The Dark Corona Character in Seedcoats of Common Bean Cosegregates with the Pink Flower Allele v^{lac}

Mark J. Bassett¹

Horticultural Sciences Department, IFAS, University of Florida Gainesville, FL 32611

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Abstract. Crosses were made with two common bean (*Phaseolus vulgaris* L.) parents that have pink flowers ($v^{lac}/-$) and mineral-brown seedcoats with dark corona, viz., $v^{lac}BC_35-593$ (derived from Lamprecht V0491) and F_3v^{lac} dark corona (derived from Lamprecht M0048). The third parent vBC_25-593 had white flowers (v/v) and mineral-brown seedcoats without dark corona (derived from Lamprecht M0056). The F_2 progenies of the crosses $vBC_25-593 \times v^{lac}BC_35-593$ and F_3v^{lac} dark corona $\times vBC_25-593$ segregated for only two phenotypic classes: either pink flowers and seeds with dark corona or white flowers and seeds without dark corona. Thus, it was demonstrated that the dark corona character (*Cor*) is either tightly linked to v^{lac} (<4 map units) or is a pleiotropic effect of v^{lac} . Pleiotropy is more probable, but tight linkage cannot be ruled out. A linkage of 15 map units between *Cor* and *R* (currently, *R* is known to be tightly linked with *C*) reported by Lamprecht was not found by subsequent authors, and the linkage map of common bean should be revised accordingly, i.e., no linkage exists between *V* (*Cor*) and *C*.

In 1985 a program was initiated at the Univ. of Florida to develop genetic stocks with selected marker genes of common bean (*Phaseolus vulgaris*) in a common genetic background by backcrossing to a recurrent parent. The recurrent parent was a Florida dry bean breeding line, 5-593, that had been developed before 1985. One of the recessive marker characters that was backcrossed into 5-593 was the pink (laelia) flower trait controlled by the v^{lac} allele, where the dominant allele *V* produces bishops violet (purple) flowers and the fully recessive allele *v* produces pure white flowers (Lamprecht, 1935; Prakken, 1970). The genetic stock carrying the pink flower allele v^{lac} was discovered to have a dark (black) corona around the hilum scar of the seeds, which had a mineral-brown color over the remainder of the seedcoat. The dark corona character was reported by Lamprecht (1934) to be controlled by the *Cor* locus. This paper describes the results of an investigation to test the hypothesis that the v^{lac} allele has an unreported pleiotropic effect on seedcoat color pattern, viz., production of a dark corona.

Materials and Methods

Florida dry bean breeding line 5-593 has determinate habit, purple flowers, and shiny, pure black seedcoats. The seedcoat genotype of 5-593 is *T P [C r] D J G B V Rk* (Bassett, 1994; Prakken, 1970, 1972).

Following the usual recurrent backcross procedure for developing the genetic stocks referred to above, there was strong selection in the F_2 generation of each successive backcross for the phenotype of the recurrent parent. The goal was to create a genetic stock that fully recovered the appearance of 5-593 except for the selected marker trait, i.e., there was strong selection against all other marker traits. When the allele *v* was substituted into the genetic background of 5-593, giving *P C D J G B v*, it had two pleiotropic effects, viz., changing purple flowers to pure white and changing pure black seedcoats to mineral brown (Prakken, 1970). The source of the *v* allele was Lamprecht Line M0056 (now PI527830),

which is known to carry *v* (Bassett et al., 1990). The resulting stock was designated vBC_25-593 , indicating two backcrosses to 5-593 with strong selection for the recurrent parent phenotype. The genetic stock $v^{lac}BC_35-593$ was created in a similar manner, using Lamprecht Line V0491 (now PI 527745) as the source of the v^{lac} allele. When the v^{lac} allele was substituted into the genetic background of 5-593, giving *P C D J G B v^{lac}*, it had two well-established pleiotropic effects, viz., changing purple flowers to pink and changing pure black seedcoat color to mineral brown (Prakken, 1970). The resulting stock was designated $v^{lac}BC_35-593$, indicating three backcrosses to 5-593 with strong selection for the recurrent parent phenotype. However, $v^{lac}BC_35-593$ had an additional trait not reported in previous literature, viz., a dark (black) corona. The corona phenotypes of $v^{lac}BC_35-593$ (dark corona) and vBC_25-593 (no dark corona) are illustrated in a drawing of the seedcoat patterns on the ventral side of the seeds (Fig. 1). The corona phenotype of vBC_25-593 was slightly darker brown than the surrounding mineral-brown seedcoat and was described as having no dark corona (Fig. 1). For additional illustration and discussion of the corona character a good current reference was provided by Leakey (1988).

