Population Differentiation in the Chestnut Blight Fungus, *Cryphonectria parasitica,* in Eastern North America

Michael G. Milgroom and Susanne E. Lipari

Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

We thank L. Shain for collecting samples of *C. parasitica* from Kentucky; A. Webb, E. Seligmann, M. Double, and W. MacDonald for sending isolates from Maryland; G. Boland for sending isolates from Ontario; B. Hillman for help collecting in New Jersey; and R. Marra for sharing isolates from West Virginia. We also thank M. Slatkin for sharing a computer program and J. B. Anderson, S. B. Goodwin, and anonymous reviewers for making helpful suggestions on earlier drafts. This research was supported in part by USDA NRI Competitive Grants 91-37303-5939 and 93-37303-9035 and McIntyre-Stennis project NYC-153553.

Accepted for publication 11 October 1994.

ABSTRACT

Milgroom, M. G., and Lipari, S. E. 1995. Population differentiation in the chestnut blight fungus, *Cryphonectria parasitica,* in eastern North America. Phytopathology 85:155-160.

Isolates of *Cryphonectria parasitica* were sampled from American chestnut trees in 13 locations in eastern North America to study genetic differentiation and gene flow among subpopulations of this pathogen. Using data from six unlinked restriction fragment length polymorphism loci, we found significant differences in allele frequencies at all loci among the 13 subpopulations. Thirty-one percent of the total gene diversity was attributed to differences among subpopulations (G_{ST} = 0.31). Genetic differentiation was examined for two subsets of data: one from between two subpopulations in Michigan, which were outside the natural range of the host, and the other from among nine subpopulations from ecologically similar sites (disturbed sites resulting from clearcutting and burning were eliminated). There were significant differences in allele frequencies at two loci between the two Michigan subpopulations, even though these sites were only 16 km apart. Differentiation was high between these subpopulations because two loci were fixed or nearly fixed for different alleles (G_{ST} = 0.81). There was also differentiation among the nine subpopulations from ecologically similar sites (G_{ST} = 0.20); significant differences in allele frequencies were found at five of the six loci. We attribute this level of differentiation to restricted gene flow among subpopulations. We did not, however, estimate gene flow quantitatively from these data because populations did not appear to be at equilibrium. There was no correlation between the estimated average number of migrants per generation between each pair of nine subpopulations and geographic distance. This correlation would be expected to be negative if populations were at equilibrium and gene flow was restricted to short distances (isolation by distance). The lack of correlation was interpreted as non-equilibrium conditions; therefore, gene flow would be overestimated.

Additional keywords: *Endothia parasitica,* population genetic structure, population structure, population subdivision.

Population differentiation, which is defined in terms of differences in allele frequencies among subpopulations, can be caused by both selection and genetic drift (32,33). When there is selection for different alleles or genotypes in different subpopulations, they will diverge genetically unless there is sufficient gene flow to prevent differentiation. An example of this in plant pathogens is selection for different pathotypes on cultivars with different resistance genes. Although there may be a large number of pathogen propagules dispersing among fields with different cultivars, pathotypes that are not compatible will not survive in resistant host populations, and, therefore, gene flow will be minimal.

In addition, subpopulations may diverge genetically by random genetic drift when gene flow is restricted among subpopulations. Migration of only a few individuals per generation is sufficient to prevent random fixation of neutral alleles in different subpopulations (32,33,37). If there is sufficient recurrent gene flow, subpopulations will remain relatively homogeneous because of the mixing of alleles among subpopulations. Therefore, inferences can be made about gene flow and drift from the extent of population differentiation or subdivision observed. To make meaningful inferences, however, there are at least two conditions that must be met. First, selectively neutral genetic markers, which are not linked to loci under selection, must be used to minimize the effects of selection on subdivision. Second, for making quantitative estimates of average gene flow per generation, populations must have reached an equilibrium between genetic drift and gene flow (32,33). If populations are not at equilibrium, gene flow is likely to be overestimated because of the effects of gene flow in the past, e.g., colonization events that cannot be distinguished from current gene flow when differentiation among subpopulations is analyzed (32,33).

