Genetic Control of Horizontal Virus Transmission in the Chestnut Blight Fungus, *Cryphonectria parasitica*

Paolo Cortesi,* Charles E. McCulloch,[†,1] Haiyue Song,[†] Haiqun Lin[†,2] and Michael G. Milgroom[‡]

**Istituto di Patologia Vegetale, Università degli Studi di Milano, 20133 Milan, Italy, [†]Departments of Statistical Science and Biometrics, Cornell University, Ithaca, New York 14853 and [‡]Department of Plant Pathology, Cornell University, Ithaca, New York 14853*

Manuscript received March 4, 2001
Accepted for publication June 21, 2001

ABSTRACT

Vegetative incompatibility in fungi has long been known to reduce the transmission of viruses between individuals, but the barrier to transmission is incomplete. In replicated laboratory assays, we showed conclusively that the transmission of viruses between individuals of the chestnut blight fungus *Cryphonectria parasitica* is controlled primarily by vegetative incompatibility (*vic*) genes. By replicating *vic* genotypes in independent fungal isolates, we quantified the effect of heteroallelism at each of six *vic* loci on virus transmission. Transmission occurs with 100% frequency when donor and recipient isolates have the same *vic* genotypes, but heteroallelism at one or more *vic* loci generally reduces virus transmission. Transmission was variable among single heteroallelic loci. At the extremes, heteroallelism at *vic4* had no effect on virus transmission, but transmission occurred in only 21% of pairings that were heteroallelic at *vic2*. Intermediate frequencies of transmission were observed when *vic3* and *vic6* were heteroallelic (76 and 32%, respectively). When *vic1*, *vic2*, and *vic7* were heteroallelic, the frequency of transmission depended on which alleles were present in the donor and the recipient. The effect of heteroallelism at two *vic* loci was mostly additive, although small but statistically significant interactions (epistasis) were observed in four pairs of *vic* loci. A logistic regression model was developed to predict the probability of virus transmission between *vic* genotypes. Heteroallelism at *vic* loci, asymmetry, and epistasis were the dominant factors controlling transmission, but host genetic background also was statistically significant, indicating that *vic* genes alone cannot explain all the variation in virus transmission. Predictions from the logistic regression model were highly correlated to independent transmission tests with field isolates. Our model can be used to estimate horizontal transmission rates as a function of host genetics in natural populations of *C. parasitica*.

THE transmission of pathogens between host individuals is a key factor that affects the invasion of pathogens in host populations (Anderson and May 1986) and the evolution of virulence (Levin 1996; Lipsitch and Moxon 1997). Transmission is often heterogeneous, resulting in diverse epidemiological dynamics because of factors like variation in susceptibility, spatial or behavioral isolation, or a combination of these factors (Read *et al.* 1995). In filamentous fungi, horizontal transmission is markedly heterogeneous because it depends on the genetics of the interacting host individuals. Infectious agents or other extranuclear genetic elements (*e.g.*, viruses, plasmids, and dysfunctional mitochondria) can be transmitted between fungal individuals after contact and cell fusion (hyphal anastomosis; Caten 1972; Collins and Saville 1990; Griffiths *et al.* 1990; Hoekstra 1996; van Diepeningen *et al.* 1997; van der Gaag *et al.* 1998). However, the persistence of hyphal anastomoses is controlled by genes at multiple vegetative incompatibility (*vic*) loci (reviewed recently in Glass *et al.* 2000; Saupe 2000; Saupe *et al.* 2000). Individuals are vegetatively compatible and can form stable heterokaryons only if they share the same alleles at all *vic* loci [also called *het* (heterokaryon incompatibility) loci in some species]. Heterokaryons formed by anastomosis between incompatible individuals, *i.e.*, with different alleles (heteroallelic) at any *vic* locus, are transient, and the fused cell eventually dies. Cell death prevents heterokaryon formation and often restricts the transmission of pathogens from the cytoplasm of one individual to another, analogous to the way the localized cell death in plants (hypersensitive response) prevents the invasion of pathogens (Hammond-Kosack and Jones 2000). However, vegetative incompatibility is an imperfect ("leaky") barrier in fungi, and there is ample anecdotal evidence of transmission of various genetic elements between incompatible individuals (Anagnostakis and Day 1979; Anagnostakis and Waggoner 1981; Anagnostakis 1983; Brasier 1984; Coenen *et al.* 1994, 1997; Debets *et al.* 1994; Huber 1996; Liu and Milgroom 1996; Poplawski *et al.* 1997; He *et al.* 1998; Baidyaroy *et al.* 2000). From an epidemiological per-

Corresponding author: Michael G. Milgroom, Department of Plant Pathology, Cornell University, 334 Plant Science Bldg., Ithaca, NY 14853-4203. E-mail: mgm5@cornell.edu

[1] *Present address:* Division of Biostatistics, Department of Epidemiology and Biostatistics, University of California, San Francisco, CA 94143.

[2] *Present address:* Division of Biostatistics, Yale University School of Medicine, New Haven, CT 06520.

spective, predicting the invasion of pathogens in a population requires an understanding of the genetic control of transmission affecting the variation in horizontal transmission rates among different individuals. For fungal populations, therefore, we would need to quantify the effects of *vic* genes on the probability of transmission. To our knowledge, this type of transmission probability has not been estimated rigorously in previous studies.

The role of fungal vegetative incompatibility in the horizontal transmission of pathogens has been studied most extensively in the ascomycete chestnut blight fungus, *Cryphonectria parasitica*. This system has attracted attention because of the potential for biological control of chestnut blight by viruses in the family Hypoviridae (Van Alfen *et al.* 1975; Anagnostakis 1982; MacDonald and Fulbright 1991; Nuss 1992; Hillman *et al.* 2000). Early studies showed that hypoviruses are transmitted without restriction between individuals with the same vegetative compatibility (vc) type (the phenotype conferred by a multilocus *vic* genotype) and with high probability between incompatible individuals heteroallelic at single *vic* loci (Anagnostakis and Day 1979; Anagnostakis and Waggoner 1981; Anagnostakis 1983). Later studies extended these observations in two ways. First, Huber and Fulbright (1994) and Huber (1996) investigated the genetics of virus transmission on a small set of strains with known *vic* genotypes. They found that heteroallelism at some *vic* loci strongly inhibits virus transmission, while no inhibition is apparent at others. Interestingly, the effects of *vic* genes on the horizontal transmission of a plasmid in the same strains of *C. parasitica* are very similar to those on viruses (Baidyaroy *et al.* 2000). Second, taking a complementary population-oriented approach, Liu and Milgroom (1996) analyzed a large number of field isolates and showed that, on average, transmission between vc types decreased as the number of heteroallelic *vic* loci increased. Both groups (Huber and Fulbright 1994; Huber 1996; Liu and Milgroom 1996) demonstrated that virus transmission can be markedly asymmetrical; *i.e.*, the frequency of transmission within some pairs of *vic* genotypes depends on which isolate is the donor and which is the recipient.

Although horizontal transmission in *C. parasitica* is among the best-studied examples among fungi, our understanding of the genetics of this process is incomplete. Previous studies (Huber and Fulbright 1994; Huber 1996; Baidyaroy *et al.* 2000) on the effects of specific *vic* genes on virus (and plasmid) transmission are based on a small set of laboratory isolates derived from a few crosses. These studies had few or no replications with independent isolates of the same *vic* genotypes; therefore, effects ascribed to *vic* genes may be confounded with the effects of other genes in the particular isolates studied, *i.e.*, genetic background effects. Testing of independent isolates is needed to determine conclusively whether *vic* genes are the primary determinants of virus transmission and to estimate the probabilities of transmission among the different *vic* genotypes. Furthermore, two *vic* loci, *vic6* and *vic7*, were recently identified in *C. parasitica* (Cortesi and Milgroom 1998) and have not yet been studied with respect to virus transmission.

