Genetics and Physiology of Primary Infection by Erysiphe graminis

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The application of genetics to the study of disease physiology can be discussed in many ways. One could begin by asking questions related to the inheritance of virulence and resistance, or to biochemical changes related to virulence or resistance. Results would be recorded as infection types or different chemical analyses. These data would be *correlated* with what one observes to happen to the plants at some time after inoculation with the pathogen. One could also take the correlated phenomena and critically evaluate, experimentally, the bases and significance of the correlations. The length of time that elapses between inoculation and when the observations are made greatly affects the interpretations that can be made. It seems most logical to the author that, to study parasitism (the basic capability of one organism to live on and at the expense of another organism), it is necessary to begin with the hour of inoculation, and make observations of the earliest interactions until compatible, functional relationships are established between host and parasite. It should be easier to get estimates of the number of critical systems, and cause-and-effect relationships during the synthesis of compatible, functional relations between a host and a parasite, than by comparative studies in advanced stages of disease development. Furthermore, excellent near-isogenic host and pathogen strains are now available for critically evaluating all stages of interactions between host and parasite.

I wish to present here the ways in which both genetic and physiological arguments and procedures have been used to study the biological process of primary infection of wheat and barley by *Erysiphe graminis* DC.

The approach used with *E. graminis* assumes that there are a series of distinct steps or stages from the moment a parasite unit is placed on a host until a compatible, functional relationship is established. The interactions may eventually reach a climax in an infection type or an indeterminant status of the diseased condition. The infection type is an indication of whether particular genetic units are involved in the interaction. The inheritance of infection type gives an indication of the number of genetic units involved in the interaction and, therefore, an indication of the number of systems involved in determining the compatibility between host and parasite. Does each genetic unit act in its own unique manner? Or do all act by the same mechanism? Is incompatibility between host and parasite expressed early in interactions between host and parasite?

Before attempting to answer these questions, a brief review of the concept of the gene-for-gene relationship and the process of primary infection—from deposition of conidia on a host leaf to the formation of secondary hyphae capable of initiating secondary infections—is given below to put into perspective the arguments and results that follow.

Gene-for-gene concept.—In gene-for-gene relationships, there are at least two alleles in the host and two alleles in the pathogen for each corresponding pair of genes. A resistant *(R)* gene in a plant can be identified and observed to segregate in a cross with a susceptible *(r)* plant only by inoculations with a pathogen that is avirulent; i.e., a pathogen that contains a *P* gene that can interact with the *R* gene. The parasite/host genotype *P/R* specifies resistance by the host, or avirulence by the pathogen, or, to use the terminology of this paper, an incompatible relationship between host and parasite. All other combinations of the alleles of these two genes; i.e., *P/r, p/R,* and *p/r* (parasite/host genotype) confer susceptibility by the host or virulence by the pathogen, or, to use the terminology of this paper, a compatible relationship between host and parasite. The parasite gene *P* and the corresponding host gene *R* together are called a corresponding pair of genes or a gene pair. The interactions are very specific in that *P1* recognizes only *R1; P2* only *R2; P3* only *R3*; etc. *P1* will not interact with *R2* or *R3* to give an incompatible relationship between host and parasite. The four possible parasite/host genotypes for a single pair of genes constitutes what is called the "quadratic check".

The process of primary infection.—Morphology of primary infection.—The process of primary infection consists of a number of morphologically identifiable stages (Fig. 1): spore germination; formation of appressorial initials (swelling of germ tubes); maturation of appressoria; formation of a penetration peg which penetrates the host cuticle and epidermal cell wall; formation of a haustorium in the epidermal cell of the host; and the formation of secondary hyphae which elongate and are capable of initiating secondary appressoria and haustoria (3, 4, 8). The formation of elongating secondary hyphae capable of initiating secondary infections is taken as evidence that the host and parasite have established compatible, functional relations. If the host and parasite have established compatible, functional relations, then all the criteria for parasitism are satisfied. All events necessary for establishment of parasitism, therefore, must have already occurred.

