Genetic Analysis of the Gene-for-Gene Interaction Between Lettuce (*Lactuca sativa*) and *Bremia lactucae*

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ABSTRACT

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Possible complexities of the gene-for-gene theory of host-parasite specificity were investigated in a genetic study of the interaction between *Lactuca sativa* (lettuce) and *Bremia lactucae* (downy mildew). Crosses between pathogen isolates were made to test whether virulence loci matching a single host resistance gene were always allelic, whether dominant inhibitor loci or other modifier genes affected the expression of avirulence loci, and whether avirulence loci were linked. The segregation data corresponded closely to the predictions of the gene-for-gene theory. Specific virulence to match resistance genes in lettuce was determined at the same loci in isolates of geographically diverse origins. Complete inhibition of avirulence loci by inhibitor genes appeared to be rare in *B. lactucae*, but partial modifications of incompatible interactions between particular cultivars and isolates were observed. No tight linkage was detected between loci controlling avirulence. Previous results that were apparently inconsistent with a gene-for-gene interaction were often explained by the presence of uncharacterized resistance genes or by polyploidy in some pathogen isolates. The action of genes modifying avirulence was difficult to characterize unambiguously. The implications of the data for molecular studies of gene-for-gene interactions are discussed.

Additional keywords: biotroph, host-pathogen interaction, oomycete, Peronosporales.

A gene-for-gene relationship between host cultivars and pathogen isolates has been proposed as the determinant of specificity in more than 30 host-pathogen associations (reviewed in 2). In its simplest form, the gene-for-gene theory proposed by Flor (12) states that each locus conditioning specific host resistance or susceptibility is matched by a complementary locus controlling specific avirulence or virulence in the pathogen. In the interaction between flax and flax rust studied by Flor, an incompatible interaction phenotype occurred when any one host resistance allele was matched by the corresponding pathogen avirulence allele. Resistance and avirulence were nearly always dominant. These general observations apply to other host-parasite associations for which gene-for-gene relationships have been demonstrated. Molecular interpretations of gene-for-gene specificity have been presented on the basis of the genetic data (9,17,18). These propose that incompatibility results from an interaction between components of host and pathogen specified in some way by the complementary alleles for resistance and avirulence; compatibility results when at least one of these alleles is absent and such an interaction does not occur. These and other models have been reviewed by Crute et al (3). No active functions are attributed to alleles for susceptibility or virulence, and in some cases, a nonfunctional homologue may not exist. Use of the term "virulence allele" to indicate the absence of an avirulence allele must be interpreted with these considerations in mind.

The basic genetic principles of the gene-for-gene theory seem to be an oversimplification, however, as they cannot fully accommodate all results from genetic studies on gene-for-gene relationships. Both Crute (2) and Barrett (1) review cases in which a strict one-to-one complementarity of resistance and avirulence loci does not seem to apply. In some pathogens, including *Melampsora lini* (20), the expression of an avirulence allele can apparently be suppressed due to the presence of a inhibitor allele at a second locus in the pathogen. There are other reports (e.g., 8,32) of virulence to a single host resistance gene controlled by two pathogen loci, but in some cases these could have resulted from additional uncharacterized resistance genes in host lines (e.g., 31,33,34), inhibitor alleles, or distortion of segregation ratios due to selection on genes linked to virulence loci. Incomplete dominance of resistance and avirulence, recessive resistance, and modifier genes affecting incompatible or compatible interaction phenotypes have also been reported (reviewed in 2,20).

The genetics of few diseases have been studied extensively and most investigations have involved only a few host cultivars and pathogen isolates. A detailed analysis of a gene-for-gene relationship may therefore reveal exceptions to the relationship between host and pathogen alleles predicted by the basic gene-for-gene theory. For example, it is often assumed that virulence to a specific resistance gene always maps to the same pathogen locus. If, however, a pathogen component determining incompatibility was the end product of a multistep biosynthetic pathway, mutations in any one of several genes could result in virulence to a single resistance gene; virulence would then map to different loci in different pathogen isolates. Some crosses between isolates virulent against a specific resistance gene would result in avirulent progeny due to complementation. Detailed experiments to test this possibility have not been undertaken. Another possible complexity of a gene-for-gene interaction is allelism of avirulence genes corresponding to resistance alleles at different loci; this situation would occur if the product of an allele for virulence to one resistance allele conditioned an incompatible interaction with another. Modifications of the gene-for-gene theory would be necessary to account for any such observations.

Any modification of the gene-for-gene theory has implications for biochemical interpretations of the mechanisms of specificity. Ellingboe (9,10) has argued that interaction between the primary products of resistance and avirulence alleles, perhaps by formation of a structural dimer, is directly responsible for incompatibility rather than being an initial event in the induction of further processes leading to host resistance. He contends that if secondary

products were involved, deviations from a one-to-one complementarity of host and pathogen genes would sometimes be observed. That they have not been frequently reported to date, however, may merely reflect the lack of sufficiently detailed investigations of host-pathogen genetics.

The biochemical nature of gene-for-gene specificity will probably only be resolved by the application of recombinant DNA techniques. For these methods to be successful, a thorough knowledge of host-pathogen genetics is essential. For example, when attempting to transform a virulent isolate with a putative avirulence allele, one must assay the transformant on a host expressing the correct resistance gene. Similarly, such attempts would be fruitless if the recipient were virulent because of an inhibitor allele. Also, modifier genes in host or pathogen could affect the expression of introduced genes.

The interaction between lettuce, *Lactuca sativa*, and the downy mildew fungus, *Bremia lactucae*, is one of the best characterized gene-for-gene relationships (4,11,25–27). Thirteen resistance genes (*Dm*) in the host matched by complementary pathogen avirulence genes (*Avr*) have been described to date and further incompletely characterized resistance factors are also known (5). *B. lactucae* is a heterothallic, diploid Oomycete fungus (21,22), and genetic studies have usually shown avirulence to be dominant and virulence recessive. Minor modifications of the basic gene-for-gene theory have been reported. Gene dosage effects can sometimes result in incomplete dominance of resistance alleles (7). Inhibitor genes similar to those in *M. lini* have been proposed (25,26). Modifier genes affecting aspects of the interaction have been implicated, for example, in cases of limited pathogen sporulation associated with host necrosis (25). The interaction of lettuce and *B. lactucae* is a candidate for studies of specificity at a molecular level: classical genetic analyses of both partners are routine; detailed genetic maps based on DNA markers (restriction fragment-length polymorphisms, RFLPs) are being prepared for both the host (19) and the pathogen (13); and the host is amenable to transformation (24).

The object of the present study was to analyze critically the genetics of the interaction between *L. sativa* and *B. lactucae*. This article focuses on data from the pathogen; a companion paper describing the simultaneous studies made on the host has been published elsewhere (11). The assumption that virulence to a single resistance gene is always determined at the same locus was examined in complementation tests involving isolates from geographically different pathogen populations. Further evidence for the action of inhibitor loci and modifier genes was sought. Analyses of linkage between virulence genes were extended to include avirulence to recently described resistance genes (11) and previously untested combinations of avirulence loci. The data resulted in a more complete understanding of the interaction and are thus a precursor to molecular studies. They also highlighted problems that can arise in the interpretation of genetic data on host-parasite associations.

