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TWO CROSSES WITH THE "NEBULOSUS-MOTTLED" VARIETY CONTENDER (Ane Ane, from the recessive character anebulosus)

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In 1964 Lamprecht tried to analyse "Die Vererbung eines neuen Typs von Marmorierung der Samen von Phaseolus vulgaris L." (1), based on the analysis of two crosses with the variety Contender. The seedcoat of Contender is "cloudy grayish brown mottled" (veined) on a cartridge buff background (Ane Ane, l.c. Fig. 9); it has a brown hilumring (the "hilumring factor" D is hypostatic in relation to the "shine factor" J, that produces a brown hilumring too), and it is shiny and afterdarkening. Genotype: (PP TT) c^uc^u (dd) JJ Ane Ane. It is known that in the c^uc^u-background type the modifying genes G (yellowbrown factor), B (gray-greenish brown factor) and V (violet factor) are almost without influence; the violet flower colour shows that it is VV. In 1968 Nakayama and Saito (2) also tried to analyse two Contender-crosses.

The articles of both authors, Lamprecht and Nakayama c.s., are extremely difficult to penetrate, mainly depending upon the fact that Lamprecht, and in agreement with him Nakayama too, wrongly considered the genes G and B (see above) as colour genes, each of which, together with the basic gene P, should produce a brown hilumring, leaving the remaining part of the seedcoat almost uncoloured. Prakken (3) extensively showed that G and B are not colour genes but merely modifying ones and therefore, together with P only, are of no influence on the (white) seedcoat colour. This misconception about the action of G and B, together with the intricacy of both articles and some difference in explanation between Lamprecht and Nakayama, had ~~with~~ the result that, after my retirement in 1969, I studied the Contender-case myself. [In a personal correspondence of Jan.-Feb. 1970 Nakayama wrote that, with respect to the yellowbrown factor G, he had come to the same conclusion as the present author and that, with respect to B, he was not yet sure; he observed that, in the Contender-article, some clear B-colours did not show a brown hilumring.] My own two analyses are discussed below.

1. The cross between Contender and "La Gaude."

The variety Contender showed the usual violet flower and nebulosus-mottled seedcoat; genotype: (PP TT) c^uc^u (dd) JJ gg BB VV Ane Ane zz (for zz [illegible] cf. below).

The variety "La Gaude" is mentioned in Annual Report of the B.I.C. 18, 1975, p. 61 (4). Its seedcoat is black striped on a cartridge buff background with strong nebulosus-mottling (Ane?), and it is shiny and afterdarkening (J). The flower is violet (V) while its standard shows sharply contrasting blackish violet veins (a character as far I know not analysed before), diverging from the narrow base to the periphery, giving, in the bud stage, the flower a zebra-striped appearance (ZZ). If the new C-allele for black (non-red) stripes upon an unchangable background (like c^uc^u) preliminary is indicated as $C^{L.G.}$, the genotype of "La Gaude" is (also on the ground of other crosses):

(PP TT) C^{LG} C^{LG} (DD) JJ gg BB VV Ane Ane ZZ.

The F_1 genotype therefore is (heterozygous loci double underlined):

(PP TT) C^{LG} c^u (Dd) JJ gg BB VV Ane Ane Zz

Its seedcoat colour was the same as in the "La Gaude" line itself (black stripes/nebulosus-mottling/cartridge buff), the flower colour violet with a, less conspicuous, zebra-veining in the standard.

The F_2-segregation actually was for $C^{L.G.}$-c^u and for Z-z only, three small families, together 81 plants:

53 $C^{L.G.}$. (exp. 60.7) + 28 c^uc^u (exp. 20.3).

The fine nebulosus-mottling in the seeds of F_2 usually was stronger than in Contender and not missing in any plant: Ane Ane. Between the black stripes of C^{LG} $C^{L.G.}$ - and C^{LG} c^u-plants no difference could be found. The accompanying colours, not to be discussed here, behaved as usual: linked with $C^{L.G.}$-c^u. Very striking was that all striped-seeded $C^{L.G.}$-plants more or less clearly showed the blackish violet zebra-like veins in the standard (Z.), but that in all c^uc^u-plants they were completely lacking; conclusion: Z is lying very near the complex locus C or may even belong to it. Further studies are concentrated upon this question.

