THE RELATIONSHIP OF GENES FOR PATHOGENICITY AND CERTAIN OTHER CHARACTERS IN VENTURIA INAEQUALIS (CKE.) WINT.[1]

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VENTURIA inaequalis (Cke.) Wint., the ascomycetous pathogen of apple scab, has been shown to have adaptations similar to the eight-spored Neurospora species for use in genetic analyses (KEITT and LANGFORD 1941; SHAY and KEITT 1945; KEITT *et al.* 1948; BOONE and KEITT 1956). It has, in addition, two very important advantages over Neurospora: 1) It is pathogenic and can be studied *in vivo*; and 2) it is uninucleate in its vegetative stage and, thus, is free from the confounding effects of heterocaryosis. Pathogenicity in *V. inaequalis* is variable and each instance of virulence that has been studied adequately has been shown to be controlled primarily by a single gene (KEITT 1952; SHAY and HOUGH 1952; SHAY and WILLIAMS 1956). Many physiological races could be recognized on the basis of pathogenicity on apple varieties, but, so far, there has been no advantage in naming more than three (SHAY and WILLIAMS 1956). The purpose of this paper is to present the relationships of the pathogenicity genes differentiating the three recorded races of the organism. A preliminary account has been published (SHAY and WILLIAMS 1953).

MATERIALS AND METHODS

The Host: All pathogenicity determinations were made on clonal selections of apple in the greenhouse during the months of February, March and April in the years 1953–56. These selections include the varieties Dolgo and Geneva, a selection (MA 1363-24) of *Malus sikkimensis*, and four seedlings, OR42T141, OR42T142, OR42T145, and OR42T173. These seedlings were selected for their uniform susceptibility to a race of the pathogen and are of the parentage, McIntosh × (R12740-7A × Delicious). Since these seedlings gave similar disease reactions in all tests, they are grouped together in this paper under the name "Russian", to denote the parent, R12740-7A, a seedling received from Russia (DAYTON *et al.* 1953).

The selections included in this study have been classified by SHAY and HOUGH (1952) and SHAY and WILLIAMS (1956) with respect to leaf symptoms incited by isolates carrying the pathogenicity factors under consideration. These reaction classes are as follows: 1 = numerous or few minute pits, no sporulation; 2 = irregular or regular necrotic or chlorotic lesions, no sporulation; 3 = restricted necrotic lesions with sporulation, no defoliation; 4 = extensive lesions with abundant sporulation, leaves may abscise.

The Pathogen—Description of Characters: The pathogenicity factors under consideration were found by SHAY and HOUGH (1952) and SHAY and WILLIAMS (1956) to be controlled by single genes in the fungus. On the basis of these results, the sym-

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bols listed below are assigned to these factors. This method of classifying and numbering the pathogenicity genes in *Venturia inaequalis* was established in conjunction with DRS. BOONE and KEITT of the University of Wisconsin. Through mutual agreement, the symbols *p-1*$^+$ through *p-7*$^+$ were reserved for genes controlling pathogenicity on commercial varieties under observation at Wisconsin. In this system of classification, the suffix "+" is used to denote full virulence on the stated variety. In some cases, this is the commonly occurring type and in others it is rare or not yet obtained.

p-8$^+$/*p-8:* *p-8*$^+$ conditions the 3 reaction class on Dolgo (Plate 1, B); while the allele, *p-8*, incites the 2 reaction class (Plate 1, A). It was discovered in the course of the investigation that another gene (unidentified) in the fungus intensifies the effect of *p-8*$^+$, conditioning a 4 reaction on Dolgo. As the effect of *p-8*$^+$ is always expressed and the intensifier gene is not, only this gene is considered in the present study.

p-9$^+$/*p-9:* *p-9*$^+$ conditions the 4 reaction class on Russian (Plate 1, D); while the allele, *p-9*, conditions the 2 reaction class (Plate 1, C).

