THE GENETICS OF REACTION TO PEPPER VEIN-BANDING VIRUS

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PEPPER vein-banding virus causes a troublesome disease of cultivated peppers (*Capsicum* spp.) in Puerto Rico (ADSUAR 1940) and in Trinidad. A rather similar disease occurs in Hawaii (KIKUTA 1947). Serological investigations (PEREZ and ADSUAR 1955) indicate that the virus responsible for the West Indian disease is a strain of potato virus Y. In Trinidad, DALE (1954) found that three distinct seedling reactions were shown by various strains of cultivated pepper and this observation prompted the genetical investigation reported here.

MATERIALS AND METHODS

Four pepper varieties were used:

1. 'Ford Hook' (FH): a commercial sweet pepper from the U.S.A., much grown in Trinidad from imported seed. A strain of *Capsicum annuum* L.
2. 'Puerto Rico Wonder' (PRW): *Capsicum annuum* L., bred in Puerto Rico for resistance to virus and introduced thence to Trinidad by W. T. DALE. ROQUE and ADSUAR (1941) and RIOLLANO, ADSUAR and RODRIGUEZ (1948) have described the breeding of PRW from the susceptible 'California Wonder' crossed by the resistant Mexican pepper 'Cuaresmeno'.
3. 'Large Bell Hot' (LBH): a commercial hot pepper from the U.S.A. introduced to Trinidad by W. T. DALE. A strain of *Capsicum annuum*.
4. 'Hot Yellow' (HY): a shrubby pepper cultivated in Trinidad, acquired from DR. F. J. SIMMONDS. An extremely hardy and long-lived type that bears a large crop of subglobular, orange-yellow, hot fruits. Probably a strain of *Capsicum sinense* Jacquin. The name, 'Hot Yellow', was invented by the writers for the variety, which was received unnamed.

Tests of virus reaction were made on seedling plants in the greenhouse unless otherwise stated. Inoculation was done with the juice of infected 'Ford Hook' plants which was rubbed onto the leaves with the fingers; carborundum powder (grade 400) was used. Seedlings were inoculated first at the 2-4 leaf stage and survivors were reinoculated after about two weeks; sometimes a second reinoculation was carried out about two weeks after the first.

Pollinations were mostly made on plants in pots growing in full sunlight because plants in the greenhouse, though they made good growth, flowered and set poorly. Emasculation was performed just before the buds opened and flowers were protected with pinned pieces of fine muslin or muslin bags which were supported on stakes in rainy weather. Most crosses were about 20 percent successful and

showed no marked reciprocal differences in this respect; an apparent exception to this was LBH $\times$ HY in which 20 crosses failed with HY as female parent but in which LBH yielded seeds at the first attempt. This agrees with the results of SMITH (personal communication) who found that the cross *C. annuum* $\times$ *C. sinense* could only readily be made with *C. annuum* as the female parent.

RESULTS

Symptoms (cf. DALE 1954)

Four seedling reactions were distinguished, thus:

1. Necrotic (N): veinal necrosis and leaf abscission; plants commonly rather drooping and flaccid; infection easy and symptoms early (5–6 days); the plant is swiftly reduced to a bare stalk with necrotic streaks; lethal.
2. Necrotic-mosaic (NM): some veinal necrosis and incomplete leaf abscission; surviving leaves show necrotic flecks and severe vein-banding (mosaic); infection difficult and symptoms slower (8–9 days); sublethal, but plants infected as seedlings rarely set fruit. The necrotic symptom is sometimes much delayed (15–20 days).
3. Severe mosaic (SM): severe vein-banding, visible first in youngest leaves, accompanied by some wrinkling, distortion and reduction of size; infection easy, symptoms appearing in 8–9 days; nonlethal but growth very poor.
4. Mild mosaic (MM): mild vein-banding, so mild as to be difficult to recognize in some instances; little or no wrinkling and distortion; infection difficult, symptoms slow (12 or more days); growth little affected. Plants in which no symptoms could be induced were perforce placed in this category, though some of them (see below) were probably of NM genotype.