The overall goal of this research was to comprehensively analyze the effects of *vic* genes on virus transmission in *C. parasitica*. We had five specific objectives: (1) to estimate the effect of heteroallelism at each of six *vic* loci on the probability of transmission; (2) to test for asymmetric transmission associated with each *vic* locus; (3) to test for independence of *vic* loci with respect to virus transmission; (4) to determine whether variation in virus transmission is associated with the genetic background of host isolates, independent of *vic* genes; and (5) to model the probability of virus transmission between *vic* genotypes found in field populations. We approached these objectives in an integrated manner through laboratory testing of virus transmission and the development of a logistic regression model to estimate the probability of transmission among *vic* genotypes. We compared model predictions, based on results from laboratory strains, with virus transmission observed between isolates randomly sampled from field populations.

MATERIALS AND METHODS

The genetics of vegetative incompatibility in *C. parasitica* were described recently for all vc types found in Italy (Cortesi and Milgroom 1998) and the majority of vc types from three populations in the eastern United States (Milgroom and Cortesi 1999). Six polymorphic *vic* loci, each with two alleles, have been identified in Europe (Cortesi and Milgroom 1998), although additional polymorphic *vic* loci are likely to exist in Europe (Robin *et al.* 2000) and in Asia (Y.-C. Liu and M. G. Milgroom, unpublished data). We use the nomenclature for *vic* genotypes described by Cortesi and Milgroom (1998), where the full genotype is abbreviated by the allele numbers only, *1* and *2* (Table 1). For example, the genotype *vic1-2, vic2-2, vic3-1, vic4-2, vic6-1, vic7-2* for six *vic* loci is denoted simply as *2212-12*. No allele is designated for locus *vic5* (shown as - in the genotype abbreviation), because polymorphism at this locus cannot be detected using our assay, and *vic5* has no detectable effect on transmission (Huber 1996; Baidyaroy *et al.* 2000). Heteroallelism at three *vic* loci has been shown to prevent heterokaryon formation in *C. parasitica* (Huber 1996).

Virus transmission assays: Virus transmission was assayed by growing donor and recipient isolates together on solid medium as described previously (Liu and Milgroom 1996). Assays were performed in petri plates containing a modified potato dextrose agar [1 liter contains 24 g potato dextrose broth (Difco, Detroit), 20 g agar no. 1 (Oxoid, Basingstoke, Hampshire, UK), 2 g yeast extract, 7 g malt extract (Difco), and 0.8 g tannic acid (Fluka Chemie, Buchs, Switzerland)]. A mycelial plug from a virus-infected donor was placed on the medium adjacent to a mycelial plug of a virus-free donor and incubated for 7 days at 24° in the dark. Host isolates infected with Cryphonectria *hypovirus 1* (CHV-1) typically produce mycelium with markedly less pigmentation when grown

under low light (Hillman *et al.* 1990). Virus transmission from donor to recipient isolates is detected by the growth of mycelium with less pigmentation in the recipient, while lack of transmission is evident when the recipient isolate maintains an orange-pigmented colony typical of virus-free isolates (Figure 1).

Initially, two CHV-1-infected isolates were used as sources of viruses in this study. E13 was isolated from Valesone (Domodossola, Italy) in 1976 (Bisiach *et al.* 1988); TE9 was isolated from Teano, Italy (Cortesi *et al.* 1996). On the basis of previous studies comparing different hypovirus species (Huber 1996; Liu and Milgroom 1996) and preliminary observations (results not shown), we assumed that virus transmission was not affected by virus strains. Viruses were transmitted into virus-free laboratory isolates to create a set of virus-infected isolates to be used as donors. Transmission was not always possible directly from the original source isolates to every laboratory isolate. In some cases we transmitted viruses into other laboratory isolates first and then from these isolates to other donor isolates (Anagnostakis 1983; Peever *et al.* 2000). Most isolates were single ascospore cultures (Cortesi and Milgroom 1998), which we assumed were virus free because of pigmented phenotypes and because CHV-1 is not transmitted into ascospores (Elliston 1985; Anagnostakis 1988). In a few cases we used field isolates (Cortesi *et al.* 1996).

Effect of heteroallelism at each *vic* locus: For each *vic* locus, we assayed virus transmission between at least three pairs of *vic* genotypes, with each pair heteroallelic only at the *vic* locus being tested. With few exceptions, at least 15 independent trials (1 per petri dish) were performed for each pair of isolates, although many tests had 20 or more (Table 1). To provide independent replication of the same *vic* genotypes, assays were repeated using isolates derived from different crosses or field isolates from different populations.

Asymmetric virus transmission: For most pairs of isolates used for estimating the effects of *vic* alleles on transmission, each isolate was used as both a donor and recipient (reciprocal transmission) to determine whether transmission was the same in both directions.

Interactions between *vic* loci: To test for independent effects at different *vic* loci, we assayed virus transmission between isolates heteroallelic at two loci; higher-order interactions were not investigated. For each two-locus combination, we paired *vic* genotypes to account for all possible two-gene differences. Donors with two-locus genotypes *11*, *12*, *21*, and *22* (at the two *vic* loci being studied) were paired with recipients with two-locus genotypes *22*, *21*, *12*, and *11*, respectively (all other *vic* alleles were held constant between donors and recipients in any given pairing). For each two-locus combination (with the exception of those involving *vic4*) we replicated at least three sets of *vic* genotypes for each two-allele pairing (Table 1). Fewer combinations were tested for *vic4* because this locus had little effect on virus transmission (Huber 1996; Huber and Fulbright 1994).

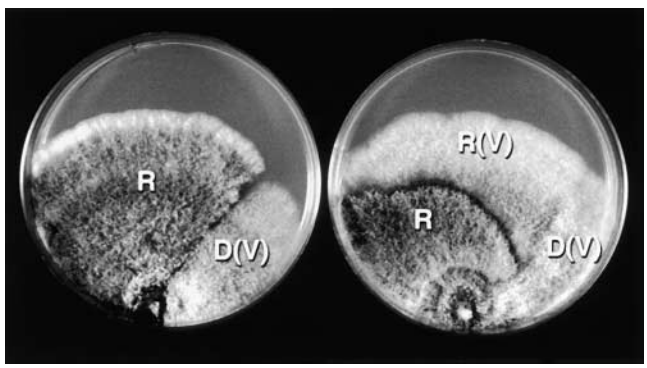

Figure 1.—Virus transmission assays in *Cryphonectria parasitica*. Virus-infected donors, D(V), and virus-free recipients, R, are co-cultured on solid medium in petri plates. Lack of virus transmission is evident in the plate on the left from the uniformly pigmented mycelium in the recipient colony. In contrast, successful virus transmission is evident in the plate on the right because the virus-infected recipient, R(V), produces mycelium with less pigmentation after transmission.

Logistic regression model: A common model for describing the presence or absence of a trait is the logistic regression model (Hosmer and Lemeshow 1989). Logistic regression posits a nonlinear model for the probability of the trait and can flexibly incorporate categorical or continuous predictors. We thus model the probability of virus transmission between a pair of isolates as a function of the heteroallelic *vic* genes, *i.e.*, as fixed effects. In addition, different isolates of the fungus were used, which may differ with regard to genes other than at the six *vic* loci; this is defined as the genetic background effect and was modeled as being selected from a distribution, *i.e.*, as random effects.

