

Genetics of Vegetative Incompatibility in *Cryphonectria parasitica*

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Vegetative incompatibility in the chestnut blight fungus, *Cryphonectria parasitica*, in Europe is controlled by six unlinked *vic* loci, each with two alleles. Four previously identified *vic* loci (*vic1*, *vic2*, *vic3*, and *vic4*) were polymorphic in European vegetative compatibility (vc) types. Two new loci, *vic6* and *vic7*, also were identified among European vc types. In one cross, *vic* genes segregated independently at five loci, and 194 progeny were assigned to 32 vc types; none of these loci were linked. A total of 64 vc types were identified from all crosses. All 64 genotypes possible from six *vic* loci, each with two alleles ($2^6 = 64$), were identified and assigned to vc types. Based on our model, vc types v-c 5 and v-c 10, which had been used in previous genetic studies, differ by only five *vic* genes. Future studies of vc types in *C. parasitica* can use knowledge of *vic* genotypes for analysis of population genetic structure based on *vic* allele frequencies and to determine the effect of each *vic* gene on virus transmission between vc types.

Vegetative (or heterokaryon) incompatibility is a self-nonself recognition system in filamentous fungi that regulates the formation of heterokaryons (5, 13, 19) and the transmission of cytoplasmic elements between strains (8, 12, 21). In most filamentous ascomycetes, incompatibility is controlled by allelic interactions; two strains are incompatible when they have different alleles at one or more vegetative incompatibility (*vic* [or *het* for heterokaryon incompatibility]) loci (5, 13, 19). Eight to 17 *het* loci have been found in *Neurospora crassa*, *Aspergillus nidulans*, and *Podospora anserina* (reviewed in references 5, 13, and 19), while segregation of large numbers of vegetative compatibility (vc) types suggests the existence of multiple *vic* loci in other ascomycetes as well (2, 7, 14, 16, 24). Among ascomycetes, most *vic* loci have only two alleles, although multiple alleles have been found for some loci in *A. nidulans* and *N. crassa* (11, 15).

Vegetative incompatibility has been a valuable phenotype for studying genetic diversity and population biology in fungi (reviewed in references 13 and 19). Although vc type diversity has been determined in numerous populations, more-detailed analyses would be possible if the *vic* genotypes of vc types were known. However, with the exception of a few vc types used in laboratory genetic studies, there has been no attempt to assign *vic* genotypes to vc types. If *vic* genotypes were known for most of the vc types in a population, population genetic analyses that require estimates of allele frequencies would be possible by using vc type data. For example, the multilocus genetic structure of populations could be analyzed to make inferences about recombination (23), or differentiation between populations could be estimated to study gene flow (22). Furthermore, in fungi in which mycoviruses may be a significant factor in population biology, as in the chestnut blight fungus, *Cryphonectria parasitica* (27, 32), the potential for virus transmission at the population level could be assessed (17, 21), especially if knowledge of the effect of each *vic* gene on virus transmission were available (16, 17). Without *vic* genotype data, however, it

is difficult to link laboratory findings on virus transmission to field populations.

The simplest populations in which to determine *vic* genotypes are those in which vc type diversity is relatively low. In *C. parasitica*, only 31 vc types were found in samples of over 1,000 isolates from Italy and Switzerland, and most subpopulations had 10 or fewer vc types (10). This limited number of vc types could be explained by a minimum of five polymorphic *vic* loci (assuming two alleles for each locus). Vegetative incompatibility in *C. parasitica* is postulated to be controlled by allelic interactions at five to seven *vic* loci (1–3, 16). Anagnostakis (2) clearly identified two *vic* loci (*vic1* and *vic2*), and Huber (16) recently identified three more (*vic3*, *vic4*, and *vic5*). Our objectives in this study were to determine the number of polymorphic *vic* loci in *C. parasitica* populations in Europe, to identify all possible *vic* genotypes, and to determine the genotypes of vc types used in previous genetic studies.

MATERIALS AND METHODS

The 31 vc types found to date in Europe are referred to as EU-1 to EU-31 (10). Single-conidial field isolates of vc types EU-1 to EU-20 were used as vc testers (9). These tester isolates, and additional single-conidial field isolates, were used as parents in crosses (Table 1); names for field isolates are preceded by two-letter codes (PC, VO, TE, LI, FI, VA, and SA). Isolate JA17, used as a parent in cross MJ1, is a single-conidial field isolate from Japan (26). Field isolate vc testers were later replaced with ascospore isolates of the same vc type; ascospore isolates with novel recombinant vc types were given new EU numbers. Cross numbers (except MJ1) were designated with the letter P (Table 1). Ascospore isolates were designated with the letter P followed by the cross number and the ascospore isolate from that cross (e.g., P1-11 is ascospore isolate 11 from cross P1).

