GENETICS OF STEM, FLOWER, AND POD COLOR IN *PHASEOLUS VULGARIS* L. AND *PHASEOLUS COCCINEUS* L.

by

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Genetics of Stem, Flower, and Pod Color in *Phaseolus vulgaris* L. and *Phaseolus coccineus* L.[1]

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Abstract. Intraspecific crosses with *Phaseolus vulgaris* show that the dark purple pod color of the cultivar 'Royal Burgundy' is due to an interaction of 2 unlinked genes: *P* and *Prp*, the latter gene completely described here for the first time. Interspecific test crosses between different genotypes of *P. vulgaris* and of *P. coccineus* revealed that white flower in PI 175858 of the latter species is due to *p* and that in a *P. coccineus* heterozygote, *Pp*, the *p* male gamete appears to function preferentially in the interspecific cross with *P. vulgaris*. Further corroborating evidence was obtained that the scarlet flower color of *P. coccineus* is determined by 2 dominant, unlinked genes in this interspecific cross.

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[1]Received for publication . . . . .

[2]Graduate student and Professor respectively.

Inheritance of immature pod color in Phaseolus vulgaris has been studied in several investigations. Lamprecht (11) hypothesized that 2 unlinked genes, Pur and Ro, controlled the inheritance of purple pod color. His scheme can be summarized as follows:

	Immature pod color	
	Ro	ro
Pur	dark purple	green
pur	rose	green

Using a somewhat different scheme, Moraes and Vieira (13) reported that pod color depended upon the interaction of 2 unlinked loci, each with 3 alleles:

		Immature pod color		
Flower Color		A	a^a	a
Purple	V	dark purple	striped purple	dark pink
Pink	v^{lae}	red	yellow	yellow
White	v	red	yellow	yellow

According to them A and a^a correspond to Lamprecht's Ro and ro; however, they did not use Lamprecht's symbols because V a^a gave pods striped in purple, while Lamprecht's corresponding genotype, Pur ro, had green pods. Further, Moraes and Vieira (13) were not able to equate V and v^{lae} or v to Lamprecht's Pur and pur, respectively, because Lamprecht (11) had not studied flower color segregation in his pod color crosses.

More recently, Frag (3) proposed that dark purple pod color was due to an interaction of 2 unlinked genes, V and Prp, which interacted to produce purple pods in two different shades of intensity.

The genetic control of flower color in P. vulgaris is due to a series of 3 alleles, V, v^{lae}, and v at one locus. These produce the colors Bishop's violet, lilac, and white, respectively (7, 9, 14). Within P. coccineus the commonest flower colors are white and scarlet, with white being due to a single, recessive gene inhibiting production of flower color (5, 10). According to Lamprecht (10, 12), 4 genes are present in P. coccineus, all of which are required to produce, by interaction, scarlet flower color, as determined by interspecific crosses of this species with P. vulgaris. On the other hand, Frag's work (3) on the same cross, but with a purple-flowered P. vulgaris, suggested that the scarlet flower color of P. coccineus appeared to be due to 2 dominant, unlinked genes which are both epistatic to the dominant allele for the purple flower color of P. vulgaris.

In light of the disagreement among these workers regarding the mode of flower color inheritance in this interspecific cross, as well as of pod color within P. vulgaris, we felt further study of both characters was needed.

Materials and Methods

Two cultivars of Phaseolus vulgaris, 'Royal Burgundy' and 'Bush Blue Lake 290', and one of Phaseolus coccineus, PI 175858, were used in the investigation. 'Royal Burgundy' was obtained from the Stokes Seed Company, Buffalo, New York and has purple stem, purple flowers, and purple pods. 'Bush Blue Lake 290' was received from Asgrow Seed Company, Kalamazoo, Michigan and has green stem, white flowers, and green pods. The P. coccineus, PI 175858, was from the W-6 U.S.D.A. Plant Introduction

Station, Pullman, Washington. It has green stem, scarlet or white flowers, and green pods.

Emasculation and pollination techniques were the rubbing and hooking methods described by Buishand (2). Pollinations were made between 8:00 a.m. and 10:00 a.m. Interspecific hybridization was attempted only in one direction since most other workers (1, 4, 5, 6, 8, 16, 17) have been unsuccessful in employing P. coccineus as the seed parent in crosses with P. vulgaris.

For describing colors, the "Royal Horticulture Society Colour Chart" (15) was used.

Results and Discussion

Segregation for stem, flower, and pod color in Phaseolus vulgaris. Phenotypes obtained and their respective frequencies are summarized in Table 1 for F_1 and F_2 progenies.