Let p_{ij} be the probability of transmission from donor isolate i to recipient isolate j. Our logistic regression model is

$$\log[p_{ij}/(1 - p_{ij})] = \mu + \sum_k \beta_k \mathrm{HTA}_{ijk} + \sum_k \gamma_k \mathrm{ASY}_{ijk} + \sum_k \sum_l \theta_{kl} \mathrm{EPI}_{ijkl} + \mathrm{donor}_i + \mathrm{recip}_j, \quad (1)$$

where $\mathrm{HTA}_{ijk} = 1$ if alleles in isolates i and j are heteroallelic at *vic* locus k and 0 otherwise; $\mathrm{ASY}_{ijk} = -\frac{1}{2}$ if locus k is heteroallelic for isolates i and j with allele *1* in the recipient, $\frac{1}{2}$ if locus k is heteroallelic with allele *2* in the recipient, and 0 if alleles at locus k are the same; and EPI_{ijkl} is an epistasis (or interaction) indicator variable that is equal to 1 if the pairing of isolates i and j is heteroallelic at both loci k and l and is 0 otherwise. The interpretation of the parameters is as follows: μ is the intercept, β_k is the effect of heteroallelism for locus k, θ_{kl} is the effect of epistasis (interaction) between heteroallelic loci k and l, and γ_k is the effect of asymmetry for locus k (*i.e.*, the difference between heteroallelism with a recipient with allele *1* and a recipient with allele *2*). The rationale for using $\frac{1}{2}$ and $-\frac{1}{2}$ for asymmetry indicator variables is to estimate the average effect of heteroallelism at a *vic* locus, with the effects of different alleles in the recipients canceling out, and, at the same time, the differences between alleles can be estimated. In other words, the average effect (on the logit scale) for the kth locus is averaged over the two possible alleles in the recipients as

$$[(\mu + \beta_k + \gamma_k/2) + (\mu + \beta_k - \gamma_k/2)]/2 = \mu + \beta_k,$$

while the difference between recipients with allele *1* and allele *2* is

$$(\mu + \beta_k + \gamma_k/2) - (\mu + \beta_k - \gamma_k/2) = \gamma_k.$$

The donor and recipient effects are random factors and we model them as selected from normal distributions:

$$\mathrm{donor}_i \sim \mathrm{Normal}(0, \sigma_D^2)$$
$$\mathrm{recip}_j \sim \mathrm{Normal}(0, \sigma_R^2). \quad (2)$$

This parameterization allows for estimation of a correlation among responses measured on the same isolate used as a donor or recipient.

Estimation was performed by maximum likelihood using a simulation-based maximization technique (McCulloch 1997).

Tests were performed by simulation-based likelihood-ratio tests (Geyer and Thompson 1992). All computations were programmed and performed in MATLAB (Version 5, 1999; The MathWorks, Natick, MA).

Tests of the individual coefficients and groups of coefficients were performed to assess the influence of each heteroallelic *vic* locus, asymmetry, and epistasis effects, using a likelihood-ratio test. For example, to test for epistasis, the likelihoods of the fitted model above and the model with all the θ_{kl} set to zero were compared. Because the likelihood cannot be calculated explicitly, a simulation was performed to approximate the value of the likelihood-ratio test (McCulloch 1997).

To test if there are other genes affecting transmission we tested whether there is any residual variation in the probabilities associated with the donors and recipients not captured by the heteroallelism, asymmetry, and epistasis variables. That is, if there are no other genes affecting the transmission of the virus, then all isolates with a given set of *vic* genes (fixed effects) will behave the same. This is implemented by testing H_0: $\sigma_D^2 = 0$, $\sigma_R^2 = 0$, again with a likelihood-ratio test.

Independent test of the regression model: To evaluate model predictions, virus transmission was tested empirically between field isolates sampled from populations from Italy and the United States. We used isolates from previous collections for which *vic* genotype data were available (Cortesi *et al.* 1996; Liu *et al.* 1996; Milgroom and Cortesi 1999). We sampled with replacement to form 20 pairs of isolates from each of three populations in northern Italy (Bergamo, Pigna, and Crevoladossola) and 40 pairs of isolates from Maryland. The *vic* alleles are in gametic equilibrium in the Maryland population (Milgroom and Cortesi 1999); therefore, we assume that *vic* genes are randomly associated with any genetic background effects in this population. The same assumption is only partially satisfied for the Italian populations, but they are relatively diverse with respect to *vic* genotypes and show some evidence for recombination (Milgroom and Cortesi 1999).

All field isolates were screened for CHV-1 by colony morphology and by the presence of double-stranded RNA (dsRNA) to ensure they were virus free. Virus screening was done by immunoblotting (Peever *et al.* 1997) or by miniprep for dsRNA (Morris *et al.* 1983). Virus-free isolates were obtained from virus-infected isolates by sampling single-conidial isolates when necessary.

One member of each isolate pair was randomly designated as the donor and was infected with CHV-1 as described above; the other isolate was designated as the recipient. Virus transmission tests were conducted between these isolates as described above to estimate the proportion of trials with successful virus transmission; 10 or more trials were conducted for each pair of field isolates. The observed proportion of trials with successful virus transmission was compared to model predictions on the basis of the *vic* genotypes of donors and recipients. The predicted probability of transmission between individuals i and j was calculated from the logistic regression model by back transformation of the predicted $\log[p_{ij}/(1 - p_{ij})]$ from Equation 1 for each pair of *vic* genotypes. We also compared virus transmission results from a published report by Bissegger *et al.* (1997), for which *vic* genotype data are available (Cortesi *et al.* 1998), to predictions from logistic regression.

RESULTS

Virus transmission results between laboratory isolates are shown in Table 1. Transmission occurred successfully in all 120 trials between isolates with the same *vic* genotype. Relatively few pairings were done between isolates with the same *vic* genotypes because this same result has been documented repeatedly in *C. parasitica* (Anagnostakis and Day 1979; Anagnostakis 1983; Huber and Fulbright 1994; Huber 1996; Liu and Milgroom 1996). Virus transmission was relatively consistent among pairs of genotypes heteroallelic at some *vic* loci but was highly variable among loci (Figure 2). At the extremes, heteroallelism at *vic4* had no effect on virus transmission, whereas heteroallelism at *vic2* resulted in strong inhibition of transmission (Figure 2). Intermediate transmission was observed with heteroallelism at *vic3* and *vic6*. Both *vic1* and *vic7* showed marked asymmetry in virus transmission, in which the proportion of successful transmissions depended on which allele at the heteroallelic locus was present in the donor or recipient. For example, when *vic1* was heteroallelic, transmission occurred in 98% of the trials (162 successes in 165 trials) when *vic1-1* was in the recipient isolate (and *vic1-2* in the donor) but only 8% (13/165) in the reciprocal pairing (Table 1).

Transmission between isolates heteroallelic at two *vic* loci was generally less frequent than for single-locus differences (Table 1). Asymmetric transmission was still evident with two-locus differences. For example, pairs that were heteroallelic at *vic3* and *vic7* showed strong asymmetry: low transmission (9/90, *i.e.*, 9 successes in 90 trials, pooled from six pairs of *vic* genotypes) occurred when recipients had allele *vic7-1*, but high transmission occurred (80/90) when they had *vic7-2*, regardless of alleles at *vic3*. This pattern of asymmetry is consistent with heteroallelism at single *vic* loci (Figure 2). Similarly, heteroallelism at *vic1* and *vic7*, both of which were strongly asymmetric when heteroallelic by themselves, together displayed marked asymmetry. Transmission was strongly inhibited (6/125), as in single-locus heteroallelism, whenever recipients had *vic1-2*, regardless of the allele at *vic*7. Intermediate transmission (35/60) occurred when recipients had *vic1-1* and *vic7-1*, but no inhibition (60/60) was observed when recipients had *vic1-1* and *vic7-2*, as expected from single-locus results (Figure 2).

Logistic regression: The parameter estimates for the full logistic regression model are shown in Table 2. The predicted probability of virus transmission between two isolates with the same *vic* genotypes is 0.98. Heteroallelism at almost any *vic* locus results in a decrease in this probability, as evidenced by the fact that five estimates of β_i (all but β_4) are significantly less than zero, *i.e.*, greater than two standard errors less than zero (Table 2). Because *vic4* had no effect on transmission, we estimated parameters for a reduced model in which heteroallelism at *vic4* was not considered (Table 2). Magnitudes of the parameter estimates reflect the variation in the effect of each *vic* locus on virus transmission; *e.g.*, $\beta_2 = -5.37$ while $\beta_7 = -1.49$, showing a stronger average effect of *vic2* than *vic7* (Figure 2). Significant asym-

TABLE 1

Results of virus transmission tests between isolates of *C. parasitica* with different *vic* genotypes