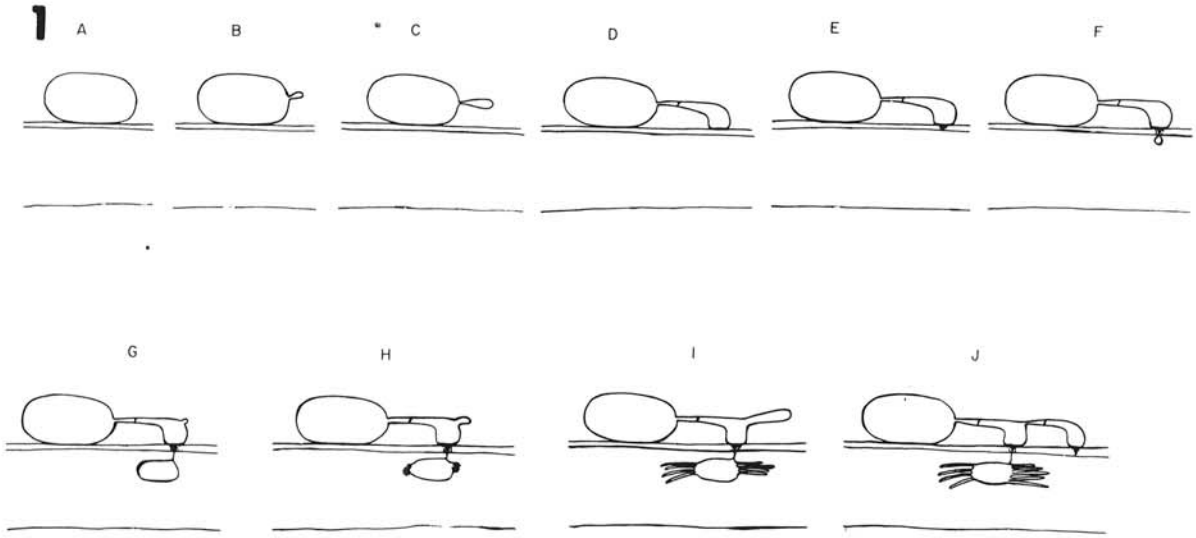

Fig. 1. Morphological stages of primary infection by *Erysiphe graminis*.

Infection efficiency.—The importance of critically defining the biological phenomena one wishes to study cannot be overemphasized. The possibility of misinterpreting data is greatly increased if a rather detailed knowledge of exactly what the organisms are doing is not available. For example, conidia of *E. graminis* produced during a period of 2-4 hr in a controlled environment might be assumed to be physiologically uniform, but they are not (8). Conidia differ in their capacity to germinate in different environments. It was shown (8) that ca. 60% of the conidia germinated in 4 hr at 18 C and at a relative humidity (RH) approaching 100%. Approximately 40% germinated in 4 hr at 22 C and 65% RH. Significant increases in the percentages over the subsequent 20 hr were not observed with a single environment. One could assume that only two-thirds of those germinating under the first set of conditions could germinate under the second set of conditions (Fig. 2-a). Almost all conidia were induced to germinate, however, if given 1 hr of 18 C and 100% RH followed by 3 hr of 22 C and 65% RH. The more probable interpretation is that the spores were of two or more distinct physiological types, each capable of responding to distinct environmental conditions (Fig. 2-b). There is essentially little or no overlap between the two populations (8).

If 95% of the conidia in a population can be induced to germinate, and therefore are viable, then any experimental procedure (e.g., change in parasite/host genotype which results in a reduction of the percentage of germinating spores to 20%) must have obtained at least 70% of the conidial population from the category of those spores capable of germination if given a parasite/host genotype which specifies compatibility between host and parasite (Fig. 2-c). In such an experiment, there is only one significant variable; namely, genotype. If, in another experiment, the per cent germination of conidia is 10% for treatment A and 1% for treatment B, it would be impossible to determine if the 1% that germinated with treatment B is a part of the 10% that germinated with the treatment A (Fig. 2-d), or if the 1% that germinated with treatment B represents a sample from a different portion of the population than the 10% that germinated with treatment A (Fig. 2-e). The two variables in such an experiment are the treatment and the physiologically different portions of the parasite spores.