Reactions of parents and F₁ hybrids

In the seedling stage, parents and F₁ hybrids reacted as follows: showing a necrotic (N) reaction—LBH, LBH $\times$ FH, LBH $\times$ PRW, LBH $\times$ HY; showing a severe mosaic (SM) reaction—FH, FH $\times$ PRW; showing a mild mosaic reaction—PRW, HY. The necrosis shown by LBH $\times$ HY F₁ was milder than that shown by LBH itself, suggesting the occurrence of incomplete dominance. Although all HY seedlings tested reacted with a mild mosaic, genetical results described below show that the necrotic mosaic phenotype also occurs in this strain. Evidently, genes controlling the necrotic reaction are dominant to all others; the NM reaction was not recognized in the parents of F₁ hybrids (though probably it occurred in the HY strain).

Reactions of healthy mature plants inoculated with the disease were much more difficult to classify and gave clear signs of incomplete dominance thus:

LBH: Vein necrosis, abscission of young leaves and fruits; sublethal.

FH: Severe mosaic with much leaf crinkling and distortion; growth poor.

PRW: Mild (occasionally moderately severe) mosaic; resistant to infection; growth good.

LBH $\times$ FH: Variably severe mosaic with leaf curling; some tip die-back; little necrosis; growth poor.

LBH $\times$ PRW: Variable mosaic and vein necrosis; much abscission of young leaves and fruits; growth poor.

FH $\times$ PRW: Severe mosaic with some leaf crinkling and distortion; growth fair.

Genetical results

Results are summarized in Table 1. They show that two loci are concerned with resistance to disease; the constitutions of plants having the various disease reactions may be symbolized as follows: N— V_1V_2 (e.g. LBH); NM— $V_1v_2v_2$ (e.g. HY, in part); SM— $v_1v_1V_2$ (e.g. FH); MM— $v_1v_1v_2v_2$ (e.g. PRW, HY in part).

The only complication was caused by difficulties of classification of F_2 families of PRW $\times$ LBH (line 3 of Table 1). The results for these dihybrid families are given in more detail in Table 2.

The 953 plants tested fell into five groups each of which was raised and tested over a comparatively short period of time (about 4–6 weeks). All the plants within

TABLE 1
Segregations in peppers

Parentage	No. of families	Frequencies				Ratio	$\chi^2[n]$	P
		N	NM	SM	MM			
LBH $\times$ FH, F_2	8	209	...	73	...	3:1	0.12[1]	0.7–0.8*
FH $\times$ PRW, F_2	5	...	...	105	33	3:1	0.09[1]	0.7–0.8†
LBH $\times$ PRW, F_2	15	526	90	232	105	9:3:3:1	94.78[3]	<0.001‡
NM types selfed, F_3	2	...	22	...	9	3:1	0.27[1]	0.5–0.7
(LBH $\times$ HY) F_1 $\times$ PRW	2	8	16	...	...	1:1	2.67[1]	0.1–0.2
(LBH $\times$ HY) F_1 $\times$ PRW	1	7	5	5	7	1:1:1:1	0.67[3]	0.7–0.8

* Six families with LBH female, two reciprocal; no heterogeneity.

† Three families with FH female, two reciprocal; no heterogeneity.

‡ See Tables 2 and 3 and analysis in text.

TABLE 2
Dihybrid segregations in peppers.
Groups ranged in descending order of NM class proportions

Group (year)	Frequencies				Totals	Families	$\chi^2[n]^*$
	N	NM	SM	MM			
I (1952)	41	14	18	10	83	1	
II (1951)	122	35	46	18	221	5	11.77[12]
III (1958–59)	115	20	55	10	200	5	9.48[12]
IV (1954)	89	11	34	26	160	1	
V (1952)	159	10	79	41	289	3	1.23[6]
Totals	526	90	232	105	953	.	

* The values of χ^2 in the last column test heterogeneity between the different families included in one group; none is significant.

any one group were therefore subjected to approximately uniform treatment. Table 2 shows that families within groups were homogeneous with respect to reaction to virus. Between groups, however, treatment was not uniform for there was, perforce, variation in weather, source of inoculum, inoculating operator and observer; it is therefore not surprising to find that the groups are heterogeneous ($\chi^2[12] = 46.02$, $P < 0.001$). Four families were tested on two or more occasions and can thus be used as checks on the occurrence of nongenetic variability of classification. Three showed no significant heterogeneity between occasions (though two χ^2 were suggestively large) and one gave clear evidence of heterogeneity. Thus family 342/1 was grown twice in 1952 (see Table 2) with these results (frequencies of N, NM, SM and MM): Group I—41, 14, 18, 10; Group V—50, 3, 25, 11; heterogeneity $\chi^2[3] = 10.72$ and $P = 0.01 - 0.02$.