Table 1. Segregation for stem, flower, and pod color in Phaseolus vulgaris.

	Observed frequencies for color[z] of					
	Stem: DP	MP	LP	G		
	Flower: DP	MP	LP	W		
	Pod: DP	MP	G	G		
Generation	Seed: PT	LT	DT	W	χ^2*	P
P_1 ('Bush Blue Lake 290')				3		
P_2 ('Royal Burgundy')	7					
F_1 (P_1 x P_2)		8				
F_2 (P_1 x P_2)	50	119	71	79	7.78	>.95

[z]DP = dark purple (77A), MP = medium purple (77B), LP = light purple (84B), W = white (155D), G = green, PT = pale tan, LT = light tan, DT = dark tan. Values in parenthesis are colors of corolla standards of freshly opened flowers, based on the Royal Horticulture Society Colour Chart (15).
*Chi square was calculated to fit a ratio of 3:6:3:4

These data suggest a segregation resulting from the action of 2 pairs of genes, with both pairs controlling stem, flower, and pod color. We propose that the genes involved in this cross are P-p and a second pair which we shall initially designate as "X-x." The genotypes of the two parents would then be:

'Bush Blue Lake 290' ppxx

'Royal Burgundy' PPXX

The interactions between the two pairs of genes can be explained by the following scheme:

		Immature pod color	
Flower Color		P	p
Dark purple	XX	dark purple	green
Medium purple	Xx	medium purple	green
Light purple	xx	green	green

Based on our results, the following conclusions can be drawn.

1. Green stem and pod and white flower color are most probably due to pp, because complete pleiotropy was observed for total absence of anthocyanin in stem, flower, pod, and seed; and p is the only basic color factor that causes this combination of characters, especially the white-seeded phenotype (18). In addition, Yarnell (18) has noted that nearly all white-seeded and white-flowered cultivars are pp.

2. 'Royal Burgundy' is presumably dominant for all genes concerned, since it has dark purple stem, flower, and pod and colored seed. This means that it would be PPVV. If it is the same as

Lamprecht's (11) purple-podded Phaseolus vulgaris, then 'Royal Burgundy' would also be Ro Ro Pur Pur. Then, "X" would equal Ro, and both 'Royal Burgundy' and 'Bush Blue Lake 290' would be Pur Pur. This latter conclusion follows because no rose-podded phenotypes were observed in our F_2. Accordingly, 'Bush Blue Lake 290' would be p p V V Pur Pur ro ro. We doubt that "x" equals ro because Lamprecht (11) did not observe gradation in purple pod color, while we recognized two distinct shades of purple pod color: dark purple and medium purple. We also observed that "X-x" gave three phenotypes each for stem and flower color; however, Lamprecht (11) did not report any effect of Ro-ro on characters other than pod color.

3. If we consider Moraes and Vieira's (13) scheme, where A is proposed to be approximately equal to Ro and a^a or a to ro of Lamprecht (11), 'Royal Burgundy' would be P P V V A A. Then "X" would equal A. Accordingly, 'Bush Blue Lake 290' would be p p V V a^a a^a or p p V V a a. We doubt that "x" is the same as a^a or a because according to Moraes and Vieira (13), V-$a^a a^a$ and V-a a phenotypes gave striped purple and dark pink pods, respectively, but our equivalent genotype, "P-x x", was green-podded like Lamprecht's (11). Moreover, we observed that "X-x" affects flower color segregation, while Moraes and Vieira (13) found no flower color segregation for A versus a^a or a. Instead, they reported that of the two loci producing purple pod color, the alleles V, v^{lae}, and v controlled purple, pink, and white flower colors, respectively.

If "x" corresponds to v^{lae}, since the "x x" genotype had light purple flowers, then 'Royal Burgundy' would be P P V V A A and 'Bush

Blue Lake 290' $\underline{p}\,\underline{p}\,\underline{v}^{lae}\,\underline{v}^{lae}\,\underline{A}\,\underline{A}$. This is not possible, because $\underline{P}$-$\underline{v}^{lae}\,\underline{v}^{lae}\,\underline{A}$- F_2 segregants from this cross should have rose-colored pods, and no plants of this phenotype were obtained.

4. Frag (3) reported on the same cross as ours, using the identical parental cultivars, but he did not observe the effect of "$\underline{X}$-$\underline{x}$" on stem and flower color. Also, he did not realize that completely anthocyaninless segregants were due to $\underline{p}$. Consequently, while he found it necessary to describe a new gene, $\underline{Prp}$-$\underline{prp}$, to account for two shades of purple pods, his F_2 ratio did not agree with ours and his description of $\underline{Prp}$-$\underline{prp}$ was incomplete.

