DAY

GENETICS OF HOST-PARASITE INTERACTION

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Peter R. Day
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PREFACE

Had breeding for resistance been a universally successful and reliable means of controlling plant parasites, there would be no reason for this book. But parasites vary and many have circumvented host-plant resistance. There are many papers describing such variation in parasites and even more on breeding for resistance in crop plants, but there are very few general accounts which deal with the genetics of both plant hosts and parasites and their interactions. I have tried to remedy this deficiency. Although my discussion is based largely on fungal parasites, I also draw on insects, nematodes, bacteria, and viruses. My chief concern is the work of the last ten years, although I have included some historical perspectives. My approach is speculative. This is especially true of Chapter 5, which explores gene function, an area in which our knowledge is surprisingly slim and where conceptual frameworks help to focus ideas. I have written for an audience of advanced undergraduates, research students, and researchers in genetics, plant breeding, plant pathology, entomology, and related fields. I have assumed a basic knowledge of genetics in my reader, but since information on the parasites is scattered, I have tried to introduce the nonspecialist to each major group that I treat. Important

developments in the study of plant-parasite interaction include the extension of Flor's gene-for-gene concept to a range of interactions, the use of isogenic lines and temperature-sensitive genes in the study of the biochemistry of disease resistance, and the heightened interest in biological controls arising from dissatisfaction with pesticides. These and other topics are discussed.

Wise and intelligent methods of crop protection can only be based on an appreciation of the principles of host-parasite interaction and the consequences of interfering with it. Entomologists, plant pathologists, and plant breeders have learned much from each other, but will do so more readily with a clearer understanding of the genetic basis of their problems.

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April 1973 *Peter R. Day*

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THE HOST-PARASITE INTERACTION

Each host-parasite interaction is a struggle for survival between two organisms. The host plant, already competing with other plants for space, light, and food materials, has the added burden of supporting another organism. The parasite not only usurps its host's food but also actively impairs its ability to make more food by invading and destroying host tissue. The consequences for the host may be trivial, or if it dies before reproduction, may threaten its survival as a species. The parasite must produce many offspring so that at least one may encounter a fresh host at an opportune time to ensure survival. The parasite must resist or avoid the defense mechanisms which ward off the myriads of other organisms with which the host comes in contact. If the parasite is specialized so that it will reproduce only on certain host species, it must not be so destructive that it eliminates the host individuals entirely or it will die out along with them.

In nature a state of balance exists, and surviving plants and their parasites are capable of coexistence. If parasites reduce both inter- and intraspecific host competition, then they may have some adaptive value to their hosts. However, much that we shall learn of their genetic flexibility

suggests that during evolution parasites have been kept in check by the requirement to conserve their hosts for their future survival.

The genetic basis of this plant-parasite interaction is the subject of this book. Most of our knowledge of this interaction arose as a result of a need to cope with the consequences of our altering the balance between host and parasite by growing crops for food and fiber. Large-scale culture of genetically uniform crops is unnatural and leads, inevitably, to the possibility of equally large-scale crop destruction by parasites. To keep the balance in our favor, we developed the sciences of plant pathology and entomology to understand parasites better, know their limitations, and be able to contain and control them. Breeding for resistance became a powerful tool to protect crops, but it revealed a corresponding ability to fight back on the part of the parasites. The resulting perturbations and fluctuations, which were only minor on an evolutionary scale, caused major epidemics and much human suffering. Man prepared his crops in a way that seemed almost to invite their mass destruction. Knowledge of parasites and parasitism, still far from complete, has become much clearer in the last 30 years. Pest control is still far from satisfactory, but without it life as we know it today would be very different. The increasing demands made on agriculture as the population of the world continues to grow make it imperative to find better, and ecologically sound, methods of protecting crops.