Heteroallelic loci[a]	Donor		Recipient		Transmission		Reciprocal transmission[d]	
	Isolate[b]	*vic* genotype[c]	Isolate	*vic* genotype	No. trials	No. successes	No. trials	No. successes
—[e]	P20-2	*1111-21*	P32-4	*1111-21*	20	20	20	20
	P16-1	*1112-11*	P19-1	*1112-11*	20	20	20	20
	P8-3	*2112-12*	P9-1	*2112-12*	20	20	20	20
vic1	P32-33	*1111-11*	VO59	*2111-11*	15	3	15	15
	P19-4	*1111-11*	P26-1	*2111-11*	40	1	40	38
	P30-9	*1111-12*	P35-3	*2111-12*	40	0	40	40
	CH18	*1111-12*	P67-8	*2111-12*	15	6	15	15
	P20-2	*1111-21*	P32-3	*2111-21*	40	0	40	39
	P32-4	*1111-21*	P67-4	*2111-21*	15	3	15	15
vic2	P1-2	*1111-22*	P1-5	*1211-22*	40	6	40	2
	LI13	*1111-22*	P38-4	*1211-22*	15	1	15	7
	P1-6	*2111-22*	P1-11	*2211-22*	40	2	40	7
	VA26	*2111-22*	VA22	*2211-22*	15	7	15	9
	P2-4	*2112-22*	P4-4	*2212-22*	40	3	40	16
	P9-11	*2112-22*	VA1	*2212-22*	15	2	15	5
vic3	P19-4	*1111-11*	P25-27	*1121-11*	40	30	40	21
	P32-33	*1111-11*	P70-7	*1121-11*	15	15	15	15
	P16-1	*1112-11*	P16-7	*1122-11*	40	29	40	27
	P19-1	*1112-11*	TE108	*1122-11*	15	15	15	13
	P26-1	*2111-11*	P25-6	*2121-11*	40	30	40	26
	VO59	*2111-11*	P33-1	*2121-11*	15	15	15	15
vic4	P25-27	*1121-11*	P16-7	*1122-11*	45	45	40	40
	P25-6	*2121-11*	P16-2	*2122-11*	40	40	40	40
	P10-18	*2211-12*	P5-2	*2212-12*	40	40	40	40
vic6	P16-1	*1112-11*	P17-2	*1112-21*	40	8	40	18
	P19-1	*1112-11*	P20-3	*1112-21*	15	11	15	15
	P9-13	*1112-12*	P1-4	*1112-22*	40	2	40	19
	P13-23	*1112-12*	P3-7	*1112-22*	15	4	15	15
	P12-39	*1212-12*	P3-3	*1212-22*	15	5	15	14
	P9-2	*1212-12*	P1-16	*1212-22*	40	5	70	3
vic7	P16-1	*1112-11*	P9-13	*1112-12*	40	40	40	17
	P19-1	*1112-11*	P13-23	*1112-12*	15	15	15	15
	P26-1	*2111-11*	P35-3	*2111-12*	40	39	60	17
	VO59	*2111-11*	P67-8	*2111-12*	15	15	15	13
	P32-3	*2111-21*	P1-6	*2111-22*	40	40	40	4
	P67-4	*2111-21*	VA26	*2111-22*	15	15	15	10
vic1, *vic2*	P1-2	*1111-22*	P1-11	*2211-22*	95	0	80	8
	P9-13	*1112-12*	P5-2	*2212-12*	80	5	80	8
	P1-4	*1112-22*	P4-4	*2212-22*	100	2	80	6
	P1-5	*1211-22*	P1-6	*2111-22*	80	5	85	2
	P9-2	*1212-12*	P8-3	*2112-12*	60	2	60	2
	P1-16	*1212-22*	P2-4	*2112-22*	80	4	59	2
vic1, *vic3*	P9-13	*1112-12*	P21-5	*2122-12*	15	0	15	14
	P17-2	*1112-21*	P21-8	*2122-21*	15	3	15	9
	P27-1	*1211-21*	P78-1	*2221-21*	15	2	14	14
	P16-7	*1122-11*	P16-6	*2112-11*	15	0	15	15
	P79-6	*1221-22*	P1-11	*2211-22*	15	0	15	14
	P45-4	*1222-21*	P17-7	*2212-21*	15	2	15	15
vic1, *vic4*	P25-27	*1121-11*	P16-2	*2122-11*	3	0	3	3
	P1-5	*1211-22*	P4-4	*2212-22*	10	1	10	10
	P1-4	*1112-22*	P1-6	*2111-22*	10	1	3	3
	P16-7	*1122-11*	P25-6	*2121-11*	3	0	3	3

(*continued*)

TABLE 1

(Continued)

Heteroallelic loci[a]	Donor		Recipient		Transmission		Reciprocal transmission[d]	
	Isolate[b]	*vic* genotype[c]	Isolate	*vic* genotype	No. trials	No. successes	No. trials	No. successes
vic1, *vic6*	P19-4	*1111-11*	P32-3	*2111-21*	20	2	20	0
	P30-9	*1111-12*	P1-6	*2111-22*	20	0	20	1
	P16-1	*1112-11*	P17-4	*2112-21*	40	2	40	1
	P29-1	*1211-12*	P1-11	*2211-22*	20	0	20	2
	P9-2	*1212-12*	P4-4	*2212-22*	20	0	20	2
	P20-2	*1111-21*	P26-1	*2111-11*	40	1	40	4
	P1-2	*1111-22*	P35-3	*2111-12*	5	0	—[f]	
	P1-4	*1112-22*	P8-3	*2112-12*	20	0	20	3
	P1-5	*1211-22*	P10-18	*2211-12*	40	1	40	6
	P1-16	*1212-22*	P5-2	*2212-12*	20	1	20	4
vic1, *vic7*	P20-2	*1111-21*	P1-6	*2111-22*	20	0	20	11
	P16-1	*1112-11*	P8-3	*2112-12*	20	0	20	15
	P17-2	*1112-21*	P2-4	*2112-22*	20	2	20	9
	P30-9	*1111-12*	P26-1	*2111-11*	20	2	20	20
	P1-2	*1111-22*	P32-3	*2111-21*	25	0	20	20
	P1-4	*1112-22*	P17-4	*2112-21*	20	2	20	20
vic2, *vic3*	P20-2	*1111-21*	P76-6	*1221-21*	15	0	15	1
	P32-2	*2111-21*	P78-1	*2221-21*	15	0	15	2
	P8-3	*2112-12*	P22-6	*2222-12*	15	4	15	2
	P57-5	*1121-12*	P29-1	*1211-12*	15	0	15	2
	P59-9	*1121-21*	P27-1	*1211-21*	15	0	15	2
	P21-5	*2122-12*	P5-2	*2212-12*	15	2	15	3
vic2, *vic4*	P1-2	*1111-22*	P1-16	*1212-22*	5	0	—	
	P1-6	*2111-22*	P4-4	*2212-22*	10	0	—	
	P2-4	*2112-22*	P1-11	*2211-22*	15	0	—	
vic2, *vic6*	P30-9	*1111-12*	P1-5	*1211-22*	35	1	15	1
	P35-3	*2111-12*	P1-11	*2211-22*	15	3	15	0
	P8-3	*2112-12*	P4-4	*2212-22*	25	0	15	0
	P1-2	*1111-22*	P29-1	*1211-12*	5	0	—	
	P1-4	*1112-22*	P9-2	*1212-12*	25	0	—	
	P1-6	*2111-22*	P10-18	*2211-12*	20	0	15	0
	P17-4	*2112-21*	P21-16	*2212-11*	15	0	15	0
	P2-4	*2112-22*	P5-2	*2212-12*	15	0	15	1
vic2, *vic7*	P16-1	*1112-11*	P9-2	*1212-12*	15	1	15	1
	P17-2	*1112-21*	P1-16	*1212-22*	15	2	15	0
	P17-4	*2112-21*	P4-4	*2212-22*	10	0	—	
	P16-2	*2122-11*	P22-6	*2222-12*	15	1	15	1
	P1-2	*1111-22*	P27-1	*1211-21*	20	0	15	1
	P1-6	*2111-22*	P24-33	*2211-21*	5	0	—	
	P8-3	*2112-12*	P21-16	*2212-11*	15	0	15	3
	P2-4	*2112-22*	P17-7	*2212-21*	15	1	15	3
vic3, *vic6*	P30-9	*1111-12*	P73-24	*1121-22*	15	0	15	4
	P9-2	*1212-12*	P60-3	*1222-22*	15	0	15	0
	P5-2	*2212-12*	P21-2	*2222-22*	15	0	15	2
	P32-3	*2111-21*	P25-6	*2121-11*	15	0	15	0
	P17-4	*2112-21*	P16-2	*2122-11*	15	0	15	1
	P4-4	*2212-22*	P22-6	*2222-12*	15	2	15	1

(*continued*)

metry was found at three of the six *vic* loci. As described above, asymmetric virus transmission was most evident for *vic1* and *vic7*; asymmetry at *vic2* is significant, but it is markedly weaker (Table 2).