Studies of fungal plant pathogens have shown that there is variation in the amount of genetic differentiation of populations depending on the biology and history of particular species in different areas and the scales at which comparisons are made (5–7,10–12,17,19,21,30). Fungal plant pathogens that have been introduced into new areas with crop species tend to show relatively little differentiation among subpopulations (5,7,10,19). For example, *Phytophthora infestans* (Mont.) de Bary populations are moderately subdivided in Mexico, where this pathogen is native (11), but much less differentiated in the Netherlands, where it is an introduced pathogen (7). The lack of subdivision may be caused by high levels of current gene flow but more likely reflects recent colonization (historical gene flow), especially if there has not been enough time for populations to reach equilibrium (32–34). Pathogens studied in their native areas have shown both high and low levels of differentiation among subpopulations. Some of the highest levels of differentiation were found among subpopulations on different host species (18,39), probably the results of selection by the hosts for specialization. In contrast, one native forest pathogen showed very little subdivision, presumably because current levels of gene flow are relatively high (6) (and assuming the population is at equilibrium).

The scale of the distances between subpopulations can be important for understanding population structure. For example, a population of *Fusarium oxysporum* Schlechtend.Fr. was found to be highly structured on a small scale (meters) in a native soil but not genetically different from a population in a nearby agricultural field (12). A similar degree of fine-scale diversity was found for *Septoria tritici* Roberge in Desmaz. within fields (22),

whereas there were only small differences between populations separated by 750 km (5). In contrast, other pathogens showed more differentiation when subpopulations from greater distances were compared, e.g., when comparisons were made within and between continents (10,30). Differentiation increases with distance when organisms disperse relatively short distances in spatially continuous populations that are not delimited in discrete subpopulations (as opposed to subpopulations arbitrarily defined by sampling locations). This concept is known as isolation by distance (38). Isolation by distance can be detected in populations that are in equilibrium for genetic drift and gene flow (34).