THE ROLE OF GENETICS

All biological phenomena are based ultimately on genetic controls. Experimental genetics is the science of dissecting and describing the function of these controls. We can expect genetics to define the components of host-parasite interaction by isolating effects that, as far as we can tell, are due to single genes in host or parasite. A difference between two reactions known to be determined by alleles at a single locus is intrinsically more amenable to explanation than one that has not been so defined. Reason and intuition tell us that it is likely to be simpler than a difference determined by alleles at two or more loci, but even in such terms a complete explanation of the difference between resistance and susceptibility is still a difficult and, in most cases, intractable problem. The biggest obstacle is the unequivocal identification of single-gene effects. However, such an analytical approach has led to our present understanding of the nature of resistance and susceptibility of crop plants. It should, in turn, lead to better ways of controlling the parasites that destroy our crops.

For most of this book I shall be considering higher plants as the hosts. Although their parasites include viruses, mycoplasms, bacteria, fungi, other higher plants, nematodes, and insects, my discussion will be

concerned mainly with fungal pathogens. Relatively little work has been done on the genetics of the other plant parasites, but what is known about them suggests that in their interaction with their hosts, all these parasites are remarkably alike. The examination of parasites other than fungi will be to demonstrate this similarity. To describe the interaction we need terms with precise meanings. Some of these are outlined in the following pages. The others I will discuss later in their proper contexts.

SOME DEFINITIONS

The distinction between pathogen and parasite is sometimes useful. A *parasite* lives in or on another organism from which it obtains nutriment. A *pathogen* is a parasite which produces a disease in its host. In this context I define *disease* as a departure from normal metabolism, reducing the normal potentiality for growth and reproduction of the host, caused by the presence of a pathogen. When a host plant exposed to a pathogen is invaded and becomes diseased, we say the host is *susceptible* and the pathogen, or incitant, *pathogenic*. A host plant which does not become invaded and is not diseased is *resistant*, and the potential pathogen is *nonpathogenic* towards that host. We may conveniently distinguish here between two kinds of resistance. One is *nonhost resistance*, such as that shown by wheat to the potato late blight organism *Phytophthora infestans* or by potato to the stem rust organism *Puccinia graminis tritici*. The other, *host resistance*, is the result of genetic modifications of the host which render it resistant to pathogens that would otherwise grow on it. Although nonhost resistance may be more complicated than host resistance, both could in fact be due to the same mechanisms. However, a simple test of this idea will have to wait on our developing the skills needed to cross wheat and potatoes.

This book is concerned almost entirely with host resistance, and rather than use the term nonpathogenic to describe a frustrated pathogen, I will use the term *avirulent*. The term *virulent* will mean ability to produce disease on a resistant host (Day 1960). As I show later, the distinctions between these alternatives are not very clear-cut, and there are degrees of resistance and degrees of pathogenicity. When we examine the differences between resistance and susceptibility, the reason for gradation will become apparent. When resistance is absolute, so that no pathogen development occurs at the expense of the host, it is sometimes defined as *immunity*. In practice, the term is often used if there is no macroscopic symptom of infection following exposure to the pathogen. There is a danger in confusing the term immunity in plants with immunity in animals where it implies interaction of antigens and antibodies. There is little or no evidence of comparable responses in plants (see page 149), and immunity here only

refers to a symptomless phenotype. We must always remember that we are describing an interaction between two organisms, and the terms resistant and susceptible describe it only from the host's point of view. To emphasize this point, Loegering (1966) has suggested the terms "low infection type" (to describe a resistant or nonpathogenic interaction) and "high infection type" (to describe a susceptible or pathogenic interaction). The terms "incompatible" and "compatible" also describe these interactions. Such terms are useful since they deal with host and pathogen together. As with the other terms, however, the boundary between them must be defined, and this is not always easy to do.

Parasites belong to two general classes: *obligate* and *facultative*. Obligate parasites cannot multiply in nature without their hosts. For example, viruses cannot multiply outside their host cells. Until recently the rust fungi were considered to be obligate parasites, but some races of certain rusts have been cultured on defined media (see pages 64–66). However, even these are still obligate parasites, since a host plant is necessary for multiplication in nature. No doubt other obligately parasitic fungi, such as the downy mildews (*Plasmopara*), powdery mildews (*Erysiphe*), and other rusts, will be grown in axenic culture as soon as their growth requirements have been established. Facultative parasites, on the other hand, can grow and live on substrates other than living host tissue and can usually be cultured on relatively simple media.