Vegetative incompatibility was assayed as the appearance of dark discoloration and/or barrage formation between colonies on agar medium as described previously (9, 28). Crosses were made on autoclaved chestnut (*Castanea sativa* or *C. dentata*) stems embedded in water agar as described previously (1, 20). For most crosses, we picked approximately 20 to 50 random ascospore progeny, although 194 ascospores (ca. 50 from each of four perithecia) were analyzed in cross MJ1.

Our strategy for determining *vic* genotypes was to start with vc types that had been assigned genotypes at four *vic* loci. D. Huber gave us isolates representing 13 of 16 possible genotypes determined by *vic1*, *vic2*, *vic3*, and *vic4* (16, 17). Six of these isolates were of vc types v-c 5, v-c 8, v-c 16, v-c 39, v-c 56, and v-c 71 which had been analyzed previously (1, 2, 29, 30). Our initial set of crosses was done to determine genotypes at *vic1* to *vic4* for the three remaining vc types that had not been assigned genotypes. Subsequent crosses were made to analyze the *vic* genotypes of additional vc types. Based on previous studies of *C. parasitica* (2, 16), we expected to find 2^n vc types in the progeny of each cross, with alleles segregating at *n* *vic* loci. Therefore, we used the number of progeny vc types to

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TABLE 1. Crosses of *C. parasitica* for studying the genetics of vegetative incompatibility

Cross no.	Parent 1 vc type	Isolate no.	Parent 2 vc type	Isolate no.	No. of progeny	<i>vic</i> loci ^a
P1	EU-4	PC17 ^b	EU-5	PC7	41	1, 2, 4
P2	EU-1	VO54	EU-2	VO56	42	2
P3	EU-1	VO54	EU-4	PC6	42	1,2
P4	EU-1	VO54	EU-5	PC7	42	4
P5	EU-1	VO54	EU-3	VO64	42	6
P8	EU-2	VO1	EU-3	VO29	40	2, 6
P9	EU-3	VO64	EU-4	PC17	41	1, 2, 6
P10	EU-3	VO29	EU-5	PC7	39	4, 6
P11	EU-5	P1-11	EU-26	P3-3	41	1, 4
P12	EU-3	P8-7	EU-26	P3-3	45	1, 6
P13	EU-4	P1-4	EU-14	P9-1	39	1, 6
P16	EU-10	TE56	EU-12	SA16	44	1, 3
P17	EU-1	P5-1	EU-12	SA31	45	1, 2, 6, 7
P19	EU-12	SA16	EU-13	PC83	45	2, 4
P20	EU-12	SA16	EU-19	FI38	43	2, 4, 6
P21	EU-1	P5-1	EU-10	TE63	42	2, 3, 6, 7
P24	EU-1	P4-4	EU-13	SA26	45	1, 4, 6, 7
P25	EU-10	TE63	EU-13	PC83	41	1, 2, 3, 4
P26	EU-9	PC39	EU-13	PC83	44	1, 2
P27	EU-13	SA25	EU-19	VA35	12	6
P28	EU-4	P3-7	EU-31	P1-5	10	2, 4
P29	EU-21	P10-18	EU-30	P12-39	12	1, 4
P30	EU-31	P11-23	EU-29	P13-23	42	2, 4, 6
P32	EU-9	PC39	EU-19	VA35	41	1, 2, 6
P33	EU-9	PC39	EU-10	TE56	19	3, 4
P35	EU-14	P8-3	EU-21	P10-18	12	2, 4
P36	EU-2	P9-11	EU-17	P16-6	24	6, 7
P37	EU-8	P17-2	EU-11	P17-25	22	2, 6
P38	EU-19	P27-5	EU-24	P29-1	24	6, 7
P40	EU-17	P16-6	EU-42	P19-4	24	1, 4
P42	EU-5	P1-11	EU-20	LI13	37	1, 2
P45	EU-25	P24-9	EU-45	P25-13	20	3, 6
P47	EU-30	P9-2	EU-45	P25-13	20	3, 7
P48	EU-3	P8-7	EU-33	P21-11	20	3, 7
P49	EU-10	P21-9	EU-14	P8-3	20	3, 7
P50	EU-21	P10-18	EU-46	P25-24	20	3, 7
P52	EU-45	P25-13	EU-46	P25-24	19	1, 4
P54	EU-10	P16-2	EU-22	P17-4	20	3, 6
P55	EU-13	P27-1	EU-37	P25-12	20	3
P56	EU-7	P17-7	EU-33	P25-9	20	3, 6
P57	EU-34	P25-27	EU-27	P30-9	20	3, 7
P58	EU-8	P17-2	EU-40	P16-7	40	3, 6
P59	EU-34	P57-2	EU-43	P32-4	20	3, 6
P60	EU-47	P45-5	EU-48	P47-14	20	6, 7
P61	EU-32	P49-6	EU-39	P54-5	20	6, 7
P62	EU-35	P22-6	EU-41	P21-35	20	6, 7
P64	EU-36	P25-6	EU-39	P21-18	20	4, 6
P65	EU-42	P19-4	EU-50	P57-5	39	3, 7
P66	EU-36	P33-1	EU-23	P35-3	20	3, 7
P67	EU-6	P1-6	EU-36	P25-6	36	3, 6, 7
P69	EU-29	P9-13	EU-40	P58-22	20	3, 7
P70	EU-49	P58-13	EU-50	P57-5	39	4, 6, 7
P72	EU-13	P43-3	EU-31	P11-23	20	6, 7
P73	EU-50	P65-1	EU-53	P59-12	30	6, 7
P74	EU-5	P1-11	EU-51	P50-16	20	3, 6
P76	EU-19	P72-6	EU-37	P25-12	20	3, 6
P78	EU-46	P50-4	EU-60	P74-7	20	6, 7
P79	EU-31	P1-5	EU-63	P76-13	20	3, 7
P80	EU-50	P65-1	EU-51	P74-10	20	1, 2
MJ1	EU-22	P17-8	EU-24	JA17	194	1, 2, 4, 6, 7