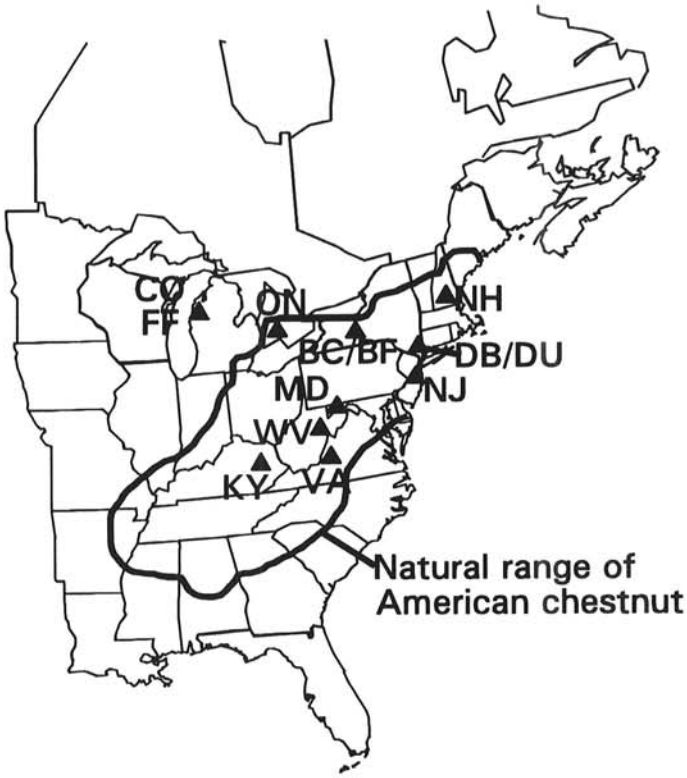

Fig. 1. Locations of subpopulations of *Cryphonectria parasitica* sampled for analysis of population genetic differentiation and gene flow. DU/DB = understory and burned site, respectively, Depot Hill, Dutchess County, New York; BC/BF = clearcut and understory, respectively, Danby, Tompkins County, New York; KY = Natural Bridge State Park, Powell County, Kentucky; MD = Finzel, Allegany County, Maryland; NH = Concord, Merrimack County, New Hampshire; ON = Wardsville, Middlesex County, Ontario, Canada; NJ = Five Points, Monmouth County, New Jersey; VA = Mountain Lake, Giles County, Virginia; WV = Parsons, Tucker County, West Virginia; CO = County Line, Manistee County, Michigan; and FF = Frankfort, Benzie County, Michigan. The approximate range of the American chestnut is also shown as described by Anagnostakis (1).

The chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr (formerly *Endothia parasitica*), was first identified in North America on American chestnut trees, *Castanea dentata* (Marsh.) Borkh., in 1904 (23), although it may have been introduced into the eastern United States from Japan much earlier on imported chestnut seedlings (3). *C. parasitica* reproduces by windborne ascospores and conidia that are dispersed by water, insects, birds, and other animals (1). After 1904, *C. parasitica* spread rapidly, at an average rate of 37 km per year, killing nearly every American chestnut tree throughout eastern North America (1,13). American chestnuts were formerly abundant and, except for understory saplings, are now quite rare because of chestnut blight (1,13). Consequently, *C. parasitica* populations were large as the pathogen initially spread through an abundant host population but are now relatively small on the few remaining chestnuts. Therefore, genetic drift may potentially be important unless there is significant gene flow still occurring among subpopulations. With relatively sparse host populations, the probability of successful gene flow between subpopulations may be reduced. Drift and restricted gene flow together have the potential to result in genetically differentiated subpopulations. However, because of the recent colonization of eastern North America by this fungus, differentiation may not yet be very marked.

The objective of this study was to determine the extent of genetic differentiation among subpopulations of *C. parasitica* and to make inferences about gene flow in eastern North America. On the basis of its recent colonization and the low degree of subdivision observed in other introduced fungal plant pathogens, we predicted that the *C. parasitica* population in eastern North America should have little population subdivision. We can not make unbiased estimates of gene flow because we can not assume the population is in equilibrium (32,33). We can, however, test whether patterns of genetic diversity in *C. parasitica* are consistent with what would be expected under isolation by distance (34,38). This analysis may also provide some clues as to whether populations are approaching equilibrium between drift and gene flow (34).

MATERIALS AND METHODS

Sampling. *C. parasitica* was obtained from 13 locations (subpopulations) in eastern North America (Fig. 1 and Table 1). Sample sizes ranged from 15 to 64. In VA, bark samples were taken from every canker within a small plot (25 × 25 m) (26). In BC, BF, DB, and DU, all cankers were identified within plots (approximately 20 × 20 m or larger), and random samples were taken with the restriction that isolates with identical DNA

TABLE 1. Subpopulation samples of *Cryphonectria parasitica* in eastern North America

Location	Subpopulation abbreviation	Date collected	Sample size	Collector[a]
Depot Hill, Dutchess County, New York				
Understory	DU	November 1990	70	...
Burned site	DB	November 1990	48	...
Danby, Tompkins County, New York				
Understory	BF	October 1991	49	...
Clearcut	BC	October 1991	49	...
Natural Bridge State Park, Powell County, Kentucky	KY	July 1993	23	L. Shain
Finzel, Allegany County, Maryland	MD	July 1991	56	A. Webb, E. Seligmann, M. Double, and W. MacDonald
Concord, Merrimack County, New Hampshire	NH	January 1993	21	...
Wardsville, Middlesex County, Ontario, Canada	ON	November 1993	35	G. Boland
Five Points, Monmouth County, New Jersey	NJ	June 1994	29	B. Hillman and M. Milgroom
Mountain Lake, Giles County, Virginia	VA	April 1989	39	Milgroom et al (26)
Parsons, Tucker County, West Virginia	WV	October 1993	30	R. Marra
County Line, Manistee County, Michigan	CO	June 1986	15	A. Michna (24)
Frankfort, Benzie County, Michigan	FF	June 1986	20	A. Michna (24)