The range of plants which serve as hosts for parasites varies greatly. Many pathogens are highly specialized and limited to a single species or even race of host plant, whereas others are unspecialized and may infect plants in many different genera. Some pathogens, such as certain rusts, complete different phases of their life cycles on unrelated host species which belong to different families or even different phyla. The crop plant or economic host is sometimes known as the *primary host*. The host on which the life cycle is completed is known as the *secondary* or *alternate host*.

METHODS

The detailed methods of studying the genetics of plant parasites and their host interactions are as varied as the organisms themselves. I shall consider them briefly under the three headings of host, pathogen, and interaction.

The Host

The preparation of host material has to satisfy several important criteria: it must be well-grown and vigorous when produced under standard, readily

reproduced conditions and available in sufficient quantity. Host plant material is used in two ways: to study variation in resistance of the host itself and to study variation in pathogenicity of the parasite. A plant breeder uses host material in the first way. He must know whether the resistance or susceptibility he observes is a feature of juvenile tissues, such as those found in seedling plants, or of mature tissues, and he must test his plants at the appropriate stage of development. It is also important to know whether the reactions observed truly reflect the host genotype and are not due to abnormal cultural conditions. For example, tomatoes carrying the gene Cf_1 for resistance to *Cladosporium fulvum* are susceptible if grown in short winter days with low light intensities, whereas in long days and sunny conditions they are resistant to appropriate forms of the pathogen (Langford 1937).

The plant pathologist, on the other hand, uses host material in the form of a set of standard varieties to observe the variation among different isolates of a pathogen or the segregation of pathogenicity in a pathogen population. Again, uniformly optimum cultural conditions are important so that comparisons of results obtained at different times and in different places are valid. The host material can consist of germinating seeds, seedlings, and young (or mature) plants or plant parts such as detached roots, leaves, fruits, or even tissue cultures. Culture facilities range from a test tube, the laboratory bench, illuminated incubators, growth chambers, greenhouses, and test plots to the farmer's fields and orchards.

Host material is now maintained in the form of seed stocks and species and variety collections of living plants. In the future some genetic stocks will no doubt be maintained as tissue cultures. Genetic purity is vital and must be safeguarded by controls which eliminate seed admixture, pollen contamination, and other mechanical errors. At the same time, the possibility of mutation must be recognized so that if it is suspected, or likely (some recombinants occur at comparably low frequencies), it can be detected and its frequency estimated by test crosses, adequate replication, and checks (see page 23).

The Parasite

Most of the points mentioned in connection with the preparation and preservation of host material apply equally well to fungal pathogens. There are, however, several special problems. Most obligate fungal parasites must be grown on susceptible host plants. Cross-contamination is a serious problem in maintaining cultures on host plants in a greenhouse because of the difficulty of ensuring complete isolation. For rusts the problems can be minimized by storing uredospores in liquid nitrogen, where viability has

been maintained for more than 5 years (Loegering et al. 1966). The spores are collected for preservation by the use of a vacuum-operated cyclone particle collector. Each sample is sealed in a glass ampoule before freezing so that there is no risk of cross-contamination in storage.

Cross-contamination is a minor problem for fungal parasites that can be cultured, but there is the risk that on an artificial culture medium, selection will favor forms adapted to that substrate rather than the host. The consequence is either complete loss of pathogenicity or its reduction after one or more transfers. Fortunately this problem can be largely overcome by preventing mycelial growth in storage. Slant cultures in tubes may be stored at 4° C and protected from desiccation by a mineral oil layer or screw-cap closures. A more convenient alternative which works well for many fungi is to store conidia or other cells, including even hyphal fragments, mixed with granules of dry, heat-sterilized silica gel in small vials (Perkins 1962). The fungi are recovered by transferring a few crystals to a fresh culture medium, and the same storage tube may be repeatedly sampled. Storage in the form of dried infected leaves is useful for such pathogens as bacterial blight of cotton (*Xanthomonas malvacearum*) (Brinkerhoff 1963) or *Helminthosporium* leaf blights of grasses (Nelson et al. 1970). The bacterium may be recovered by placing fragments of dried leaf on agar media, but *Helminthosporium* sporulates on leaf material kept overnight in a moist chamber. Nelson et al. recovered *H. maydis* from dried leaf samples stored for up to 15 years. For the many organisms that withstand it, lyophilization is one of the most convenient and inexpensive methods for long-term storage. It is routinely used by the centers that maintain large collections of genetic stocks of organisms like *Escherichia coli, Neurospora crassa, Aspergillus nidulans,* and *Saccharomyces cerevisiae.* The method has had some use in preserving bacterial plant pathogens.