MATERIALS AND METHODS

All isolates of *B. lactucae* were derived from single conidia using the method of Michelmore and Ingram (23). The origins, sexual compatibility types, and virulence phenotypes of the isolates are presented (Table 1). Virulence genotypes of isolates, where given in the text, are those cited by Norwood and Crute (26) or Ilott et al (16) or were inferred from data collected during the present study, in which the segregation of virulence and avirulence conformed to the basic gene-for-gene theory.

Procedures for maintaining isolates on lettuce seedlings, storing isolates at −80 C, obtaining sexual progeny from crosses, and determining virulence phenotypes and sexual compatibility types of isolates have been described elsewhere (16,21,25). The differential series of resistant lettuce cultivars used to determine

TABLE 1. Origins, virulence phenotypes, and sexual compatibility types (SCT) of 27 isolates of *Bremia lactucae*

Isolate	Origin	Virulence to match *Dm* gene[a]													SCT
		1	*2*	*3*	*4*	*5/8*	*6*	*7*	*10*	*11*	*13*	*14*	*15*	*16*	
Tv	U.K.	+	+	+	+	+	+	+	+	−	+	+	−	+	B1
IM25R7	U.K.	+	−	−	+	+	+	+	+	+	+	+	+	+	B1
NL6	Netherlands	+	+	−	+	+	−	−	+	+	+	+	−	*	B1
SF3	Finland	+	+	+	−	+	−	+	−	+	+	+	+	−	B1
SF5	Finland	−	+	−	+	−	−	−	+	−	+	+	+	−	B1
S1	Sweden	+	−	+	+	+	+	+	+	−	+	+	+	+	B2
CG1	Switzerland	+	−	+	+	−	−	+	+	−	+	−	+	−	B2
CS7	Czechoslovakia	+	+	−	+	+	−	−	+	−	−	+	−	−	B1
CS9	Czechoslovakia	+	+	+	+	+	+	+	+	+	+	+	+	−	B1
CS12	Czechoslovakia	−	+	−	+	−	−	+	−	+	−	+	−	+	B1
NL6246	F_1, NL6 × C82P24	−	+	−	+	+	−	−	+	−	+	+	−	−	B2
NL6248	F_1, NL6 × C82P24	−	+	−	−	+	−	−	+	−	+	+	−	−	B2
NL6CG19	F_1, NL6 × CG1	+	−	−	+	−	−	−	+	−	+	−	−	−	B2
NL6473	F_1, NL6 × C83M47	−	+	−	+	+	−	−	−	−	+	+	−	−	B1
TvCG15	F_1, Tv × CG1	+	−	+	+	−	−	+	+	−	+	−	−	−	B2
IMOs6b	F_1, IM25R7 × CG1[b]	+	−	−	+	−	−	+	+	−	+	−	+	−	B2
IMOs7c	F_1, IM25R7 × CG1[b]	+	−	−	+	−	−	+	+	−	+	−	?	−	B1
AM	Australia	+	−	−	*	+	−	+	+	−	+	−	−	−	B2
JP1	Japan	+	+	−	*	+	−	+	+	−	+	+	−	−	B2
C83M40	California I[c]	+	−	*	−	−	+	+	+	−	+	+	−	−	B1
C85B4	California I[c]	+	−	+	−	−	+	+	+	−	+	+	−	−	B1
C85B6	California I[c]	+	−	*	−	−	+	+	+	−	+	+	+	−	B1
C82P24	California II[c]	−	+	+	−	+	+	+	+	*	+	+	−	−	B2
C83M47	California III[c]	−	+	+	+	+	+	+	−	−	+	+	−	+	B2
C84M4	California IV[d]	−	+	+	*	+	+	+	*	−	+	+	−	*	B2
C85B8	California IV[d]	−	+	+	*	+	+	+	*	−	+	+	−	*	B2
C85T1	California	−	+	+	−	+	+	+	−	−	+	+	+	+	B1
19c	California	+	+	−	+	+	+	+	+	−	+	+	−	−	B2

[a]All isolates virulent on Cobham Green (susceptible check) and Hilde (R12). R-factor 9 has yet to be satisfactorily characterized genetically (11). + = Profuse sporulation, pathogen virulent; − = no sporulation, pathogen avirulent; * = sparse sporulation with necrosis on some host genotypes; ? = virulence phenotype not known.

[b]Cross made by Michelmore et al (25).

[c]California pathotypes I, II, and III described by Ilott et al (16).

[d]California IV described by Hulbert and Michelmore (15).

virulence phenotypes is described in Table 2. The absence of sporulation on a differential cultivar 7–10 days after inoculation with an isolate of *B. lactucae* was interpreted as an incompatible interaction phenotype. Conversely, profuse sporulation implied a compatible interaction. Occasionally sporulation of a particular isolate on a differential cultivar was sparse, delayed, or accompanied by extensive host necrosis; this may have been due to the influence of modifier genes in the host or pathogen, or partial dominance of avirulence, as described later. When possible, crosses were constructed so that the test of a hypothesis relied on the presence or absence of a particular class of progeny rather than on trying to distinguish between different segregation ratios. This is because characterization of large numbers of progeny isolates is labor-intensive and segregation of deleterious alleles could have distorted segregation ratios. The probabilities of obtaining the observed results were calculated from the binomial expansion and show the strength of the evidence for each hypothesis. In most cases, strong evidence could be provided by the small progenies used.

RESULTS

Complementation tests. Isolates of *B. lactucae* that were both virulent to a specific resistance gene were crossed to test whether virulence to match a single resistance gene might be determined at different loci in different isolates. The occurrence of progeny isolates avirulent on a host cultivar that was susceptible to both parents would be good evidence for the presence of complementary, nonallelic virulence genes, especially if all the progeny were avirulent (Table 3). Such a result was not obtained in previous genetic studies with *B. lactucae*; these, however, usually involved only a small number of parental isolates collected from a single continent. If only some progeny were avirulent, heterozygous inhibitor alleles in one or both parents could be an alternative explanation (Table 4).

One hundred and twenty five tests for allelism of virulence determinants were analyzed in the progenies of 19 crosses (Table 5). Isolates of diverse geographical origins (California, Australia, Japan, and several European countries) were used to increase the opportunities for detecting different avirulence loci. In no case was avirulence expressed by all the progeny of a cross between two virulent parents. Avirulent progeny did segregate in three such crosses; however, explanations other than nonallelic avirulence appeared likely (see below). There is, therefore, no definitive evidence for nonallelic avirulence. The data do not preclude nonallelic avirulence; however, it is unlikely (Table 5). Avirulence could segregate in the progeny if at least one of the parents carried two mutations for virulence to a specific *Dm* gene (Table 3). This is, however, unlikely to occur frequently, as there would be no selective pressure for a second mutation in a virulent isolate; it would most often arise following crosses between virulent isolates carrying different mutations.

