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Analysis of Several Types of Gene Action Controlling Resistance to Bacterial Blight of Cotton by Means of a Generalized Computer Model[1]

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ABSTRACT

A computer program has been written having the capability to fit three- or four-gene models to F_2 segregation data divided into ten or fewer phenotypic classes. The various types of gene action analyzed are known to occur in the determination of bacterial blight resistance in cotton. The genes in any specific model may be independent or dependent, unequal in effect, and either dominant, partially dominant, additive, or recessive. Successive increments of gene values were limited to integers. Linkage was in five increments from 0 to 50% recombination inclusive, coupling or repulsion, and restricted to two genes in any particular model. The X^2 criterion was used to select those models printed out as possible solutions. Exact data based on hypothetical three-gene models were solved satisfactorily by the computer program. To provide a "live" test, a strain of cotton (*Gossypium hirsutum*, L.) resistant to the bacterial blight pathogen (*Xanthomonas malvacearum* [(E. F. Smith) Dowson], was crossed to a susceptible strain. Data from the segregating progenies were analyzed by the program, and feasible models for the gene action accounting for resistance were proposed. Classification into eight grades of resistance-susceptibility were shown to be statistically satisfactory for resistant and susceptible parents and for a segregating progeny in a specified environmental range of temperature and humidity. Conclusions regarding gene action are restricted to the specified environmental range. The program is best adapted for analysis of samples of 200 to 350 individuals.

Additional index words: Epistasis, *Xanthomonas*, Genetic recombination, Quantitative genetics.

DEVELOPMENT OF A COMPUTER PROGRAM FOR REALISTIC GENETIC MODELING

Flexibility Requirements of Realistic Modeling

CASTLE (4) dealt with the estimation of the number of genetic factors in quantitative inheritance based on a model restricted to independent, equivalent factors, none of which show dominance. The likelihood that such restrictions are not realistic has long been recognized. Wright (20) noted that interaction is the rule rather than the exception in quantitative systems. He also pointed out other complications of quantitative studies, including incomplete dominance, linkage, and unequal effects of genes (21). In genetic modeling, restrictions that ignore any of these factors would make a model unsatisfactory for fully reflecting the scope of real gene action.

Only a limited number of loci can be dealt with relatively free of restrictions. Three-gene systems which can be handled quite well under additive qualitative models can require complicated quantitative analysis if the genes are epistatic and greatly unequal in effect (21).

The term semi-quantitative is used in this paper to describe those genetic situations in which the genes involved have measurable individual effects, but where variation in gene action results in a segregating population which appears to represent multifactorial control.

The genetic factors in cotton (*Gossypium hirsutum* L.) that control resistance to the bacterial blight organisms [*Xanthomonas malvacearum* (E. F. Smith) Dowson] show ample range of variability in type and intensity of gene action and illustrate the utility of computer analysis.

Blight resistance genes generally vary in strength (i.e., the metrical increment of resistance conferred against a specific bacterial culture) (13). All types of interallelic interaction, with the exception of overdominance, have been reported (13). Several cases of epistasis have been documented; one type involved both major and minor "background" genes (9). The interaction of genes B_2 and B_6 conferred a level of resistance equal to any other additive combination known (13). Other genes were apparently independent in action, and weakly to strongly additive (10, 13). At least one clear-cut case of linkage (12) was observed, and one gene originally reported as a major locus is now through to be a linked complex of polygenes (11). Clearly then, a realistic analysis of bacterial blight resistance requires a very flexible model.

One of the better means of analyzing the effects of three or fewer loci is the partitioning method developed by Powers (15, 16, 17). However, Powers (15) did not attempt to analyze a situation more complex than a two-locus interaction. The partitioning model will be compared with the computerized method described in this paper.

Capacity and Limitations of the Program

The program was developed in Fortran IV for the IBM 360-50 computer and the following restrictions were designed into the analysis:

(1) The phenotype data array was limited to 10 classes.

(2) Only models with a maximum of four loci were considered.