This type of argument has been applied to most stages of primary infection (1). Under optimum conditions, over 80% of conidia applied to a wheat leaf can be induced to proceed through primary infection and to produce elongating secondary hyphae by 26 hr after inoculation with a compatible parasite/host genotype (2, 3, 4, 9). If, however, with the incompatible parasite/host genotype *P4/Pm4* only 10% of the parasite units developed to produce elongating secondary hyphae, at least 50% of the conidia that did not succeed in producing elongating secondary hyphae failed because of a single variable, namely, genotype.

Synchrony.—Synchrony is defined in these experiments to be the degree to which all members of a population of parasite units proceed through each of the stages of primary infection together. Ideally, it was considered desirable to have all of the parasite units proceed to penetrate the host epidermis at the same hour after inoculation, all possess haustoria of similar size at a given hour after inoculation, and all initiate the formation of elongating secondary hyphae at the same hour after inoculation. With a high degree of synchrony, the chances of detecting a molecular or physiological change associated with a particular stage of interactions between host and parasite are greatly increased. Furthermore, the precision with which it is possible to pinpoint a molecular change and stage of primary infection greatly affects the interpretations of data. For example, if only the parasite units that had reached the most advanced stages of development were considered in a nonsynchronized population, the interpretations of how and when different

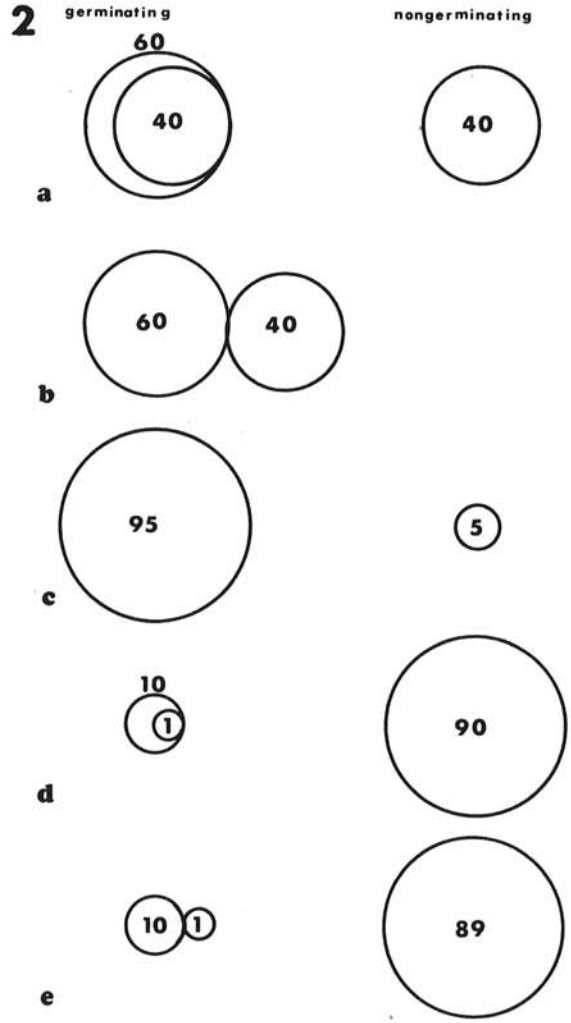

Fig. 2. a) The 40% of the spores germinating at 22 C and 65% relative humidity (RH) are two-thirds of the spores that would have germinated at 18 C and 100% RH; **b)** 40% of the spores germinating at 22 C and 65% RH are not the same spores that would have germinated at 18 C and 100% RH; **c)** 95% of the spores germinate, 5% do not; **d)** 1% of the spores germinating with one treatment is part of the 10% germinating with a different treatment; **e)** 1% of the spores germinating with one treatment is not part of the same portion of the population that gives 10% with a second treatment.

parasite/host genotypes for incompatibility act to affect the interactions between host and parasite would be, and have been, quite different from the interpretations given for data with more synchronized populations.