An amended description follows.

$\underline{Purple\ pod}$ ($\underline{Prp}$). $\underline{Prp}$, together with the basic color factors, as well as $\underline{V}$ and presumably $\underline{Ro}$ produces, when homozygous, dark purple flower color and immature pod color and strong anthocyanin pigmentation in the stems and leaves. In the presence of all dominant color factors, the $\underline{Prp}\ \underline{prp}$ genotype shows an intermediate level of pigmentation in foliage, pods, and flowers; while the $\underline{prp}\ \underline{prp}$ genotype has light purple flowers, weak purpling of the stems, and green pods. In purple-podded genotypes, exposed pods on the plant develop stronger anthocyanin pigmentation than those covered by the foliage.

This description is necessarily provisional until allele tests can be made, particularly with $\underline{Pur}$-$\underline{pur}$. Seed of Lamprecht's (11) original $\underline{Pur}\ \underline{Ro}$ line has recently been obtained for this purpose, and it is interesting to note that Lamprecht has also designated this line as being $\underline{V}$. The difficulty in obtaining known gene stocks for allele testing in $\underline{Phaseolus\ vulgaris}$ has led to some confusion in genetic nomenclature in this species, and the situation regarding purple pod is certainly an example of this problem.

Segregation for flower color in the interspecific cross P. vulgaris x P. coccineus. The cultivars used and the respective frequencies of phenotypes obtained are summarized in Table 2.

Table 2. Segregation for flower color in Phaseolus vulgaris x Phaseolus coccineus.

Generations	Observed frequencies for flower color[z]					
	S	SL	R	RP	P	W
P_1 (P. vulgaris 'Royal Burgundy')					7	
P_2 (P. vulgaris 'Bush Blue Lake 290')						3
P_3 (P. coccineus PI 175858 scarlet)	2					
P_4 (P. coccineus PI 175858 white)						7
F_1 (P_1 x P_3)		58				
F_2 (P_1 x P_3)	1					
BC_1 [P_1 x (P_1 x P_3)]			1	3		
F_1 (P_2 x P_3)						6
F_1 (P_2 x P_4)						5
F_2 (P_2 x P_4)						128

[z]Color of corolla standard of freshly opened flowers, based on the Royal Horticulture Society Colour Chart (15). S = scarlet (33A), SL = salmon (47D), R = red (52A), RP = reddish purple (70A), P = purple (80A), W = white (155D).

In the cross P_2 x P_4 (P. vulgaris 'Bush Blue Lake 290' x white-flowered P. coccineus PI 175858), all 5 F_1 plants produced white flowers. Owing to their high level of self fertility, all these plants, grown in the winter greenhouse, produced pods that set seeds naturally, giving a total of 128 plants in the F_2 generation. This total includes families from each of the 5 F_1 plants, the smallest family consisting of 14 plants. All of the F_2 plants were also white-flowered. Therefore, since only one gene for flower color is segregating in this line of P. coccineus, according to Ibrahim and Coyne (5), and since 'Bush Blue Lake 290' appears to be pp, it follows that P. coccineus is probably also pp.

Segregation in this F_2 of 90 pole to 38 bush types (indeterminate versus determinate) shows that there is no disturbed segregation for determinate habit, due to fin (12). The χ^2 value for this family was 1.5 based on a 3:1 ratio and had a probability of 95%.

The pollen parent of the cross P_2 x P_3 (P. vulgaris 'Bush Blue Lake 290' x scarlet-flowered P. coccineus PI 175858) was a single plant grown from a white seed. It therefore presumably was Pp, resulting directly from the cross of pp(♀) x P-(♂) parents in the generation previous to when we received the seed. We were unable to confirm the heterozygosity of this scarlet-flowered P. coccineus plant by selfing because it was self sterile. However, when it was crossed onto 'Bush Blue Lake 290', 6 white-flowered F_1 plants were obtained. All showed the indeterminate growth habit of the P. coccineus parent and were intermediate between the two parents in leaf shape. If 'Bush Blue Lake 290' is pp and this plant of P. coccineus is Pp, then the probability of obtaining 6 F_1 plants all white-flowered from this cross is $(\frac{1}{2})^6 = 0.0156$, an unlikely event. Using white-flowered and white-seeded 'Great Northern' cultivars,which are presumably pp, as female parents, Ibrahim and Coyne (5) observed all pink-flowered F_1 plants from this cross and all scarlet-colored F_1 plants in the reciprocal cross. They concluded that the pink-flowered F_1 plants were due to a genic-cytoplasmic interaction such that the cytoplasm of P. coccineus was necessary for complete dominance of the genes from P. coccineus for scarlet-colored flowers. The complete absence of scarlet-colored flowers in our F_1 could be due to complete recessivity of the genes for scarlet flower color in P. vulgaris 'Bush Blue Lake 290' cytoplasm. However, in the P_1 x P_3 (P. vulgaris 'Royal Burgundy' x scarlet-flowered P. coccineus) cross to be discussed below, the