^a *vic* loci at which segregation occurred in each cross. The nomenclature for these loci is explained in text.

^b Isolate designations beginning with two-letter codes are field isolates from Italy (9) from the Bergamo (PC), Crevoladossola (VO), Teano (TE), Tonara (SA), Pomino (FI), Ponte in Valtellina (VA), and Pigna (LI) populations. Isolates whose designations begin with the letter P are ascospore progeny isolates; the first number following the P is the cross number, and the second number is the ascospore isolate from that cross (e.g., P1-11 is ascospore isolate 11 from cross P1).

TABLE 2. Segregation of *vc* types defined by *vic1* to *vic4* in crosses of *C. parasitica*

vc type	Crosses ^a								Genotype ^b
	P33	P16	P52	P55	P19	P26	P40	P25	
EU-42									1111
EU-12		P			P				1112
EU-34									1121
EU-40								c	1122
EU-13					P	P	P		1211
EU-11								P	1212
EU-37					P				1221
EU-45									1222
EU-9	P						P		2111
EU-17								P	2112
EU-36									2121
EU-10	P	P						P	2122
EU-15									2211
EU-28									2212
EU-46									2221
EU-33									2222

^a Crosses are defined in Table 1. Solid cells indicate *vc* types that segregated in each cross. Parental *vc* types are indicated by the letter P. Lightly shaded cells represent the assignment of a genotype to a *vc* type in a particular cross.

^b Genotypes for *vic1* to *vic4* as defined by Huber (16).

^c EU-40 was expected but was not observed in this cross.

infer the number of *vic* genes segregating in each cross. Interpretation of crosses was done sequentially and depended on results from each previous cross.

We also compared isolates of *vc* types v-c 10, v-c 17, and v-c 40 which had been used in crosses to study *vc* genetics (1, 2) with *vc* types from our crosses to determine their *vic* genotypes. Incompatibility caused by *vic5* is weak and cannot be detected on potato dextrose agar (16) or on potato dextrose agar containing bromocresol green (9, 28; unpublished observations); therefore, this locus was not considered in our genetic analyses.

RESULTS

In our initial set of crosses among *vc* types with genotypes previously defined by *vic1* to *vic4* (Table 2) we determined the genotypes of the three remaining *vc* types. In each cross (P33, P16, and P52), three of the four *vc* types in the progeny had known *vic* genotypes, making it possible to infer the fourth genotype. Progeny *vc* types from five additional crosses conformed to those expected from Huber's model (16). For example, in cross P25, the parents (EU-10 and EU-13) differed at all four *vic* loci and 15 of the 16 predicted *vc* types were found in the 30 progeny. Eight of the 31 *vc* types from Europe were compatible with testers in this set of 16 genotypes.

In our second set of crosses, we found 48 additional *vc* types, for a total of 64, suggesting segregation at six *vic* loci (Table 3). Crosses are discussed in the order in which they appear in Table 3 to explain how each *vic* genotype was determined. Crosses with single *vic* genes segregating were found between EU-1 and EU-2 (cross P2), EU-1 and EU-5 (cross P4), and EU-13 and EU-19 (cross P27).