By achieving both a high efficiency of infection and a high degree of synchrony of the parasite population, it has been possible to determine the hour after inoculation at which the expression of the interaction of several different parasite/host genotypes for incompatibility can be detected.

The role of cuticle in primary infection.—Spores of *E. graminis* placed on either wheat or barley, when given the appropriate environment, proceed to germinate, produce appressorial initials, and produce characteristic appressoria which are called "mature" appressoria. Spores placed on agar media, synthetic membranes, and a variety of surfaces produce appressoria which are not characteristic of appressoria produced on wheat or barley, and, therefore, are called "malformed" appressoria (12). The latter differ in both size and shape from appressoria produced on wheat and barley.

Both *E. graminis* f. sp. *tritici* and *E. graminis* f. sp. *hordei* produce mature appressoria on wheat and barley seedlings. Malformed appressoria are either not formed, or are formed in very low percentages.

Normal, mature appressoria are formed (i) on the upper surface (with cuticle) of epidermal strips; (ii) on the upper (outer) surface of cuticles isolated by enzymatically separating them from epidermal cells of epidermal strips floated on extracts from *Myrothecium verracucaria;* (iii) on enzymatically isolated cuticles grafted onto the underside of epidermal strips or onto mesophyll cells; and (iv) on cuticles or epidermal strips from six wheat lines, each of which had a different *Pm* gene, and from four barley lines, each of which had a different *M1* gene.

Mostly malformed appressoria were formed (i) on the lower surface of epidermal strips; (ii) on mesophyll cells exposed after removal of epidermal strips; (iii) on the under (inner) surface of enzymatically isolated cuticles; (iv) on reconstituted cuticles after extraction of the wax layer either from isolated cuticles or intact leaves; and (v) on plants which possessed eceriferum (wax) mutations.

Since normal, mature appressoria are principally formed on what is originally the outer surface of natural cuticles, it appears that only the wax layer stimulates the formation of mature appressoria. A hypothesis that toxic substances inhibit the formation of mature appressoria in some treatments does not appear plausible. The fact that none of the *Pm* and *M1* genes affects the formation of mature appressoria on either enzymatically isolated cuticles or epidermal strips suggests that these genes do not impart any specificity to the wax layer which can interact with the *P* genes in the pathogens to affect the formation of mature appressoria.

Reconstituted wax layers do not provide what is necessary for the formation of mature appressoria. This suggests that the physical structure is more important than the chemical composition of the wax layers. Five different eceriferum mutations (*cer-J*[59], *cer-J*[71], *cer-Zd*[67], *cer-Ze*[81], *and cer-Zj*[78]) which affect the composition and physical structure of the wax layer of barley all adversely affect the formation of mature and normal appressoria. These effects are seen after inoculation of the intact plant, the epidermal strips, or the enzymatically isolated cuticles (12). From what is known about the chemical analyses and physical structure of cuticles using scanning electron microscopy, it appears that the physical structure may be more important than the chemical constituents in stimulation of *E. graminis* in the formation of mature and normal appressoria.

The formation of mature appressoria appears to be critical to survival of *E. graminis*. Malformed appressoria rarely are capable of initiating a successful primary infection.

Formation of haustoria and secondary hyphae.—Mature appressoria produce infection pegs about 10-12 hr after inoculation. Rudimentary haustoria can be first observed 12 hr after inoculation. Maximum size of the haustorial body is obtained by 18 hr after inoculation, when appendages are first beginning to form (4). A nucleus is obvious in the haustorium at about 16 hr after inoculation (6). Once full-sized haustorial bodies are produced, secondary hyphae are produced which are capable of eventually producing secondary infections. When a haustorium is not produced under the primary appressorium, secondary hyphae may begin to form on the appressorium but will not continue to elongate and initiate the formation of secondary appressoria and secondary infections (4). Therefore, the formation of elongating secondary hyphae is dependent on the existence of functional haustoria. The formation of functional haustoria and elongating secondary hyphae capable of initiating secondary infections is considered proof that compatible, functional relationships between host and parasite have been established. All the prerequisites for parasitism must have been satisfied. The maintenance of the compatible, functional relationship, however, is considered, for experimental purposes, to be a separate problem.

