Correlation Between Hypovirus Transmission and the Number of Vegetative Incompatibility (*vic*) Genes Different Among Isolates from a Natural Population of *Cryphonectria parasitica*

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ABSTRACT

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Correlation between hypovirus transmission and the number of different vegetative incompatibility (*vic*) genes among isolates of *Cryphonectria parasitica* was estimated using isolates sampled from a natural population. We tested the hypothesis that transmission of hypoviruses among isolates is negatively correlated to the number of *vic* genes that are different between vegetative compatibility (vc) groups. In a sample of 58 isolates collected from a population in Finzel, MD, eight isolates, each in a different vc group, were randomly selected and infected with each of the *Cryphonectria* hypoviruses CHV1-EP43 and CHV2-NB58. The frequency and time required for virus transmission were estimated by pairing infected donor isolates with uninfected recipient isolates in vitro. The number of *vic* genes different between vc groups was estimated by crossing the donor and recipient isolates and determining the proportion of ascospore progeny that were vegetatively compatible with either parent. Hypovirus transmission occurred between all pairs of isolates that were vegetatively compatible. The frequencies of transmission between vc groups that differed by one *vic* gene were 0.50 and 0.48 when the donor isolates were infected with CHV1-EP43 or CHV2-NB58, respectively. Transmission frequencies decreased to 0.13 for CHV1-EP43 and 0.14 for CHV2-NB58 when vc groups differed by two *vic* genes. When vc groups were different by more than two *vic* genes, transmission of hypoviruses occurred in only one out of 37 pairs (3%) and one out of 25 pairs (4%) with CHV1-EP43 and CHV2-NB58 in the donor isolates, respectively. The transmission frequency was negatively correlated to the number of *vic* genes different between isolates ($P < 0.01$). In contrast, the time taken for transmission to occur was only weakly correlated ($r = 0.40$, $P = 0.05$) to the number of *vic* genes different between vc groups. Unidirectional transmission of hypoviruses was observed between six pairs of vc groups. This study provides evidence for a significant negative correlation between the frequency of hypovirus transmission and the number of *vic* genes different between isolates of *C. parasitica* from a natural population.

Additional keywords: chestnut blight, *Endothia parasitica*, hyphal anastomosis, hypovirulence.

The presence of cytoplasmic double-stranded RNA (dsRNA) hypoviruses has been demonstrated to cause a reduction of virulence (hypovirulence) in the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr, and is associated with the biological control of this disease (10,13,17,25,31). For biological control of chestnut blight to be successful, hypoviruses must be transmitted among individuals in the pathogen population. Hypoviruses can be transmitted in two ways. First, hypoviruses can be transmitted vertically from mycelium to conidia, which then initiate new cankers (13,29). Unfortunately, the production of conidia is suppressed in virus-infected isolates (12) and hypoviruses are often transmitted to only a fraction of conidia (13,29). Hypoviruses can also be transmitted horizontally between different individuals through the mixing of cytoplasm after hyphal anastomosis (5,19,32). However, hypoviruses have not been found to be vertically transmitted to ascospores, nor have virus-infected isolates been found to produce perithecia (1,4,12,19).

Horizontal transmission of hypoviruses in *C. parasitica* may depend on the stability of hyphal anastomosis between isolates (5,14,27). Anastomoses may be stable and last for a sufficient time to allow the exchange of cytoplasmic elements between isolates (21), or transmission of cytoplasmic elements may not occur if the anastomosis is unstable and short-lived (14,21,27). Factors that govern the formation of stable hyphal anastomosis may, therefore, affect the transmission of hypoviruses and are important for understanding the potential for biological control.

Vegetative incompatibility, which limits hyphal anastomosis, has been shown to restrict hypovirus transmission (5,7,32). Vegetative incompatibility in *C. parasitica* is controlled by five to seven unlinked vegetative incompatibility (*vic*) loci (2). Isolates that share identical alleles at all *vic* loci are vegetatively compatible and belong to the same vegetative compatibility (vc) group. Stable anastomoses between vegetatively compatible isolates has been observed to allow the transmission of hypoviruses from one isolate to another (27). In contrast, isolates are vegetatively incompatible if they differ by one or more *vic* genes (2). Hyphal anastomosis between incompatible isolates may be limited because of cytoplasmic degeneration and death of heterokaryotic and heteroplasmic cells (1,21,27). Consequently, transmission of hypoviruses can be limited by the vegetative incompatibility system (5,32).

Hypovirus transmission has further been shown to be associated with the genetic relatedness of vc groups (5,7). Rapid transmission was observed between isolates in the same vc group and in some incompatible pairs. However, transmission occurred more slowly and at a lower frequency in other incompatible pairings (5). Anagnostakis and Waggoner (7) found that canker expansion rates were significantly reduced when trees were inocu-

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lated with pairs of virulent and hypovirulent isolates that were vegetatively compatible or differed by only one *vic* gene. In contrast, canker expansion rates were not reduced when isolates differed by five or more *vic* genes.

In contrast to the hypothesis that hypovirus transmission depends on the genetic relatedness between vc groups, Huber and Fulbright (18) reported that different *vic* genes have different effects on virus transmission. For example, certain one-gene differences between vc groups prevented transmission, whereas some two-gene differences did not. Therefore, hypovirus transmission does not consistently correspond to the relatedness between isolates in their vc groups, but depends on the effects of individual *vic* genes.

Estimating the relationship between hypovirus transmission and the genetic differences among vc groups at a population level may be an appropriate approach for making inferences to biological control in the field. Previous transmission studies (5,7,18) were based on small numbers of laboratory isolates, which may not represent the general behavior of hypovirus transmission in natural populations. As a complement to laboratory collections, we conducted transmission experiments on isolates randomly sampled from a natural population of *C. parasitica*. The objective of this study was to test the hypothesis that transmission of hypoviruses among isolates of *C. parasitica* from a natural population was negatively correlated to the number of different *vic* genes.