Six isolates avirulent on cultivars carrying *Dm3* occurred in a total of 13 progeny isolates from a cross between the isolates C83M40 (Californian pathotype I) (16) and C83M47 (pathotype III) that were both virulent on *Dm3*. This could have represented complementation of avirulence loci (Table 3) or have resulted from the presence of inhibitor genes and avirulence alleles in one or both isolates (Table 4). C83M47 was unlikely to possess inhibitor or avirulence alleles, however, as all nine progeny of a cross with NL6 (*Avr3Avr3*) were avirulent on cultivars with *Dm3*, an improbable result if C83M47 had the genotype *I3i3* ($P < 0.002$). Further analysis of the genotype of C83M40 was impossible using other crosses, as these proved to be infertile (16) and matings involving the progeny of the C83M40 × C83M47 cross were similarly problematic. Studies using RFLP markers subsequently demonstrated that C83M40 and several other isolates were tetraploid or heterokaryotic, "hyperploid" isolates (15). The results described above would be observed if C83M40 had the genotype *Avr3avr3avr3avr3* but was able to sporulate on cultivars with *Dm3*. Diploid progeny isolates of genotype *Avr3avr3* would be avirulent. Sporulation of C83M40 and other Californian pathotype I isolates on Dandie (*Dm3*) was often less intense than on cultivars lacking *Dm3*, suggesting that the isolates may carry an avirulence allele (*Avr3*). Other putative gene dosage effects in hyperploid isolates giving interactions that were difficult to classify as compatible or incompatible were also observed, as described below.

Avirulent progeny were also obtained in two crosses between parental isolates both originally scored as virulent on cultivars possessing *Dm4*. Six of 30 progeny from CS9 × AM and three of 30 progeny from JP1 × Tv were avirulent on the lettuce breeding line R4T57 (*Dm4*). Both AM and JP1 proved to be hyperploid isolates (15) and sporulation of both isolates on R4T57 was weak, sometimes accompanied by host necrosis. Therefore, both isolates may have one copy of the avirulence allele (*Avr4*) and three copies of the virulence allele (*avr4*). An alternate possibility, that the isolates were heterozygous at avirulence and inhibitor loci, was not supported by the results of a cross between AM and SF3 (*Avr4Avr4*), as no progeny were virulent against *Dm4*.

TABLE 2. Differential series of lettuce cultivars resistant to *Bremia lactucae*

Primary series[a]		Secondary series[b]	
Cultivar/line	*Dm* gene[c]	Cultivar/line	*Dm* gene (or R-factor)[d]
Lednicky	*1*	Blondine	*1, 13*
UCDM2	*2*	Mildura	*1, 3*
Dandie	*3*	Amplus	*2, 4*
R4T57	*4*	Liba	*1, 2*
Valmaine or Valverde	*5/8*[e]	Kordaat	*1, 3, 4*
Sabine	*6*	Avondefiance	*5/8, 6*
Mesa 659	*7,13*	Salinas or Calmar	*5/8, 7, 13*
UCDM10	*10*	Sucrine	*5/8, 10*
Capitan	*11*[f]	Fila	*2, 11*
Hilde × *L. serriola* F_4	*11*[f]	G. Winterkonig	*4, 13, 14*
Empire or Pennlake	*13*	Vanguard or Winterhaven	*7, 10, 13*
UCDM14	*14*	Kinemontepas	*10, 13, 16*
PIVT1309	*15*	Saffier	*1, 3, 7, 16*
LSE/18	*16*	Diana	*1, 3, 7, 5/8*
Cobham Green[g]	None	Hilde	(R12)

[a]Cultivars/lines with well-characterized downy mildew resistance genes (*Dm*).
[b]Cultivars/lines with combinations of well-characterized resistance genes used to confirm conclusions from the primary series, or lines with incompletely characterized R-factors.
[c]As described in Farrara et al (11).
[d]Resistance factors (R-factors) have been invoked when the resistance in a cultivar has not been fully characterized.
[e]*Dm5* and *Dm8* are the same gene (14).
[f]Two cultivars used as interactions sometimes difficult to score.
[g]Susceptible check.

The data from the allelism tests, therefore, provided no evidence for virulence to a specific resistance gene being determined at more than one locus and suggested that virulence was allelic in all the isolates analyzed. The data do not preclude, however, the possibility of different alleles for virulence at each locus.

Test crosses to detect inhibitor genes. Dominant inhibitor genes in *B. lactucae* suppressing avirulence to *Dm4* (25), *Dm1*, and *Dm5/8* (26) have been proposed previously. Crosses between isolates avirulent to specific *Dm* genes and virulent isolates were used in the present study to confirm these inhibitor loci and provide evidence for others.

The suggestion that isolate CS9 possessed an inhibitor gene, *I5/8*, epistatic to *Avr5/8* (26), was supported in this study. CG1 is *Avr5/8avr5/8* because avirulent progeny segregated from crosses between CG1 and virulent isolates other than CS9. All 17 progeny of the cross between CS9 (virulent) and CG1 (avirulent) were virulent on Valmaine (*Dm5/8*) rather than giving the 1:1 ratio of avirulent to virulent progeny expected. The evidence for an inhibitor gene was not conclusive, however, as no crosses were made with homozygous, avirulent isolates (*Avr5/8Avr5/8;* such isolates of the correct mating type were not available). The two avirulent isolates used previously to detect the presence of *I5/8* in CS9 were also heterozygotes (26). Disturbed segregation ratios could have accounted for the absence of avirulent progeny; however, the probability of all 17 progeny isolates from the cross CS9 × CG1 being virulent is low ($P < 8 \times 10^{-6}$) unless linked loci influenced the fitness of the progeny. No evidence was obtained that indicated the presence of an *I5/8* allele in any other isolate.

The allele *I1* was proposed on the basis of a disturbed segregation ratio (44 virulent to *Dm1* and 23 avirulent), differing ($P < 0.05$) from the 1:1 ratio expected when SF3 (virulent against *Dm1*) was crossed with SF5/NL5/3 (a confirmed avirulent heterozygote, *Avr1avr1*) (26). The proposed genotype of SF3 was therefore *Avr1avr1I1i1*, the avirulence and inhibitor loci being unlinked. *I1* alleles were also proposed in S1, as no avirulent

TABLE 3. Segregation of avirulent progeny from crosses between virulent isolates due to complementation between nonallelic mutations to virulence[a]

Parental genotypes	Ratio in progeny	
	Avirulent	Virulent
AAbb × *aaBB*	1 (all *AaBb*)	0
AAbb × *aaBb*	1 (*AaBb*)	1 (*Aabb*)
Aabb × *aaBb*	1 (*AaBb*)	3 (*Aabb, aaBb, aabb*)

[a]If a product responsible for pathogen avirulence results from a two-step biosynthetic pathway requiring the function of two genes, *A* and *B*, the crosses shown between virulent isolates of a diploid pathogen would produce avirulent progeny, assuming that avirulence requires dominant alleles at both loci and that the two loci are unlinked.