Genetic analysis of primary infection.—Effect of parasite/host genotypes on primary infection.—As stated earlier, none of the parasite/host genotypes for incompatibility (*P/Pm* for wheat mildew or *P/M1* for barley mildew) affects the formation of mature appressoria. Three of the four gene pairs affecting wheat mildew and all four of the gene pairs affecting barley mildew affect the percentage of parasite units which produce haustoria and, therefore, elongating secondary hyphae (Fig. 3) (2) (one gene pair, *P2/Pm2*, does not affect primary infection efficiency). The genotypes for incompatibility must act after penetration is attempted but before even small rudimentary haustoria are formed. The subsequent fate of the parasite units that do form haustoria with incompatible genotypes is also determined by the gene pairs (9). For example, two gene pairs, *P4/Pm4* and *P1/Pm1*, affect the infection efficiency. With *P4/Pm4*, collapse of the parasite units and a darkening of the area of the cell surrounding the haustorium is observed by ca. 21 to 22 hr after inoculation (Fig. 3). With *P1/Pm1*, collapse of parasite units is observed ca. 26 hr after inoculation, but with no observable darkening of the cell adjacent to the haustorium (Fig. 3). A clear effect of any of the other gene pairs for incompatibility on morphological development during primary infection; i.e., before 30 hr after inoculation (other than infection efficiency), in both wheat and barley mildew is not available now. The other gene pairs apparently affect the continued morphological development of the parasite subsequent to primary infection (after 30 hr from inoculation).

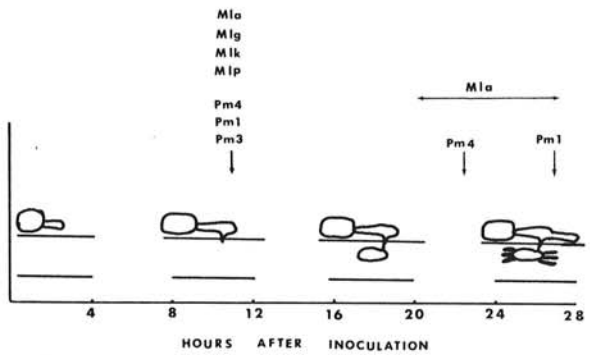

Fig. 3. The hour after inoculation when the various host genes interact with the corresponding parasite *P* genes to affect the development of *Erysiphe graminis* during primary infection.

If a high efficiency of infection and synchronized development of the parasite with compatible genotypes were not attained, the identification of the effects of the genotypes for incompatibility on primary infection would be essentially impossible. The maximum development of a limited number of parasite units is almost always the same for all parasite/host genotypes because a limited number of parasite units almost always reaches maximum development regardless of the parasite/host genotype. From our observations to date, it appears that the various genotypes for incompatibility present a series of hurdles for the parasite. A certain portion of the parasite units gets over each hurdle. The portion of the parasite population stopped by each hurdle, and the time after inoculations when the interactions between host and parasite affect the ontogeny of the parasite, are unique to each gene pair (2). For example, *P4/Pm4* affects infection efficiency at 10-12 hr after inoculation and subsequent development ca. 21 hr after inoculation. *P3/Pm3* affects infection efficiency 10-12 hr after inoculation and subsequent development sometime after 30 hr after inoculation but before sporulation would begin at about 96 hr after inoculation. *P2/Pm2* does not affect infection efficiency, but does operate somewhere between 30-72 hr after inoculation to cause collapse of infected host cells and adjacent host cells. Clearly, there must be several stages in the ontogeny of the aegricorpus.