MATERIALS AND METHODS

Population samples. We used a sample of 58 mass-hyphal isolates that were collected from a population of infected American chestnuts (*Castanea dentata* (Marshall) Borkh.) in Finzel, MD, by A. Webb and E. Seligmann in 1991. Thirty-one vc groups had been previously identified in this sample (22). Isolates were grown on potato-dextrose agar (PDA) (Difco Laboratories, Detroit) at 25°C under white fluorescent light with a 16-h photoperiod and maintained on PDA at 4°C. All isolates were screened for the presence of dsRNA using an immunoblot technique; only two isolates, MD1 and MD7, contained dsRNAs (T. L. Peever and M. G. Milgroom, *unpublished data*).

TABLE 1. Segregation of vegetative compatibility (vc) groups and estimated number of vegetative incompatibility (*vic*) genes different between vc groups of *Cryphonectria parasitica*

VC groups[a] (isolate number)		Observed vc groups among progeny			Estimated number *vic* genes different	$\chi^2_{(1)}$[b]	$\chi^2_{(2)}$[c]	$\chi^2_{(3)}$[d]
Parent 1	Parent 2	Parental 1	Parental 2	Nonparental				
A(7)	B(24)	12	13	55[e]	>2	…[f]	11.28	1.72
A(7)	D(32)	9	12	19	2	…	0.55	17.03
A(20)	D(32)	5	2	33	>2	…	17.35	2.1
A(49)	D(32)	13	8	19	2	…	1.35	18.63
A(40)	F(35)	2	4	34	>2	…	19.8	2.53
A(7)	G(12)	4	2	34	>2	…	19.8	2.53
A(7)	W(16)	2	4	34	>2	…	19.8	2.53
A(7)	BETA(8)	8	8	24	2	…	1.6	4.8
A(44)	DELTA(48)	21	19	0	1	0.1	…[g]	…[g]
A(7)	FSG2(27)	10	13	17	2	…	1.35	23.43
A(40)	FSG2(18)	13	31	38[e]	2	…	8.6	53.07
A(7)	FSG5(13)	4	5	31	>2	…	12.15	0.23
A(7)	FSG9(22)	15	25	0	1	2.5	…	…
A(7)	FSG14(50)	25	15	0	1	2.5	…	…
B(24)	C(46)	2	7	31	>2		13.35	2.63
B(24)	F(25)	9	31	0	1	12.1	…	…
B(24)	G(14)	12	10	18	2	…	0.6	19.6
B(24)	Q(6)	23	19	0	1	0.5	…	…
B(24)	X(9)	15	25	0	1	2.5	…	…
B(24)	FSG1(17)	9	4	27	>2	…	6.15	3.7
B(24)	FSG2(18)	20	20	0	1	0	…	…
B(24)	FSG4(37)	10	9	21	2	…	0.15	10.9
B(24)	FSG7(57)	19	20	1[h]	1	0.05	…	…
B(24)	FSG8(45)	11	19	48[e]	2	…	5.7	10.6
C(46)	D(32)	24	16	0	1	1.6	…	…
C(46)	W(16)	0	2	38	>2	…	32.6	8.93
C(46)	FSG1(17)	11	29	0	1	8.1	…	…
D(32)	F(2)	23	17	0	1	0.9	…	…
D(32)	F(25)	21	19	0	1	0.1	…	…
D(32)	F(34)	17	23	0	1	0.9	…	…
D(32)	F(35)	24	16	0	1	1.6	…	…
D(32)	F(39)	21	19	0	1	0.1	…	…
D(32)	G(14)	20	20	0	1	0	…	…
D(32)	N(11)	3	2	35	>2	…	22.55	3.43
D(32)	Q(6)	12	10	18	2	…	0.6	19.6
D(32)	Q(23)	7	11	22	2	…	1.2	10.13
D(32)	Q(29)	11	4	25	2	…	4.95	8.23
D(32)	W(16)	0	4	36	>2	…	26.4	6.4

(*continued on next page*)

[a] Vegetative compatibility groups A to BETA were named by vc testing with tester strains as in MacDonald and Double (24). FSG1 to FSG11 were new vc groups found in this population.

[b] Chi-square tests for goodness-of-fit to 1:1 segregation. The critical value at the 5% level of significance with df = 1 is: $\chi^2_{1,0.95} = 3.84$.

[c] Chi-square tests for goodness-of-fit to 1:1:2 segregation. The critical value at the 5% level of significance with df = 2 is: $\chi^2_{2,0.95} = 5.99$.

[d] Chi-square tests for goodness-of-fit to 1:1:6 segregation. The critical value at the 5% level of significance with df = 2 is: $\chi^2_{2,0.95} = 5.99$.

[e] Eighty ascospores were sampled and used for analyses.

[f] 1:1 model was not applicable when nonparental vc types were observed.

[g] Not calculated for this ratio because no nonparental vc types were observed.

[h] The occurrence of only one to two progeny in nonparental vc groups was considered to be the result of mutation to *vic* genes (28), and were ignored in this analysis.

Two hypovirus-infected isolates, EP43 (ATCC 38767) and NB58 (ATCC 76221), were used as sources of hypoviruses in this study. Isolate EP43 is a predominately white strain of *C. parasitica* with a French hypovirus (12); we designated this hypovirus as CHV1-EP43 because of its origin and similarities in phenotypic effects to other CHV1 viruses (16). Isolate NB58, which has dark orange-brown cultural morphology, was isolated from New Jersey and is infected with hypovirus CHV2-NB58 (16).

VC tests. VC tests were performed as described previously (22). We followed the method of Anagnostakis (4), with the modification of adding 25 drops of red food coloring to each liter of PDA to improve visualization of barrage lines. Two small cubes of agar, about 3 mm on each side, were cut from the margins of 4- to 6-day-old colonies and placed next to each other on PDA. Plates were incubated at 25°C in the dark for 6 to 7 days before scoring. The presence of a barrage was interpreted as evidence of incompatibility. Tests were performed at least three times for each pair of isolates.