Epistasis between *vic* loci could be estimated for only 11 of the 15 possible interactions in the full model and 9 of 10 interactions in the reduced model (Table 2) because of instability and lack of convergence due to

TABLE 1

(Continued)

Heteroallelic loci[a]	Donor		Recipient		Transmission		Reciprocal transmission[d]	
	Isolate[b]	*vic* genotype[c]	Isolate	*vic* genotype	No. trials	No. successes	No. trials	No. successes
vic3, *vic7*	P27-1	*1211-21*	P79-6	*1221-22*	15	12	15	4
	P16-6	*2112-11*	P21-5	*2122-12*	15	14	15	1
	P21-16	*2212-11*	P22-6	*2222-12*	15	14	15	0
	P30-9	*1111-12*	P25-27	*1121-11*	15	3	15	15
	P9-3	*1112-12*	P16-7	*1122-11*	15	0	15	13
	P4-4	*2212-22*	P21-35	*2222-21*	15	1	15	12
vic4, *vic6*	P1-2	*1111-22*	P9-13	*1112-12*	5	1	—	
vic4, *vic7*	P1-2	*1111-22*	P17-2	*1112-21*	5	0	—	
	P2-4	*2112-22*	P32-3	*2111-21*	5	1	—	
vic6, *vic7*	P16-1	*1112-11*	P1-4	*1112-22*	15	6	15	0
	P19-2	*1212-11*	P1-16	*1212-22*	15	7	15	3
	P16-2	*2122-11*	P21-4	*2122-22*	15	5	15	0
	P9-13	*1112-12*	P17-2	*1112-21*	15	1	14	5
	P35-3	*2111-12*	P32-3	*2111-21*	15	1	15	3
	p22-6	*2222-12*	P21-35	*2222-21*	15	6	15	6

[a] *vic* loci at which donor and recipient isolates have different alleles.

[b] Ascospore isolates from crosses described in Cortesi and Milgroom (1998) are denoted by "P," followed by the cross number and the progeny from that cross (*e.g.*, P20-2 is ascospore isolate 2 from cross P20). Field isolates are indicated by isolate numbers beginning with two-letter codes CH, LI, TE, VA, and VO.

[c] Abbreviations for *vic* genotypes are allele numbers (*1* or *2*) for *vic* loci *1*, *2*, *3*, *4*, *6*, and *7* (see text).

[d] Transmission results in opposite direction as presented in the "Transmission" column, *i.e.*, from isolate labeled "recipient" to isolate labeled "donor".

[e] Donor and recipient with same *vic* genotypes.

[f] Not tested.

insufficient data. Four of the 11 estimated epistasis parameters were significant; all were positive, thereby increasing the probability of virus transmission relative to the same two *vic* loci acting independently. Three of the four significant interactions involved *vic2*, which alone inhibits transmission most strongly.

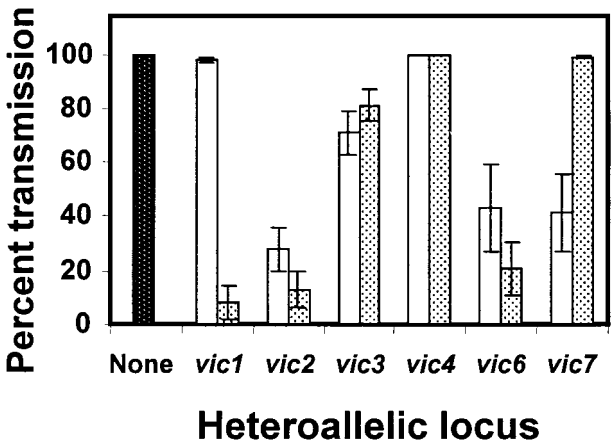

Figure 2.—Observed virus transmission between genotypes heteroallelic at single *vic* loci. Open bars represent transmission when the recipient has *vic* allele *1*; stippled bars represent transmission when the recipient has *vic* allele *2*. Transmission between individuals with the same *vic* genotype is shown by a darker stippled bar. Data are from Table 1, pooled over pairs of genotypes with single heteroallelic loci only. Error bars represent one standard error estimated among different pairs of isolates with the same heteroallelic *vic* locus.

Estimates of the variances for effects of specific donor and recipient isolates were significantly greater than zero, indicating that genes other than *vic* in the genetic background affect virus transmission (Table 2). However, variation in these effects, especially for the donor, is relatively small compared to the effects of heteroallelism and asymmetry. The variance in the recipients was greater than for donors, indicating that the genetic background of the recipient may be more important to virus transmission than that of the donor. The correlation between the ability of an individual to donate or receive virus was not significant; *i.e.*, an isolate that donates virus well (or poorly) in transmission tests is not necessarily any better (or worse) at receiving viruses.

Test of regression model: Predicted virus transmission probabilities estimated from the reduced model (Table 2) were compared to observed probabilities for laboratory isolates (Table 1) and for isolates from field populations. Overall, the observed probabilities correlated highly with predicted probabilities (Figure 3). In four field populations, the correlation for all pairs of isolates was $r = 0.93$ ($N = 100$). Transmission between isolates with the same *vic* genotype occurred successfully

TABLE 2

Parameter estimates for logistic regression of probability of virus transmission between *vic* genotypes

		Full model[a]			Reduced model		
Parameter[b]		Parameter estimate	Standard error		Parameter estimate	Standard error	
Intercept	μ	3.99	0.37	*[c]	4.16	0.33	*
Heteroallelism	β_1	−3.18	0.46	*	−3.36	0.39	*
	β_2	−5.37	0.45	*	−5.43	0.42	*
	β_3	−2.45	0.41	*	−2.67	0.35	*
	β_4	0.62	0.55	NS	—[d]		
	β_6	−3.87	0.34	*	−4.04	0.28	*
	β_7	−1.49	0.31	*	−1.56	0.29	*
Asymmetry	γ_1	−4.61	0.40	*	−4.63	0.38	*
	γ_2	−0.97	0.40	*	−0.95	0.37	*
	γ_3	0.15	0.41	NS	0.09	0.37	NS
	γ_4	0.27	0.67	NS	—		
	γ_6	0.14	0.36	NS	0.22	0.31	NS
	γ_7	3.07	0.40	*	3.11	0.40	*
Epistasis	θ_{12}	1.33	0.53	*	1.43	0.50	*
	θ_{13}	1.83	0.64	*	2.04	0.57	*
	θ_{14}	−0.72	0.81	NS	—		
	θ_{16}	−0.68	0.52	NS	−0.50	0.46	NS
	θ_{17}	0.77	0.55	NS	0.89	0.48	NS
	θ_{23}	1.30	0.60	*	1.41	0.57	*
	θ_{26}	1.21	0.63	NS	1.29	0.61	*
	θ_{27}	0.02	0.54	NS	−0.03	0.54	NS
	θ_{36}	−0.82	0.59	NS	−0.61	0.55	NS
	θ_{37}	−0.18	0.48	NS	−0.09	0.47	NS
	θ_{46}	−1.47	1.04	NS	—		
Isolate	σ^2_D	0.27	0.05	*	0.28	0.09	*
	σ^2_R	1.24	0.33	*	1.27	0.34	*

[a] Full model estimates all parameters possible given the data available. Reduced model excludes parameters associated with *vic4*, which has no effect on virus transmission.

[b] See text for definitions of parameters.

[c] Asterisk denotes an estimated standard error less than two times the absolute value of the parameter estimate; NS, not significant otherwise.