Seven of the eight gene pairs (*P/M1* and *P/Pm*) studied affect the kinetics of ^{35}S transfer from host to parasite during primary infection (7, 11). The kinetics of ^{35}S transfer for wheat mildew cannot be explained simply by the effects of the four gene pairs on infection efficiency. Three of the four gene pairs studied affected the kinetics of ^{35}S transfer before the effects on morphological development of secondary hyphae were observed.

Segregation of per cent elongating secondary hyphae, ^{35}S transfer, and infection type.—Data from three types of observations—per cent elongating secondary hyphae, kinetics of ^{35}S transfer from host to parasite, and infection types 7 days after

inoculation—have been correlated. The *Pm* and *M1* genes were first identified by their effect on infection type; so by definition, the *Pm* and *M1* genes are genetic elements affecting infection type. The preliminary evidence that the various *Pm* and *M1* genes affect the per cent elongating secondary hyphae and ^{35}S transfer was obtained by inoculating host lines that were highly isogenic except for specific *Pm* or *M1* genes (7, 11). The data suggested that the *Pm* and *M1* genes did control the formation of elongating secondary hyphae and ^{35}S transfer. If this is true, there should be an absolute association among the three types of observations in generations of the host segregating for either *Pm* or *M1* genes or of the pathogen segregating for *P* genes. If the three types of phenomena observed were controlled by separate genetic factors, segregation of the control of the separate phenomena would be expected among progenies of the crosses, and an absolute association would not be observed.

Both types of segregations have been observed in the host with the powdery mildew disease, even though highly isogenic host lines have been used in all experiments. The kinetics of the formation of secondary hyphal *initials* is clearly not controlled by the *M1* genes in barley (5). Gene differences between the highly isogenic barley lines other than the *M1* genes are clearly involved. On the other hand, there is an absolute association between the per cent of secondary hyphae which elongate (and are capable of initiating secondary infections) and the factors that control final infection type. The control of the formation of elongating secondary hyphae, however, cannot be clearly observed in F_2 populations of the host, apparently because of an influence of the heterozygous parent on the progeny (12). The per cent elongating secondary hyphae varies greatly on homozygous plants (dominant or recessive) derived from heterozygous parents. The per cent elongating secondary hyphae does not vary greatly on homozygous plants derived from homozygous F_2 plants.

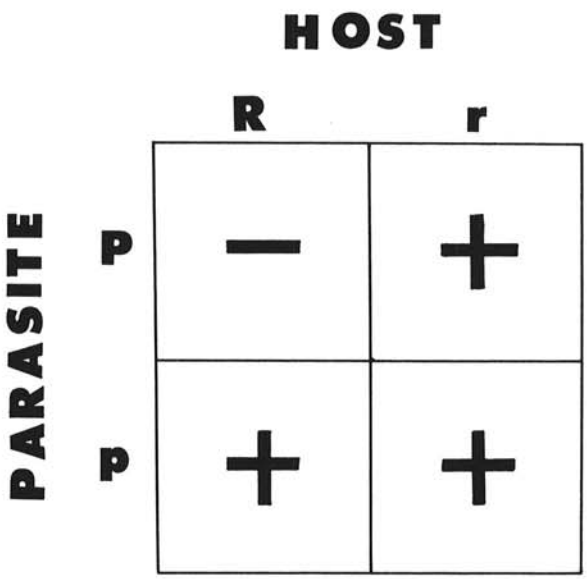

Fig. 4. The quadratic check as applied to *Erysiphe graminis* and wheat or barley: (–) = an incompatible host-parasite relationship; (+) = a compatible host-parasite relationship. The genotypes were first identified by their effects on infection type 7 days after inoculation.