[a]Collected by authors unless otherwise noted.

fingerprints (26) from the same trees be eliminated to avoid sampling clonal individuals more than once (*unpublished data*). Isolates from MD were collected from all cankers on chestnut stems ≥2.5 cm in diameter in a forest stand approximately 100 × 250 m; no isolates with identical DNA fingerprints occurred on the same trees (*unpublished data*). Single isolates were collected haphazardly from each tree with cankers in small chestnut stands in KY, NH, NJ, ON, and WV. Isolates from Michigan (CO and FF) were collected from abandoned orchards (24), which were recovering from chestnut blight because of hypovirulence (8), outside the natural range of American chestnuts. Single-conidial isolates were used from the Michigan populations (24) and from KY. Mass hyphal isolates were used from the other populations and provided unambiguous results in most cases. When there was any ambiguity, single-conidial isolations were made, and the analyses were repeated. All isolates were cultured on potato-dextrose agar (Difco, Detroit, MI) overlain with cellophane as described by Anagnostakis (2). Mycelium was harvested from the cellophane, lyophilized, and ground to a fine powder for DNA preparations.

RFLP analysis. DNA was prepared from each isolate as described by Milgroom et al (26,27). Restriction fragment length polymorphism (RFLP) analysis was conducted for six loci as described previously (25). DNA from each isolate was digested with the restriction endonuclease *Pst*I, size fractionated by electrophoresis in 0.9% agarose gels, transferred to nylon membranes, and probed with each of four probes, which hybridized to a total of six loci. Previous studies showed that alleles at all loci segregate in Mendelian (1:1) ratios, and most loci are not linked (25). Each segregating restriction fragment was considered to be an allele at its respective locus. RFLP data for the six loci in VA were reported previously (25) but are included here for comparison with other subpopulations.

Data analysis. The frequencies of alleles at each RFLP locus were determined for each subpopulation and compared by a heterogeneity chi-square test in the program BIOSYS-1 (35). Chi-square statistics from BIOSYS-1 were divided by two to correct for haploids. Gene diversity analysis was performed by estimating G_{ST}, the proportion of genetic diversity resulting from differences among subpopulations (28); corrections for small and unequal sample sizes (29) were made as described by Goodwin et al (10).

We tested whether there was isolation by distance among nine eastern subpopulations using the method described by Slatkin (34). If there is short distance gene flow among subpopulations and the population is in equilibrium, the logarithm of the average number of migrants per generation between each pair of subpopulations, $\hat{M}$, is expected to be negatively correlated with the logarithm of geographic distance between subpopulations (34). $\hat{M}$ was estimated for each pair of subpopulations as

$$\hat{M} = (1/G_{ST} - 1)/2,$$

where G_{ST} was the estimate for genetic differentiation between pairs of subpopulations (34). We used 2 in the denominator instead of 4 because *C. parasitica* is haploid. This analysis was done with a program provided by M. Slatkin that was compiled and executed on a personal computer.

RESULTS

Allele frequencies. Chi-square tests showed that allele frequencies in the 13 populations (Table 2) were significantly different at all six RFLP loci ($P < 0.001$). There was only one locus (MS29) with significantly different allele frequencies between two pairs of subpopulations, BC/BF and DB/DU, sampled from plots less than 300 m apart ($P < 0.01$ for BC/BF, and $P < 0.025$ for DB/DU). Because other sampled sites in this study were ecologically more similar to the understory sites, BF and DU, than to BC and DB, BC and DB were dropped from subsequent analysis to avoid bias resulting from the inclusion of samples from nearby locations. Excluding the Michigan subpopulations and BC and DB, allele frequencies were significantly different at five loci ($P < 0.005$, and $P > 0.10$ for locus CB15-1), and the overall chi-square test for all six loci was significant at the 0.001 level.