TABLE 4. Production of avirulent progeny isolates from crosses between virulent isolates, at least one of which is heterozygous for an inhibitor gene[a]

Parental genotypes	Ratio of virulent to avirulent in progeny
AvrAvrIi × *AvrAvrIi*	3:1
AvrAvrIi × *AvravrIi*	3:1
AvrAvrIi × *avravrIi*	3:1
AvrAvrIi × *avravrii*	1:1
AvravrIi × *AvravrIi*	13:3
AvravrIi × *avravrIi*	7:1
AvravrIi × *avravrii*	3:1

[a]If expression of an avirulence allele (*Avr*) can be inhibited by an inhibitor allele (*I*) at a second locus, the crosses shown between virulent, diploid isolates would produce avirulent progeny, assuming that avirulence loci (*Avr*) are hypostatic to inhibitor loci (*I*), inhibitor alleles (*I*) are dominant to *i*, avirulence alleles (*Avr*) are dominant to virulence alleles (*avr*), and the *Avr* and *I* loci are unlinked.

TABLE 5. Complementation tests to show allelism of virulence determinants in *B. lactucae*

Isolates crossed[a]	*Dm* genes for which virulence tested	Number of progeny[b]	Probability of all progeny being virulent if parents are	
			AAbb × aaBb	Aabb × aaBb[c]
European × European				
Tv × CG1	*1, 3, 4, 7, 13*	7	7.8×10^{-3}	1.4×10^{-1}
NL6 × CG1	*1, 4, 10, 13*	13	1.2×10^{-4}	2.4×10^{-2}
NL6 × S1	*1, 4, 5/8, 10, 13, 14*	14	6.1×10^{-5}	1.8×10^{-2}
CS9 × CG1	*1, 3, 4, 7, 10, 13*	5	3.1×10^{-2}	2.4×10^{-1}
CS9 × S1	*1, 3, 4, 5/8, 6, 7, 13*	5	3.1×10^{-2}	2.4×10^{-1}
European × Japanese				
Tv × JP1	*1, 2, 4, 5/8, 7, 10, 13, 14*	30	9.3×10^{-10}	1.8×10^{-4}
European × Californian				
Tv × C82P24	*2, 3, 5/8, 6, 7, 10, 13, 14*	14	6.1×10^{-5}	1.8×10^{-2}
NL6 × C82P24	*2, 5/8, 10, 13, 14*	14	6.1×10^{-5}	1.8×10^{-2}
Tv × C83M47	*2, 3, 4, 5/8, 6, 7, 13, 14, 16*	7	7.8×10^{-3}	1.3×10^{-1}
NL6 × C83M47	*2, 4, 5/8, 13, 14*	9	1.9×10^{-3}	7.5×10^{-2}
Tv × C84M4	*2, 3, 5/8, 6, 7, 13, 14*	10	9.8×10^{-4}	5.6×10^{-2}
19c × C85T1	*2, 5/8, 7, 13, 14*	7	7.8×10^{-3}	1.3×10^{-1}
Tv × 85B8	*2, 3, 5/8, 6, 7, 13, 14*	16	1.5×10^{-5}	1.0×10^{-2}
CS12 × 19c	*7*	17	7.6×10^{-6}	7.5×10^{-3}
Californian × Californian				
C83M40 × C83M47	*3, 6, 7, 13, 14*	13	1.2×10^{-4}	2.4×10^{-2}
C85B4 × C83M47	*3, 6, 7, 13, 14*	14	6.1×10^{-5}	1.8×10^{-2}
C85B6 × C83M47	*6, 7, 13, 14*	5	3.1×10^{-2}	2.4×10^{-1}
European × Australian				
CS9 × AM	*1, 4, 5/8, 7, 10, 13*	30	9.3×10^{-10}	1.8×10^{-4}
SF3 × AM	*1, 5/8, 7, 13*	4	6.3×10^{-2}	3.2×10^{-1}

[a]Both isolates virulent to the *Dm* genes in column 2. Avirulent progeny isolates could have arisen if virulence in the parents was determined by complementary, nonallelic virulence factors (see Table 3).

[b]All progeny were virulent against each of the listed *Dm* genes, except that some progeny were avirulent to *Dm3* from C83M40 × C83M47 and some were avirulent to *Dm4* from Tv × JP1 and CS9 × AM (see text). All parental and progeny isolates were also virulent on Cobham Green and Hilde (R12).

[c]As calculated from the binomial expansion (see Table 3). The probability of all progeny being virulent if the parents were AAbb and aaBB (the most likely genotypes) is 0.

progeny were obtained from the cross SF3 × S1, and in NL6, because of a deviation from a 1:1 ratio in the progeny of a cross with an avirulent heterozygote. The present study, however, did not support these conclusions. SF3 was crossed with C83M47, avirulent on Lednicky (*Dm1*) and Blondine (*Dm1, Dm13*). None of 40 progeny of this mating were virulent on either cultivar (Table 6), which suggested that SF3 did not possess an inhibitor of avirulence to *Dm1*. Data on the segregation of resistance in Blondine indicate that avirulence in C83M47 is conferred by *Avr1* rather than by another avirulence gene interacting with a previously uncharacterized resistance gene in Blondine; none of 200 F_2 seedlings of a cross between Blondine and R4T57 (*Dm4* only) were susceptible to C83M47 but resistant to SF5 (*Avr1Avr1*, the source of the *Avr1* allele in SF5/NL5/3), or vice versa. Therefore, a novel resistance factor was unlikely to account for the resistance of Blondine to C83M47 unless it was tightly linked to *Dm1*. More probably, the abnormal segregation in the SF3 × SF5/NL5/3 cross resulted from differential effects of linked loci on the fitness of sexual progeny, or was due to type I error. Similarly, the cross NL6 × C83M47 did not support the contention that an inhibitor allele was present in NL6 ($P = 0.002$ if NL6 was *I1i1*), and the cross NL6473 × S1 did not support the suggestion that S1 was homozygous for an inhibitor allele (*I1I1*) (Table 6).

The existence of *I4* was suggested after avirulence to *Dm4* segregated in a cross between two virulent isolates, CG1 and IMOs7C, a progeny isolate from the cross CG1 × IM25R7 (25). Genotypes were proposed to be *Avr4avr4I4i4* for CG1, *Avr4avr4I4i4* or *avr4avr4I4i4* for IMOs7C, and *avr4avr4I4I4* for IM25R7. The present study did not support these published genotypes. Progeny avirulent on R4T57 (*Dm4*) resulted from the cross IM25R7 × C82P24 (*Avr4avr4*) and all progeny from the cross CG1 × SF3 (*Avr4Avr4*) were avirulent to *Dm4* (Table 6) ($P = 1 \times 10^{-6}$, if CG1 was *I4i4*). All 29 progeny from crosses between CG1 and isolates Tv, NL6, and CS9 (*avr4avr4*) were virulent on R4T57. Hence it was unlikely that IM25R7 was homozygous for an inhibitor of *Avr4* or that CG1 had avirulence and inhibitor alleles. The segregation of avirulence in the progeny of CG1 × IMOs7C could have resulted from complementation of virulence mutations (Table 3). If this were so, however, avirulent progeny should have occurred in the cross CG1 × IM25R7 that produced IMOs7C (25). None were observed, although the number of isolates tested was small. In addition, crosses between CG1 or IM25R7 and isolates virulent to *Dm4* might have resulted in avirulent progeny, but none were obtained in complementation tests involving CG1. The possibility that IM25R7 might carry a different mutation for virulence to *Dm4* was not critically tested.