The quadratic check.—There are four possible parasite/host genotypes involving one locus in the parasite and one locus in the host (Fig. 4) (10). Only one genotype, *P1/R1*, gives an incompatible relationship between host and parasite (host "resistant" and pathogen "avirulent") as originally identified by infection type. The other three parasite/host genotypes (*P1/r1*, *p1/R1*, and *p1/r1*) condition compatibility between host and parasite. The simplest hypothesis is that specific host-parasite interactions occur to give an incompatible relationship. If specific interactions occur to give compatible relationships, there should be differences among the three genotypes which specify compatibility between host and parasite.

The quadratic check has now been completed for two gene pairs using the three criteria of infection type, per cent elongating secondary hyphae in primary infection, and ^{35}S transfer from host to parasite. Essentially identical results were obtained by all three criteria with the three genotypes *Pg/m1g*, *pg/M1g*, and *pg/m1g* affecting barley mildew. Essentially identical results were obtained with the three genotypes *P1/pm1*, *p1/Pm1*, and *p1/pm1* affecting wheat mildew by the two criteria of infection type and the per cent elongating secondary hyphae in primary infection. By the criterion of ^{35}S transfer from host to parasite during primary infection, the three genotypes for compatibility are different (11). It is tempting to speculate that the effect of transition from *pm1* to *Pm1* in the host is not completely negated by the transition from *P1* to *p1* in the parasite. On the other hand, the differences observed with the three genotypes may be due to other gene differences between the two isogenic host lines and the nonisogenic strains of the parasite.

Differences have been observed between the three genotypes for compatibility with one gene pair, but not with a second gene pair. Which is the more universal phenomenon will have to await completion of the quadratic check with many gene pairs and the use of many strains of host and parasite for a single gene pair.

Predictions of the actions of alleles.—As stated earlier, a basic premise in the research on the mildews is that there is a series of distinct steps in the ontogeny of interactions. We also assume, for experimental purposes, that the different genes in the host, together with their corresponding genes in the parasite, operate to affect unique stages of ontogeny of the interactions. The data support this assumption. If the product of a given *R* gene is important in a given stage of interactions, then one would expect the product of an allele of that gene also to be important in the same stage of interactions, but possibly with a different intensity because of the modified product. The fact that the alleles in host interact with *P* genes

in the parasite that are not allelic is not critical for this argument. Only one such test has been made with the mildews. There are three alleles (as determined by standard genetic tests) at the *Pm3* locus in wheat. One allele, *Pm3c*, is not expressed in the first leaf stage. Its expression as infection type is the most obvious on plants inoculated after the fourth or fifth leaves are produced on the plant. Therefore, only a single pair of alleles, *Pm3a* and *Pm3b*, have been tested. *Pm3b* appears to affect the ontogeny of primary infection on the first leaf essentially identical to *Pm3a*.

There are six alleles at the *M1a* locus in barley. Only the allele from the variety Algerian has been tested. If all six are true alleles, we expect that all will affect similar stages in the ontogeny of the interactions. If this chromosomal region is composed of two or more closely linked regions, two or more patterns in the effects of the genes on the ontogeny of interactions should be observed.

The ability to predict the results represents, in my opinion, a major step in determining cause and effect relationships between a number of different phenomena occurring during the early interactions between host and parasite.

Summary.—By achieving a high efficiency of infection and a high degree of synchrony of the parasite population, it is possible to detect subtle effects of experimental treatments on the early stages of interactions between host and parasite.

It is possible to combine physiological arguments with genetic arguments such as use of naturally occurring genotypic differences and induced mutations to evaluate, for example, the structure and function of the cuticle in the process of primary infection.

Naturally occurring genes in host and parasite which follow the rules of the gene-for-gene hypothesis do affect some early stages of interactions between host and parasite.

A separation or an absolute association in segregating populations between two or more observed phenomena provides evidence that the two or more phenomena are connected by a cause and effect relationship, effects of the same cause, or have no relationship.

The quadratic check provides a means to test whether the genetically controlled specific interactions are needed for the development of compatible or incompatible relationships between host and parasite.

Allelic series can be used to predict and evaluate interpretations of cause and effect relationships between correlated phenomena.

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