p-10$^+$/*p-10:* *p-10*$^+$ conditions the 4 reaction class on Geneva (Plate 1, G) while the allele, *p-10*, conditions the 2 reaction class (Plate 1, E).

p-11$^+$/*p-11:* *p-11*$^+$ conditions the 3 reaction class on Geneva (Plate 1, F) while the allele, *p-11*, conditions the 2 reaction class.

p-12/*p-12*^{1}: *p-12*^{1} incites the 1 reaction class (Pinpoint pits, Plate 1, H) on *Malus sikkimensis*; while the allele, *p-12*, incites large necrotic lesions (2 reaction class, Plate 1, I) with tan centers and red-brown margins on this selection. (The genotypes *p-12*$^+$ and *p-13*$^+$ have not been discovered.)

p-13/*p-13*^{1}: *p-13*^{1} incites pinpoint pits with slight necrosis in the base on *M. sikkimensis*; while the allele, *p-13*, incites the typical 2 reaction class described above.

In addition to the pathogenicity genes described above, two additional genes, green and mating type, were included. These genes were used as aids in identifying ascospore pairs. The position of one, green, is known (BOONE and KEITT 1956).

green (*gr*$^+$/*gr*): *gr* is characterized by a bottle green mycelial color; while the allele, *gr*$^+$, present in wild type isolates, is characterized by the typical brown-olivaceous mycelial color.

mating type (*mt*$^+$/*mt*$^-$): *mt*$^+$ was arbitrarily assigned to the type of standard isolate 356-2; while *mt*$^-$ was assigned to the type of standard tester isolate 651.

Cultural Methods: Cultures were maintained under sterile mineral oil on potato dextrose agar (PDA) in tubes. *In vitro* matings of the isolates and isolation of the ascospores were made manually with a glass needle by the method of KEITT and LANGFORD (1941). After isolation and when sufficient growth had developed, the ascospore cultures were numbered and classified according to ascospore pairs. Some transposition of spores in the ascus was observed. Only asci in which the original position of the spores could be deduced were included in the classification of segregation of factors.

To determine mating types, crosses were made between each of the ascospore isolates and one or both of the mating type tester isolates, 356-2 and 651.