An additional source of pink flowers and the v^{lac} allele was used in the investigation, viz., Lamprecht Line M0048 (now PI527829). The letter M signifies multigaris in Lamprecht's terminology, i.e., a line that is derived from the interspecific cross *P. vulgaris* $\times$ *P. coccineus* (formerly *multiflorus*). Line M0048 has dark seal brown seedcoat color and determinate habit. The dark seal brown color is hypothesized to be the expression of the genotype *P [c R] J G B v^{lac}* (M.J. Bassett, unpublished data). The cross M0048 $\times$ 5-593 was used to substitute the *[C r]* allele of 5-593 for the *[c R]* allele of M0048. Thus, an F_3 progeny was obtained that was true breeding for pink flowers and mineral-brown seedcoats with dark corona (*P [C r] J G B v^{lac}*). The line is designated F_3v^{lac} dark corona.

Two crosses were made to test the hypothesis that the dark corona character is not controlled by a gene *Cor* that is independent of *V*, but is a pleiotropic effect of v^{lac} . The first was $vBC_25-593 \times v^{lac}BC_35-593$, and the "second" was F_3v^{lac} dark corona (seven F_3 plants used) $\times vBC_25-593$. The F_2 plants from the first cross were grown in the field in Spring 1993 and the F_2 from the second cross were grown in the field in Spring 1992. Data were taken on the flower and seedcoat color of F_1 and F_2 progeny. A single seed was harvested from each plant, keeping the seeds from the two flower

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¹Professor.

color classes separate. The seeds were later examined for any indication of segregation for phenotypic classes.

I wrote letters to the curators of several common bean germplasm collections in Europe requesting seed of the two parental lines used by Lamprecht (1934) in the experiments leading to the discovery of *Cor*, viz., 'de la Chine' (Lamprecht Line 29) and 'Pariser Gelbe' (Lamprecht Line 23). Neither of the parental lines used by Lamprecht (1934) are extant in the U. S. Department of Agriculture plant introduction collection (at Pullman, Wash.) of Lamprecht's experimental materials, PI 527711 through PI 527878 (168 accessions). I was unsuccessful in obtaining seed of 'de la Chine', but I obtained 'Pariser Gelbe' (BGRC #25351) from the Institut für Pflanzenbau der Bundesforschungsanstalt für Landwirtschaft (FAL), Bundesallee 50, D-38116 Braunschweig, Germany. The seedcoat and corona colors of 'Pariser Gelbe' (#25351) are identical to the description of Lamprecht (1934), and the seed shape is also the same as his illustration. Seed of 'Pariser Gelbe' (#25351) was grown in the greenhouse at Gainesville and data were taken on the flower color.

Results and Discussion

The F_1 plants from the cross v BC₂5-593 x v^{lac} BC₃5-593 had pink flowers (v^{lac}/v) and mineral-brown seeds with dark corona (data not shown). The F_2 segregated for only two phenotypic classes rather than the four classes that would be expected if flower color and dark corona segregated independently (Table 1). Plants with pink flower color always had dark (black) corona, and plants with white flowers always had no dark corona. Thus, there must be either tight linkage between the genes controlling the two characters or pleiotropic effects originating from the v^{lac} allele.

There was full dominance for the dark corona trait, whereas Lamprecht (1934) reported partial dominance for dark corona. However, it should be noted that the parental materials of Lamprecht had seedcoat genotypes that were different from the parents in Table 1. For example, Lamprecht Line 23, 'Pariser Gelbe,' had genotype $P C j g b v$ with corona, and Line 29, 'de la Chine,' had genotype $P C j g b v$ without corona. It is probable that 'Pariser Gelbe' carried V^{lac} , but Lamprecht (1934) did not give information

on the flower color and probably omitted the superscript lac as an irrelevant distraction. This view is supported by the observation that 'Pariser Gelbe' (#2535 1) had pink flowers, which is characteristic of plants with v^{lac} .

The dark corona in v^{lac} BC₃5-593 was black, whereas the corona in 'Pariser Gelbe' was a light purple color. It is my hypothesis that the action of the recessive alleles at *G* and *B* greatly reduced the quantity of anthocyanin pigment present (M.J. Bassett, unpublished data; Prakken, 1970). Therefore, the reduction in pigment produced by the heterozygote *Cor/cor* (really v^{lac}/v) gave a much paler purple corona (Lamprecht, 1934). The genotype *cor/cor* (really v/v) had no corona (Lamprecht, 1934). The parental lines derived from 5-593 had dominant alleles at *G* and *B* and, therefore, had a much higher concentration of anthocyanin (Table 1). Thus, the heterozygote v^{lac}/v did not produce a discernible reduction in pigment, but it is probable that a reduction actually took place that is proportional to the one observed by Lamprecht (1934). It is my hypothesis that the absolute concentration of pigment must be reduced to some critical threshold level for the loss to be discernible to the naked eye. Clearly, the partial dominance of *Cor* (v^{lac}) for corona expression depends on the background genotype for visual evaluation of its expression.

