately 3 U^{st} : 1 *u* (Table 2), and the calculated recombination distance between *Pgdc* and *U* was 7 cM. The locus *Gp* was also segregating in this population, and it mapped on the opposite side of *Pgdc* from *U* in agreement with (2). These results place *U* at approximately the same position as *Fs*, within the precision of the data from crosses 1 and 2.

Cross 3 (WL1414 x WL808)

This cross contained the violet testa (*U* allele) observed in many *P. s.* ssp. *abyssinicum* lines. The initial cross gave seeds lacking anthocyanin because the maternal parent was white-flowered. The F_1 generation surprisingly produced mostly seeds lacking a solid violet testa, indeed most lacked any evidence of *U* expression with seven showing the solid violet pattern, three a partial violet testa, and 25 being either *Fs* or *fs* (a careful examination of the *Fs* phenotype was unfortunately not performed on this generation). When the F_2 generation was grown, only 25 of the 35 lines produced seed and 10 of these were white-flowered, precluding the scoring of *Fs* or *U*. Of the colored-flowered plants producing seed, none were *U* or U^{st}, nine were *Fs* and six were *fs*. One of the *Fs* and two of the *fs* plants were from seeds with a *U* or partial U phenotype. The *fs* phenotype came from the *P. s.* ssp. *abyssinicum* parent, indicating that either all six of the *fs* F_2 plants reflected recombination between *Fs* and *U* or that the *U* phenotype had been suppressed in these plants. The 9:6 ratio does not differ significantly from the expected 3:1 ratio (assuming lack of *U* expression), suggesting that segregation is not strongly distorted in this region of the genome.

Cross 4 (WL1018 x 87-19i-a)

The seed produced from the cross displayed the expected solid violet testa, as did the seed produced from the F_1. Nearly half the F_2 plants were homozygous for *a*, so that *U* and *Fs* could not be scored. Four of the F_2 plants produced at least some seed with solid violet testa, whereas four F_2 plants produced only *Fs* seed. However, two of the F_2 plants scored as *U* produced some seed on which only part of the testa was solid violet, the remaining being violet spotted. Here again it appears that *U* is often incompletely dominant in segregating populations.

Cross 5 (87-19I-a x WL1018)

The hybrid seed possessed the *Fs* phenotype characteristic of the maternal parent. The seed from the F_1 was a mixture of *U* and *U* plus *Fs* phenotypes. The *U:Fs* segregation ratio in the F_2 did not differ significantly from the expected 3:1 ratio. However nine of the plants scored as *U* gave seed that only displayed partial fusion of the anthocyanin pigmentation into a solid pattern. Some of the seed collected

from these plants displayed only the spotted pattern typical of *Fs* genotypes. One plant gave three pods, one in which all seeds were *U*, one in which all seeds were *Fs*, and one in which the seeds were *Fs* but some had a partial fusion of the spots. These results mimic the spectrum of patterns observed in some *Fs* genotypes in which the obscura phenomenon is common. In addition, several of the plants scored *Fs* had slightly larger spots on the testa of some seeds, suggestive of a U^{st} pattern in combination with *Fs*. All of the F_2 plants with an incomplete *U* pattern showed violet spots in the open regions. The lack of a double recessive phenotype can be explained by either WL1018 being *Fs*, *U* or with *Fs* and *U* being allelic.

Cross 6 (C98-54 x C98-1-12)

This cross used a line expressing the *U* phenotype (C98-54) that had produced solid violet seed coats for several generations, although the pedigree could not be traced back to either *P. sativum* ssp. *abyssinicum* or Lamprecht's type line for *U*. The hybrid seed had the expected solid violet testa. However, the seed from the F_1 was a mixture of phenotypes with some of the *U* seeds displaying only a partially violet testa and a significant proportion of the seeds exhibiting an *Fs* phenotype. Not all the F_2 plants produced seed, but of those that did, 4 produced only seeds with solid violet testa, 9 produced seeds displaying various mixtures of partially solid violet testa and 11 produced only seeds with the *Fs* phenotype, which was sometimes very faint. In order to confirm the F_2 seed testa phenotype, two seeds were taken from each F_2 and grown to produce seed from the F_3. In most cases the testa phenotype from F_3 plants corroborated that from the F_2. However, there were some notable exceptions. In two cases seed from the F_2 that possessed an *Fs* phenotype gave seed with a *U* phenotype in the next generation. In another three cases, seed from the F_2 that had barely discernable violet spots (although two had obscura markings) produced seed from the F_3 that had clear *Fs* markings. In summary, this cross gave a slight but significant ($\chi^2 = 5.5$, 1 d.f.) deficiency in the dominant (*U*) phenotype in the F_2 even after correcting for the two lines that displayed *U* in the F_3 but not in the F_2 (Table 2), and there were a number of cases in which the expression of a dominant character (*U* or *Fs*) was observed in seed from F_3 plants, when it had been lacking in the previous generation.