The Michigan subpopulations (CO and FF) were treated separately because they are disjunct subpopulations outside the natural range of American chestnuts and are recovering from chestnut blight (8). CO and FF were fixed for the same allele at three loci and had a fourth locus (MS26) that was fixed in CO and had a frequency of 0.95 for the same allele in FF (Table 2). However, CO and FF had significantly different allele frequencies at two loci: CO was fixed at loci MS29 and CB15-1 while FF had frequencies of only 0.05 and 0.10 for the same alleles at each locus, respectively (Table 2).

Gene diversity analysis. Gene diversity analysis was performed on data from three sets of subpopulations: 1) all 13 subpopulations, 2) between the two Michigan subpopulations (CO and FF), and 3) nine eastern subpopulations, excluding Michigan and BC and DB. The mean total gene diversity (H_T) for all six loci across all 13 subpopulations was 0.43. This level of diversity is relatively high, in part because only loci known previously to be polymorphic (25) were analyzed. The mean within-population gene diversity (H_S) for all six loci across all subpopulations was 0.30, resulting in a mean proportion of gene diversity caused by differences

TABLE 2. Allele frequencies for six restriction fragment length polymorphism loci in subpopulations of *Cryphonectria parasitica* in eastern North America

		Subpopulation[a,b]												
Locus	Allele[c]	BC (46)	BF (45)	DB (44)	DU (64)	KY (23)	MD (56)	NH (21)	NJ (29)	ON (35)	VA (39)	WV (30)	CO (15)	FF (20)
MS11	1	0	0	0.46	0.36	0.04	0.21	0.81	0.21	0.03	0.13	0.93	1.00	1.00
	2	1.00	1.00	0.55	0.64	0.96	0.79	0.19	0.79	0.97	0.87	0.07	0	0
MS26	1	0.65	0.80	0.57	0.45	0	0.55	0.10	0.55	0.77	0.64	0.76	1.00	0.95
	2	0.28	0.18	0.43	0.55	0.91	0.39	0.67	0.34	0.23	0.26	0.20	0	0.05
	3	0.07	0.02	0	0	0.09	0.05	0.24	0.10	0	0.08	0.03	0	0
	4	0	0	0	0	0	0	0	0	0	0.03	0	0	0
MS29	1	0.89	0.65	0.64	0.83	0.22	0.68	0.90	0.38	0.66	0.69	0.57	1.00	0.05
	2	0.11	0.35	0.36	0.17	0.78	0.32	0.10	0.62	0.34	0.31	0.43	0	0.95
CB15-1	1	0.87	0.76	0.73	0.80	0.61	0.66	0.71	0.76	0.83	0.82	0.90	0	0.90
	2	0.13	0.24	0.27	0.20	0.39	0.34	0.29	0.24	0.17	0.18	0.10	1.00	0.10
CB15-2	1	0.61	0.76	0.46	0.63	0.52	0.45	0.86	0.34	0.54	0.77	0.53	0	0
	2	0.39	0.24	0.55	0.38	0.48	0.55	0.14	0.66	0.46	0.23	0.47	1.00	1.00
CB15-3	1	0.98	0.91	0.75	0.70	1.00	0.86	0.38	0.90	1.00	0.95	0.93	1.00	1.00
	2	0.02	0.09	0.25	0.30	0	0.14	0.62	0.10	0	0.05	0.07	0	0

[a] Sampling locations and subpopulation abbreviations are defined in Table 1.
[b] Sample size for each subpopulation is shown in parentheses.
[c] Restriction fragment as defined in Milgroom et al (25).

among subpopulations (G_{ST}) of 0.31. Even though the two Michigan populations are only 16 km apart, differentiation between them was quite high ($G_{ST} = 0.81$) because of such different allele frequencies at loci MS29 and CB15-1. Moderate differentiation was evident among the nine eastern subpopulations (excluding CO, FF, DB, and BC); G_{ST} was estimated to be 0.20 (Table 3). This last estimate is the most informative since these subpopulations are most representative of *C. parasitica* in eastern North America relative to the other data sets.