No evidence for other inhibitors of avirulence in *B. lactucae* was found in any other cross during the present study (Table 6). In no case did a cross between an isolate avirulent to a specific *Dm* gene and a virulent isolate result in progeny that were all virulent. Furthermore, no avirulent isolate behaved as though it were a homozygote (*AvrAvr*) in some crosses and as a heterozygote (*Avravr*) in others, which would have indicated the segregation of inhibitor genes in the gametes of one of the virulent parents.

In conclusion, evidence for an inhibitor locus epistatic to *Avr5/8* is good but not unequivocal. The existence of inhibitor loci epistatic to *Avr1* and *Avr4* was not substantiated. Inhibitor loci are not common in *B. lactucae*. In the future, the existence of putative inhibitor loci should be confirmed using crosses to homozygous avirulent isolates.

TABLE 6. Test crosses[a] to detect inhibitor genes in *B. lactucae*

Dm gene	Virulent parent	Avirulent parent	Genotype of avirulent parent	Number of progeny[b] +	Number of progeny[b] −	Probability[c] if virulent parent is *avravrIi*
1	Tv	C82P24	*Avr1avr1*	6	8	$0.02 > P > 0.01$
	Tv	C83M47	*Avr1Avr1*	0	7	7.8×10^{-3}
	NL6	C82P24	*Avr1avr1*	5	9	$0.01 > P > 0.001$
	NL6	C83M47	*Avr1Avr1*	0	9	2.0×10^{-3}
	IMOs6b	SF5	*Avr1Avr1*	0	27	7.5×10^{-9}
	C83M40	C83M47	*Avr1Avr1*	0	14	6.1×10^{-5}
	C85B4	C83M47	*Avr1Avr1*	0	14	6.1×10^{-5}
	SF3	C83M47	*Avr1Avr1*	0	40	9.1×10^{-14}
	S1	NL6473	*Avr1avr1*	3	4	$P > 0.10$
	19c	CS12	*Avr1avr1*	7	10	$0.01 > P > 0.001$
	19c	C85T1	*Avr1Avr1*	0	7	7.8×10^{-3}
2	NL6	CG1	*Avr2Avr2*	0	11	4.9×10^{-4}
	NL6	S1	*Avr2Avr2*	0	14	6.1×10^{-5}
	CS9	S1	*Avr2Avr2*	0	5	0.031
	CS9	CG1	*Avr2Avr2*	0	5	0.031
	CS9	AM	*Avr2avr2*	11	19	$P < 0.001$
	Tv	CG1	*Avr2Avr2*	0	7	7.8×10^{-3}
	C83M47	C83M40	*Avr2avr2*	2	11	$P < 0.001$
	C82P24	IM25R7	*Avr2Avr2*	0	18	3.8×10^{-6}
	SF5	IMOs6b	*Avr2Avr2*	0	27	7.5×10^{-9}
3	CG1	NL6	*Avr3Avr3*	0	11	4.9×10^{-4}
	S1	NL6	*Avr3Avr3*	0	14	6.1×10^{-5}
	Tv	NL6246	*Avr3avr3*	8	10	$0.01 > P > 0.001$
	Tv	JP1	*Avr3avr3*	13	17	$P < 0.001$
	C82P24	SF5	*Avr3avr3*	25	15	$P > 0.10$
	C82P24	NL6	*Avr3Avr3*	0	14	$6.1 \times\times 10^{-5}$
	C82P24	IM25R7	*Avr3Avr3*	0	18	3.8×10^{-6}
	C83M47	NL6	*Avr3Avr3*	0	9	2.0×10^{-3}
	CS9	AM	*Avr3avr3*	15	15	$0.01 > P > 0.001$
4	Tv	C82P24	*Avr4avr4*	6	8	$0.02 > P > 0.01$
	NL6	C82P24	*Avr4avr4*	10	4	$P > 0.10$
	IM25R7	C82P24	*Avr4avr4*	8	10	$0.01 > P > 0.001$
	SF5	C82P24	*Avr4avr4*	14	26	$P < 0.001$
	CG1	SF3	*Avr4Avr4*	0	20	9.5×10^{-7}

(*continued on next page*)

Modifier genes in pathogen and host. Inoculation of isolate C82P24 (Californian pathotype II) (16) on some cultivars containing *Dm4* (e.g., Amplus, Gelber Winterkonig) resulted in extensive host necrosis and limited pathogen sporulation after 8–14 days. The isolate was completely avirulent, however, on other host lines containing *Dm4* (e.g., R4T57). RFLP data demonstrated that this isolate was diploid (15). The genetic basis of the necrotic phenomenon was studied in the pathogen by means of crosses involving C82P24 (Table 7). The differences in genetic background between R4T57 and cultivars expressing *Dm4* on which necrosis occurred were not investigated genetically.

The necrotic reaction seemed to be due to modification of an incompatible interaction, resulting in incomplete avirulence to *Dm4* in some host lines. In the cross C82P24 × NL6, only progeny isolates that had inherited the avirulence allele *Avr4* from C82P24 and were thus avirulent on R4T57 were necrotic on Amplus (Table 7). Necrosis did not occur in all pathogen genetic backgrounds. Progeny of the cross C82P24 × Tv that were avirulent on R4T57