2. The cross between Contender and Citroenboon.

The Contender-parent belonged to the same line as in cross 1, above.

The seeds of the Citroenboon-parent (flower pale lilac $V^{lae}V^{lae}$) are self-coloured dark pattern type (CC), (pale) greenish yellow (GG bb), without brown hilumring and non-afterdarkening. Genotype:

(PP TT) CC dd jj GG bb $V^{lae}V^{lae}$ ane ane(?) zz.

The F_1-genotype therefore is (segregating loci double underlined):

(PP TT) Cc^u (dd) Jj Gg Bb VV^{lae} Ane ane zz

Leaving out, at first, the segregation for G-g, B-b and Ane-ane, the F_2-plants showed 3 x 2 x 2 = 12 seedcoat types (Table 1).

The segregation for each of the three single gene pairs was:

Table 1. The 12 main seedcoat colour types in F_2 of Contender with Citroen; (dd) jj without brown hilumring and with a white background mottling (C[illegible] c^u) or completely white (c^uc^u).

dark pattern type, CC				heteroz. mottled type, Cc^u				background type, c^uc^u				total
J. V.	J. $V^{lae}V^{lae}$	jj V.	jj $V^{lae}V^{lae}$	J. V.	J. $V^{lae}V^{lae}$	jj V.	jj $V^{lae}V^{lae}$	J. V.	J. $V^{lae}V^{lae}$	jj V.	jj $V^{lae}V^{lae}$	
19	9	3	0	42	13	19	5	19	10	6 (W)	0 (W)	145
20.4	6.8	6.8	2.3	40.7	13.6	13.6	4.6	20.4	6.8	6.8	2.3 exp.	

for C-C^u: 31 CC + 79 Cc^u + 35 c^uc^u (exp. 36.25)
for J-j: 112 J. + 33 jj (exp. 36.25; without hilumring)
for V-V^{lae}: 108 V. + 37 $V^{lae}V^{lae}$ (exp. 36.25; pale lilac flower).

For all three therefore a good agreement with the 3:1 segregation, (probably independent from each other.

As already known the gene pairs G-g, B-b (and V-V^{lae}) are (almost) without influence on the c^uc^u background type. In the dark pattern colour of Cc^u and CC each of the three has its own characteristic influence, more clearly however in the shiny J.-types than in the mat (dd)jj-types. The complete analysis for the three modifying gene pairs, G-g, B-b and VV^{lae} will therefore not be given here. It may only be pointed out that among the 112 J.-plants all seven expected colours were represented: 1. greenish yellow (= schamois), 2. yellowbrown, 3. gray greenish brown, 4. brown, 5. (dark)blue violet, 6. (dark) brown violet and (by far the most frequent 7. and 8. black (gg B. V. + G. B. V.).

The influence of Ane is most clearly visible in the c^uc^uJ. background types. The 35 c^uc^u-plants (Table 1) appeared to consist of: 22 (dd) J. Ane. (more or less clear "nebulosus" upon cartridge buff) + 7 (dd) J. ane ane (purely cartridge buff) + 6 (dd) jj..... (purely whiteseeded; Ane not working), probably representing the ratio 9:3:4. In the selfcoloured dark pattern type CC (31 plants) the action of Ane-ane is not at all observable, nor is it in the white background mottling of the 24 (dd) jj Cc^u mottled seeded plants. In the cartridge buff background of the 55 dd J. Cc^u-plants, however, the action of Ane-ane can be observed: it appeared that (almost?) all B.-plants (with the gray-greenish brown factor) were Ane. and (almost?) all bb-plants ane ane, which is in agreement with Lamprecht's suggestion (1, p. 270) of a (rather strong) linkage between B and Ane.