Progeny from cross MJ1 (EU-22 × EU-24) were of 32 *vc* types, indicating segregation at five *vic* loci in this cross. Only eight progeny *vc* types were among the 16 genotypes defined by *vic1* to *vic4* (i.e., those shown in Table 2); these eight types all shared allele *vic3-1*. From this result, we concluded that all of the progeny in this cross had allele *vic3-1*. We also concluded that alleles were segregating at *vic1*, *vic2*, and *vic4* and at two new *vic* loci, which we designated *vic6* and *vic7*. Alleles *vic6-1* and *vic7-1* were arbitrarily assigned to the 16 genotypes defined by *vic1* to *vic4*.

TABLE 3. Segregation of vc types in crosses of *C. parviticola*

vc type	Crosses ^a																										
	P2	P4	P27	MJ1	P24	P17	P21	P5	P48	P12	P8	P3	P1	P10	P13	P9	P11	P28	P42	P35	P29	P30	P72	P38 ^b	P36	P66	
EU-42				■		P																					
EU-12				■		P																					
EU-34																											
EU-40																											
EU-13			P		P																		P				
EU-11				■																							
EU-37																											
EU-45																											
EU-9				■																						■	■
EU-17				■																						P	
EU-36																										P	P
EU-10																											
EU-15				■																							
EU-28				■																							
EU-46																											
EU-33																											
EU-27				■																							
EU-29																											
EU-50																											
EU-56																											
EU-24			P																								
EU-30																											
EU-52																											
EU-48																											
EU-23				■																							
EU-14				■																							
EU-58																											
EU-32																											
EU-21				■																							
EU-3				■																							
EU-51				■																							
EU-35																											
EU-43				■																							
EU-8				■																							
EU-53																											
EU-49																											
EU-19			P																								
EU-25				■																							
EU-63																											
EU-47																											
EU-16				■																							
EU-22																											
EU-57																											
EU-39																											
EU-18				■																							
EU-7				■																							
EU-64																											
EU-41																											
EU-20				■																							
EU-4				■																							
EU-61																											
EU-62																											
EU-31				■																							
EU-26																											
EU-55																											
EU-54																											
EU-6				■																							
EU-2			P																								
EU-59																											
EU-38																											
EU-5			P																								
EU-1			P																								
EU-60																											
EU-44																											

^a Crosses are defined in Table 1. Solid cells indicate vc types that segregated in each cross. Parental vc types are indicated by the letter P. Lightly shaded cells represent the assignment of a genotype(s) to a vc type(s) in a particular cross.

^b Crosses P38 and P65 confirm segregation results in crosses P72 and P57, respectively.

^c Crosses discussed in detail in the text but not in order.

^d Genotypes for *vic1* to *vic4*, *vic6*, and *vic7* are abbreviated with allele numbers only. The first 16 genotypes are the same as those in Table 2.

Three crosses (P24, P17, and P21) were analyzed in which there were 16 vc types in the progeny; we concluded that four *vic* genes were segregating in each cross. In each of these crosses, 4 of the 16 progeny vc types were in the subset of 16 vc

types whose genotypes are defined by *vic1* to *vic4* (Table 2). We interpreted this to mean that there was segregation at only two of loci *vic1* to *vic4* and that there was segregation at both *vic6* and *vic7*. Based on the known genotypes of progeny vc

TABLE 3—Continued

Crosses																		Genotype ^d										
P69	P57	P65 ^b	P49	P50	P47	P76	P80	P54	P74	P64	P61	P78	P79	P56 ^c	P62	P37 ^c	P20		P59	P58	P45	P60	P73	P70	P67 ^c	P32		
		P																P										1111-11
																		P										1112-11
		P																	P									1121-11
																				P								1122-11
																					P							1211-11
																						P						1212-11
																							P					1221-11
																										P		2111-11
																												2112-11
																												2121-11
																												2122-11
																												2211-11
																												2212-11
																												2221-11
																												2222-11
		P																										1111-12
																												1112-12
																												1121-12
																												1122-12
																												1211-12
																												1212-12
																												1221-12
																												1222-12
																												2111-12
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																												2222-22

types, we inferred that all of the progeny of P24 had alleles *vic2-2* and *vic3-1* because no segregation was evident at these loci; similarly, all of the progeny of P17 had *vic3-1* and *vic4-2*, and progeny of P21 had *vic1-2* and *vic4-2*. Since EU-1 was a progeny vc type in these three crosses and in cross MJ1, we determined its genotype for *vic1* to *vic4* (as described above). Because there was segregation at *vic6* and *vic7* in cross P21, and

parent EU-10 has alleles *vic6-1* and *vic7-1*, the other parent, EU-1, had alleles *vic6-2* and *vic7-2*. Therefore, EU-1 has the genotype *vic1-2 vic2-2 vic3-1 vic4-2 vic6-2 vic7-2*. To simplify the notation, genotypes hereafter are designated simply by their allele numbers for these six loci, e.g., 2212-22 for EU-1; the hyphen indicates that the allele for *vic5* is not known. Lightly shaded cells in Table 3 indicate the cross in which a genotype was assigned to a vc type.