One of the most effective methods of culture preservation is by freezing at the temperature of liquid nitrogen. The American Type Culture Collection maintains a number of plant pathogenic fungi by this means in addition to the rusts kept as frozen uredospores. Unfortunately, preservation in liquid nitrogen is presently of limited use because it is costly and inconvenient.

Mechanically transmissible viruses may be conveniently stored frozen under liquid nitrogen either in leaf tissue or as purified suspensions. Other viruses must be maintained in their host plants much in the way that obligate parasites were maintained before liquid nitrogen storage provided an alternative.

Nematodes and insects may often be maintained in populations confined, by containers or cages, on host plants grown in the greenhouse. Some can be maintained on culture media. Certain nematodes can be

grown on agar substrates with a bacterium as a food source, and others can be maintained on tissue cultures of their hosts.

The Interaction

Disease is induced in the host by inoculation or the introduction of a pathogen. For most fungal pathogens, the conditions which favor maximal rates of infection are a comparatively narrow range of temperatures, relative humidities, and light intensities. Under these conditions each spore, or other propagule, has a given probability of giving rise to an infection. The postinfection environmental conditions determine the rate of growth and development of the pathogen and the host's response to the challenge. When signs and symptoms of disease appear, they are described and recorded. Although it is often sufficient to distinguish only two classes such as high and low infection types, it may be more useful to distinguish more than two degrees of reaction. The leaf reactions of cereals to rusts, for example, are classified into 7 categories. These can in turn be grouped into low (0, 0;, 1), high (2, 3, and 4) and variable (X—the so-called mesothetic reaction) classes to simplify analysis. The scoring of disease reactions in genetic studies is generally more a qualitative than a quantitative evaluation. Pathogen inoculum density can be standardized so that comparisons of discrete lesion phenotypes are possible. As we shall see later, however, the probability of infection, the rate of lesion growth, the final size of the lesions, the degree of sporulation, and a range of other effects on the host are equally important as parameters of the infection.

Inoculation experiments commonly include control plants known to be susceptible to the inoculum. Their reactions confirm that the conditions employed favored disease expression. When the control plants respond in the way expected, the investigator has confidence in the test plant results. It is sometimes necessary to test single plants with several different races of a pathogen to discriminate their resistance phenotypes. When infection is localized, the inocula can be applied to different leaves, different leaflets, or different regions of the same leaf. Alternatively, single plants can be inoculated consecutively, allowing time for symptom expression between successive inoculations. When infection is systemic, the reaction of a single plant to several different isolates may have to be deduced by testing its progeny. An example of this is described later on page 98.

Root and stem infecting pathogens require other techniques. For example, the host plant may need to have its root system or vascular tissue exposed for inoculation. Some wilt diseases can be produced by placing a leafy host shoot in a suspension of pathogen cells. The cells taken up in the transpiration stream grow and produce wilting symptoms.

The use of detached leaves or organs sometimes make possible an increase in the scale of experiments, allowing testing of a wider range of temperatures or light intensities, or a larger number of hosts or pathogens, under conditions where growth-chamber or greenhouse facilities are limited. Some instances are known where the host plant can even be dispensed with altogether, and pathogenic and nonpathogenic isolates may be distinguished by their phenotypes on artificial culture media (see page 75, *Ustilago maydis*, and page 147, *Erwinia aroideae*).

Insect parasites are kept in contact with test populations of host plants by using cages or other barriers in the greenhouse or in the field. Infestation is established by ensuring that environmental conditions are optimum for parasite development. The experience gained in handling greenhouse or laboratory parasite populations is useful here.