(3) Two restrictions were placed on the increment of resistance contributed by each gene: (i) fractional increments were excluded; and (ii) models which would generate a theoretical genotype greater than 10 (the maximum observable phenotype +1) were disallowed. For example, in an array of data with a maximum observed phenotype of +9, three genes may contribute increments of +3 each (or +4, +3, +2, etc.) but the fourth gene is then limited to +1 making up the maximum allowable genotype of +10. Allowing the genotype to slightly exceed the phenotype seemed justified because of the certainty that a plant cannot be phenotypically classified higher than "immune" regardless of the numbers and/or the strength of the resistance genes it carries.

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(4) No restriction was placed on degree of dominance. The genotypes at each locus were coded by number of dominant factors (i.e. D2 = 2 dominant alleles, D1 = 1 dominant, 1 recessive allele, D0 = homozygous recessive) and the increment of resistance allotted to each. The following sample model will suffice to show complete dominance (genes 1 and 2), partial dominance (gene 3), and recessiveness (gene 4).

Gene 1 (A)	*Gene 2 (B)*	*Gene 3 (C)*	*Gene 4 (D)*
D2=2	D2=1	D2=4	D2=0
D1=2	D1=1	D1=3	D1=0
D0=0	D0=0	D0=0	D0=3

The iteration of values begins with all genes recessive with a base value of 1 (D0=1) and moves through all possible combinations of completely recessive models. Then gene 1 is set at a base dominant value of 1 (D2=1, D1=1), and all allowable recessive combinations are tried with the other three genes. Successively higher values are iterated for the first dominant gene until the possibilities are exhausted. Then a second gene is set at the base dominant value and the procedure is repeated.

Overdominance was simulated by a special combination effect. By specifying genes 1 (A) and 2 (B) to be linked in repulsion at 0% recombination, the total effect of gene 1 (A) and gene 2 (B) combination locus is an overdominant effect, i.e., D2=2, D1=3, D0=1.

(5) Epistatic interactions of two types were generated. The sample model specified above can be used as follows: (i) In the complementary model any of the four genes can be specified as dependent upon any other gene. For example, with gene 1 (A) specified independent; gene 2 (B) specified as dependent upon gene 1 (A), then the genotype A_B_ produces a +3 phenotype, A_bb a +2 phenotype, but aaB_ equals 0 since gene 2 (B) has no independent value of its own. (ii) A locus that exhibits both independent action and epistasis can be specified by absolute coupling phase linkage involving a dependence specific gene (as gene B above) and another independent locus.

With a four-gene model, 81 different genotypes can be recovered. The program computes the phenotype and expected frequency of each gene combination. Analysis involved chi-square comparison of frequency data (observed) to the hypothetical segregation as generated by each computer model (expected). No correction for continuity was applied in order to preserve a more powerful test.

(6) Linkage was restricted to any two of the loci for any specific model and was limited to increments of 12.5%; i.e. 0, 12.5, 25, 37.5, and 50% recombination. It developed that if a given model provided a solution with two loci linked at 25%, similar models differing from the first only in specifying linkage between the same two loci at 12.5%, 37.5%, or both were often also feasible solutions. Both coupling and repulsion linkage were considered by the computer program. Repulsion linkage allowed for the generation of models requiring the contribution of a major resistance gene from the susceptible parent. Repulsion models, therefore, were usually rejected a priori as artifact solutions. Exceptions to this rule will be explained in the section on genetic interpretations.

The number of discrete models generated and tested by the program in 30 minutes must be between 1 and 5×10^4. A precise estimate is impossible because the number will vary from one array of data to another, becoming generally greater as the number of classes increases. The program logics also react to the skewness of the data array; only models having some degree of probability of fitting the extremes of the data are generated fully. Some of the models are not discrete but are permutations of other solutions. The speed with which acceptable models fitting any set of 10-class experimental data are obtained is generally less than 15 minutes execution time on the IBM 360-50 computer.