Inoculation Methods: Inoculum was produced by the method of KEITT and PAL-

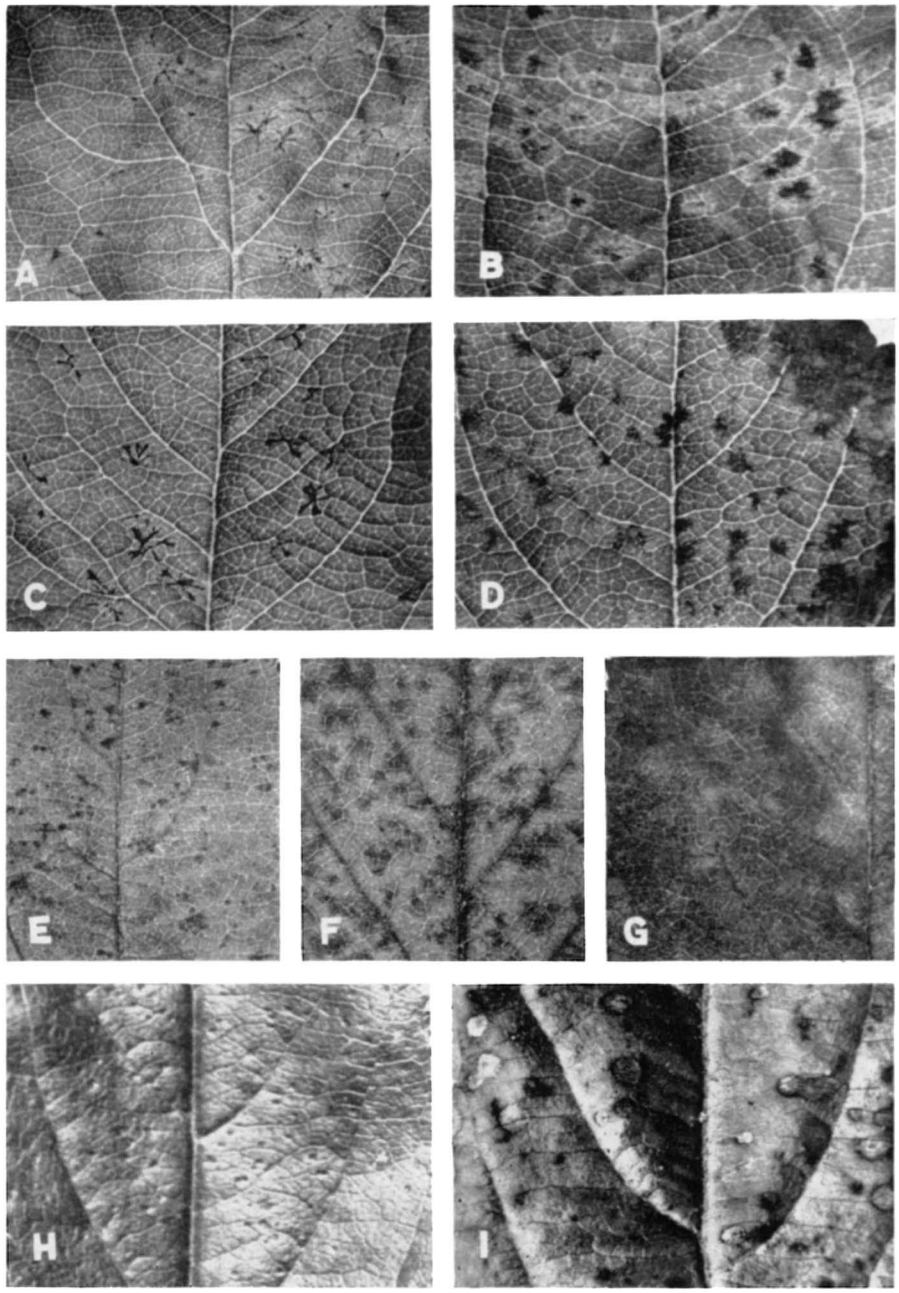

PLATE 1:—A. Reaction class 2 incited on Dolgo by isolates with genotype *p-8*. B. Reaction class 3 incited on Dolgo by isolates with genotype *p-8*$^{+}$. C. Reaction class 2 incited on Russian differentials by isolates with genotype *p-9*. D. Reaction class 4 incited on Russian differentials by isolates with genotype *p-9*$^{+}$. E. Reaction class 2 incited on Geneva by isolates with genotype *p-10* and/or *p-11*. F. Reaction class 3 incited on Geneva by isolates with genotype *p-11*$^{+}$. G. Reaction class 4 incited on Geneva by isolates with genotype *p-10*$^{+}$. H. Reaction class 1 incited on *M. sikkimensis* by isolates with the genotypes *p-12*^{1} and/or *p-13*^{1}. I. Reaction class 2 incited on *M. sikkimensis* by isolates with the genotypes *p-12* and/or *p-13*.

MITER (1938) modified to the extent that cheesecloth wicks in 4 ounce prescription bottles were used. Inoculum was diluted to provide approximately 100 conidia per low power (100×) microscope field and was applied to the terminal leaves of the plant by means of a number 15 DeVilbis atomizer attached to an airline providing 15 pounds pressure. The inoculations were made in a plastic covered chamber in a room removed from the greenhouse. Between inoculations, the interior was washed down with a water spray. The inoculated plants were kept for 44 hours in a moist chamber consisting of a frame covered with wet muslin. Infection data were taken two weeks after inoculation. The plants were then returned to the moist-chamber for another 44 hours to enhance maximum symptom development. Readings were taken again immediately upon removal.