Estimation of hypovirus transmission. Transmission tests were performed as described by Anagnostakis and Day (5) with the modification that cellophane was not used on the surface of the agar, and biotin and methionine were not added in the medium. Eight isolates, each in a different vc group, were randomly selected from the 58 isolates and infected separately with each of the hypoviruses CHV1-EP43 and CHV2-NB58. Transmission of hypoviruses into the eight isolates was first attempted directly from EP43 and NB58. For some isolates, hypoviruses were transferred to other isolates in our culture collection to find a successful pathway for transmission. The resulting 16 virus-infected isolates were used as donor strains and paired with the 58 isolates in the original sample (recipient strains) to test for hypovirus transmission. Morphological changes of the recipient isolates, similar to cultural characteristics of the donor isolates (5), were used to confirm hypovirus transmission. Transmission tests were performed three times for each pair of isolates; transmission was considered successful for each pair of isolates if hypoviruses were transmitted in at least two of the three replicates.

Time taken for transmission to occur was only analyzed for CHV1-EP43. To estimate when hypovirus transmission occurred, a small piece of mycelium was sampled from the growing margin

TABLE 1. (*continued from preceding page*)

VC groups[a] (isolate number)		Observed vc groups among progeny			Estimated number *vic* genes different	$\chi^2_{(1)}$[b]	$\chi^2_{(2)}$[c]	$\chi^2_{(3)}$[d]
Parent 1	Parent 2	Parental 1	Parental 2	Nonparental				
D(32)	X(4)	4	2	34	>2	...	19.8	2.53
D(32)	X(9)	3	10	27	>2	...	7.35	6.10
D(32)	DELTA(48)	12	11	17	2	...	0.95	22.63
D(32)	FSG1(17)	17	10	53[e]	>2	...	9.68	5.72
D(32)	FSG1(41)	10	9	21	2	...	0.15	10.90
D(32)	FSG2(18)	8	11	21	2	...	0.55	11.70
D(32)	FSG4(37)	17	22	1[h]	1	0.65	...	...
D(32)	FSG5(13)	6	4	30	>2	...	10.20	0.40
D(32)	FSG6(1)	4	3	33	>2	...	16.95	1.30
D(32)	FSG8(45)	3	14	23	2	...	6.95	18.63
D(32)	FSG10(31)	7	9	24	2	...	1.8	5.2
D(32)	FSG12(38)	16	14	10	2	...	10.2	53.73
D(32)	FSG13(42)	24	16	0	1	1.6	...	...
F(35)	G(12)	11	6	23	2	...	2.15	9.03
F(35)	W(16)	5	6	29	>2	...	8.15	0.23
F(35)	X(10)	7	16	17	2	...	4.95	30.63
F(35)	Y(43)	20	18	2[h]	1	0.2	...	...
F(35)	BETA(8)	22	16	2[h]	1	1.0	...	...
F(51)	FSG1(17)	13	25	2[h]	1	3.7	...	...
F(2)	FSG2(18)	9	9	22	2	...	0.4	8.53
F(35)	FSG3(28)	19	21	0	1	0.1	...	...
F(35)	FSG5(13)	10	9	21	2	...	0.15	10.90
G(12)	X(9)	11	7	22	2	...	1.2	10.13
G(12)	FSG2(18)	4	3	33	>2	...	16.95	1.3
G(14)	FSG2(18)	12	6	22	2	...	2.2	12.13
P(15)	FSG2(18)	2	7	31	>2	...	13.35	2.63
W(16)	Q(6)	7	6	27	>2	...	4.95	1.30
W(16)	DELTA(48)	6	6	28	>2	...	6.40	0.53
W(16)	FSG2(18)	1	4	35	>2	...	22.95	4.23
W(16)	FSG4(37)	1	0	39	>2	...	36.15	10.90
X(9)	Y(43)	1	3	36	>2	...	25.80	5.2
X(4)	FSG2(18)	3	7	30	>2	...	10.80	1.6
X(10)	FSG2(18)	7	1	32	>2	...	16.20	4.13
X(9)	FSG3(28)	24	16	0	1	1.6	...	...
X(9)	FSG5(13)	6	10	24	2	...	2.40	6.40
Y(3)	FSG2(18)	11	13	56[e]	>2	...	12.90	1.27
BETA(8)	X(9)	7	2	31	>2	...	13.35	2.63
BETA(8)	FSG1(17)	10	6	24	2	...	2.40	6.40
BETA(8)	FSG2(18)	12	9	19	2	...	0.55	17.03
FSG1(17)	FSG2(27)	8	10	22	2	...	0.60	8.93
FSG1(17)	FSG5(13)	8	1	31	>2	...	14.55	5.03
FSG1(17)	FSG14(50)	24	15	1[h]	1	2.05	...	...
FSG2(18)	FSG3(28)	12	12	16	2	...	1.60	26.13
FSG2(18)	FSG5(13)	17	10	53[e]	>2	...	9.68	5.72
FSG2(18)	FSG6(1)	12	9	19	2	...	0.55	17.03
FSG2(18)	FSG8(19)	4	3	33	>2	...	16.95	1.30
FSG2(18)	FSG8(21)	2	3	35	>2	...	22.50	3.43
FSG2(18)	FSG9(22)	5	0	35	>2	...	13.75	5.83
FSG2(18)	FSG11(33)	3	5	32	>2	...	14.60	0.93

of the recipient colony each day for 7 days and transferred to a new PDA plate. Cultural morphology of each subculture was compared to a virus-free recipient isolate of the same culture age to determine the presence of hypoviruses.