Cross 7 (WL 1238 x JI 261)

This cross gave the expected U^{st}, *fs* phenotype on the one seed produced. The hybrid plant was semi sterile, and only four seeds were obtained. All these seeds displayed both the typical U^{st} streaks in a background of small violet spots, indicating that both U^{st} and *Fs* were expressed. I was not able to detect on the seed from the F_1 generation the small streaks that characterize the U^{st} phenotype of JI 261. Rather all four seeds had the large blotches characteristic of WL 1238 together with many round spots attributable to *Fs* from JI 261.

Cross 8 (Marx 15241 x Marx 15098)

This cross was complicated slightly by the presence of recessive alleles at *Oh* (testa reddish-brown) and *B* (petals pink) segregating in the population. However, the critical finding for the purposes of this paper was that both seeds with the *U* phenotype and seeds with the *Fs* phenotype were produced from the hybrid plant. The known genotype of the hybrid was *u/U*, *b/B*, *oh/Oh*. I am uncertain whether Marx 15098 has violet spots obscured by the solid violet color of the testa. However, the seeds from the hybrid would all be expected to display the *U* phenotype. The presence of seeds with only violet spots further indicate that *U* is not 100% penetrant in some crosses. Seeds collected from two F_2 plants derived from seeds with *U* phenotype and two F_2 plants derived from seeds with *Fs* phenotype were, respectively, U, pale U (with no violet spots), *fs* with a faint obscura, and *fs* with a faint pinkish hue. All four F_2 had wildtype (*B*) flowers. Finally, when a typical *U* phenotype seed from the first of the F_2 plants mentioned was used to produce F_3 seed, this seed was uniformly *Fs*. Thus, in this cross the *U* phenotype was lost by the third filial generation, appearing to transform into an *Fs* pattern despite the *U* allele having been

derived from a line that stably expressed the *U* phenotype for several generations. The loss of the *Fs* phenotype in some lines when going from the F_2 to the F_3 generation is also interesting, although it could be explained by simple segregation, or interactions with *b* or *oh*.

Cross 9 (C01-1b x A03-123)

C01-1b was an F_1 plant produced from the cross B5 (*U*) x B77-257 (*fs*, *u*). The line B5 shared the same *U* allele as C98-54 in cross 6. The cross of this hybrid with A03-123 (*Fs*) gave two seeds both of which display a testa that was partially solid violet and partially violet spots. Selfed seed from C01-1b was typical *U* (completely solid violet). The two hybrid seeds were planted, and one produced all *U* seeds, whereas the other produced all *Fs* seed. This last results can be interpreted as being consistent with the segregation at *U* from C01-1b. However, because the original hybrid seeds were produced on a plant that was heterozygous at *U*, both of these seeds should have been uniformly solid violet. It again appears that *U* is not completely penetrant in some genetic backgrounds.

The results from the above crosses clearly demonstrate that the solid violet testa phenotype described for the *U* allele is not completely penetrant in many crosses. This behavior does not appear to be dependent on the source of the *U* allele, for lines from the Weibullsholm collection, the Marx genetic stocks collection, my own collection and the taxon *P. s.* ssp. *abyssinicum* all showed incomplete expression in at least one cross. At present, the specific genetic background (if any) that produces the incomplete expression is not obvious.