[d] Not estimated in reduced model.

in all but 1 of 240 trials (10 trials per 24 pairs of isolates). The correlation between predicted and observed probabilities was slightly lower for transmission between different *vic* genotypes ($r = 0.85$, $N = 76$). We also compared field data from a published report (Bissegger *et al.* 1997) and found that model predictions correlated highly with observed transmission for all pairs of isolates (Figure 3C; $r = 0.97$, $N = 36$) and transmission between different *vic* genotypes ($r = 0.95$, $N = 30$).

Although the model performed well on average, some noticeable outliers also occurred. Two pairs of isolates from Bergamo, heteroallelic only at *vic4* [*vic* genotype pairs (*2111-22*, *2112-22*) and (*2211-22*, *2212-22*)], were predicted to have transmission probabilities close to 1, but instead had observed transmission frequencies of 0.2 and 0.3 (see bottom right corner of Figure 3B). We repeated transmission assays and *vic* genotyping with these isolates to confirm these results. In contrast, 100% of the trials showed virus transmission for six other pairs of field isolates heteroallelic only at *vic4*, as predicted. Two other pairs of isolates, each heteroallelic only at *vic6* (but with different alleles in the recipients of the two pairs), had predicted transmission probabilities of 0.5 and 0.55 but observed transmissions of 0 and 0.1 (Figure 3B). Several other outliers, with both overestimates and underestimates of transmission probabilities, were heteroallelic at two to four *vic* loci.

DISCUSSION

We demonstrated conclusively that virus transmission between individuals of *C. parasitica* is controlled primarily by *vic* genes, with only small effects attributable to other genes in the genetic background of the fungus. Unlike most previous studies on horizontal transmission in fungi, we conducted extensive replication with inde-

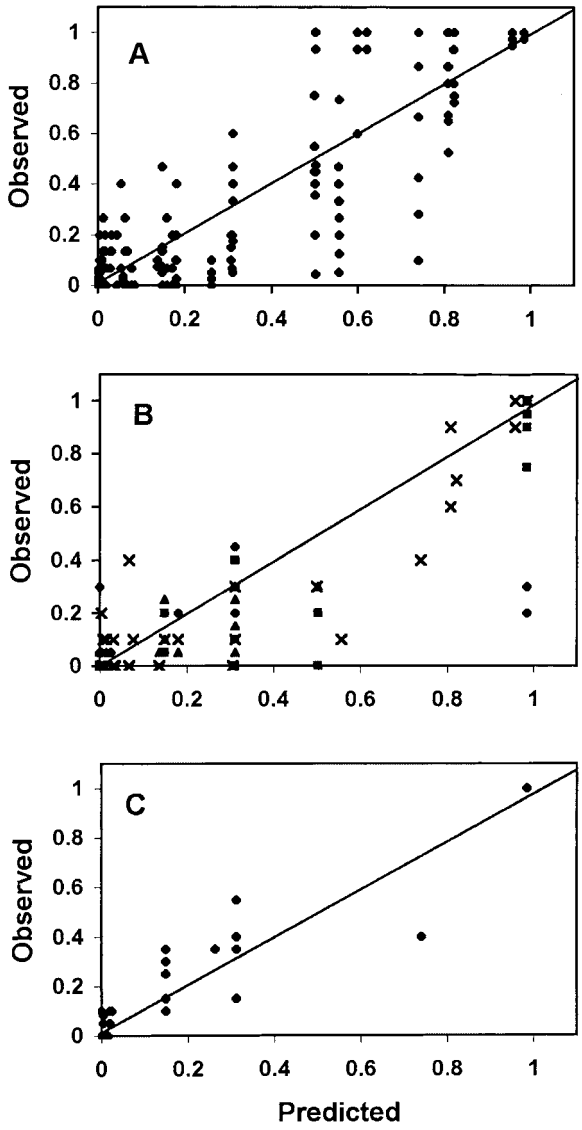

FIGURE 3.—Predicted and observed probabilities of virus transmission. Predicted probabilities were calculated from estimates of the reduced model (see Table 2 and text for explanation). Observed transmission probabilities are the proportions of trials between pairs of isolates in which virus was transmitted from donor to recipient. (A) Observed data used for estimating parameters in logistic regression (Table 1); data are plotted for those pairs of isolates with 10 or more trials. (B) Pairs of isolates randomly sampled from four natural populations: ◆, Bergamo; g, Crevaldossola; ▲, Pigna; and r, Maryland (MILGROOM and CORTESI 1999). (C) Data from Table 2 in BISSEGGER *et al.* (1997).

pendent strains to quantify the effects associated with heteroallelism at different *vic* loci, independent of host genetic background. We found marked variation in effects among six *vic* loci on virus transmission in *C. parasitica*, from strong inhibition to no effect (Figure 2). Some *vic* loci exhibited significant asymmetries such that the probability of transmission between *vic* genotypes depended on which alleles were in the donors and recipients. Furthermore, heteroallelism at different *vic* loci generally had independent effects; significant epistasis was observed in four cases but the magnitude of the interactions was generally small (Table 2). Quantitative estimates of these detailed genetic effects were integrated in a logistic regression model that accurately predicts the probability of virus transmission between any two *vic* genotypes defined by the six *vic* loci studied.

We observed marked variation among *vic* loci and significant asymmetry of effects for alleles at three loci. ANAGNOSTAKIS and DAY (1979) and ANAGNOSTAKIS (1987) speculated that viruses may be transmitted between some pairs of vc types more easily than others because of differences in the rate of cell death after anastomosis. Rapid cell death is likely to prevent virus movement between individuals more effectively than delayed cell death. Therefore, differences in transmission between *vic* loci, or asymmetry in transmission between *vic* genotypes heteroallelic at one locus, may be caused by variation in cell death rates. Preliminary cytological studies suggest that individuals that are poor virus recipients in asymmetric transmission exhibit cell death earlier than recipients that are more easily infected (S. BIELLA, J. AIST, P. CORTESI and M. MILGROOM, unpublished data). We also hypothesize that the variation in magnitude of the asymmetry at different *vic* loci is correlated to the differences in the average cell death rates. The *vic* genes in *C. parasitica* that have little effect on virus transmission (*e.g.*, *vic4*) may be analogous to partial heterokaryon incompatibility genes found in *Aspergillus nidulans* that do not restrict horizontal transmission of viruses or mitochondria (COENEN *et al.* 1994, 1997). However, to our knowledge, studies to demonstrate partial incompatibility (*e.g.*, using auxotrophs to force heterokaryons) have not been conducted in *C. parasitica*.

The effects of *vic* genes at different loci were generally additive (Table 2), even though we observed statistically significant interactions with four pairs of *vic* loci. Most of the significant interactions were between *vic* loci at which heteroallelism already had large average effects, reducing virus transmission (*e.g.*, $\beta_1 = -3.36$, $\beta_2 = -5.43$, and $\theta_{12} = 1.43$ in the reduced model). Although we could detect significant epistasis between some loci, it does not appear to be a dominant feature of this system. Similarly, other studies on horizontal transmission in fungi have also shown additive effects of *vic* or *het* genes (COENEN *et al.* 1994, 1997; HUBER 1996). In contrast to these other studies, however, we could test this hypothesis statistically because of independent replications of *vic* genotypes.

To isolate the effect of *vic* genes on virus transmission (independent of other genes in the genetic background of the fungus), we replicated transmission tests with the same *vic* genotypes and between different sets of *vic* genotypes heteroallelic at specific loci. Previous studies estimating the effects of *vic* genes on virus transmission

in *C. parasitica* (Huber and Fulbright 1994; Huber 1996) could not isolate these effects conclusively because they lacked sufficient replication and investigated a small number of genetically related strains. Although most variation in virus transmission could be explained by heteroallelism at *vic* loci, we found significant variation in virus transmission associated with both donor and recipient isolates and concluded that transmission is not controlled solely by *vic* genes. The larger variance in genetic background effect found in recipient isolates (compared to donor isolates) may reflect differences in genes associated with cell death in the recipient downstream of effects associated with *vic* genes *per se*. Tests of these hypotheses await further cytological and/or biochemical investigation.