Estimation of number of *vic* genes different between vc groups. The numbers of *vic* genes different between donor and recipient isolates were estimated by crossing the eight donor isolates with the recipient isolates and determining the proportion of ascospore progeny that were vegetatively compatible with either parent. Two of the donor isolates with opposite mating types (MD18 and MD32) were crossed with all recipient isolates to determine their mating types (22); subsequent crosses with the other six donor isolates were made only with recipient isolates of compatible mating types. Crosses were made on autoclaved, dormant chestnut-stem pieces (4), one per petri plate, in sterile water agar (2%) amended with methionine (100 mg/liter); 0.5 ml of biotin (0.4 mg/ml) was applied on each stem before it was inoculated with the recipient isolate. Plates were incubated at room temperature for 2 weeks. To effect fertilization, a suspension of conidia from a compatible donor isolate was distributed over the stem. Plates were then incubated for 6 to 10 weeks at 18°C.

The number of *vic* genes different between vc groups was not estimated for all pairs of vc groups, because of mating type incompatibility and the large sample size used in this study. Ascospores were first sampled from most (44 out of 57) of the successful crosses between isolates MD18 or MD32 and the recipient isolates; fewer plates were sampled from crosses between the other six donor isolates and the recipient isolates. For each donor isolate, we randomly sampled crosses with recipient isolates into which viruses were successfully transmitted, and other crosses with those recipients for which virus transmission did not occur. Two perithecia were sampled from each of the crosses analyzed, with 40 single ascospores sampled per perithecium. A total of 87 crosses were analyzed.

The ratio of parental and nonparental vc types among progeny was used to determine the number of *vic* genes different between isolates. Forty ascospore progeny (20 from each perithecium) were paired with each of the parents for vc testing. Parental vc types were determined to be different by one *vic* gene when progeny segregated 1:1 for the parental vc types, and no nonparental vc types were found. When progeny segregated in a ratio of 1:1:2 for the two parental and nonparental vc types, respectively, parents were considered to differ by two *vic* genes. A greater proportion of progeny with nonparental vc types was considered to indicate more than two *vic* genes different between parental vc types. Chi-square goodness-of-fit statistics were estimated for expected ratios 1:1:2 and 1:1:6, for the two-gene and greater than two-gene differences, respectively. Another 20 ascospores were

TABLE 2. Hypovirus transmission and estimated number of vegetative incompatibility (*vic*) genes different between eight isolates of *Cryphonectria parasitica* paired with 57 isolates in different vegetative compatibility (vc) groups

Recipient isolates		Hypovirus-infected isolates (donors)																					
		vc groups (isolate)																					
		A (MD7)			B (MD24)			D (MD32)			F (MD35)			W (MD16)		X (MD9)		FSG1 (MD17)			FSG2 (MD18)		
VC group	Isolate	*i*[a]	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43[b]	*i*	CHV1-EP43[b]	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58
A	MD7	0	+(1.6)[c]	+	>2	–[d]	–	2	–	–	>2	–	–	>2	–	[e]	–		+(1.3)	+	2	+(1.6)	+
	MD20		+(2.0)	+		–	–		–	–		–	–		–		–		+(1.6)	+		+(1.6)	+
	MD40		–	–		–	–		–	–		–	–		–		–		–	–		–	–
	MD44		+(1.3)	+		–	–		–	–		–	–		–		–		+(1.6)	+		+(2.6)	+
	MD49		+(1.6)	+		–	–		–	–		–	–		–		–		+(1.0)	+		+(2.0)	+
B	MD24	>2	–	–	0	+	+		–	–	1	+(2.6)	+		–	1	–		–	–	1	+(2.0)	+
C	MD46		–	(+)[f]	>2	–	–	1	–	–		–	–	>2	–		–	1	+(2.6)	–		–	–
D	MD32	2	–	–		–	–	0	+(1.6)	+	1	–	–	>2	–	>2	–		–	–	2	–	+
F	MD2	>2	–	–	1	–	–	1	+(1.6)	+	0	+(1.3)	+	>2	–		–	1	–	–		+(1.6)	+
	MD25		–	–		–	–		+(1.6)	+		+(1.6)	+		–		–		–	–		+(1.6)	+
	MD34		–	–		–	–		+(1.3)	–		+(1.6)	+		–		–		–	–		+(2.0)	+
	MD35		–	–		–	–		+(1.6)	–		+(1.6)	+		–		–		–	–		+(1.3)	+
	MD36		–	–		–	–		+(2.0)	+		+(1.3)	+		–		–		–	–		+(1.6)	+
	MD39		–	–		–	–		+(2.0)	+		+(1.3)	+		–		–		–	–		+(1.6)	+
	MD47		–	–		–	–		+(1.6)	+		+(1.3)	+		–		–		–	–		+(1.6)	+
	MD51		–	–		–	–		+(2.0)	+		+(1.3)	+		–		–		–	–		+(1.6)	+
G	MD12	>2	–	(+)	2	(+)	–	1	+(3.0)	+	2	–	–		–		–		–	–	>2	–	–
	MD14		–	–		–	–		+(2.0)	+		+(1.6)	–		–		–		–	–		–	–
N	MD11		–	–		–	–	>2	–	–		–	–		–		–		–	–		–	–
P	MD15		+(3.0)	–		–	–		–	–		–	–		–		–		–	–	>2	–	–
Q	MD6		–	–	1	+(1.3)	+	2	–	–		+(2.6)	+		–		–		–	–		+(2.0)	+
	MD23		–	–		+(1.6)	+		–	–		+(2.6)	+		–		–		–	–		+(1.6)	+
	MD29		–	–		+(2.0)	+		–	–		+(2.6)	+		–		–		–	–		+(2.3)	+
W	MD16	>2	–	–		–	–	>2	–	–	>2	–	–	0	+(1.3)		+(2.0)		–	–	>2	–	–
X	MD4		–	–	1	–	–	>2	–	–		–	–		–	0	+(1.3)		–	–	>2	–	–
	MD9		–	–		–	–		–	–		–	–		–		+(1.6)		–	–		–	–
	MD10		–	–		–	–		–	–		–	–		–		+(1.0)		–	–		–	–

(*continued on next page*)

[a] Number of *vic* genes different between vegetative compatibility groups (estimates from Table 1).
[b] CHV2-NB58 was not tested because hypovirus morphology was not stable when isolates MD9 and MD16 were infected with this hypovirus.
[c] Plus sign indicates hypovirus transmission occurred in at least two of three replicates. Numbers in parentheses indicate the average number of days taken for transmission to occur in CHV1-EP43 only.
[d] Lack of hypovirus transmission in all replicates.
[e] A blank space indicates no genetic data available for *vic* gene differences between these isolates.
[f] Plus signs within parentheses indicate hypovirus transmission occurred in only one of three replicates.

sampled from each of the two perithecia when ratios were ambiguous. To decide between two-gene and greater than two-gene differences, we chose the model with the smaller chi-square value. We did not attempt to estimate the exact number of different *vic* genes when it was greater than two, since the statistical power would be too small because of the limited sample size.