The program is adaptable to F_2 data for any metric trait that can be measured in 10 or fewer classes if the frequency of occurrence of extreme parental classes is sufficient to demonstrate that all or nearly all of the variability can be attributable to four or fewer loci.

EVALUATION OF THE COMPUTER PROGRAM

The program was tested on a series of data derived from the expansion of an exact three-gene model. In each case the total number of observations (n) equals 256. The generalized form for each of these trial models was as follows: gene 1, a major additive gene (D2=4, D1=2, D0=0); gene 2, a minor complete dominant (D2=1, D1=1, D0=0); and gene 3, a complete recessive (D2=0, D1=0, D0=2). The data sets derived from seven simple variants of this generalized model and the chi-square fit obtained by the program are listed in Table 1. All models falling within the limitations of the program were fitted exactly, resulting in a chi-square of 0.0. These models were useful in showing the program to be free of defects over a wide range of operations.

Not just any set of data will result in acceptable solutions. Model #110 in Table 1 is a complex three-

Table 1. Computer solutions to three-gene hypothetical models.

Model No.	Model Type	Phase	Linked genes	% recomb.	Independence/ interaction	Data arrays: Disease reaction class 0	1	2	3	4	5	6	7	Best X^2 fit
I. Models falling within program limitations														
115	Standard (see text)	Repulsion	1 and 2	25	All indep.	3	45	19	93	33	47	9	7	0.0
116	Standard	Repulsion	1 and 2	25	1 dep. on 2	48	45	16	93	0	47	0	7	0.0
117	Standard	Repulsion	1 and 2	25	3 dep. on 1	4	45	24	93	36	47	0	7	0.0
123	Standard	Binomial	None	--	All indep.	12	36	28	84	20	60	4	12	0.0
125	Standard	Binomial	None	--	3 dep. on 2	16	36	32	84	16	60	0	12	0.0
127	Standard	Binomial	None	--	Duplicate	12	36	112	32	48	16	0	0	0.0
126	Standard	Binomial	None	--	Partial dup.	12	36	28	92	36	40	12	0	0.0
II. Models falling outside program limits														
115A	Standard except for Gene 1: D2 = 3.5	Repulsion	1 and 2	25	Indep.	3	45	19	106	30	41	8	4	1.04*
115B	Standard except for Gene 1: D1 = 2.5	Repulsion	1 and 2	25	Indep.	3	45	10	63	69	47	12	7	5.33†
115C	Standard except for Gene 3: D0 = 1.5	Repulsion	1 and 2	25	Indep.	3	45	26	89	43	36	8	4	2.64†
118	Standard except for Gene 1: D1 = 2.5	Repulsion	1 and 2	25	Indep.	4	45	12	66	75	34	13	7	6.10†
110	Homozygous locus (see text)	Coupling	1 and 2	25	3 dep. on 1	0	36	24	28	80	28	34	36	17.76‡

* Solution leading to misinterpretation. † Solution giving excellent approximation of model. ‡ Solution outside range of acceptability.

gene case in point. In this model, the third gene was specified as homozygous and partially dependent upon gene 1. This set of specifications produced a model that falls outside the capabilities of the program, and no solutions acceptable at the .05 level were found.

Apparent artifact models may sometimes have a logical interpretation. The solution of model 126 in Table 1 is a case in point. In this three-gene model, the genes were assigned the values of the generalized form above. This was a hypothetical hybrid model; gene 3 was assigned duplicate action while genes 1 and 2 were additive. No solution was expected since a hybrid-type model was involved. The demands of the theoretical model required that gene 3 have an increment value of +1 when acting additively to gene 1, but an independent value of +2 when acting alone.

This effect was achieved in the program solution by splitting the complementary and duplicate functions of gene 3 between two genes (3 and 4 in the example below).