There was considerable variation in G_{ST} estimates among the six loci for the nine eastern subpopulations (Table 3). Two loci (CB15-1 and CB15-2) showed little genetic differentiation; G_{ST} estimates were 0.01 and 0.08, respectively. This is consistent with the lack of heterogeneity for allele frequencies at CB15-1 among these same subpopulations. Estimates of G_{ST} at the other four loci were 0.15–0.48 (Table 3).

Isolation by distance. When Slatkin's (34) test was used, there was no evidence for isolation by distance among the nine eastern subpopulations. The average number of migrants per generation between each pair of subpopulations, $\hat{M}$, was greater than one for all 36 pairwise comparisons between the nine eastern subpopulations (Fig. 2). $\hat{M}$ from this analysis provides an indication of the relative levels of genetic similarity among the different subpopulations (34). When $\hat{M}$ is approximately equal to one, then gene flow and drift are assumed to be roughly balanced (32,33). If populations are at equilibrium and there is isolation by distance, the logarithm of $\hat{M}$ would be negatively correlated to the logarithm of geographic distance (34). However, there was no correlation between log($\hat{M}$) and log(geographic distance) in *C. parasitica* ($r = -0.10$, and $P > 0.50$) (Fig. 2).

DISCUSSION

Subpopulations of *C. parasitica* in eastern North America have significantly different allele frequencies and, therefore, are probably not panmictic. Significant differences in allele frequencies indicate that there is some restriction in gene flow. We estimated that approximately 20% of the genetic diversity at six RFLP loci is attributable to differentiation among subpopulations ($G_{ST} = 0.20$). This result is contrary to our prediction that a recently introduced pathogen would have relatively little or no genetic differentiation. Some introduced plant-pathogenic fungi have little genetic differentiation ($G_{ST} = 0.04$–0.06) (5,7,19). In contrast, other introduced pathogens are more differentiated on comparable geographic scales (10,30).

The causes of the observed subdivision in *C. parasitica* cannot be determined unequivocally from these data. There are several possible explanations, one of which is selection for different alleles in different locations. If differentiation is caused by selection, we would expect there to be some loci with high estimates of G_{ST}. The variation in G_{ST} estimates observed among loci does not give any indication of selection acting on any particular locus, because four of the six loci showed relatively high levels of differentiation ($G_{ST} \geq 0.15$) (Table 3). It is unlikely that there would be selection on all four of these loci since we used anonymous RFLP probes with no known function, which in other studies have been shown to be nearly neutral (16). Also, *C. parasitica* reproduces predominantly sexually in eastern North America (20,26; *unpublished data*), so selection on linked loci is not likely to affect these loci unless the linkage is extremely tight. Assuming selection on these loci is negligible, we are left with genetic drift as the most probable cause of differentiation.

Genetic drift causes random changes in allele frequencies, especially in small populations, and causes differentiation because random genetic changes occur differently in each subpopulation. There are two different processes that may contribute to genetic drift in populations of *C. parasitica* in eastern North America. First, founder effects have been shown previously to have caused reduced genetic diversity in the United States compared with China, where *C. parasitica* is native (25). Although it is more likely that *C. parasitica* was introduced from Japan (3,36), the role of founder effects in the introduction of *C. parasitica* onto a new continent is still valid. Founder effects could also have affected *C. parasitica* after its introduction as it colonized eastern North America. As it spread throughout the range of American chestnuts, some areas, by chance, may have been colonized by different genotypes, causing random differences in allele frequencies.