Joint segregation analysis between the locus *Pgdc* and either *Fs* or *U* revealed nearly identical recombination frequencies between the isozyme marker and each of the seed testa phenotypes, placing both *Fs* and *U* distal to *Pgdc* (in agreement with previous studies) and within 1 to 2 cM of each other. This result is in disagreement with the position of U on LG V on certain linkage maps for pea (1) but agrees well with some of Lamprecht's data (3, 4). When the two markers are separated on LG V, *U* is always placed distal to *Fs*, and usually forms the most distal marker on that arm of the linkage group. I suggest that most recombinants identified between *Fs* and *U* in earlier studies are a result of incomplete expression of the *U* phenotype and are apparent recombinants rather than real. The only evidence for a significant recombination frequency between *Fs* and *U* is the appearance of the *fs* phenotype on seeds in the F_2 of cross 3. Such seed could be interpreted as being produced from a plant that was recombinant between *Fs* and *U* on both homologous chromosomes. However, the F_1 of cross 3 already displayed significant loss of *U* expression, suggesting that a general loss or suppression of *U* expression might be a more conservative explanation for the *fs* phenotype. Indeed, a study of a large population produced from backcrossing a *P. sativum* ssp *abyssinicum* x 'Sparkle' hybrid (*U*/*u*, *fs*/*fs*) to other white-flowered cultivars (*u*/*u*) revealed a complete absence of *U* phenotypes in the seed from 26 BC_1F_3 plants that displayed violet flowers (data not presented). The only anthocyanin markings on testa of seed from this generation were very faint obscura patterns in some lines. This result suggests that the solid violet testa in *P. s.* ssp. *abyssinicum* is not particularly stable in crosses and possibly can be transformed to obscura, a phenotype associated with the *Fs* locus.

As heterozygotes from all sources of *U* can show incomplete penetrance, the mapping of *U* and the use of *U* as an anchor marker become problematic. If *U* maps near *Fs*, an allelism test is called for, yet with *Fs* being hypostatic to *U*, with the capability of *Fs* converting to an obscura phenotype very similar to an incomplete expression of *U*, and with the expression of *U* being erratic in many crosses, it becomes difficult perform such a test. To my knowledge, the only lines used in the above experiments that are definitely *fs*, *U* are the violet testa *P. s.* ssp. *abyssinicum* accessions, and this taxon displays the greatest loss of penetrance of the *U* phenotype in seed from F_1 plants. In all other crosses between a line with an *Fs* phenotype and a line with a *U* phenotype I have yet to isolate a derivative with an *fs* phenotype lacking

some type of obscura pattern. My review of the literature also fails to identify an allelism test in which the problems of incomplete penetrance and the obscura phenomenon have been clearly overcome.

It has always been the responsibility of the investigator, when describing a new gene, to perform the necessary allelism tests. In the case of *Fs* and *U*, both symbols are already accepted in the literature despite an apparent absence of a rigorous allelism tests, due to the complications described above. Again because of these complications, I have been unable to provide clear evidence that the two phenotypes represent alleles at the same locus. However, given the facts that (1) the phenotypic expression of both *Fs* and *U* is unstable and produces similar 'off types' and (2) in independent crosses phenotypic segregation places *Fs* and *U* within the same 1-3 cM region on LG V, the most conservative alternative at present is to treat *Fs* and *U* as the same locus. Hence, I recommend that *Fs* be referred to as U^{fs} until a clear demonstration is available that it represents a distinct locus.

1. Blixt, S. 1972. Agri Hort. Genet. 30: 1–293.
2. Irzykowska, L., Wolko, B. and Święcicki, W.K. 2001. Pisum Genetics 33: 13-18.
3. Kajanus, B. and Berg, S.O.. 1919. Ark. f. Botanik 15: 1–18.
4. Lamprecht, H. 1942a. Hereditas 28: 143–156.
5. Lamprecht, H. 1942b. Hereditas 28: 157–164.
6. Lamprecht, H. 1961. Agri Hort. Genet. 19: 360–401.
7. Tschermak, E. von. 1912. Z. ind. Abst. u. Vererbungsl. 7: 81–234.
8. Weeden, N.F., Ellis, T.H.N., Timmerman-Vaughan, G.M., Święcicki, W.K., Rozov, S.M. and Berdnikov, V.A. 1998. Pisum Genetics 30: 1–4.
9. White, O.E. 1917. Proc. Amer. Phil Soc. 56: 487–588.