Correlation between frequency of transmission and number of *vic* genes different. We tested for a linear trend (30) between the frequency of transmission, defined as the proportion of vc group pairs with successful hypovirus transmission, and the number of *vic* genes different between vc groups. We tested the null hypothesis that the proportion of pairs with successful hypovirus transmission between vc groups does not decrease as the number of *vic* genes different increases.

The correlation between time for transmission to occur and the number of *vic* genes different between isolates was estimated by linear regression analysis; only data from vc group pairs in which hypovirus transmission occurred within 7 days were used to estimate the correlation coefficient (r).

RESULTS

One hundred ninety-five out of a total 802 pairs of isolates (24%) had successful hypovirus transmission among two or three of the replicates. Fourteen pairs of isolates had transmission in only one of the three replicates and were not scored as successful transmissions. The estimated number of *vic* genes different between vc groups are shown in Table 1. Some pairs of isolates showed ambiguous ratios of parental and nonparental vc types (Table 1), therefore, 80 ascospore progeny were used for these crosses. Twenty-four pairs of vc groups were different by one *vic* gene, 29 pairs by two *vic* genes, and 34 pairs by more than two *vic* genes.

Hypovirus transmission was very similar for CHV1-EP43 and CHV2-NB58 (Table 2). With the same recipient and donor isolates, transmission of CHV1-EP43 occurred in 97% of the pairs of isolates in which transmission of CHV2-NB58 also occurred (Table 2). Transmission tests with CHV2-NB58 were only performed with six donor isolates because two of the eight randomly selected isolates, MD9 and MD16, did not show stable cultural morphology when infected with CHV2-NB58. Similar instability was also observed when ascospore progeny in the same vc groups as isolates MD9 and MD16 were experimentally infected with CHV2-NB58. Hypovirus transmission also occurred consistently within vc groups. If a donor transmitted the hypovirus to a recipient isolate, it usually also transmitted the hypovirus to other isolates in the same vc group (Table 2).

The frequency of transmission between isolates was inversely proportional to the number of *vic* genes that were different for both hypoviruses CHV1-EP43 and CHV2-NB58 (Fig. 1).

TABLE 2. (*continued from preceding page*)

Recipient isolates		Hypovirus-infected isolates (donors)																					
		vc groups (isolate)																					
		A (MD7)			B (MD24)			D (MD32)			F (MD35)			W (MD16)	X (MD9)		FSG1 (MD17)			FSG2 (MD18)		
VC group	Isolate	*i*[a]	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43[b]	*i*	CHV1-EP43[b]	*i*	CHV1-EP43	CHV2-NB58	*i*	CHV1-EP43	CHV2-NB58
Y	MD3		+(1.6)	+		–	–		–	–	1	–	–		–	>2	–		+(2.0)	+	>2	–	–
	MD43		+(1.3)	+		–	–		–	–		–	–		–		–		+(1.6)	+		–	–
β	MD8	2	–	–		–	–		–	(+)	1	+(2.3)	+		–	>2	–	2	–	–	2	+(1.6)	+
δ	MD48	1	+(2.0)	+		–	–	2	–	–		–	–		–		–		+(2)	+		–	–
FSG1	MD17		+(1.0)	+		–	–		–	–	1	–	–		–		–	0	+(1.3)	+	2	–	–
	MD41		+(1.6)	+		–	–		–	–		–	–		–		–		+(1.3)	+		–	–
FSG2	MD18	2	(+)	–	1	–	–	2	–	–		+(1.6)	+	>2	–	>2	–	2	–	–	0	+(2.0)	+
	MD27		–	–		–	–		–	(+)		+(1.6)	+		–		–		–	–		+(1.6)	+
	MD53		–	–		–	–		–	–		+(1.3)	+		–		–		–	–		+(2.0)	+
FSG3	MD28		–	–		–	–		–	–	1	–	–		–	1	–		–	–	2	–	–
	MD58		–	–		–	–		–	–		(+)	–		–		(+)		–	–		–	–
FSG4	MD37		+(2.0)	–	2	–	–	1	–	–		–	–	>2	–		–		+(2.0)	+		–	–
FSG5	MD13	>2	–	–		–	(+)	>2	+(2.3)	+	2	+(2.0)	–		–		–	>2	–	–	>2	–	–
FSG6	MD1		+(2.0)	–		–	–	>2	–	–		–	–		–		–		–	–	2	–	–
FSG7	MD5		–	–	1	+(2.6)	+		–	–		+(3.0)	–		–		–		–	–		+(3.0)	+
	MD30		+(3.0)	–		+(2.3)	+		–	–		+(2.3)	+		–		–		–	–		+(2.3)	+
	MD57		–	–		+(1.6)	+		–	–		+(2.6)	+		–		+(3.0)		–	–		+(2.0)	+
FSG8	MD19		+(2.0)	–	2	–	–		–	–		–	–		–		–		+(2.6)	+	>2	–	–
	MD21		–	+		–	–		–	–		–	–		–		–		+(2.0)	+		–	–
	MD45		–	–		(+)	–		–	–		–	–		–		–		+(2.0)	+		–	–
FSG9	MD22	1	+(3.3)	+		–	–		–	–		–	–		–		–		–	+	>2	–	–
FSG10	MD31		–	–		–	–	2	–	–		–	–		–		–		–	–		–	–
FSG11	MD33		–	–		–	–		–	–		–	–		–		–		–	–	>2	–	–
	MD56		–	–		–	–		–	–		–	–		–		–		–	–		–	–
FSG12	MD38		–	+		–	–		–	(+)		–	–		–		–		+(2.0)	–		–	–
FSG13	MD42					–	–	1	–	–		+(2.0)	+		–		–		–	–		+(2.0)	+
FSG14	MD50	1	+(1.5)	+		–	(+)		–	–		–	–		–		–	1	+(2.0)	+		–	(+)
FSG15	MD52		–	–		+(2.0)	+		–	–		+(2.0)	+		–		–		–	–		+(2.0)	+
FSG16	MD54		–	–		–	–		–	–		–	–		–		+(2.0)		–	–		–	–
FSG17	MD55		–	(+)		–	–		–	–		–	–		–		–		–	–		–	–