The second way in which drift may have affected *C. parasitica* in eastern North America is bottlenecks, where local populations are reduced to small sizes (a concept closely related to founder effects). In understory chestnut stands in Virginia and West Virginia, the average blight incidence was approximately 38%, although two plots had only 0 and 4% blight incidence (14). Because the understory plots in Griffin's (14) study were less than 100 m from clearcut sites, which had average disease incidence of 95% and presumably produced high levels of inoculum, Griffin's (14) study may have overestimated the size of *C. parasitica* populations in understory stands that were not adjacent to clearcuts. Chestnut blight epidemics in clearcut or disturbed sites have been hypothesized to have cycles that reflect the abundance of hosts, much like predator-prey cycles, where the pathogen population crashes when the host population is destroyed by blight (1). The early stages of epidemic cycles are characterized by low disease incidence (4,15). Small population sizes in understory populations, or after a crash in disturbed sites, make genetic drift a potentially powerful force in shaping the genetic structure of populations.

TABLE 3. Gene diversity analysis of nine subpopulations of *Cryphonectria parasitica* in eastern North America[a]

Locus	H_S[b]	H_T[c]	G_{ST}[d]
MS11	0.22	0.42	0.48
MS26	0.44	0.56	0.22
MS29	0.40	0.47	0.15
CB15-1	0.36	0.37	0.01
CB15-2	0.44	0.48	0.08
CB15-3	0.20	0.26	0.25
Mean	0.34	0.43	0.20

[a]The Michigan subpopulations (CO and FF) and BC and DB were not included in this analysis.
[b]Mean within-population gene diversity corrected for small samples as described by Nei and Chesser (29).
[c]Total gene diversity corrected for small sample sizes (29).
[d]$G_{ST} = (H_T - H_S)/H_T$. Rounding errors are responsible for apparent discrepancies; G_{ST} was calculated before rounding off.

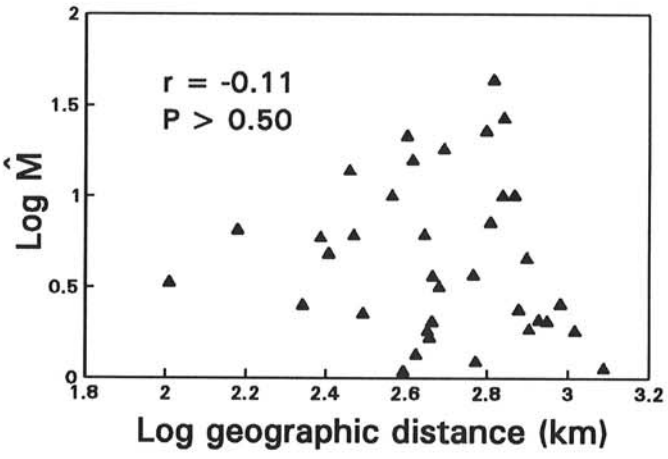

Fig. 2. The relationship between the estimated average number of migrants per generation between each pair of subpopulations, $\hat{M}$, and geographic distance for *Cryphonectria parasitica* in eastern North America. There is no significant correlation between log($\hat{M}$) and log(geographic distance) between nine subpopulations of *C. parasitica* (BF, DU, KY, MD, NH, NJ, ON, VA, and WV). The Michigan populations (CO and FF) and BC and DB were excluded from this analysis.

In contrast to genetic drift, gene flow is a potentially homogenizing force that can prevent genetic differentiation if there is sufficient migration among subpopulations (32,33,37). The fact that we observed a significant degree of genetic differentiation in *C. parasitica* means that gene flow is at least partially restricted in eastern North America. The current level of gene flow in *C. parasitica* is difficult to estimate accurately because it is likely to be affected by recent colonization (32–34). We know from historical data that *C. parasitica* spread across the range of the American chestnut at an average rate of 37 km per year (1,13), suggesting that *C. parasitica* has the potential for long-distance gene flow. However, this description of rapid colonization of eastern North America gives a biased view of the current potential for gene flow in *C. parasitica.* The initial epidemic earlier in this century occurred in an abundant host population. Before *C. parasitica* was introduced, the American chestnut was one of the most common trees in eastern deciduous forests, while today it is rare except as an understory shrub (1,13). The present day small host population reduces the probability of successful migration among subpopulations since a propagule will have less chance of encountering another chestnut host than it would have had in previously large host populations.