Predicted probabilities of virus transmission generally correlated well with observed transmission among field isolates (assayed in the laboratory; Figure 3). However, the lack of fit between predicted and observed transmission probabilities in some pairings may be another indication that genes other than *vic* genes affect virus transmission. For example, we observed much less transmission (20 and 30%) between two pairs of field isolates heteroallelic at *vic4*, which showed no inhibition in laboratory studies (Huber 1996; this study, Figure 2), and therefore 100% transmission was expected. Interestingly, plasmid transmission occurred in only 3 of 18 trials between one pair of isolates heteroallelic only at *vic4* and in 22 of 29 trials in another pair of isolates (Baidyaroy *et al.* 2000). Genetic background effects are treated as unknown random factors and cannot be accounted for when predicting transmission between field isolates. Therefore, some discrepancies between observed and predicted transmissions are inherent in this model. Nonetheless, our model can predict transmission among the 64 *vic* genotypes defined by six *vic* loci (Cortesi and Milgroom 1998). Using this model will enable us to estimate the average virus transmission probability within populations where *vic* genotypes are known, *e.g.*, most populations in Europe and some populations in the eastern United States (Milgroom and Cortesi 1999). However, estimated transmission probabilities are derived strictly from laboratory assays; whether these estimates are accurate predictors of actual transmission under natural conditions remains to be determined.

A recent study of *Neurospora* spp. suggests that *het* genes may be under balancing selection (Wu *et al.* 1998). A potential mechanism for balancing selection is the advantage accorded to individuals with rare *het* (or *vic*) alleles because they are less likely to encounter compatible individuals from which they could acquire deleterious cytoplasmic elements (Hartl *et al.* 1975; Nauta and Hoekstra 1994; Milgroom 1999). In this case, balancing selection would favor intermediate *vic* allele frequencies and high *vic* genotype diversity. However, balancing selection due to viruses (or other extranuclear elements) may be weak if the incompatibility system allows transmission between *vic* genotypes, as in *C. parasitica*. Simple models predict that viruses will invade fungal populations (at equilibrium) if transmission rates are greater than zero (Shaw 1994; Taylor *et al.* 1998). This theory challenges the hypothesis that deleterious cytoplasmic elements might be exerting balancing selection on *vic* loci over the long term. However, contrary to simple models, a spatially explicit numerical model of hypovirus invasion showed that vc type diversity had profound effects on virus invasion (Liu *et al.* 2000) and that viruses can select for intermediate *vic* genotype frequencies under some conditions (Y.-C. Liu and M. G. Milgroom, unpublished results). *Vic* allele frequencies in *C. parasitica* populations in Europe and eastern North America, except at one *vic* locus, were generally not intermediate (Milgroom and Cortesi 1999), as expected at equilibrium under balancing selection (Hartl *et al.* 1975; Nauta and Hoekstra 1994). However, the one locus with consistently intermediate allele frequencies was *vic2*, which also exhibits the strongest overall inhibition on virus transmission (Figure 2). Whether this is coincidence or can be explained by balancing selection is not known. The fact that allele frequencies were intermediate at only one *vic* locus is an inconclusive test for balancing selection because these populations may not have reached equilibrium in the relatively short time since *C. parasitica* was introduced from Asia. Even if balancing selection cannot be tested adequately in introduced populations in Europe and North America, we cannot rule out the hypothesis that viruses exert balancing selection on *vic* genes in native populations. However, estimating *vic* allele frequencies in native populations of *C. parasitica* in Japan and China is not currently possible because of much greater diversity of *vic* genotypes than in Europe and the lack of knowledge of *vic* genetics in these populations (Y.-C. Liu and M. G. Milgroom, unpublished data). This question could be addressed by reconstructing *vic* gene genealogies at different loci or comparing nonsynonymous to synonymous substitution rates for *vic* genes that have large effects on virus transmission with those that have little effect. If viruses are responsible for balancing selection, we would expect to see stronger evidence for balancing selection from genealogies at loci with strong effects on virus transmission than at loci with weak effects on virus transmission. Until several *vic* genes are cloned from *C. parasitica*, however, these types of analyses are not possible.

We thank Luca Rancati and Gwendoline Izzo for help with virus transmission assays and Marco Bisiach, Antonio De Martino, and Mario Intropido for sharing isolate E13. This study was funded by U.S. Department of Agriculture National Research Initiative Competitive Grants Program grant no. 97-35303-4536 to M.G.M, Italian Ministry of University Competitive Grants Program 40% 1998 to P.C., a North Atlantic Treaty Organization Cooperative Research grant no. 930930 to M.G.M and P.C., and U.S. National Science Foundation grant no. DMS 9625476 to C.E.M.

LITERATURE CITED

Anagnostakis, S. L., 1982 Biological control of chestnut blight. Science **215:** 466–471.

Anagnostakis, S. L., 1983 Conversion to curative morphology in *Endothia parasitica* and its restriction by vegetative compatibility. Mycologia **75:** 777–780.

Anagnostakis, S. L., 1987 Chestnut blight: the classical problem of an introduced pathogen. Mycologia **79:** 23–37.

Anagnostakis, S. L., 1988 *Cryphonectria parasitica*, cause of chestnut blight. Adv. Plant Pathol. **6:** 123–136.

Anagnostakis, S. L., and P. R. Day, 1979 Hypovirulence conversion in *Endothia parasitica*. Phytopathology **69:** 1226–1229.

Anagnostakis, S. L., and P. E. Waggoner, 1981 Hypovirulence, vegetative incompatibility, and the growth of cankers of chestnut blight. Phytopathology **71:** 1198–1202.

Anderson, R. M., and R. M. May, 1986 The invasion, persistence and spread of infectious diseases within animal and plant communities. Philos. Trans. R. Soc. Lond. B **314:** 533–570.

Baidyaroy, D., J. M. Glynn and H. Bertrand, 2000 Dynamics of asexual transmission of a mitochondrial plasmid in *Cryphonectria parasitica*. Curr. Genet. **37:** 257–267.

Bisiach, M., A. D. Martino, E. Gobbi, M. Intropido and G. Vegetti, 1988 Studies on chestnut blight: activity report. Riv. Patol. Veg. **24:** 3–13.

Bissegger, M., D. Rigling and U. Heiniger, 1997 Population structure and disease development of *Cryphonectria parasitica* in European chestnut forests in the presence of natural hypovirulence. Phytopathology **87:** 50–59.

Brasier, C. M., 1984 Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological properties and ecological importance, pp. 451–497 in *The Ecology and Physiology of the Fungal Mycelium*, edited by D. Jennings and A. D. M. Rayner. Cambridge University Press, Cambridge, UK.

Caten, C. E., 1972 Vegetative incompatibility and cytoplasmic infection in fungi. J. Gen. Microbiol. **72:** 221–229.

Coenen, A., F. Debets and R. F. Hoekstra, 1994 Additive action of partial heterokaryon incompatibility (*partial-het*) genes in *Aspergillus nidulans*. Curr. Genet. **26:** 233–237.

Coenen, A., F. Kevei and R. F. Hoekstra, 1997 Factors affecting the spread of double-stranded RNA viruses in *Aspergillus nidulans*. Genet. Res. **69:** 1–10.

Collins, R. A., and B. J. Saville, 1990 Independent transfer of mitochondrial chromosomes and plasmids during unstable vegetative fusion in *Neurospora*. Nature **345:** 177–179.

Cortesi, P., and M. G. Milgroom, 1998 Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Appl. Environ. Microbiol. **64:** 2988–2994.

Cortesi, P., M. G. Milgroom and M. Bisiach, 1996 Distribution and diversity of vegetative compatibility types in subpopulations of *Cryphonectria parasitica* in Italy. Mycol. Res. **100:** 1087–1093.

Cortesi, P., D. Rigling and U. Heiniger, 1998 Comparison of vegetative compatibility types in Italian and Swiss populations of *Cryphonectria parasitica*. Eur. J. For. Pathol. **28:** 167–176.

Debets, F., X. Yang and A. J. F. Griffiths, 1994 Vegetative incompatibility in Neurospora: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. Curr. Genet. **26:** 113–119.