Taken jointly the five groups diverge significantly from a 9:3:3:1 ratio ($\chi^2[3] = 94.78$, $P < 0.001$) but they jointly agree with 9:7, thus: deviation $\chi^2[1] = 0.43$, $P = 0.5 - 0.7$; heterogeneity $\chi^2[4] = 1.58$, $P = 0.8 - 0.9$.

The frequency of the N class is not at fault and this suggests that linkage, if it is involved in the problem, is not tight. The contingency χ^2 appropriate to detect linkage in such data (MATHER 1938) is highly significant ($\chi^2[1] = 36.65$, $P < 0.001$). However, calculations, which need not be set out here, show that no reasonable hypothesis based on linkage explains the over-all discrepancy from a 9:3:3:1 ratio. The data of Table 2, however, are heterogeneous and deviations from 9:3:3:1 ratios range from nonsignificant in group I to great in group V. We have seen above that the frequency of the N class accords with expectation and that the discrepancies therefore lie in the other three classes; scrutiny of Table 2 shows that there is a general tendency, increasing down the table, towards deficiency of NM and excess of SM and MM.

All the facts so far adduced tend to show that we are dealing with a 9:3:3:1 ratio sporadically disturbed by environmental influences that cause misclassification of plants other than those of N genotype. The misclassification is twofold: first, NM plants often show very weak and/or late necrotic symptoms and, if none were detected, would have to be classified SM; second, it is known (see below) that v_2v_2 genotypes are much more difficult to infect than genotypes that carry V_2 so that NM plants tend to escape infection, being thus misclassified MM. Obviously, these two errors will jointly tend to produce the observed discrepancy, namely deficiency of NM and excess of SM and MM.

It may be helpful to note here that the obvious method of testing the misclassification hypothesis, namely to raise and test F_3 s of plants scored NM, SM and MM in the F_2 , is not practicable because NM and SM seedlings either die or are so stunted as to be virtually sterile in later life. Attempts to score whole F_2 families on the basis of F_3 progenies likewise failed because natural virus infection of the F_2 made it impossible to achieve an unbiased sample. Direct proof of the misclassification hypothesis is not therefore available.

The problem may be analyzed as follows. Let p be the probability of misclassifi-

cation of an NM plant as SM and q the probability of failure of infection of an NM plant, then the F_2 will give:

Class	N	NM	SM	MM	Total
Obs.	a	b	c	d	n
Exp.	$\frac{9n}{16}$	$\frac{3n(1-p-q)}{16}$	$\frac{3n(1+p)}{16}$	$\frac{n(1+3q)}{16}$	n

By application of the method of maximum likelihood or, more directly, from the statement

$$\frac{3(1-p-q)}{b} = \frac{3(1+p)}{c} = \frac{(1+3q)}{d}$$

it can be shown that (where $m = b+c+d$)

$$p = \frac{4c-3b-3d}{3m} \text{ and } q = \frac{6d-b-c}{3m}$$

of which the variances and covariance are

$$\begin{aligned} V_p &= \frac{(1+p)(4-3p)}{3(b+c+d)} = \frac{49}{9m^3} \cdot c(b+d) \\ V_q &= \frac{(1+3q)(2-q)}{3(b+c+d)} = \frac{49}{9m^3} \cdot d(b+c) \\ W_{pq} &= -\frac{(1+3q)(1+p)}{3(b+c+d)} = -\frac{49}{9m^3} \cdot cd \end{aligned}$$

Calculation of the estimates of p and q absorbs two degrees of freedom so that one remains to test goodness of fit. Since p and q depend only on b , c and d , the remaining degree of freedom attaches to the comparison of the frequency of the N class with the other three classes combined—i.e., it applies, in effect, to the test of a 9:7 ratio. Table 3 shows that agreement of calculated with observed values

TABLE 3
*Dihybrid segregations in peppers. Estimates of p and q (see text)
and calculated segregations (see Table 2)*