The F_2-results as described above were in agreement with the results of 14 F_3-families, with 16-32 plants per family.

(Presence of the shine-factor J is usually called necessary for the action of Ane; but about an eventual action in D. jj nothing can be concluded, as in the two crosses of all three authors, Lamprecht, Nakayama and Prakken,

the one cross was dd JJ (Cont.) with ... JJ (all F_2-plants JJ) and
the other cross was dd JJ (Cont.) with dd jj (all F_2-plants dd)).

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CROSSES WITH SOME *Phaseolus* VARIETIES THAT ARE "CONSTANTLY PATTERNED WITH A DARK PATTERN COLOUR"

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In B.I.C. Annual Report 18, 1975, the present author wrote a note about A "forgotten group" of seedcoat colours in *Phaseolus vulgaris* L., viz. those varieties with a "constant pattern in the dark-pattern-colour of the ever-segregating-mottling" (6).

Since the beginning of the century two types of mottling have been distinguished, viz. a. ever-segregating-mottling and b. constant mottling.
a/ The ever-segregating type of mottling is in the colours from pale (greenish) yellow to black. It is usually "explained" by accepting that the heterozygote Cc (or c^u) is mottled:
~~and~~ a darker colour (like that in the homozygous CC = dark pattern colour and a paler colour (like that in the homozygous cc = background colour).

An old alternative explanation is to ascribe the ever-segregating mottling to two genes, (almost) absolutely linked, Yz and yZ, or in more recent symbols Cm and cM, M locally suppressing the darker colour produced by C.

If the basic gene P and the colour gene J (shine factor) are present, the P.CC J. "dark pattern colours", caused by the action of the modifying genes G (yellow brown f.), B (gray-greenish brown f.) and V (violet f.) are usually sharply distinguishable:

1. P. CC J. gg bb vv = pale greenish yellow
2. " " " G. bb vv = yellow brown
3. " " " gg B. vv = gray-greenish brown
4. " " " G. B. vv = (coffee) brown
5. " " " gg bb V. = dark blue violet
6. " " " G. bb V. = dark brown violet
7. " " " gg B. V.
8. " " " G. B. V. } = black

the corresponding paler "background colours" (cc) will not be discussed here.

b/ In the constant mottling (striping, etc. see Lamprecht (1)), the starting colour of the series 1-8 is not pale greenish yellow but (lilac) red. The red colour is usually ascribed to the multiple allelic series of R: R (selfcoloured red), $R^{m(a)}$ (red mottled), R^{st} (red striped) etc., see Lamprecht 1947 (1). In case of R^{st} and again in the presence of basic gene P and colour gene J, the colour series (of the stripes), caused by the modifying genes G, B, and V is:

1. P. R^{st} . J. gg bb vv = (lilac) red striped
2. " " " G. bb vv = orange to br. red str.
3. " " " gg B. vv = grayish violet str.
4. " " " G. B. vv = dark (black) brown str.
5. " " " gg bb V. = reddish dark purple str.
6. " " " G. bb V. = more br. dark purple str.
7. " " " gg B. V. } = blackish str.
8. " " " G. B. V. }

} background always the same: pale buff

Crosses of "dark pattern type" <u>CC (rr)</u> with e.g. "red striped", $\underline{c^u c^u R^{st} R^{st}}$ produce F_1-plants with three-coloured seeds: ever-segregating-mottled and "red" striped: $\underline{Cc^u\ R^{st} r}$. <u>C</u> and $\underline{R^{st}}$ appear, like C and M above, (almost) absolutely linked:

F_1, $\overset{\frown}{Cr}\ \overset{\frown}{cR^{st}}$, segr. in F_2 into
- 1/4 $\overset{\frown}{Cr}\ \overset{\frown}{Cr}$ (homoz. dark pattern col.)
- 2/4 $\overset{\frown}{Cr}\ \overset{\frown}{cR^{st}}$ (heteroz., three-coloured)
- 1/4 $\overset{\frown}{cR^{st}}\ \overset{\frown}{cR^{st}}$ (homoz. red striped)

These (almost?) absolute linkages between <u>C-M-R-St-.....</u> make that some authors considered all "alleles" as belonging to one "locus" <u>C</u>. Up to now of this "<u>locus C</u>" about 20 "alleles" are known, and systematic designation of new "<u>alleles</u>" becomes more and more difficult (cf. below).