Elliston, J. E., 1985 Further evidence for two cytoplasmic hypovirulence agents in a strain of *Endothia parasitica* from western Michigan. Phytopathology **75:** 1405–1413.

Geyer, C. J., and E. A. Thompson, 1992 Constrained Monte Carlo maximum likelihood for dependent data. J. R. Stat. Soc. B **54:** 657–699.

Glass, N. L., D. J. Jacobson and P. K. T. Shiu, 2000 The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu. Rev. Genet. **34:** 165–186.

Griffiths, A. J. F., S. R. Kraus, R. Barton, D. A. Court, C. J. Myers *et al.*, 1990 Heterokaryotic transmission of senescence plasmid DNA in *Neurospora*. Curr. Genet. **17:** 139–145.

Hammond-Kosack, K., and J. Jones, 2000 Responses to plant pathogens, pp. 1102–1156 in *Biochemistry and Molecular Biology of Plants*, edited by B. Buchanan, W. Gruissem and R. Jones. American Society of Plant Physiologists, Rockville, MD.

Hartl, D. L., E. R. Dempster and S. W. Brown, 1975 Adaptive significance of vegetative incompatibility in *Neurospora crassa*. Genetics **81:** 553–569.

He, C., A. G. Rusu, A. M. Poplawski, J. A. Irwin and J. M. Manners, 1998 Transfer of a supernumerary chromosome between vegetatively incompatible biotypes of the fungus *Colletotrichum gloeosporioides*. Genetics **150:** 1459–1466.

Hillman, B. I., R. Shapira and D. L. Nuss, 1990 Hypovirulence-associated suppression of host functions in *Cryphonectria parasitica* can be partially relieved by high light intensity. Phytopathology **80:** 950–956.

Hillman, B. I., D. W. Fulbright, D. L. Nuss and N. K. Van Alfen, 2000 *Hypoviridae*, pp. 515–520 in *Virus Taxonomy: Seventh Report of the International Committee for the Taxonomy of Viruses*, edited by M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes *et al.* Academic Press, San Diego.

Hoekstra, R. F., 1996 Horizontal transmission in fungal populations, pp. 337–348 in *Fungal Genetics: Principles and Practice*, edited by C. J. Bos. Marcel Dekker, New York.

Hosmer, D. W., and S. Lemeshow, 1989 *Applied Logistic Regression*. Wiley, New York.

Huber, D. H., 1996 Genetic analysis of vegetative incompatibility polymorphisms and horizontal transmission in the chestnut blight fungus *Cryphonectria parasitica*. Ph.D. Thesis, Michigan State University, East Lansing, MI.

Huber, D. H., and D. W. Fulbright, 1994 Preliminary investigations on the effect of individual *vic* genes upon the transmission of dsRNA in *Cryphonectria parasitica*, pp. 15–19 in *Proceedings of the International Chestnut Conference*, edited by M. L. Double and W. L. MacDonald. West Virginia University Press, Morgantown, WV.

Levin, B. R., 1996 The evolution and maintenance of virulence in microparasites. Emerg. Infect. Dis. **2:** 93–102.

Lipsitch, M., and E. R. Moxon, 1997 Virulence and transmissibility of pathogens: what is the relationship? Trends Microbiol. **5:** 31–37.

Liu, Y.-C., and M. G. Milgroom, 1996 Correlation between hypovirus transmission and the number of vegetative incompatibility (*vic*) genes different among isolates from a natural population of *Cryphonectria parasitica*. Phytopathology **86:** 79–86.

Liu, Y.-C., P. Cortesi, M. L. Double, W. L. MacDonald and M. G. Milgroom, 1996 Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*. Phytopathology **86:** 1344–1451.

Liu, Y.-C., R. Durrett and M. G. Milgroom, 2000 A spatially-structured stochastic model to simulate heterogeneous transmission of viruses in fungal populations. Ecol. Modell. **127:** 291–308.

MacDonald, W. L., and D. W. Fulbright, 1991 Biological control of chestnut blight: use and limitations of transmissible hypovirulence. Plant Dis. **75:** 656–661.

McCulloch, C. E., 1997 Maximum likelihood algorithms for generalized linear mixed models. J. Am. Stat. Assoc. **92:** 162–170.

Milgroom, M. G., 1999 Populations of fungi and their viruses, pp. 283–305 in *Structure and Dynamics of Fungal Populations*, edited by J. J. Worrall. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Milgroom, M. G., and P. Cortesi, 1999 Analysis of population structure of the chestnut blight fungus based on vegetative incompatibility genotypes. Proc. Natl. Acad. Sci. USA **96:** 10518–10523.

Morris, T. J., J. A. Dodds, B. Hillman, R. L. Jordan, S. A. Lommel *et al.*, 1983 Viral specific dsRNA: diagnostic value for plant virus disease identification. Plant Mol. Biol. Rep. **1:** 27–30.

Nauta, M. J., and R. F. Hoekstra, 1994 Evolution of vegetative incompatibility in filamentous ascomycetes. I. Deterministic models. Evolution **48:** 979–995.

Nuss, D. L., 1992 Biological control of chestnut blight: an example of virus-mediated attenuation of fungal pathogenesis. Microbiol. Rev. **56:** 561–576.

Peever, T. L., Y.-C. Liu and M. G. Milgroom, 1997 Diversity of hypoviruses and other double-stranded RNAs in *Cryphonectria parasitica* in North America. Phytopathology **87:** 1026–1033.

Peever, T. L., Y.-C. Liu, P. Cortesi and M. G. Milgroom, 2000 Variation in tolerance and virulence in the chestnut blight fungus-hypovirus interaction. Appl. Environ. Microbiol. **66:** 4863–4869.

Poplawski, A. M., C. He, J. A. Irwin and J. M. Manners, 1997 Transfer of an autonomously replicating vector between vegetatively incompatible biotypes of *Colletotrichum gloeosporioides*. Curr. Genet. **32:** 66–72.

Read, A. F., S. D. Albon, J. Antonovics, V. Apanius, G. Dwyer *et*

al., 1995 Group report: genetics and evolution of infectious diseases in natural populations, pp. 450–477 in *Ecology of Infectious Diseases in Natural Populations*, edited by B. T. Grenfell and A. P. Dobson. Cambridge University Press, Cambridge, UK.

Robin, C., C. Anziani and P. Cortesi, 2000 Relationship between biological control, incidence of hypovirulence, and diversity of vegetative compatibility types of *Cryphonectria parasitica* in France. Phytopathology **90:** 730–737.

Saupe, S. J., 2000 Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. **64:** 489–502.

Saupe, S. J., C. Clavé and J. Béguret, 2000 Vegetative incompatibility in filamentous fungi: *Podospora* and *Neurospora* provide some clues. Curr. Opin. Microbiol. **3:** 608–612.

Shaw, M. W., 1994 Seasonally induced chaotic dynamics and their implications in models of plant disease. Plant Pathol. **43:** 790–801.

Taylor, D. R., A. M. Jarosz, R. E. Lenski and D. W. Fulbright, 1998 The acquisition of hypovirulence in host-pathogen systems with three trophic levels. Am. Nat. **151:** 343–355.

Van Alfen, N. K., R. A. Jaynes, S. L. Anagnostakis and P. R. Day, 1975 Chestnut blight: biological control by transmissible hypovirulence in *Endothia parasitica*. Science **189:** 890–891.

van der Gaag, M., A. J. M. Debets, H. D. Osiewacz and R. F. Hoekstra, 1998 The dynamics of pAL2–1 homologous linear plasmids in *Podospora anserina*. Mol. Gen. Genet. **258:** 521–529.

van Diepeningen, A. D., A. J. M. Debets and R. F. Hoekstra, 1997 Heterokaryon incompatibility blocks virus transfer among natural isolates of black *Aspergilli*. Curr. Genet. **32:** 209–217.

Wu, J., S. J. Saupe and N. L. Glass, 1998 Evidence for balancing selection operating at the *het-c* heterokaryon incompatibility locus in a group of filamentous fungi. Proc. Natl. Acad. Sci. USA **95:** 12398–12403.

Communicating editor: R. H. Davis