TABLE 6. *continued*

Dm gene	Virulent parent	Avirulent parent	Genotype of avirulent parent	Number of progeny[b]		Probability[c] if virulent parent is *avravrIi*
				+	−	
5/8	CS9	CG1	*Avr5avr5*	17	0	7.5×10^{-3}
	Tv	CG1	*Avr5avr5*	3	4	$P > 0.10$
	NL6	CG1	*Avr5avr5*	7	4	$P > 0.10$
	C83M47	C83M40	*Avr5avr5*	1	11	2.0×10^{-6}
	C83M47	C85B4	*Avr5avr5*	8	6	$P > 0.10$
	C82P24	SF5	*Avr5avr5*	21	19	$0.01 > P > 0.001$
	19c	CS12	*Avr5avr5*	7	10	$0.01 > P > 0.001$
6	Tv	CG1	*Avr6Avr6*	0	7	7.8×10^{-3}
	Tv	NL6246	*Avr6avr6*	6	12	$P < 0.001$
	Tv	JP1	*Avr6Avr6*	0	30	9.3×10^{-10}
	S1	NL6	*Avr6avr6*	7	7	$0.10 > P > 0.05$
	C82P24	SF5	*Avr6avr6*	24	16	$0.05 > P > 0.02$
	C82P24	NL6	*Avr6avr6*	7	7	$0.10 > P > 0.05$
	CS9	CG1	*Avr6Avr6*	0	5	0.031
	C83M47	NL6	*Avr6avr6*	4	5	$0.10 > P > 0.05$
	19c	CS12	*Avr6Avr6*	0	17	7.6×10^{-6}
	19c	CS7	*Avr6Avr6*	0	7	7.8×10^{-3}
	CS9	AM	*Avr6avr6*	11	18	$P < 0.001$
7	Tv	NL6246	*Avr7avr7*	6	12	$P < 0.001$
	C82P24	SF5	*Avr7avr7*	18	22	$P < 0.001$
	C82P24	NL6	*Avr7Avr7*	0	14	6.1×10^{-5}
	CG1	NL6	*Avr7Avr7*	0	9	2.0×10^{-3}
	S1	NL6	*Avr7Avr7*	0	14	6.1×10^{-5}
	C83M47	NL6	*Avr7Avr7*	0	9	2.0×10^{-3}
	IMOs6b	SF5	*Avr7avr7*	13	14	$0.01 > P > 0.001$
	19c	CS7	*Avr7Avr7*	0	7	7.8×10^{-3}
10	NL6	C83M47	*Avr10Avr10*	0	9	2.0×10^{-3}
	Tv	C83M47	*Avr10Avr10*	0	7	7.8×10^{-3}
	S1	NL6473	*Avr10avr10*	3	4	$P > 0.10$
	C83M40	C83M47	*Avr10Avr10*	0	13	1.2×10^{-4}
	C85B4	C83M47	*Avr10Avr10*	0	14	6.1×10^{-5}
11	CS9	S1	Not known	0	5	0.031 or 9.8×10^{-4}
13	19c	CS12	*Avr13avr13*	8	9	$0.02 > P > 0.01$
14	Tv	CG1	*Avr14Avr14*	0	7	7.8×10^{-3}
	NL6	CG1	*Avr14Avr14*	0	9	2.0×10^{-3}
	CS9	CG1	*Avr14Avr14*	0	5	0.031
	SF5	IMOs6b	*Avr14avr14*	16	15	$0.01 > P > 0.001$
15	CG1	NL6	*Avr15Avr15*	0	11	4.9×10^{-4}
	CG1	Tv	*Avr15avr15*	3	4	$P > 0.10$
	S1	NL6	*Avr15Avr15*	0	7	7.8×10^{-3}
	SF5	C82P24	*Avr15avr15*	16	24	$P < 0.001$
	CS9	AM	*Avr15avr15*	15	10	$P > 0.01$
16	Tv	C82P24	*Avr16avr16*	8	6	$P > 0.01$
	Tv	NL6246	*Avr16Avr16*	0	18	3.8×10^{-6}
	Tv	CG1	*Avr16Avr16*	0	7	7.8×10^{-3}
	Tv	JP1	*Avr16Avr16*	0	30	9.3×10^{-10}
	C83M47	C85B4	*Avr16Avr16*	0	7	7.8×10^{-3}
	C83M47	NL6	*Avr16avr16*	2	6	$0.01 > P > 0.001$
	S1	CS9	*Avr16Avr16*	0	5	0.031
	S1	NL6	*Avr16avr16*	4	10	$P < 0.001$
	CS12	19c	*Avr16avr16*	11	6	$P > 0.10$

[a]Isolates virulent against individual *Dm* genes were crossed with avirulent isolates. If the virulent isolate is homozygous for an allele inhibiting avirulence (*II*), only virulent progeny should be obtained. If the virulent isolate is heterozygous at such a locus (*Ii*), avirulence should segregate in the progeny (see Table 4).

[b]+ = Virulent, − = avirulent.

[c]Probability of obtaining a deviation from expected ratio (1:1 or 1:3, avirulent to virulent) at least as great as that shown. Number of virulent progeny was always less than that expected if the progeny isolate was *avravrIi*. The probability of avirulent progeny is 0 if the virulent progeny is *II*. Virulent genotypes *AvrAvrIi* and *AvravrIi* are excluded, as they would have been detected in complementation tests.

were also avirulent, with no necrosis, on Amplus. Only two of nine progeny from the cross C82P24 × CS9 that were avirulent on R4T57 induced necrosis on Amplus.

The incomplete avirulence on Amplus could have been due to the *Avr4* allele in C82P24 or due to modification of the expression of *Avr4* by linked loci. This *Avr4* allele from C82P24 may have exhibited partial dominance in C82P24 and the heterozygous progeny. Other isolates that were heterozygous for *Avr4*, however, did not exhibit the partial phenotype. Alternatively, if modifier gene(s) were involved, these could be interpreted as partial inhibitors of avirulence.

Further complexities involving the necrotic phenotype were observed. NL6, SF5, and IM25R7 were completely avirulent on cultivars containing *Dm3*; however, isolates that inherited the *Avr4* allele from C82P24 and an *Avr3* allele from NL6, SF5, or IM25R7 had a necrotic, partially incompatible phenotype on Dandie (*Dm3*) but were completely incompatible on Mildura (*Dm1, Dm3*) with no necrosis. The modification of avirulence to *Dm4*, therefore, also influenced avirulence to *Dm3*, the modification again depending on host genetic background. The necrotic interaction phenotype was not merely due to the expression of both *Avr3* and *Avr4* in the same isolate; C82P24 itself had no *Avr3* allele and progeny isolates from the cross NL6248 × Tv that possessed the *Avr4* allele from C82P24 and the *Avr3* allele from NL6 were not necrotic on Dandie or Amplus. Also, there was not a general modification of the incompatible response in progeny from C82P24. Avirulence on Amplus (*Dm2, Dm4*) due to *Avr2* was not modified in these experiments; progeny of C82P24 × IM25R7 (*Avr2Avr2*) were not necrotic on Amplus but were on Gelber Winterkonig (*Dm4, Dm13, Dm14*).

Similar necrotic phenotypes were also observed in some interactions involving *Dm16* from LSE/18 (Table 8). As with *Dm4*, the incompatible interaction was clearly being modified; sparse sporulation and extensive necrosis on LSE/18 only occurred when isolates were completely avirulent on Kinemontepas (*Dm10, Dm13, Dm16*). As with *Dm4*, not all isolates heterozygous for *Avr16* showed a necrotic phenotype.

It is probably invalid to propose simple genetic models to explain these partially incompatible phenotypes. The numbers of observations were limited. The amount of necrosis and sporulation in a necrotic interaction was variable and grouping all partial interactions together might obscure more complex genetic events. Also, as both pathogen and host genotypes influence the expression of the necrotic phenotype, the genetic control of this phenomenon is likely to be complex.

Most avirulence genes exhibited complete dominance in the present study; the heterozygotes induced the same incompatible response as the homozygotes (*AvrAvr*). Heterozygotes at certain loci (e.g., *Avr3* and *Avr4*), however, seemed more likely to determine incomplete avirulence in some genetic backgrounds. Incomplete interactions were not observed in isolates homozygous at these loci. Similarly, incomplete interactions may be common at some loci when only one copy is present in hyperploid isolates. In a simultaneous study using RFLP markers, California pathotype IV was shown to be a somatic fusion of pathotypes II and III (15). Pathotype II is heterozygous at *Avr4* and *Avr16*, whereas pathotype III is homozygous virulent at both these loci. The somatic hybrid therefore has only one *Avr* and three *avr* alleles at several loci. This resulted in a modification of the incompatible phenotype; pathotype IV isolates sporulate weakly on Amplus (*Dm2Dm4*), induce extensive necrosis on R4T57 (*Dm4*), and also give necrotic reactions on LSE/18 (*Dm16*). Genetic analysis of other hyperploid isolates (California pathotype I, AM, JP1) has also indicated that incomplete avirulence may result from a single copy of *Avr3* or *Avr4* in these isolates.