In Prakken IV, 1974 (5), he tried to find the solution in more or less the reversed way. In Fig. 1 (l.c. p. 31) he tentatively pictures the "<u>complex locus C</u>" as consisting of (at least) <u>six sub-loci.</u> He found a few cases of crossing-over, localised between sub-locus <u>M</u> (for ever-segregating mottling) and $\underline{c^u}$ (one of the <u>C-alleles</u> s. s.) and thereby he received a new complex gene, indicated as $\underline{C^{m'}}$ (fig. 1, to the right), containing <u>M Pr Acc C r st,</u> and, after homozygotising the new complex locus, it appeared to produce <u>a "constant mottled type"</u>, <u>with the mottling in dark pattern colour:</u> $\underline{\overset{\frown}{CM}\ \overset{\frown}{CM}}$.

[Nakayama in art. VIII, 1965 (2) received, according to me, in an F_2-family with ever-segregating mottling, the complementary "allele", $\underline{\overset{\frown}{cm}}$, that must be present in the selfcoloured F_2 dark pattern plant that "monofactorially" segregated into 3/4 selfcoloured dark pattern type + 1/4 background type, i.e. <u>no mottled types</u>: F_2-plant $\underline{\overset{\frown}{Cm}\ \overset{\frown}{cm}}$ segregated 1/4 $\underline{\overset{\frown}{cm}\ \overset{\frown}{cm}}$. Nakayama named the new "allele" C^{cr}, from <u>c</u>ompletely <u>r</u>ecessive.]

In his above mentioned note on <u>A "forgotten group" of seedcoat colours,</u> the present author (6) shortly mentioned, safe for his own (<u>mottled</u>) <u>CM CM</u> cross-over type, two more varieties that are "patterned with a dark pattern (colour":

(constant

"<u>La Gaude</u>", black <u>striped</u> on cartridge buff (cf. preceding art.).
"<u>Pinto</u>", greenish brown <u>dotted</u> on cartridge buff (i.e. the stripes broken up into shorter elements). From their crosses with selfcoloured dark pattern types like "Wagenaar" and "Citroen" (seed of F_1 mottled with dark pattern colour, the pattern one-coloured and of intermediate extension between the selfcoloured and the patterned parent), their genotypes were concluded to be:

"La Gaude": $\underline{(PP\ TT)\ C^{LG}C^{LG}\ DD\ JJ\ gg\ BB\ VV\ (Ane\ Ane\ ZZ)}$;
"Pinto" (F) : $\underline{(PP\ TT)\ C^{Pi}C^{Pi}\ DD\ JJ\ gg\ BB\ vv\ (.?\ .?\ ZZ)}$.

The "Pinto" flower was first looked at as <u>vv</u>, <u>pure white</u>, but in an F_2 with $\underline{v^{lae}v^{lae}}$, clear zebra-striping was found, <u>Z.</u>; close inspection of "Pinto" later on learned that it really is <u>ZZ</u>, with the <u>ZZ</u>-striping in the standard however, because of vv, restricted to a small lilac stip with short extensions into the veins, at the basis of the standard. Not yet feeling quite sure to use the six-(sub-)~~loci~~-indication, I preliminary gave the new "C-alleles" a symbol derived from the name of the variety: C^{LG} resp. C^{Pi}, cf. the genotypes above.