Theoretical model	*Solution*
Gene 1 D2=4, D1=2, D0=0, Independent	Gene 1 D2=4, D1=2, D0=0, Independent
Gene 2 D2=1, D1=1, D0=0, Independent	Gene 2 D2=1, D1=1, D0=0, Independent
Gene 3 D2=0, D1=0, {D0=2; if gene 1=D0, D0=1; if gene 1={D2, D1}, Independent	Gene 3 D2=0, D1=0, D0=1, Dependent
	Gene 4 D2=0, D1=0, D0=1, Independent

Specifications: (A) Gene 1 and gene 3 linked, in coupling, 0% recombination; (B) Gene 3 dependent upon gene 4.

Gene 3 has been assigned the locus of gene 1, creating a supergene with the values D2=4, D1=2, and D0=1. The presence of the dominant forms restrict gene 4 to a value of one; the recessive form of this compound locus is dependent on gene 4 and increases its value by one.

This indirect type of solution indicates that those solutions that are meaningless artifacts at first glance may be further analyzed to reveal a real or often plausible solution.

The computer program was also tested against several sets of live data with sample sizes varying from 100 to 1000. These tests showed what might be expected of type I and II errors arising from chi-square discrimination. With the maximum number of classes fixed at 10, we are more or less bound to a certain range of sample size (5). If n is too small the results cannot be too credible; if n is too large classes with expectations greater than 50 will be common and there will be appreciable loss of power in the test. In actual practice we found that in sets of data where n=100, type II error is likely to be unmanageable. Several samples of n=100 were run, and usually 100 or more solutions were generated that would be acceptable at the P.05 level. By contrast, three sets of data with n>400 were run, and not a single acceptable solution was found. Samples of 200 to 350 appear to be optimum for this particular procedure.

Table 2 shows previously published data used in challenging the program. Two of the data sets had been analyzed by the partitioning method so that a comparison could be made to this type of genetic analysis, even though the data often had to be compressed into fewer classes in order to make the adaptation to the computer program.

A single analysis of the genetics of maturity date in Ramona × Baart 46 wheat, a cross that has been explored in depth by Allard and co-workers, is taken from F_2 data presented in graphical form in the paper by Crumpacker and Allard (6), and is compared to a series of three experiments by Allard's group (Table 2). The computer indications are somewhat better than the early generation analysis and the diallel, but markedly inferior to the inbred backcross line technique. Wehrhahn and Allard (19) estimated that the latter procedure was sufficiently rigorous to detect a locus contributing only 2% of the total additive variance. According to the precise estimates of Wehrhahn and Allard our computer program accurately estimated the effects of the major gene, but considerably overestimated the effects of two minor genes.

USE OF THE COMPUTER PROGRAM TO ANALYZE BLIGHT RESISTANCE

Materials and Methods

A number of progenies were used in the testing and development of the genetic analysis program at the New Mexico Agri-

Table 2. Computer solutions compared to published partitioning, diallel, F_2, and inbred backcross analyses.

Author	Type of analysis	Multigenic trait	Phenotypic classes: Author's No.	Author's Units	Computer no.	Author's solution	X^2	Computer solution: Pref. model
Powers (16)	Partitioning	No. locules in tomatoes	11	Log. trans.	10	2 effective loci with interaction	11.158	3 major loci; 2 partially dom., 1 dependent recessive.
Powers (16)	Partitioning	No. locules in tomatoes	15	Locule single	10	3 effective loci; 2 partially dom. for fewer locules, 1 partially dom. for more locules	23.961*	3 major loci; 2 partial dom. for more locules, 1 dom. for fewer locules.
Allard and Harding (1)	Early generation freq.	Heading date in Ramona ×		Graphical heading date × freq.	10	1 dominant locus contributing about 16 days diff.		4 loci; 2 dom, 1 partial dom; 1 recessive; contributing 16, 4, 8, 8 days to diff in maturity.
	Late generation freq.	Baart 46 wheat		Graphical heading date × freq.	10	At least 4 loci		4 loci; 2 dom, 1 partial dom; 1 recessive; contributing 16, 4, 8, 8 days to diff in maturity.
Crumpacker and Allard (6)	Diallel, F_2, and backcross	Dom.		Graphical heading date × freq.	10	1 major dom. locus for earliness		4 loci; 2 dom, 1 partial dom; 1 recessive; contributing 16, 4, 8, 8 days to diff in maturity.
Wehrhahn and Allard (19)	Inbred backcross line analysis	Dom.		Graphical heading date × freq.	10	4 loci; contributing about 14, 4, 3, and 4 days to early maturity. Other loci present.	14.522	4 loci; 2 dom, 1 partial dom; 1 recessive; contributing 16, 4, 8, 8 days to diff in maturity.
Saunders (18)	F_2 not analyzed in detail	Hairiness in cotton	9	Visual scale	9	Major dominant dep. on major recessive + unknown factors. (pedigree expectation)	34.188*	Minor dom. dep. on major recessive, plus major independent recessive locus.