Methods Used for Computing Map Distances: The location of genes with respect to centromere was calculated by the method developed by LINDEGREN (1933) based on the percent of asci showing second division segregation of the gene-pair (alleles) concerned.

Tests for homogeneity of data were made in the manner of BARRATT and GARNJOBST (1949). Using the percent of second division segregation of all crosses for a single factor as a base, Chi square values were calculated for each cross. These values were totaled and P was calculated for the total Chi square value.

The tests for independence of factors were based on the hypothesis that the factors are inherited independently of each other. Chi square values were calculated for the ratio of parental and nonparental ditype asci. As pointed out by PERKINS (1953) values obtained by this method are not obscured by excessive numbers of tetratype asci.

No attempts were made to correct for multiple crossovers or for possible chiasma or chromatid interference.

The limits in which the true values of the map distance might be expected to lie were determined from the 95 percent confidence limit curves of BARRATT, *et al.* (1954, Fig. 8).

TABLE 1

Summary of the data on segregation of the gene pairs included in this investigation

Gene symbols	Total asci no.	Second division segregation		Test for homogeneity of data*			Distance from centromere	95% confidence range†
		No.	Percent	df	χ^2	P		
p-8$^+$/*p-8*	316	211	66.8	17	15.8	0.54	—	30.7 to —
p-9$^+$/*p-9*	191	122	63.9	12	13.7	0.33	—	28.5 to —
p-10$^+$/*p-10*	123	78	63.4	10	5.6	0.85	—	27.0 to —
p-11$^+$/*p-11*	78	46	59.0	3	3.6	0.32	—	22.5 to —
p-12/*p-12*^{1}	33	15	45.5	4	3.1	0.55	22.7	13.8 to 31.7
p-13/*p-13*^{1}	24	17	70.8	3	3.5	0.33	—	27.0 to —
mt$^+$/*mt*$^-$	289	187	64.7	18	9.4	0.95	—	27.0 to —
gr$^+$/*gr*	312	215	68.9	12	8.4	0.75	—	31.5 to —

* Tests for homogeneity made in the manner of BARRATT and GARNJOBST (1949).

† Based on 95 percent confidence limit curve of BARRATT *et al.* (1954, Fig. 8).

RESULTS

Gene to Centromere Distance: Chi square tests for homogeneity for each factor indicate that the data for all crosses involving a particular factor are homogeneous and can be combined. Presented in table 1 is a summary of the data relating to the location of these factors with relation to their centromeres as determined by LINDEGREN'S (1933) method. All of the genes under consideration are distant from their centromeres and only one, *p-12*^{1}, deviates significantly from random assortment and, on the basis of these data, is located at 22.7 crossover units from its centromere. The 95 percent confidence range limits calculated for this gene indicate that *p-12*^{1} apparently lies at a point between 13.8 and 31.7 units from the centromere.

On the basis of percent of second division segregation of the factors controlling pathogenicity on Geneva, *p-10*$^+$ and *p-11*$^+$ might be judged to be allelic. However, progeny recombinations from crosses involving these two genes (see table 2) indicate that they are not allelic and apparently not even linked. The relationship of *p-12*^{1} and *p-13*^{1} is less distinct. Crosses involving the two genes have not been available. However, as the recombination data between these genes and *p-8*$^+$ (see table 2) indicate a marked difference, *p-12*^{1} and *p-13*^{1} are considered, tentatively, as being nonallelic.