Crosses to test linkage of avirulence loci. The linkage relationships of avirulence loci were tested by crossing isolates heterozygous at several avirulence loci with isolates that had homozygous recessive alleles for virulence at these loci. Highly heterozygous parental isolates were created specifically for this purpose by crossing isolates each avirulent to a number of different *Dm* genes. The use of these heterozygous isolates allowed the segregation of many virulence alleles to be followed in the F_1 progeny of individual crosses. Chi-squared tests for independent segregation and maximum likelihood estimates of recombination values were calculated to determine linkage relationships between loci using the computer program QUICKLINK (35).

Close linkage between loci controlling avirulence was not found in this study. Table 9 summarizes all the available segregation data. Only independent segregation or loose linkage has been demonstrated for the pairs of avirulence loci shown; no tight linkage has been observed. Numbers of progeny were usually insufficient to permit detection of loose linkage, but the presence of all four possible progeny classes, even in small progenies, was good evidence that loci were not tightly linked. Data that initially indicated cosegregation of avirulence to two cultivars was always subsequently explained by the presence of a common *Dm* gene in both (11). Independent segregation of most pairs of virulence loci has now been demonstrated (Table 9) (the more recently characterized *Avr* genes have yet to be studied in detail). This is in contrast to the complementary *Dm* genes, which are clustered in only four linkage groups (11,14).

The linkage analyses tested a possible complication of the gene-for-gene theory. If a mutation conferring virulence to a specific resistance gene resulted in a modified product that subsequently conditioned an incompatible interaction with a different host

TABLE 7. Modification of incompatible interactions involving *Dm3* and *Dm4*

	Interaction phenotypes[a] with lettuce lines			
	R4T57 (*Dm4*)	Amplus (*Dm2, Dm4*)	Dandie (*Dm3*)	UCDM2 (*Dm2*)
Parental isolates				
C82P24 (*avr2avr2, avr3avr3, Avr4avr4*)	−	*[b]	+	+
NL6 (*avr2avr2, Avr3Avr3, avr4avr4*)	+	+	−	+
IM25R7 (*Avr2Avr2, Avr3Avr3, avr4avr4*)	+	−	−	+
Tv (*avr2avr2, avr3avr3, avr4avr4*)	+	+	+	+
Progeny of crosses				
C82P24 × Tv				
6 isolates	−	−	+	+[b]
8 isolates	+	+	+	+
C82P24 × NL6				
20 isolates	+	+	−	+
15 isolates	−	*	*	+
C82P24 × IM25R7				
10 isolates	−	−	*	−
8 isolates	+	−	−	−

[a] + = Profuse sporulation, pathogen virulent; − = no sporulation, pathogen avirulent; * = sparse sporulation with necrosis.
[b] Progeny phenotypes other than those presented here did not occur.

resistance gene, avirulence to the two resistance genes would be allelic. As no such association was found, it seems that no known *Dm* gene can detect the product of an *avr* allele.

DISCUSSION

The basic tenets of the gene-for-gene theory were sufficient to explain the majority of specific interactions between *L. sativa* and *B. lactucae*. In most cases, a single host resistance locus was clearly matched by one pathogen locus determining avirulence and virulence. Complementation of virulence alleles, indicating that mutations to virulence to a single *Dm* gene had occurred at different loci, was not observed. Dominant inhibitor genes affecting the expression of avirulence appeared to be infrequent in *B. lactucae*; good evidence was obtained for only one, *I5/8*. Linkage analyses showed that avirulence corresponding to different resistance genes was always nonallelic and that avirulence loci were not tightly linked. Avirulence was usually completely dominant, although the expression of certain alleles could be modified (particularly when present in a single copy), depending on the genetic backgrounds of host and pathogen.

TABLE 8. Modification of incompatible interactions involving *Dm16*

Isolates and proposed genotypes		Interaction phenotypes[a] with lettuce lines: LSE/18 (*Dm16*)	Kinemontepas (*Dm10, Dm13, Dm16*)
Parental isolates			
Tv	(*avr16avr16*)	+	+
NL6	(*Avr16avr16*)	*	−
C82P24	(*Avr16avr16*)	−	−
CG1	(*Avr16Avr16*)	−	−
C83M47	(*avr16avr16*)	+	−[b]
S1	(*avr16avr16*)	+	+
NL6CG19	(*Avr16Avr16*)	*	−
NL6248	(*Avr16avr16*)	−	−
NL6246	(*Avr16Avr16*)	−	−
TvCG15	(*Avr16avr16*)	*	−
Progeny of crosses			
Tv × C83M47			
All isolates		+	−[b,c]
Tv × C82P24			
5 isolates		+	+
5 isolates		−	−
NL6 × C82P24			
5 isolates		+	+
5 isolates		*	−
3 isolates		−	−
NL6 × CG1			
5 isolates		−	−
3 isolates		*	−
NL6 × S1			
3 isolates		+	+
5 isolates		*	−
Tv × CG1			
4 isolates		*	−
3 isolates		−	−
NL6CG19 × Tv			
All isolates		*	−
NL6246 × Tv			
5 isolates		*	−
9 isolates		−	−
NL6248 × Tv			
9 isolates		−	−
5 isolates		+	+
TvCG15 × Tv			
8 isolates		+	+
2 isolates		*	−
3 isolates		−	−

[a] + = Profuse sporulation, pathogen virulent; − = no sporulation, pathogen avirulent; * = sparse sporulation with necrosis.
[b] Due to *Avr10*.
[c] Progeny phenotypes other than those presented did not occur.

The present study highlighted the difficulties involved in interpreting genetic data from gene-for-gene interactions. Genetic analysis of one partner requires assumptions about the genes being expressed in the other. Results that do not apparently conform to the basic gene-for-gene theory may be due to additional genes in the host or in the pathogen or both. It is therefore important to study both host and pathogen simultaneously. It is also often difficult to generate and characterize large progenies of these pathogens. We tried, therefore, to test hypotheses by erecting crosses from which wholly avirulent or wholly virulent progeny were expected rather than trying to distinguish between different segregation ratios.

Incorrect assumptions about resistance genes can compromise interpretations of pathogen linkage analyses and of the relationship between resistance and avirulence genes. Cultivars may carry uncharacterized resistance genes; many lettuce cultivars have been shown to possess resistance genes in addition to those previously described (6,11,14). Avirulence of *B. lactucae* to *Dm11* seemed to be determined by a dominant allele at either of two loci, but the apparent deviation from a one-to-one complementarity of resistance and avirulence genes might have been due to two tightly linked resistance genes (25). Attempts to separate *Dm11* into two components have been unsuccessful; however, recombination between any of the *Dm* genes in linkage group III (*Dm4, Dm7, Dm11*) has yet to be detected. Pairs of genes have been reported to determine virulence to a single host gene in interactions between *M. lini* and flax (33) and between *Puccinia recondita* f. sp. *tritici* and wheat (34); this could also be explained by uncharacterized resistance genes in host cultivars. As resistance genes are frequently tightly clustered in the genome (11,14,28–30), multiple resistance genes in a host cultivar may not be easy to detect. Genetic analyses of virulence in diverse pathogen isolates may be necessary to characterize the resistance genes in host lines (11).