* "First approximation" solution outside range of acceptability.

cultural Experiment Station. The cross (8373S × T582) has been developed in detail to show both the limitations and potential of our method of computerized analysis. Source 8373S has been used as a donor of blight resistance genes in the development of two commercial varieties. The T582 material is from a backcross marker stock line obtained from Texas A and M University. Three different single crosses of 8373S × T582 are reported in this paper. All were progeny tested in the same controlled greenhouse unit under similar conditions but at dfiferent times.

Xanthomonas malvacearum, race NM2, was furnished as inoculum by Mr. C. F. Chew, USDA-ARS plant pathologist. Race NM2, as defined by Chew, is a stable culture virulent on the cultivars 'Pima 32,' 'Stoneville 2B,' and 'Stoneville 20;' avirulent on 'Acala 1517BR-2,' 'Mebane B-1,' and 101-102 B under normal greenhouse conditions.

Cultures taken from 5-day-old agar slants and dispersed in water to an approximate 1:120 dilution served as inoculum. Two-week-old cotton seedlings were inoculated on the underside of each cotyledon with a smooth scratch about 3 cm long. A modified speedball A-5 pen, with points shortened, blunted, and bent apart to form a notch 1.5 mm wide was used to cut the tissue and deliver an uninterrupted flow of bacterial suspension into the wound.

The plants were grown and inoculated in a greenhouse with thermostatic controls on the heating and cooling systems and vents. These controls were adequate only to hold day and night temperatures to a range between 36 and 24 C. Relative humidity ranged from 35 to 40% (mid-afternoon) to 75% (mornings, after watering). Under this humidity and temperature regime the expression of resistance may have been enhanced (3). The segregating genotypes were well differentiated.

The microenvironment, however, has proved to be much more treacherous. Our own results (unpublished) have shown that those plants in the center of densely planted flats grade more susceptible than plants on the edge of the flat or plants growing in individual pots. The optimum differentiation of segregating genotypes has been obtained by growing plants in small pots (2¼ to 3 inches square) packed tightly together in groups. Plants at the ends of the rows are more exposed to open air and have atypical more-resistant reaction; very young plants germinating a week or more after the bulk of the population tended to be atypically susceptible in reaction. Neither of these extremes are usable for data. Ideally, the entire population should emerge the same day and be identical in size at inoculation. In practice, the age spread may be as great as seven days. It is doubtful if complete uniformity could ever be expected in a segregating population.

Adapting the Disease Grading System to the Computer

Various grading scales have been used to measure the intensity of blight reaction on cotton. From two to as many as 12 grades have been used to portray the stepwise sequence from immunity to full susceptibility. Visual estimation of size, shape, color, and wetness of the lesions are most often used (8).

Most authors recognize a threshold point on their numerical scales where a division between resistant and susceptible is drawn. A threshold point indicates a possible need for separate growth functions to characterize disease development in the resistant and susceptible grades (14). Wright (20) has pointed out that growth factors generally contribute percentage increments rather than absolute ones, and logarithms of measurements may be more appropriate than the measurements themselves. Gates (7) pointed out that there is some arbitrariness involved in choosing any scale. Further complexities arise when the measurements are the result of two interacting genetic systems, such as host and pathogen (2).