Crosses of "La Gaude" and "Pinto" with resp. CC (dark pattern)-, cc (background)- and c^uc^u (unchangeable background)-types were intensely studied. The characteristics of their F_1 and F_2 however, will not be discussed here, but only the striking results of crosses with the red striped "kievitsboon" will be given, in general terms, based upon detailed analysis:

$C^{LG}C^{LG}$ (black striped "La Gaude") } with $C^{st}C^{st}$ brown-red striped kiev-
$C^{Pi}C^{Pi}$ (gr. brown dotted "Pinto") } itsboon (PP TT) $C^{st}C^{st}$. JJ GG bb $v^{lae}v^{lae}$

The F_1 genotypes of the two crosses (heteroz. loci double underlined) are:
"La Gaude" × "kievit": (PP TT) $C^{LG}C^{st}$. JJ Gg Bb Vv^{lae};
"Pinto" × "kievit": (PP TT) $C^{Pi}C^{st}$ .. JJ Gg Bb $v^{lae}v$.

Important is that the seeds of both F_1's showed, more or less clearly, ! the patterns of both parents, each pattern in about its own form and colour:
$C^{LG}C^{st}$ dark pattern striping + "red" striping;
$C^{Pi}C^{st}$ dark pattern dotting + "red" striping.

Not before having studied the F_2-segregations, the existence of this "double pattern" became quite clear. Generally spoken the F_2's contained:
1/4 of the one parent type (striped resp. dotted in dark pattern colour,
1/4 of the other parent type (striped resp. dotted in "red" colour), plus
2/4 of the F_1-type (double patterned, in d. p. and "red'colour).
(Each of these three types showed it's specific "accompanying colours" in cotyledons, hypocotyl, plant, flower and fruit, but this is further left out.)

In the seedcoat of the double patterned heterozygotes the parallel influence of the modifying genes G, B and V could, within each colour series, be followed as combination of the two corresponding homozygotes. The exact localisation of the two colours has to be studied further, but the impression is that C^{LG} resp. C^{Pi} is dominant over (or may be better: is epistatic over) C^{st}. See the colour series 1-8 on pp. 35 and 36.

If one compares the two colour series 1-8 given above, it immediately becomes clear that the contrast between the two colours in the heterozygotes is:
very conspicuous in 1 and 2,
medium clear in 3 and 4,
still less clear in 5 and 6 and
not or hardly observable in 7 and 8.

An accurate reader will observe the strong resemblance of my results with those of Nakayama and Saito, art. XI, 1975 (3). They cross two "narrow striped" types (both certainly of the "red" C^{st}-type) with a "broad striped" one, indicated as $C^{BS}C^{BS}$. The seeds of F_1 showed both patterns, each in their own colour (l.c. Fig. 1). The colour of the "broad striped" type is dark violet purple, i.e. the dark pattern colour (PP TT) CC .. JJ gg bb VV. The F_2 normally segregates into 1/4 $C^{BS}C^{BS}$ + 2/4 $C^{BS}C^{s(t)}$ + 1/4 $C^{s(t)}C^{s(t)}$. F_1 being VV^{lae}, in F_2 there appear about 1/4 $V^{lae}V^{lae}$, of which 1/4 $C^{BS}C^{BS}$ $V^{lae}V^{lae}$. This F_2-"broad striped" type shows a rather "intriguing" colour, indicated as Terra-cotta. The investigators apparently are not quite certain whether it belongs to the greenish yellow to black "dark pattern series" or to the red to blackish "red series". My (very tentative) supposition is that it may be the dark pattern colour yellowbrown (PP TT) CC .. JJ GG bb $V^{lae}V^{lae}$, that during two or three years afterdarkens to a colour very near Terra-cotta. The broad striped parent $C^{BS}C^{BS}$ then needs to have been not gg bb VV, dark violet purple, but GG bb VV, i.e. slightly more brownish; this is a not very surprising supposition, for in the same article the authors write about "the difficulty of dividing G.-plants from gg-ones on the seedcoat colour." With the remark of Nakayama and Saito that "when the two types are supposed to overlap each other, the colour of the broad stripe dominates", my own observations seem to be quite in agreement, though further detailed study is necessary.

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