TABLE 2

Summary of the data on recombination of genes for pathogenictiy and certain other characters

Segregating characters*	Total no. asci	Observed tetrad numbers†			Test for independence‡ P	Percent recombination	Conclusions
		PD	NPD	T			
p-8$^+$, *p-9*$^+$	173	130	0	43	<0.01	12.4	Linked
p-8$^+$, *p-10*$^+$	97	21	15	61	0.32	46.9	Independent
p-8$^+$, *mt*$^+$	234	31	33	170	0.89	50.4	Independent
p-8$^+$, *gr*	181	33	23	125	0.19	47.2	Independent
p-8$^+$, *p-12*^{1}	32	16	0	16	<0.01	25.0	Linked
p-8$^+$, *p-13*^{1}	23	4	4	15	0.99	50.0	Independent
p-8$^+$, *p-11*$^+$	71	15	8	48	0.15	45.1	Independent
p-9$^+$, *mt*$^+$	168	26	25	117	0.89	49.7	Independent
p-9$^+$, *p-10*$^+$	76	13	8	55	0.27	46.7	Independent
p-9$^+$, *p-12*^{1}	29	9	2	18	0.04	37.9	Linked
p-9$^+$, *gr*	116	22	13	81	0.13	46.1	Independent
p-9$^+$, *p-11*$^+$	80	15	7	58	0.09	45.0	Independent
p-10$^+$, *p-12*^{1}	12	2	0	10	0.16	41.7	Independent
p-10$^+$, *mt*$^+$	89	13	13	63	0.99	50.0	Independent
p-10$^+$, *gr*	52	12	10	30	0.68	48.1	Independent
p-10$^+$, *p-11*$^+$	25	5	8	12	0.42	56.0	Independent
p-11$^+$, *gr*	78	10	11	57	0.84	50.6	Independent
p-11$^+$, *mt*$^+$	78	13	10	55	0.54	48.1	Independent
p-12^1, *mt*$^+$	28	4	7	17	0.38	55.4	Independent
p-13^1, *gr*	24	3	8	13	0.14	60.4	Independent
p-13^1, *mt*$^+$	15	3	0	12	0.09	40.0	Independent
gr, *mt*$^+$	147	27	26	94	0.89	49.7	Independent

* See text for description of characters.

† PD = parental ditype asci; NPD = non-parental asci; and T = tetratype asci.

‡ Test for independence based on PD:NPD deviation from 1:1.

TABLE 3

Linkage Group A

Cross	Number asci tested	Gene to gene distance*	95% Confidence range limits†
p-8$^+$, *p-9*$^+$ × *p-8*, *p-9*	173	12.4	9.0 to 15.0
p-8$^+$, *p-12* × *p-8*, *p-12*^{1}	32	25.0	15.5 to 34.0
p-9$^+$, *p-12* × *p-9*, *p-12*^{1}	29	31.0	21.9 to 40.5

* Corrected distance based on the method of BARRATT *et al.* (1954) in which one half the percentage of tetratype asci is taken as the uncorrected distance in crossover units between the genes.

† Based on BARRATT *et al.* (1954, Fig. 8) method of determining 95 percent confidence range interval.

The factor green has been included in BOONE and KEITT's (1956) inheritance studies with color mutants. Through linkage studies, these workers have located this gene at a point between 45 and 55 crossover units from the centromere.

Gene to Gene Distance: The results of the 22 combinations of characters studied are listed in table 2. The tests for independence, made in the manner suggested by PERKINS (1953) based on parental ditype:nonparental ditype ratios, indicate that *p-8*$^+$ is linked with *p-12*^{1} and *p-9*$^+$. All other combinations studied were not significantly different from a 1:1 ratio and can be considered as not linked. Six gene pair combinations were not tested. These include *p-13*^{1} with *p-9*$^+$, *p-10*$^+$, *p-11*$^+$ and *p-12*^{1}; and *p-12*^{1} with *p-11*$^+$ and *gr*. As *p-12*^{1} can be located with respect to its centromere (see table 1) and with the genes, *p-8*$^+$ and *p-9*$^+$, and as *p-8*$^+$ and *p-9*$^+$ have been shown to be inherited independently of *p-11*$^+$ and *gr*, it can be assumed that the latter genes are not linked with *p-12*^{1}.