The influence of temperature and humidity (3) and light (2) in modifying the expression of any specific genotype are recognized. With precise environmental growth chambers it should be possible to devise experiments that will reveal semi-quantitative gene action with a high degree of accuracy. The very necessity of precise environmental controls limits the range in which the obtained results have an assured validity. On the other hand, the magnitude of the effects of microclimate with respect to plant age and spacing place a practical limit on the capacity for accurate subdivision into grades of resistance or susceptibility. Familiarity with the developmental pattern of the specific trait under study is a prerequisite for successful analysis.

Table 3. Description of blight reaction grading system.

Reaction type	Grade	
Immune	7*	No discoloration of inoculation scratch.
Highly resistant	6	Inoculation scratch only slightly discolored; no evidence of spread of infection beyond the scratch.
Resistant	5	Inoculation scratch largely discolored with evidence of very slight (pin point) spreading in spots; lesion completely dry.
Moderately resistant	4	Spreading of infection up to 0.5 mm away from scratch; lesion essentially dry.
Intermediate	3	Spreading of infection up to 1 mm away from scratch; lesion still moist in spots, but dark and not 'waxy' in appearance.
Moderately susceptible	2	Lesion spreading 1-3 mm; the entire margin moist and partially waxy in appearance.
Susceptible	1	Lesion spreading 3-5 mm, moist and waxy throughout.
Highly susceptible	0	Abnormally large, moist, waxy lesions.

* The highest numerical grade was assigned to the highest level of resistance; since resistance genes are best handled on the computer as contributing positive increments to the resistance phenotype.

Table 4. Statistical analysis of disease reaction grading using cotyledons for paired samples in an eight class system.

Source population	Disease reaction	n pairs	t	P	sd^-
8373	Resistant	93	+1.8013	0.05-0.10	0.08
8373 × T582	Segregating	134	+0.9445	0.30-0.40	0.08
T582	Susceptible	87	-0.7607	0.40-0.50	0.07

Blight resistance phenotypes were divided into 8 classes (fully susceptible class 0 to immune class 7) (Table 3) in the example cited in this paper. Grade three is the threshold grade between resistance and susceptibility. Below this grade the incompatibility reaction between host and pathogen may be more important than the mere multiplication of pathogenic cells and the enlargement of lesions. Above this grade, growth and enlargement occur on a geometric, or possibly logarithmic scale. Because the true quantitative disease expression is not known, this scale was adopted after repeated attempts as the most realistic and practical scale available. Table 4 gives estimates of precision and repeatability achieved with this system of measurement. Cotyledon lesions were scored independently and at random on both resistant and susceptible parents and a segregating progeny. The results were tabulated, with a listing of paired cotyledon readings for each plant, and analyzed. In no case were the paired readings significantly different. Readings on the susceptible source were more consistent than those on the resistant source, which may indicate that differences in moistness and color of the lesion (resistant range of grades 4 to 6) are harder to classify accurately or are more sensitive to minute environmental differences than differences in lesion size (susceptible range of grades 0 to 2). Because the 8373S readings verge on significance, it was not justified to further subdivide the disease reaction scale into more than eight classes.

Table 5. Resistant, susceptible, F_1, and segregating populations of cotton classified into eight grades of resistance to bacterial blight.

Source	Type	Disease reaction class frequency: Susceptible classes 0	1	2	Intermediate classes 3	4	Resistant classes 5	6	7
A. Progeny test 1.									
T582	sus.	2	15	2	1	1	0	0	0
8373-13	rst.	0	0	0	0	2	7	6	0
8373-13 × T582	F_1	0	0	2	2	6	5	0	0
B. Progeny test 2.									
T582	sus.	16	33	37	4	0	0	0	0
8373-13	rst.	0	1	10	26	26	26	6	0
8373-13 × T582	F_2#1	3	29	37	20	21	23	2	0
C. Progeny test 3.									
8373-32 × T582	F_2#2	5	12	18	9	34	17	5	0
8373-12 × T582	F_2#3	2	18	24	21	23	8	4	0

Analysis and Interpretation of Blight Resistance in Sources 8373S and T582

Distribution of resistant and susceptible populations and segregating progenies into seven disease reactions (the immune class is not represented) is shown in Table 5. The mean for the resistant parent 8373S-13 is 3.87, almost exactly 2.5 grades higher than the susceptible parent at 1.32. Selfed progeny of resistant parent 8373S-13 show an excessive spread in disease reaction that is probably due to variations in micro-environment.