Transmission occurred in all pairs of isolates that were vegetatively compatible (zero *vic* genes different). The frequencies of transmission between vc groups that differed by one and two *vic* genes were 0.50 and 0.13 with hypovirus CHV1-EP43, and 0.48 and 0.14 with hypovirus CHV2-NB58, respectively (Fig. 1). When vc groups were different by more than two *vic* genes, transmission occurred in only one of 37 (3%) and one of 25 (4%) pairs of vc groups for CHV1-EP43 and CHV2-NB58, respectively. The null hypothesis that the frequency of hypovirus transmission between vc groups does not decrease with the increasing number of *vic* genes different between them was rejected for both CHV1-EP43 and CHV2-NB58 ($P < 0.001$). The time for transmission to occur was slightly greater between different vc groups than within vc groups. There was only a weak correlation ($r = 0.40$, $P = 0.05$) between transmission time of CHV1-EP43 and the number of *vic* genes different between vc groups (Fig. 2).

Unidirectional transmission of hypoviruses, as described by Huber and Fulbright (18), was observed between six pairs of vc groups in this study (Table 2). For both CHV1-EP43 and CHV2-NB58, MD24 (in vc group B) failed to transmit hypoviruses to any of the recipient isolates in vc groups F and FSG2, but did transmit successfully to three other vc groups (Q, FSG7, and FSG15). In contrast, hypoviruses were transmitted from donor isolates in vc groups F and FSG2 into MD24 (Table 2). Isolates MD18 in vc group FSG2 and MD32 in vc group D transmitted hypoviruses to isolates in vc groups A and F, respectively. However, transmission did not occur when isolates in vc groups A and F functioned as the donor isolates (Table 2). Similar results were observed between vc groups W and X, for which data were available on transmission of CHV1-EP43 only. While isolate MD16 in vc group W could receive CHV1-EP43 hypovirus from isolate MD9 in vc group X, none of the three isolates in vc group X, including isolate MD9, received CHV1-EP43 when isolate MD16 acted as the donor isolate (Table 2).

DISCUSSION

This study showed that the frequency of hypovirus transmission with CHV1-EP43 and CHV2-NB58 decreased with an increasing number of *vic* genes different between vc groups of donor and recipient strains. When vc groups differed by more than two *vic* genes, a very low level of transmission (3 to 4%) occurred. Although we did not distinguish between pairs different by three, four, or more *vic* genes, virus transmission was too rare to warrant more detailed genetic analysis. Similar results have been observed in other fungi in which transfer of cytoplasmic factors was more frequent between isolates that differed by fewer *vic* (or *het*) genes (8,9). In contrast to results reported by Anagnostakis and Day (5), that transmission occurred more slowly among incompatible isolates, we found only a weak correlation between time taken for transmission to occur and the number of *vic* genes different (Fig. 2).

Although previous studies using laboratory strains showed that hypovirus transmission always occurred between compatible isolates and less frequently when isolates were incompatible (5,7), the transmission dynamics of hypoviruses in natural populations of *C. parasitica* remained unknown. The correlation estimated in this study was based on a large sample of isolates ($N = 58$), representing 31 vc groups from a single population. With multiple

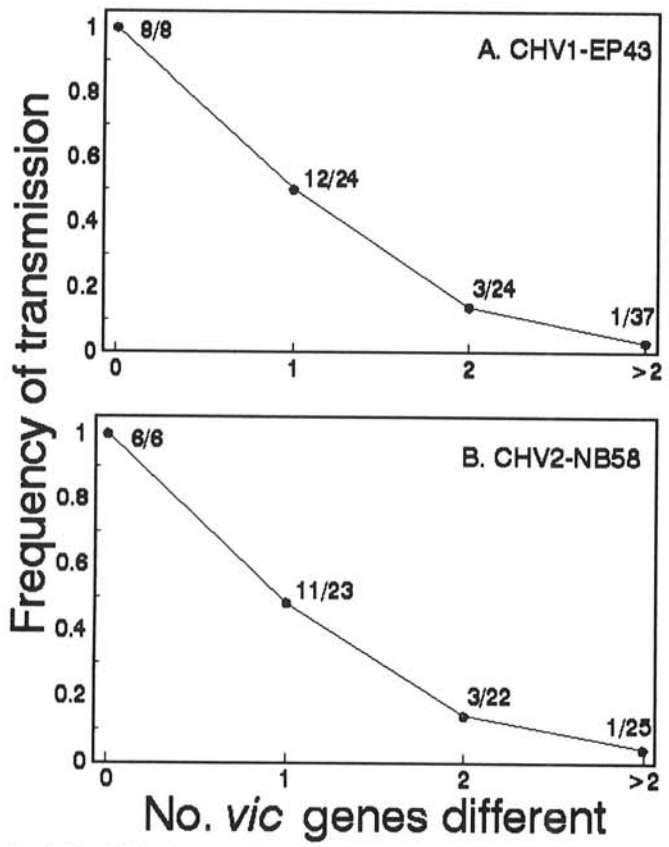

Fig. 1. Correlation between the frequency of hypovirus transmission and the number of vegetative incompatibility (*vic*) genes different between vegetative compatibility (vc) groups of *Cryphonectria parasitica* sampled from a natural population. **A,** CHV1-EP43 and **B,** CHV2-NB58. The value beside each point represents the number of hypovirus transmissions over the total number of pairs of vc groups tested.