In cross P5, EU-1 and EU-3 were found to differ at one *vic* locus. By the same reasoning as that used for EU-1, EU-3 has genotype 2212--- because it was found in crosses MJ1, P24, P17, and P21; therefore, segregation must have occurred at either *vic6* or *vic7* in cross P5. Since *vic6* and *vic7* were not yet defined separately, we arbitrarily assigned alleles *vic6-1* and *vic7-2* to EU-3. When EU-3 (2212-12) was crossed (in cross P48) with EU-33 (2222-11), two *vic* genes segregated, at *vic3* and *vic7*, allowing us to assign genotype 2222-12 to recombinant vc type EU-35 because the other recombinant genotype, 2212-11, was already assigned to EU-28. For most of the remaining crosses (not discussed explicitly), we assigned genotypes to vc types in this way; i.e., from crosses in which all but one progeny vc type had genotypes already determined from previous crosses (Table 3). Exceptions to this strategy are described below.

Cross P12. When EU-3 (2212-12) was crossed with EU-26 (-212---), segregation at *vic6* was evident because EU-1 (2212-22), which has *vic6-2*, was a recombinant vc type. Therefore, *vic6-2* was assigned to parent EU-26 and *vic6-1* was assigned to the other recombinant type, EU-30. EU-30 could not also have had allele *vic7-1* because it is not among the 16 vc types defined by *vic1* to *vic4* that have both *vic6-1* and *vic7-1* (Table 2). Therefore, *vic7-2* was assigned to both EU-26 and EU-30. With four different progeny vc types in cross P12, there must have been segregation at *vic1* in addition to *vic6* because *vic2-2*, *vic3-1*, *vic4-2*, and *vic7-2* were common to both parents. Therefore, genotypes 1212-22 and 1212-12 were assigned to EU-26 and EU-30, respectively.

Cross P8. We deduced the genotypes for EU-2 and EU-14 from cross P8 by using a rationale similar to that used for cross P12. From crosses MJ1, P17, and P21, we knew that EU-2 and EU-14 both had alleles *vic1-2*, *vic3-1*, and *vic4-2*. As in P12, segregation at *vic6* was evident because EU-1, which has *vic6-2*, was a recombinant vc type (2212-22), while parent EU-3 (2212-12) had *vic6-1*. Therefore, *vic6-2* was assigned to parent EU-2, while *vic6-1* was assigned to the other recombinant type, EU-14. EU-14 could not have had allele *vic7-1* because it was not among the 16 vc types defined by *vic1* to *vic4* that have both *vic6-1* and *vic7-1* (Table 2). Therefore, *vic7-2* was assigned to both EU-2 and EU-14. Since there were four progeny types, alleles must have segregated at *vic2* in addition to *vic6*, and *vic2-1* was assigned to both EU-2 and EU-14. Thus, genotypes 2112-22 and 2112-12 were assigned to EU-2 and EU-14, respectively.

Cross P10. Parent EU-3 (2212-12) and recombinant type EU-1 (2212-22) differ at *vic6*, demonstrating segregation at this locus. Therefore, parent EU-5 must have had *vic6-2*, while recombinant EU-21 must have *vic6-1*. From crosses MJ1 and P24, we knew that parent EU-5 had alleles *vic2-2* and *vic3-1*. After determining the genotype of EU-4 in cross P3, we deduced from cross P1 that EU-5 had *vic1-2* because EU-4, the other parent in cross P1, had *vic1-1*, but there was segregation at *vic1* (Table 3). EU-21 could not have had allele *vic7-1* because it was not among the 16 vc types defined by *vic1* to *vic4* that have both *vic6-1* and *vic7-1* (Table 2). Therefore, *vic7-2* was assigned to both EU-5 and EU-21. Since there were four progeny types in cross P10, alleles must have segregated at *vic4* in addition to *vic6*, and *vic4-1* was assigned to both EU-5 and EU-21. Thus, genotypes 2211-22 and 2211-12 were assigned to EU-5 and EU-21, respectively.

Cross P56. Parent EU-33 (2222-11) and recombinant type EU-28 (2212-11) differ at *vic3*, demonstrating segregation at this locus. Therefore, parent EU-7 had *vic3-1*, while recombinant EU-41 had *vic3-2*. One parent (EU-7) and one recombinant type (EU-41) were not among the 16 vc types defined by

vic1 to *vic4* that have both *vic6-1* and *vic7-1* (Table 2). Therefore, alleles segregated at either *vic6* or *vic7* in addition to *vic3*. However, genotypes 2212-12 and 2222-12 were already defined, leaving genotypes 2212-21 and 2222-21 to be assigned to EU-7 and EU-41, respectively.