The chi-square tests for heterogeneity applied to the three F_2 segregating progenies in Table 5 showed that segregating progeny number 2 (8373S-32 × T582), which has a much higher frequency of plants in grades 4 to 6, actually represents a different population. Progenies 1 and 3 gave a nonsignificant test for homogeneity; these two segregating progenies represent the same population-environment complex. There was no indication of which complex most closely represents the 'normal' population environment complex. Therefore both sets of data were analyzed and compared.

Table 6 gives gene action solutions to data sets 8373S-32 × T582 and 8373S-12 and 13 (pooled) × T582. Two major genes account for most of the resistance increment. At least one of these shows partial dominance; the other may be either full or partially dominant. One gene is probably augmented by epistatic interaction, either in combination with the other major locus or with the third locus, a minor recessive contributing an increment of between 1 and 2 resistance. Because of the small amount of resistance in the susceptible parent, the minor gene in the model may possibly be derived from this source.

DISCUSSION

Considerable care must be given to several factors in analysis by computer, particularly any aspect of nongenetic variability and scaling. Wright (21) deems understanding and/or control of nongenetic variability the first essential to successful analysis of quantitative factors, noting that different strains, even parental strains and F_1's, may be nonuniform in their response to different environments. On the other hand, gene action cannot be studied or defined in the absence of a cellular environment or an external environment. The only practicable solution is to specify the limits for the environmental parameters

Table 6. Computer analysis of segregating progenies of 8373 blight resistant × T582 blight susceptible.

		Gene 1	Gene 2	Gene 3	
I. F_2 seg. #2 (8373-32 × T582)					
Model #1	D2	3*	1 (2)†	0	
	D1	2*	1 (2)	0	x^2 = 10.692
	D0	0	0	1	
Model #2	D2	2*	2 (3)	0	
	D1	2*	1 (2)	0	x^2 = 11.840
	D0	0	0	1	
II. F_2 seg. #1 and 3. (8373-12 and 13 × T582)					
Model #3	D2	3	2	0	
	D1	1	1	0	x^2 = 12.409
	D0	0	0	2	
Model #4	D2	3 (4)	2	0	
	D1	1 (2)	1	0	x^2 = 12.554
	D0	0 (0)	0	1*	

* Denotes key independent gene of a complementary pair of loci that increases the value of the dependent gene whenever the active forms of both are present. † Numbers in parentheses denote the increased value of a dependent gene when it is present together with the active form of the key independent gene.

(e.g. temperature, humidity). A proposed model of gene action is valid only for the specified host tissue and range of environment (2).

The number of combinations possible with so few unequal loci makes the computer a virtual necessity in further probing gene action in semi-quantitative traits. Despite the number of trial models fitted to the data in the course of iteration, several examples show the rigorous discrimination in deciding which of the conventional models that occur as solutions may indicate interesting new possibilities for study. "Conventional" gene action is merely what we have observed and/or understood about gene action thus far. Genes obviously are not limited by our scope of understanding, and means of gathering new insights may prove most valuable.

The method of analysis reported in this paper could not be expected to give estimates of gene action as precise as those obtained by partitioning methods, diallel, or inbred backcross techniques.

The time, numbers, and effort required for computer analysis are much less than in any of the methods mentioned above. Tests using F_2 class frequency data taken from experiments analyzed by the partitioning method have generally given similar results when analyzed by the computer program. Therefore, a good tentative hypothesis of gene action in a given cross can be arrived at with only a fraction of the effort. Data carefully taken from F_2's of 200 to 350 plants have given good estimates. The method reported here should serve quite well in preliminary studies, especially if a number of crosses are to be screened.

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