The linkage group formed by genes *p-12*^{1}, *p-8*$^+$ and *p-9*$^+$ is designated as Linkage Group A (see table 3) until it is determined where it fits in BOONE and KEITT's (1956) gene linkage classification. Based on the method of computing corrected gene to gene distance as one half the percentage of tetratype asci (BARRATT *et al.* 1954), *p-12*^{1} and *p-8*$^+$ are placed at 25 crossover units apart; *p-8*$^+$ and *p-9*$^+$ at approximately 12 units; and *p-12*^{1} and *p-9*$^+$ at 31 units. These distances are uncorrected for multiple crossovers. While the number of asci included in this test are not large, these data indicate that the *p-12*^{1}, *p-8*$^+$, and *p-9*$^+$ are arranged on the chromosome in that order with *p-12*^{1} being nearest to the centromere.

DISCUSSION

It has been pointed out by BLACK (1952) that the identification and determination of the relationship of genes for pathogenicity in a microorganism are important not only from the standpoint of use in a breeding program but also as an aid in determining biosynthetic pathways and ultimately in working out the nature of resistance in the host.

The study of host-parasite relationship is complicated with pathogens such as rusts and smuts. This is in part due to the dicaryotic condition of nuclei in the vegetative stage. However, *Venturia inaequalis*, with its uninucleate vegetative cells, is singularly adapted for use in such a detailed study. This character, in addition to suitability of the host for asexual propagation affording a continued supply of genet-

ically identical host material, presents a favorable basis for future studies on host-parasite interaction.

Pathogenicity in *V. inaequalis* has been shown to be controlled primarily by a single gene (KEITT 1952; SHAY and WILLIAMS 1956). Resistance in the host has not been completely worked out. However, there are indications that, with the host material included in this study, resistance may be controlled by single genes (SHAY *et al.* 1953; DAYTON *et al.* 1953).

The pathogenicity gene symbol classification employed in this study is designed to facilitate the orderly presentation of data as further studies with this organism are completed. Furthermore, for the same reason, it is believed that a comparable method of naming genes for resistance in the host would be advantageous. For instance, these genes can be identified as R_1, R_2, R_3, etc., with each differential resistance gene being given the corresponding pathogenicity number as it is isolated in the gene population.

Based on this system of pathogenicity gene classification, isolates of the races described by SHAY and WILLIAMS (1956) can be assigned the following pathogenicity genotypes: *Race 1* isolates are nonvirulent on Dolgo, Russian, and Geneva, and, thus, would have the genotype *p-8*, *p-9*, *p-10*, *p-11*; *Race 2* isolates are virulent on the three varieties and would have the genotype *p-8*$^+$, *p-9*$^+$, *p-10*, *p-11*$^+$; while *Race 3* isolates are virulent only on Geneva and have the genotype *p-8*, *p-9*, *p-10*$^+$, *p-11*.

It is of interest that all pathogenicity genes under consideration are located at considerable distances from their respective centromeres. KEITT and BOONE (1952) have described six gene pairs conditioning pathogenicity on commercial varieties of Malus. Of these, only one, Haralson$_2$, was found to be close enough to the centromere to determine map distance by second division segregation. This gene and the gene, *p-12*^{1}, conditioning pin-point pits on *M. sikkimensis*, are approximately the same distance from their respective centromeres.

SUMMARY

The four factors controlling pathogenicity in the three named races of *Venturia inaequalis*, two factors conditioning reduced virulence against *Malus sikkimensis*, and single factors controlling green mycelial color and sex compatibility in the fungus, were studied with respect to linkage relationship and centromere distance. Three factors, *p-12*^{1}, conditioning reduced virulence symptoms on *M. sikkimensis*, *p-8*$^+$, conditioning pathogenicity against *M. baccata* var. Dolgo, *p-9*$^+$ conditioning pathogenicity against a differential selection of *M. pumila* # R12740-7A, and the centromere were found to be loosely linked. All other factors were inherited independently of each other and of their respective centromeres.

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