Cross P37. Parent EU-11 (1212-11) and recombinant type EU-12 (1112-11) differ at *vic2*, demonstrating segregation at this locus. Therefore, the other parent, EU-8, had *vic2-1*, while recombinant EU-25 had *vic2-2*. EU-8 and EU-25 were both assigned *vic6-2* because 32 vc types had already been assigned genotypes with *vic6-1*. With segregation at only two loci (*vic2* and *vic6*), there could not have been segregation at any other locus. Therefore, genotypes 1112-21 and 1212-21 were assigned to EU-8 and EU-25, respectively.

Cross P67. Six of the eight progeny vc types, including both parents (EU-6 and EU-36), had known genotypes; genotypes were not yet known for EU-16 and EU-59. The only progeny genotypes in this cross that were not already assigned to vc types were 2111-21 and 2121-22. Because EU-16 was a progeny vc type in cross MJ1 and EU-59 was not, EU-16 had *vic3-1* and was assigned genotype 2111-21, leaving 2121-22 to be assigned to EU-59.

All 64 possible *vic* genotypes, vc types, and tester isolates are summarized and cross-listed with vc types and testers from previous genetic studies in Table 4. vc types v-c 10, v-c 17, and v-c 40, which were previously used in genetic studies but without known *vic* genotypes, were compatible with EU-1, EU-26, and EU-5, respectively.

There was no evidence for linkage among any of the six *vic* loci (Table 5). Data from multiple crosses with segregation at the same two *vic* loci were tested for homogeneity ($\alpha = 0.10$) before pooling (31).

DISCUSSION

Genetic analyses of vc types in *C. parasitica* in Europe are consistent with a model of six unlinked *vic* loci, each with two alleles. Our results confirm that vegetative incompatibility is controlled by allelic interactions. Four of the *vic* loci we identified in this study were previously described by Huber (16); therefore, we have identified two additional *vic* loci. We concur with Huber (16) that—except for *vic1* and *vic2*—the tentative genotype assignments made by Anagnostakis (1) and Rizwana and Powell (29, 30) should be disregarded and replaced by Huber's nomenclature (16). Therefore, we called the two new *vic* loci found in this study *vic6* and *vic7*. Incompatibility caused by an additional *vic* locus, *vic5*, cannot be detected on agar media (16; unpublished results) and was therefore disregarded in this study.

There was no evidence for multiple alleles at any *vic* locus for the vc types found in the field in Europe (EU-1 to EU-31). Thirty of the 31 European vc types were among the progeny of cross MJ1, in which alleles segregated at each of five *vic* loci; there was no segregation at *vic3* in this cross. EU-10 was the only vc type found in the field in Europe that was not found among the progeny of cross MJ1 (Table 3). EU-10 was shown to have allele *vic3-2* (cross P33, Table 2), but all of the other European vc types had allele *vic3-1* (Table 2). Therefore, since all 64 vc types were either found in the field or derived from field isolates, only two alleles were found for each *vic* locus.

Our results (Table 2) are fully in agreement with Huber's (16) and agree with six of the nine crosses reported by Anagnostakis (1, 2). However, our results disagree with three of Anagnostakis' crosses. In general, we predict fewer vc types segregating than were observed in some crosses. We speculate that the excess vc types found in previous studies were artifacts

TABLE 4. Genotypes and tester isolates of 64 *C. parasitica* vc types in this and previous studies