Group	p	q	Calculated values				$\chi^2[n=1]$	P
			N	NM	SM	MM		
I	0.0000 ± 0.1782	0.2222 ± 0.1534	46.7	12.1	15.6	8.7	1.58	0.2-0.3
II	0.0842 ± 0.1171	0.0909 ± 0.0905	124.3	34.2	44.9	17.6	0.98	0.3-0.5
III	0.5098 ± 0.1210	-0.0588 ± 0.0815	112.5	20.6	56.6	10.3	0.13	0.7-0.8
IV	0.1174 ± 0.1383	0.5211 ± 0.1334	90.0	10.9	33.5	25.6	0.03	0.8-0.9
V	0.4179 ± 0.0999	0.4026 ± 0.0951	162.6	9.7	76.8	39.9	0.18	0.5-0.7

(1) Values of p and q written in *italics* differ from zero by more than twice the appropriate standard error.
(2) The values of χ^2 in the penultimate column test agreement of observed frequencies (Table 2) with calculated values given here; each has $3-2=1$ degree of freedom and all are nonsignificant.

is satisfactory. Values of p and q , it will be noted, vary widely and apparently independently—as would be expected, because there is no obvious reason to expect that failure of NM plants to develop necrotic symptoms should be correlated with failure of infection.

In summary, the dihybrid F_2 data agree with the hypothesis that two independent loci are concerned but that segregation is disturbed in a more or less orderly (or, at least, calculable) fashion by difficulties of classification.

The statement made above that v_2v_2 genotypes are resistant to infection needs justification. Table 4 summarizes information on ease of infection of plants subsequently classified by disease reaction. The data came from the records of inoculation and infection made by one operator over a limited period of time and are not necessarily generally representative but they illustrate the point at issue. Uninfected plants have been excluded on the grounds that they must have included some MM types; and the SM class probably contained a few NM types if the interpretation of aberrant F_2 ratios proposed above is correct. Thus resistance to infection has probably been somewhat overestimated for the NM class. The proportion of this class in the sample of Table 4 that remained uninfected after two inoculations was evidently, therefore, somewhat greater than 12.5 per cent; this estimate may be compared with the values of q recorded in Table 3 to which, it will be seen, it is roughly median.

Combining columns 2 and 3 in Table 4, we have: for the V_1v_1 locus, $\chi^2 [1] = 0.50$, $P = 0.5$; for the V_2v_2 locus, $\chi^2 [1] = 99.02$, $P < 0.001$; and for the residual interaction, $\chi^2 [1] = 17.28$, $P < 0.001$. The V_2 locus is the most important factor, v_2v_2 genotypes being much more resistant to infection than V_2 . Elimination of the errors mentioned in the preceding paragraph would strengthen this conclusion.

Distribution of resistance

The susceptible (N and SM) peppers worked with in this study both belong to *Capsicum annum*. In a survey of pepper varieties in Puerto Rico, ROQUE and ADSUAR (1941) and RIOLLANO *et al.* (1948) found that 82 out of 84 strains tested were susceptible to the disease; it is not known, however, how many *Capsicum* species were represented in this collection and, therefore, whether reaction to virus was related to botanical origins.

TABLE 4
Resistance to infection

Reaction	Genotype	Number of plants	Percentage infected at inoculation number		
			1	2	3
N	V_1V_2	261	77.0	19.6	3.4
NM	V_1v_2	24	54.2	33.3	12.5*
SM	v_1V_2	252	84.5	13.9	1.6†
MM	v_1v_2	49	12.2	53.1	34.7*

* Resistance probably slightly underestimated.

† Overestimated (see text).

In a preliminary attempt to define the distribution of resistance in the genus, eight strains representing four *Capsicum* species were tested with the Trinidad virus. Seeds were supplied by DR. P. G. SMITH as follows (for taxonomic discussion see HEISER and SMITH 1948; SMITH, RICK and HEISER 1951; SMITH and HEISER 1951; and HEISER and SMITH 1953. In the list that follows, strains are distinguished by DR. SMITH's accession numbers; reaction to pepper virus is indicated by "+" for MM reaction in some at least of the four to ten plants tested and by "-" for "no reaction". All eight strains were probably $v_1v_1v_2v_2$. The list is: *C. sinense* Jacq. (1725 from Suriname, +; 1774 from Puerto Rico, -); *C. pubescens* R. and P. (1789 from Peru, +?); *C. frutescens* L. (965 from Costa Rica, +; 1583 from Nicaragua, +); *C. pendulum* Willd. (1244 from Chile, -; 1762 from Peru, -; 1786 from Peru, -).