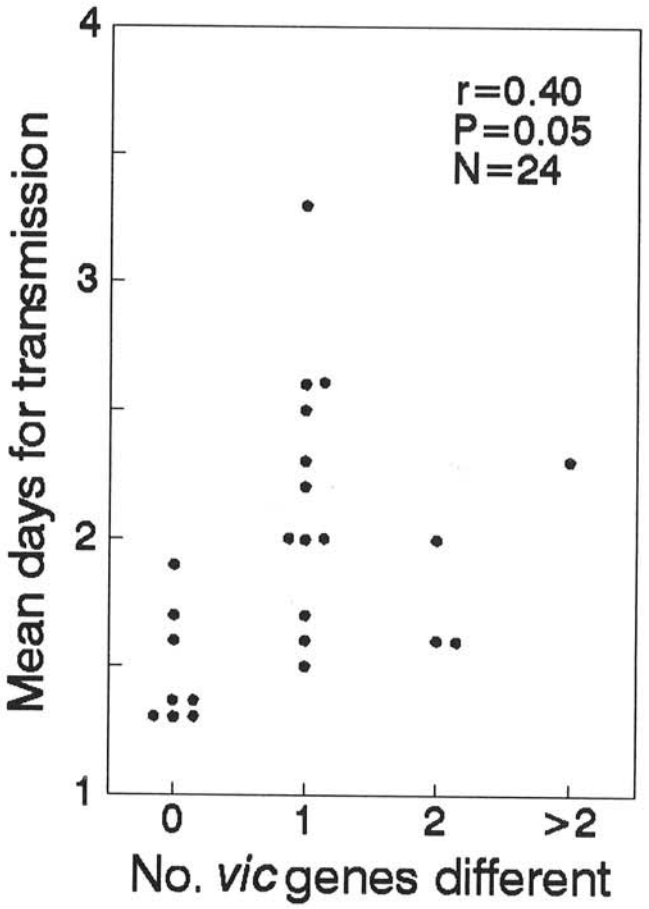

Fig. 2. Relationship between time for hypovirus transmission to occur and the number of vegetative incompatibility (*vic*) genes different between vegetative compatibility (vc) groups. Correlation coefficient (r) was estimated using data from pairs of vc groups in which hypovirus transmission occurred within 7 days. Data from 12, 20, and 35 pairs of vc groups which differed by one, two, and greater than two *vic* genes, respectively, are not shown because hypovirus transmission was not observed within 7 days.

Transmission occurred in all pairs of isolates that were vegetatively compatible (zero *vic* genes different). The frequencies of transmission between vc groups that differed by one and two *vic* genes were 0.50 and 0.13 with hypovirus CHV1-EP43, and 0.48 and 0.14 with hypovirus CHV2-NB58, respectively (Fig. 1). When vc groups were different by more than two *vic* genes, transmission occurred in only one of 37 (3%) and one of 25 (4%) pairs of vc groups for CHV1-EP43 and CHV2-NB58, respectively. The null hypothesis that the frequency of hypovirus transmission between vc groups does not decrease with the increasing number of *vic* genes different between them was rejected for both CHV1-EP43 and CHV2-NB58 ($P < 0.001$). The time for transmission to occur was slightly greater between different vc groups than within vc groups. There was only a weak correlation ($r = 0.40$, $P = 0.05$) between transmission time of CHV1-EP43 and the number of *vic* genes different between vc groups (Fig. 2).

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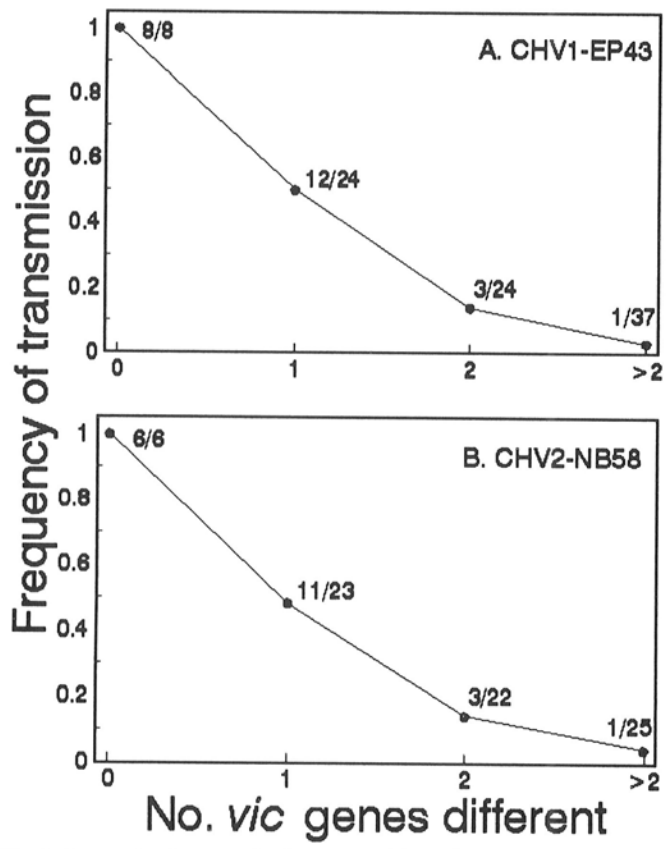