vic geno- type ^a	Results of:						ATCC no.
	This study			Previous studies			
	EU type ^b	Isolate no.	Cross ^c	vc type ^d	Isolate no.		
1111-11	EU-42	P19-4	— ^e	v-c 5	EP389, 389.7	38980	
1112-11	EU-12	P16-1	—	v-c 56	EP243, F3.2		
1121-11	EU-34	P25-27	—		N1.9		
1122-11	EU-40	P16-7	P16				
1211-11	EU-13	P1-5	—	v-c 71	J2.43		
1212-11	EU-11	P19-2	—		J2.6		
1221-11	EU-37	P25-12	—		M1.6		
1222-11	EU-45	P25-13	P52				
2111-11	EU-9	P26-1	—	v-c 39	EP388	38979	
2112-11	EU-17	P16-6	—		F3.13		
2121-11	EU-36	P25-6	—		K1.43		
2122-11	EU-10	P16-2	P33				
2211-11	EU-15	P26-10	—	v-c 8	22508 ^f , A1.8	22508	
2212-11	EU-28	P21-16	—		J2.20, L1.16		
2221-11	EU-46	P25-24	—	v-c 16	EP29, K2.30	38754	
2222-11	EU-33	P21-11	—		L1.39		
1111-12	EU-27	P30-9	P30				
1112-12	EU-29	P9-13	P13				
1121-12	EU-50	P57-5	P57				
1122-12	EU-56	P69-5	P69				
1211-12	EU-24	P29-1	P29				
1212-12	EU-30	P9-2	P12				
1221-12	EU-52	P80-1	P80				
1222-12	EU-48	P47-14	P47				
2111-12	EU-23	P35-3	P35				
2112-12	EU-14	P8-3	P8				
2121-12	EU-58	P66-9	P66				
2122-12	EU-32	P21-5	P49				
2211-12	EU-21	P10-18	P10				
2212-12	EU-3	P5-2	P48				
2221-12	EU-51	P50-3	P50				
2222-12	EU-35	P22-6	P48				
1111-21	EU-43	P20-2	P20				
1112-21	EU-8	P17-2	P37				
1121-21	EU-53	P59-9	P59				
1122-21	EU-49	P58-13	P58				
1211-21	EU-19	P27-1	P72				
1212-21	EU-25	P24-9	P37				
1221-21	EU-63	P76-6	P76				
1222-21	EU-47	P45-4	P45				
2111-21	EU-16	P32-3	P67				
2112-21	EU-22	P17-4	P36				
2121-21	EU-57	P64-2	P64				
2122-21	EU-39	P21-18	P54				
2211-21	EU-18	P24-33	P32				
2212-21	EU-7	P17-7	P56				
2221-21	EU-64	P78-1	P78				
2222-21	EU-41	P21-35	P56				
1111-22	EU-20	P1-2	P28				
1112-22	EU-4	P1-4	P3				
1121-22	EU-61	P73-24	P73				
1122-22	EU-62	P70-11	P70				
1211-22	EU-31	P1-5	P11				
1212-22	EU-26	P1-16	P12	v-c 17	EP78	38752	
1221-22	EU-55	P79-6	P79				
1222-22	EU-54	P60-3	P60				
2111-22	EU-6	P1-6	P42				
2112-22	EU-2	P2-4	P8				
2121-22	EU-59	P67-7	P67				
2122-22	EU-38	P21-14	P61				
2211-22	EU-5	P1-11	P10	v-c 40	EP155	38755	
2212-22	EU-1	P4-4	P21	v-c 10	EP67	38753	
2221-22	EU-60	P74-3	P74				
2222-22	EU-44	P21-1	P62				

^a Genotypes for vc types were based on four *vic* loci (*vic1* to *vic4*) named by Huber (16), followed by two new loci found in this study (*vic6* and *vic7*). Only the allele numbers for each locus are shown. The hyphen after the allele at the fourth locus is to signify no data for *vic5*.

^b Nomenclature for EU types is based on that of Cortesi et al. (10). Isolate numbers refer to tester isolates derived from the crosses listed in Table 1.

^c Cross number in which a *vic* genotype was assigned to a vc type.

^d The v-c nomenclature is based on that of Anagnostakis (1, 3). ATCC (American Type Culture Collection) numbers correspond to isolate numbers beginning with EP; all other isolates were from Huber (16).

^e These genotypes were determined previously by Huber (16).

^f No EP number is known for this isolate; Huber (16) used the ATCC number instead.

TABLE 5. Analysis of linkages among six *vic* loci identified in *C. parasitica*

No. of crosses pooled ^a	n ^b	<i>vic</i> loci ^c		No. of progeny with <i>vic</i> genotypes ^d				Chi square ^e
		<i>i</i>	<i>j</i>	<i>vic</i> genotypes ^d				
				1, 1	1, 2	2, 1	2, 2	
10	546	1	2	148	135	133	130	1.4
2	85	1	3	26	16	16	27	5.2
8	417	1	4	113	95	105	104	1.6
7	450	1	6	114	115	120	101	1.8
3	284	1	7	70	74	71	69	0.2
2	83	2	3	16	26	22	19	2.6
8	428	2	4	108	117	108	95	2.3
9	470	2	6	121	113	125	111	1.1
3	236	2	7	56	65	62	53	1.5
2	60	3	4	11	15	17	17	1.6
9	196	3	6	46	52	46	52	0.7
11	235	3	7	63	62	51	59	1.5
7	383	4	6	100	97	97	89	0.7
3	233	4	7	64	59	55	55	0.9
14	534	6	7	133	132	137	132	0.1

^a Number of crosses pooled for each pair of loci. Chi square tests of homogeneity were conducted before pooling (see text).

^b Total number of progeny from all crosses analyzed for each locus pair.

^c Pairs of *vic* loci, *i* and *j*, for which linkage analyses were done.

^d Genotypes were defined as allele 1 or 2 at loci *i* and *j*.