The action of additional genes that influence virulence is difficult to demonstrate unambiguously. The segregation of both avirulent and virulent progeny in a cross between two virulent isolates can be explained by several different phenomena, e.g., inhibitor genes (Table 4) or complementation of virulence alleles (Table 3); a heterokaryotic or polyploid parental isolate could also be responsible, if a genotype such as *Avr,avr,avr,avr* determined a virulent phenotype. Inhibitor loci can only be confirmed if all progeny of a cross between a virulent isolate and an avirulent homozygote (*AvrAvr*) are virulent. Such confirmation would be difficult to obtain if the avirulence and inhibitor alleles segregating in the cross of virulent isolates were matching an uncharacterized resistance factor. Complementation of virulence loci may be equally difficult to prove. The only conclusive demonstration of complementation is a cross between virulent isolates that produces progeny that are all avirulent (Table 3). Synthesis of appropriate pathogen genotypes might be essential to achieve this confirmation, which could be a laborious process in a biotrophic pathogen. Distinguishing between the effects of complementation and inhibitor loci is important, however, because of the implications of inhibitor genes for the interpretation of virulence surveys (16,26), for studies of somatic variation that might employ inhibitor alleles as dominant selectable markers, and for molecular investigations of specificity.

Deviations from expected ratios of virulent and avirulent isolates obtained from crosses involving heterozygous parental isolates are not conclusive evidence of exceptions to the basic gene-for-gene theory. An excess of one progeny class may simply be caused by effects on germination or pathogenicity of genes linked to virulence loci or by polyploidy of parental isolates. Hypotheses derived from apparently unusual segregation data generally require confirmation by further crosses of both host and pathogen. For example, the necrotic effect involving the alleles *Dm4* from Amplus and *Avr4* from C82P24 might have been interpreted as a simple case of partial dominance of avirulence, but additional studies, using different host lines and pathogen isolates, indicated that host and pathogen genetic background effects were important in determining the interaction phenotype

TABLE 9. Summary of segregation data for avirulence loci in *B. lactucae*[a]

Avirulence loci	Avirulence loci												
	1	*2*	*3*	*4*	*5/8*	*6*	*7*	*10*	*11*	*13*	*14*	*15*	*16*
1		ac[b]	cd	ad	cd	d	cd	d	acd	d	n	d	d
2	97		d	a	cd	d	cd	n	e	n	d	d	d
3	101	21		d	bcd	bd	cd	d	cd	n	d	d	d
4	115	38	16		n	d	d	n	a	n	n	d	d
5/8	84	99	216	···		bd	bcd	n	bcd	d	d	d	d
6	34	22	144	16	144		d	d	bd	n	d	d	d
7	101	83	144	16	169	118		d	cd	n	d	d	d
10	7	···	7	···	···	27	7		n	n	n	n	n
11	117	97	88	102	123	43	98	···		d	d	d	n
13	17	···	···	···	17	···	···	···	17		n	f	n
14	···	36	52	···	49	53	22	···	31	···		d	d
15	86	57	55	91	52	69	56	···	17	17	34		d
16	27	12	13	21	13	24	13	···	···	···	12	25	

[a]The upper, right-hand portion of the table shows the origin of the data and whether loose linkage or independent segregation was observed. The lower, left-hand portion shows the total number of informative progeny isolates analyzed for each pair of loci.
[b]Explanation of codes: a = Independent segregation demonstrated by Norwood et al (27). b = Independent segregation demonstrated by Michelmore et al (25). c = Independent segregation demonstrated by Norwood and Crute (26). d = Independent segregation demonstrated or confirmed by the present authors. e = Loose linkage detected by Norwood et al (27) and Norwood and Crute (26). f = Independent segregation not confirmed due to insufficient progeny; not all genotypes detected. n = Independent segregation not yet tested.

and that interactions with *Dm3* were also influenced.

The basic gene-for-gene theory therefore appears to be an adequate genetic description of most differential interactions in host-parasite associations, such as that between *B. lactucae* and *L. sativa*, that are controlled by a gene-for-gene relationship. Apparent exceptions may result from inadequate genetic analysis of an interaction. Nevertheless, there may be complexities such as inhibitor and modifier alleles superimposed on the one-to-one complementarity of host and pathogen genes; however, they do not invalidate the basic theory. In the *B. lactucae-L. sativa* association, virulence alleles at a particular locus were always sufficient to overcome a specific resistance gene; modifier genes only affected incompatible interactions. Compatible interactions may sometimes be influenced in gene-for-gene systems, however; Wolfe (36) discusses evidence for relationships between resistance genes in barley and noncorresponding virulence genes in *Erysiphe graminis* f. sp. *hordei*. It is also important to recognize that not all features of a host-pathogen association are necessarily controlled by a gene-for-gene system. Genes determining the ability of the pathogen species to cause disease on that host species (basic pathogenicity genes) and genes determining the vigor of the isolate (aggressiveness genes) are unlikely to be the genes primarily involved in a gene-for-gene interaction.

Classical genetic studies are insufficient to confirm or refute hypotheses of the biochemical mechanisms underlying gene-for-gene resistance. For instance, the lack of complementation between alleles for pathogen virulence and host susceptibility has been used (9,10) to argue against molecular models of specificity that invoke the triggering (specified by particular combinations of resistance and avirulence alleles) of a generalized resistance response. The absence of evidence for complementation of virulence loci (for example in the lettuce-*B. lactucae* system) is not proof, however, that incompatibility always results from an interaction that involves the immediate product of an allele for pathogen avirulence. In a two-step pathway leading to a product responsible for avirulence (Table 3), mutations to virulence might be much more frequent at one locus or less deleterious than at the other. Selection for virulent mutants is likely to be intense in the presence of host resistance, so that one genotype may quickly dominate the pathogen population on a particular cultivar (1) and isolates with other mutations to virulence would be rare. Unless many separate pathogen populations were analyzed, therefore, detection of nonallelic virulence would be unlikely. Such a situation could occur in pathways of more than two steps. In the host, complementation of alleles for susceptibility has not been reported. Lines that lacked resistant responses may have been eliminated in breeding programs, however, so that studies using commercial cultivars would not reveal any such complexity. Even cultivars thought to be "universally susceptible" may possess resistance genes effective against rare isolates (5). Further difficulties of attaching a mechanistic interpretation to the gene-for-gene theory were discussed by Barrett (1), who criticized molecular models of specificity based on dominance relationships at loci determining resistance and avirulence. The gene-for-gene theory, however, provides a robust genetic framework for constructing hypotheses on the nature of specificity that can be tested using biochemical and molecular methods.

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