The F_1 plants from the cross $F_3 v^{lac}$ dark coronaxvBC25-593 had pink flowers and mineral-brown seed with dark corona (data not shown). In the F_2 progeny, only two phenotypic classes were observed, viz., plants with pink flowers and seeds with dark corona on mineral brown or plants with white flowers and seeds with no dark corona on mineral brown (Table 1). As with the previous cross, there was no independent segregation of flower color and corona color. One must conclude that either the genes are linked or the two characters are pleiotropic effects of a single locus, *V*. Considering the data from both crosses (Table 1), the possible linkage would be less than four map units, as estimated by adding a single (hypothetical) crossover event to the observed data and using the maximum likelihood equations of Allard (1956). One way to symbolically represent such tight linkage is to use the bracket convention, where two or more tightly linked genes are written with their gene symbols enclosed in brackets (Bassett, 1991). For the materials used in the above experiments, the

Fig. 1. The seed on the left (v BC₂₅-593) has no dark corona (light shading indicates a slightly darker brown than the surrounding mineral brown), whereas the seed on the right has dark (black) corona (v^{lac} BC₃₅-593).

Table 1. Segregation for flower and seed coat color in the F₂ from the crosses v BC₂5-593 x v^{lac} BC₃5-593, and F₃ v^{lac} dark corona x v BC₂5-593.

Cross no.	Pink flowers, mineral-brown seeds with dark corona ^z ($v^{lac}/-$)	White flowers, mineral-brown seeds with no dark corona ^y (v/v)	χ^2 3:1	P value
1	269	107	2.397	0.12
2	1247	650	86.84	<0.001

^zThe dark corona is black in those genotypes because of dominant *B*. In other genetic backgrounds, v^{lac} produces various shades of purple in the corona, e.g., with recessive *b* or *g b*.

^yThe corona is slightly darker brown than the surrounding mineral brown of the remainder of the seedcoat.

symbols would be [v^{lac} *Cor*] for plants with pink flowers and dark corona and [v *cor*] for plants with white flowers and no corona (Table 1).

The segregation for pink and white flowers in the F₂ from the cross F₃ v^{lac} dark corona x v BC₂5-593 was highly disturbed in relation to the expected 3:1 ratio for pink to white, respectively (Table 1). That result is not surprising, considering that the F₃ v^{lac} dark corona parent was derived from M0048. The initial cross M0048 x 5-593 also had a highly disturbed 3:1 F₂ segregation at the *V* locus for purple to pink flowers, respectively (data not shown). Disturbed segregation ratios in the progenies from interspecific crosses involving *P. vulgaris* and *P. coccineus* are common and have been frequently reported (as reviewed by Smartt, 1970).

Lamprecht (1961) reported a linkage of 15 map units between *Cor* and *R* and incorporated that linkage into his linkage group I. The current view is that *R* is tightly linked in the complex *C* locus (Bassett, 1991; Prakken, 1974). If *Cor* is tightly linked to v^{lac} as demonstrated by the data (Table 1), then the linkage of Lamprecht (1961) translates into a linkage of 15 map units between *V* and *C*. No such linkage was found in the extensive work by Nakayama (1960, 1964, 1965, 1968) and Prakken (1972) with materials segregating jointly at *V* and *C*. Linkage group I should be revised accordingly, viz., splitting linkage group I into two separate linkages: *D* with *V* and *C* with *tri*. The *D* locus controls hilum ring color, *C* controls seedcoat color, and *tri* controls tricotyledony (Bassett, 1991).

The data presented above are sufficient to demonstrate tight linkage between dark corona color and pink flower color, but other observations suggest that the better hypothesis is that dark corona is a pleiotropic effect of v^{lac} . I have observed many seedcoat

genotypes in the course of my career and have never observed either of the putative crossover phenotypes, e.g. (v *Cor*) with pure white flowers and mineral-brown seeds with dark corona due to the genotype *T P C J G B v*, or (v^{lac} *cor*) with pink flowers and mineral-brown seedcoats with no dark corona due to the genotype *T P C J G B v^{lac}*. Until such recombinant have been found and verified, the more probable hypothesis is pleiotropy.

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