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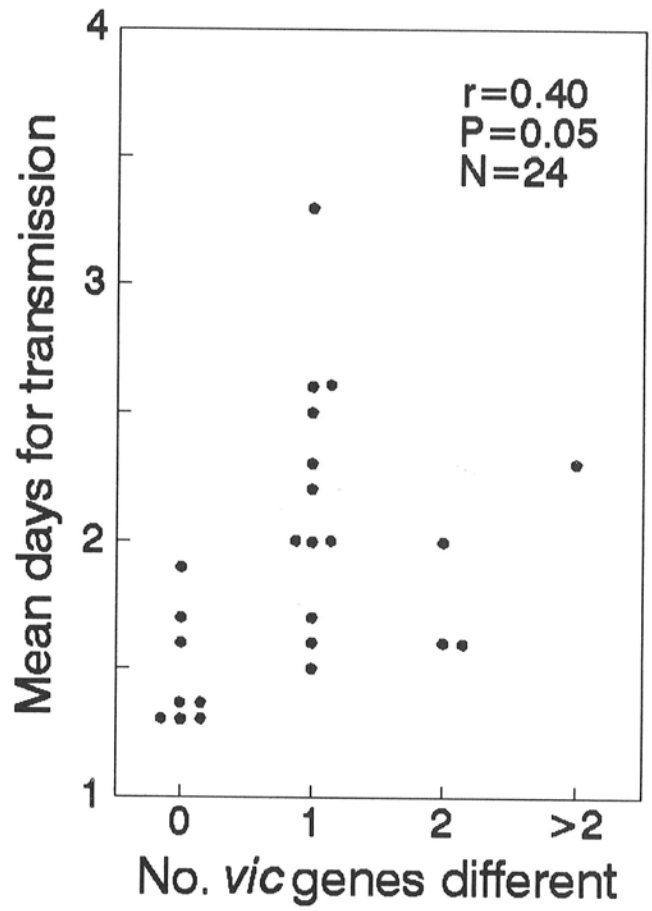

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isolates within some vc groups, replicated transmission tests were made for some pairs of vc groups. The results showed that, with the same donor isolates, hypovirus transmission was usually consistent among multiple recipient isolates in the same vc group. A previous study of the genetic structure of this population showed that individuals within vc groups were not closely related for DNA fingerprints (22). The use of genetically diverse isolates in the same vc group reinforced the hypothesis that differences in *vic* genes affected hypovirus transmission; other genes would be expected to be randomized to vc groups by recombination unless they are tightly linked to *vic* loci.

Unidirectional transmission of hypoviruses was found in six pairs of vc groups in this study. A similar phenomenon has been found with laboratory strains and is controlled by specific *vic* alleles (18). Whether the unidirectional transmission of hypoviruses was governed by the same *vic* genes as described by Huber and Fulbright (18) is unknown. The observation of unidirectional transmission of hypovirus in our study suggested that these or similar genes occurred in natural populations of *C. parasitica.* However, the frequencies of genes affecting unidirectional or bidirectional transmission were not clear since relatively few pairings were made for testing unidirectional transmission, and genotypes of vc groups are unknown except for a few laboratory strains (7,18).

It is not clear why hypovirus transmission occurred between some vc groups but not others, even though they were different by the same number of *vic* genes. One explanation is that individual *vic* genes have different effects on virus transmission (18). Hypoviruses may be transmitted frequently between vegetatively compatible isolates, as well as between isolates that differ at only one *vic* locus. Unidirectional transmission could be another explanation for this phenomenon. We did not attempt to analyze the effects of individual *vic* genes on hypovirus transmission between specific isolates; instead, the average effect of *vic* genes that were different among individuals in a population was estimated. At the population level, the average frequency of hypovirus transmission was correlated to genetic differences between vc groups, regardless of the effects of individual *vic* genes. However, this correlation may be different in other populations because of differences in *vic* allele frequencies.

The successful establishment of hypoviruses in Europe and the limited spread in the United States may be explained, in part, by the correlation between transmission of hypoviruses and number of *vic* genes different between isolates. Diversity of vc groups is usually low in the European *C. parasitica* populations in which very few vc groups are found (6,15,22). However, genetic relatedness among vc groups in these populations is not known. If a few vc groups are closely related, with alleles differing at only one or two *vic* loci, the introduction of a hypovirus into one vc group may result in a relatively high frequency of transmission to other vc groups. In contrast, vc group diversity is generally high in United States populations (6,22,24). Although hypoviruses can be transmitted between some incompatible isolates, there is a much smaller probability that transmission will occur in more diverse populations because many isolates are likely to differ by more than two *vic* genes. Based on what we know about the distribution (22) and genetic relatedness of vc groups in Finzel, MD, the average probability of virus transmission between any two randomly chosen individuals in this population is estimated to be 0.18. However, this estimate is biased because the crosses analyzed were not a random sample of all possible pairs of vc groups in this population. Although virus transmission occurred between similar proportions of vc group pairs in those sampled for genetic analyses as in the overall sample, the distribution of number of *vic* genes different is biased because of nonrandom sampling.

The results of this study represent the qualitative relationship between hypovirus transmission and the genetic relatedness between vc groups. However, it should not be used as a quantitative prediction in the field because hypovirus transmission in the field may be different from what was observed in the laboratory. Double (11) showed that hypovirus transmission between vegetatively incompatible isolates occurred more frequently in field inoculations. Canker expansion was reduced by hypovirulence when chestnut trees were inoculated with pairs of virulent and hypovirulent isolates that were vegetatively incompatible, even though hypovirus transmission was not observed with these same isolates in vitro (11).

This study suggests that the establishment of biocontrol with hypoviruses may depend on genetic relatedness among vc groups, which is a function of the number of polymorphic *vic* loci and their allele frequencies in the population. When populations contain high diversities of vc groups, transmission of hypoviruses may be limited by genetic differences among vc groups. Hypoviruses probably can, eventually, spread into all vc groups in a population through a network of different vc groups, as hypothesized by Anagnostakis (3) and Kuhlman et al. (20). However, the transmission of hypoviruses in *C. parasitica* populations may also depend on the dispersal of spores between cankers and the reproductive biology of the fungus (22,23,26).

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