^e Chi square goodness-of-fit statistics (three degrees of freedom) for a genotype ratio of 1:1:1:1.

caused by vc assays. Recent improvements in vc testing techniques have reduced these ambiguities considerably (9, 28). First, Anagnostakis (1) reported finding only parental vc types in a cross between v-c 5 and v-c 16. In contrast, Rizwana and Powell (29) found 17 nonparental vc types among the progeny of a cross between v-c 5 and v-c 16; however, the ratios of progeny vc types in this cross were highly irregular. According to our interpretation, v-c 5 and v-c 16 are compatible with EU-42 and EU-46, respectively, which differ by three *vic* genes and should produce progeny in eight vc types; Huber (16) also found that these two vc types differed by *vic1*, *vic2*, and *vic3*. The second cross Anagnostakis (1) reported that differed from our results was between v-c 8 and v-c 17 (EU-15 and EU-26, respectively). Anagnostakis reported finding 22 vc types in the progeny, but we would predict only 16 because the parents differ at four *vic* loci (Table 4). Finally, the third cross, with which our results are not fully consistent, is between v-c 5 and v-c 10 (2). This cross has often been cited because it was the basis for estimating the minimum number of *vic* loci controlling vc types in *C. parasitica*. Anagnostakis (2) estimated segregation at seven *vic* loci after observing 106 vc types in the progeny of this cross. A lower estimate of five *vic* loci, however, was based on the observation that approximately 1/25 (11 of 263 and 37 of 973) of the progeny from this same cross were of one parental type. One thirty-second of the progeny would have been expected to be compatible with each parent if alleles had segregated independently at five *vic* loci (2, 3), whereas 1/128 would have been expected to be compatible if alleles had segregated at seven loci. Our results show that v-c 5 and v-c 10 (compatible with EU-42 and EU-1, respectively; Table 4) differ by only five, not seven, *vic* genes.

Six *vic* loci, each with two alleles, may be sufficient to explain much of the diversity of *vic* genotypes, or vc types, found in populations of *C. parasitica* in North America. Anagnostakis and Kranz (4) found 48 vc types in a small forest plot in Connecticut; other studies have shown similar levels of vc type diversity in North America (18, 20, 25). Our preliminary stud-

ies have shown that most of the 31 *vc* types found in one population in Maryland (20) are compatible with testers in the 64 genotypes found in this study (unpublished results). More testing is required to determine if additional *vic* loci are polymorphic in North America or if multiple alleles occur at known loci. In Asia, there are probably additional polymorphic *vic* loci since 131 *vc* types were identified among 231 isolates of *C. parasitica* (33). Furthermore, only two of the 71 *vc* types found in Japan are compatible with the 64 types from this study (21a). Unfortunately, the effort required to identify additional *vic* genotypes doubles with every locus that is identified. For example, one additional *vic* locus in *C. parasitica* would bring the total number of possible *vc* types to 128 and two loci would raise it to 256. Alternatively, a third allele at any *vic* locus would increase the number of possible genotypes by 50%. Multiple alleles at several loci—in relatively high frequencies in populations—would be necessary to explain the diversity of *vc* types observed in Asia and the lack of compatibility with the 64 *vc* types we found. However, we found no evidence for multiple alleles in European populations.

Our study has significantly extended the findings of previous genetic studies on *vc* types in *C. parasitica* because we have successfully assigned 64 (2^6) *vic* genotypes to *vc* types (Table 4). To our knowledge, such extensive information on *vic* genotypes is not available for any other fungus. All 31 of the European *vc* types (EU-1 to EU-31) found in the field to date (10) have been assigned a *vic* genotype. Thirty of the 32 possible *vc* types (all except EU-42 and EU-43) defined by five *vic* loci (*vic1*, *vic2*, *vic4*, *vic6*, and *vic7*) have been found in the field. Interestingly, allele *vic3-2* was only found in two populations in southern Italy, in *vc* type EU-10 (9, 10); all of the other *vc* types found in Europe have *vic3-1*. Knowledge of *vic* genotypes will allow us to reanalyze *vc* type survey data more thoroughly (e.g., references 6, 9, and 10). For example, we will be able to test whether *vc* types occur at frequencies that would be expected from random mating; this analysis requires estimates of *vic* allele frequencies along with *vc* type frequencies (see reference 23). In addition, comparison of the 64 *vc* types from this study with *vc* types found in the United States is revealing a high proportion of successful matches (unpublished results), which opens the possibility of reanalyzing *vc* type population data in the United States based on *vic* genotypes. Finally, with our knowledge of *vic* genotypes, we can now examine the effect of each *vic* allele on virus transmission (17, 21; unpublished results) to make inferences about the potential of virus transmission in populations of *C. parasitica*.

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