genes for scarlet flower color were expressed in this P. vulgaris cytoplasm, since the flowers of all F_1 plants were salmon colored (Table 2), and this has also been observed by Lamprecht (10). Consequently, we feel that preferential fertilization of the P. vulgaris parent by p gametes from the P. coccineus parent is a more likely explanation. The discrepancy between normal Mendelian expectations for this cross on the one hand and the differing but abnormal results obtained by Ibrahim and Coyne (5) and by us on the other hand clearly indicate the need for further study. It should be noted that our F_1 plants of this cross, which we presume should be pp and identical to those produced from our cross of $P_2 \times P_4$ (P. vulgaris 'Bush Blue Lake 290' x white-flowered P. coccineus), were completely self sterile and would not set seed naturally or by artificial self pollination, while the F_1 plants from the $P_2 \times P_4$ cross were highly fertile. We have no explanation for this.

In the cross $P_1 \times P_3$ (P. vulgaris 'Royal Burgundy' x scarlet-flowered P. coccineus PI 175858), due to previous difficulty by Frag (3) in obtaining segregating generations from this cross, a large F_1 consisting of 58 plants was produced in order to have greater opportunity for fertility in selfs, sib crosses, and backcrosses. However, all F_1 plants were self sterile, and from over 130 sib crosses made only 1 F_2 plant was obtained. Two techniques described by Ibrahim and Coyne (5) to reduce embryo abortion in the F_1 were employed but with no success. These are the "Ziploc" plastic bag technique and partial breakage of the pedicels to prevent the translocation of embryo resorption substances from the leaves to the pods. Also, the application of a mixture of indoleacetic acid and parachlorophenoxyacetic acid on the pedicels, as suggested by Al-Yasiri and

Coyne (1), to obtain large embryos by delaying pod abscission and embryo abortion failed. However, the F_1 plants were used successfully as pollen donors in backcrosses to P. vulgaris 'Royal Burgundy'. Although only 4 backcross plants were obtained (Table 2), the observed 3:1 (purple versus red flower color) segregation ratio agrees with Frag's (3) proposal, namely, that 2 pairs of dominant, unlinked genes from P. coccineus control the inheritance of its scarlet flower color.

Our results do not agree with the single gene inheritance described for this cross by Ibrahim and Coyne (5). As we have observed above, what they observed was probably not a color gene per se but the color inhibitor, p. Our F_1 flowers were fully pigmented (salmon), while theirs were diluted pink (geranium pink). The salmon-colored F_1 we observed indicates nearly complete epistasis of the scarlet color genes from P. coccineus over the V allele from 'Royal Burgundy'.

The present results also do not show any indication that 4 different dominant genes from P. coccineus are required for the production of scarlet flowers as hypothesized by Lamprecht (10, 12). Although we did not recover the scarlet color of P. coccineus or the similar salmon color of the F_1 in the backcross, our single scarlet-flowered F_2 plant and the backcross red-flowered segregant (Table 2) are consistent with the color shift from orange red to purplish red observed upon interspecific backcross transfer of genes for scarlet corolla from Sinningia cardinalis H. E. Moore into Sinningia eumorpha H. E. Moore (Clayberg, unpublished). The BC_1 color shift is probably due to background modifiers in 'Royal Burgundy', but further backcrosses and sibcrosses are needed to verify this assumption.

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ABSTRACT

Intraspecific crosses with P. vulgaris show that the dark purple color of the cultivar 'Royal Burgundy' is due to an interaction of 2 unlinked genes: P and Prp, the latter gene completely described here for the first time. Interspecific test crosses between different genotypes of P. vulgaris and of P. coccineus revealed that white flower in PI 175858 of the latter species is due to p and that in a P. coccineus heterozygote, Pp, the p male gamete appears to function preferentially in the interspecific cross with P. vulgaris. Further corroborating evidence was obtained that the scarlet flower color of P. coccineus is determined by 2 dominant, unlinked genes in this interspecific cross.