A note on tobacco mosaic

HOLMES (1934, 1937; and see LUTES 1954) has described genetically controlled variability of reaction to tobacco mosaic in peppers. It was clearly of some interest to determine whether either one of the loci recognized in the course of the present study was the same as the *L* locus of HOLMES. Evidence that different loci were involved in resistance to the two diseases was obtained by inoculating LBH, FH and PRW with tobacco mosaic virus; all seedlings responded with an ill-defined mosaic (presumably the "systemic chlorosis" of HOLMES) and were therefore presumably all *ll*. In view of the facts that tobacco mosaic and pepper virus are known to be quite different viruses and that genes conferring resistance to one virus are not effective against others (HOLMES 1958), this finding is perhaps not very surprising.

CONCLUSIONS

The activities of the two loci in response to pepper vein-banding virus may be stated as follows:

V_1 causes vein necrosis and leaf abscission; also mosaic in the surviving leaves.

It is neutral (or nearly so) with respect to resistance to infection. Dominance (judged by results of infection of mature plants and difficulties of F_2 classification) is incomplete.

V_2 causes mosaic, enhances the necrotic-abscission action of V_1 and increases susceptibility to infection. Dominance is virtually complete.

From the plant breeding viewpoint the $v_1v_1v_2v_2$ system offers an extremely useful resistance to the disease, combining, as it does, resistance to and tolerance of infection. In Trinidad, 'Ford Hook' ($v_1v_1V_2V_2$) plants are virtually always infected long before their period of useful production is over and there is every reason to replace them by the resistant 'Puerto Rico Wonder'. The vigor and long bearing life of 'Hot Yellow' is surely at least a partial consequence of its resistance to virus. (The situation is complicated by the fact that it is also resistant to attack by *Cercospora capsici* which is responsible for some defoliation of FH, LBH and PRW). It is interesting that V_1 survives in this strain even though $V_1v_2v_2$ plants,

if infected, are severely handicapped; the inference must be that v_2v_2 , by reason of its control of infection, is of crucial importance in conferring field resistance, thus protecting the sublethal V_1 from elimination.

The symptoms of the disease described from Puerto Rico do not exactly agree with those known in Trinidad but the fact that 'Puerto Rico Wonder', bred in the island, is also resistant to the Trinidad virus is good evidence that the disease is indeed the same, minor differences in symptoms notwithstanding. This, the problem of identifying a virus disease, is a recurrent one (especially in a family such as the Solanaceae) and the genes described here offer a useful means of diagnosis (cf. HOLMES 1958).

Observations recorded above suggest that susceptible (i.e. V_2 and V_1V_2) genotypes occur and may be general in *Capsicum annum*; but that other *Capsicum* species are predominantly resistant (v_1v_2 or V_1v_2). This is, at present, only a suggestion for clearly a much wider survey of botanically known materials is required.

Of the three virus diseases of *Capsicum* of which the genetics of host reaction has been investigated, one provides an instance of dominant resistance (tobacco mosaic—references above), one an instance of recessive resistance (tobacco etch virus—GREENLEAF 1956) and one an instance of complementary recessive resistance (pepper vein-banding virus—the present work). All three systems offer useful material alike to the plant breeder and to the pathologist in search of diagnostic test plants.

SUMMARY

Two loci control reaction to vein-banding virus in pepper (*Capsicum*) seedlings. There is no evidence of linkage between the two loci concerned. The reactions are: V_1V_2 —easily infected, vein-necrosis, leaf abscission and death; $V_1v_2v_2$ —resistant to infection, vein-necrosis, mosaic, partial abscission, stunted; $v_1v_1V_2$ —easily infected, severe mosaic, poor growth; $v_1v_1v_2v_2$ —resistant to infection, mild mosaic, good growth. Dominance of V_1 is incomplete and this, together with resistance to infection conferred by v_2v_2 , causes some difficulties in classification.

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