The current potential for long-distance gene flow is difficult to assess directly. Chestnut blight has only recently been observed in a disjunct stand of American chestnuts in southwestern Wisconsin (31), hundreds of kilometers outside the natural range. *C. parasitica* presumably dispersed to Wisconsin from blighted trees a long distance away. This type of long-distance gene flow may be quite rare, however, since chestnut blight was first reported in this stand only recently, even though chestnuts were originally planted there approximately 100 yr ago (31).

The highly differentiated Michigan populations ($G_{ST} = 0.81$) that are only 16 km apart (CO and FF) provide additional evidence for restricted gene flow in *C. parasitica.* With even a modicum of gene flow, they would not be nearly fixed for different alleles at two different loci (Table 2). However, these subpopulations are not representative of those in the forests in the natural range of American chestnut. The chestnut populations at CO and FF are remnants of orchards planted for nut production. The fungus populations at these sites are primarily hypovirulent, and chestnut trees are recovering from blight (8). Furthermore, the multilocus genetic structure within each of these populations is highly clonal (20), and no perithecia were observed in a study of *C. parasitica* in a nearby site (9). The lack of ascospores, which are windborne and potentially dispersed longer distances than conidia (1), may contribute to the lack of gene flow among nearby populations.

Although there was significant genetic differentiation, and therefore restricted gene flow, among subpopulations of *C. parasitica,* there was no evidence for isolation by distance (Fig. 2). A pattern of isolation by distance would be expected if there were only short-distance gene flow among subpopulations and if the population had had time to reach equilibrium (34). In contrast to *C. parasitica,* the Scots pine pathogen, *Crumenulopsis sororia* (P. Karst.) Groves, showed a small but significant degree of genetic differentiation ($G_{ST} = 0.04$) among six subpopulations in Scotland for three out of four isozyme loci (6). However, using the same analysis (34) on the allele frequency data and distances between subpopulations for *C. sororia* published by Ennos and Swales (6), we found a significant negative correlation between $\log(\hat{M})$ and log(geographic distance) between the six subpopulations ($r = -0.51$, and $P = 0.05$) (Fig. 3), indicating isolation by distance (34). Not surprisingly, this pathogen is native to Scotland (6), and, therefore, populations may have been established long enough for *C. sororia* to approach equilibrium conditions and for isolation by distance to be evident.

There are two ways to explain the lack of isolation by distance in *C. parasitica* in eastern North America. Either long-distance migration prevents isolation by distance, or if migration is predominantly short-distance, populations have not reached equilibrium between gene flow and drift. Independent information showing that long-distance migration is impossible or rare would be necessary to distinguish between these possibilities (34). However, from colonization events in sites such as those in Wisconsin, which are hundreds of kilometers from the nearest naturally occurring chestnuts, we know that long-distance migration can occur. However, the rarity of long-distance migration could be argued because this stand remained blight-free throughout the time when epidemics raged across the entire range of American chestnuts earlier this century. Additional support for the rarity of long-distance gene flow is the degree of differentiation among subpopulations. If long-distance migration were common, we might not expect to observe as much variation in allele frequencies as we did. Therefore, the relatively large values in Figure 2 could be interpreted as effects of recent colonization of these areas (34) and overestimates of current gene flow. These gene flow estimates would then be biased because they do not distinguish between current and historical events (32,33).

This study highlights some of the difficulties in interpreting data on population genetic structure. We can conclude with little reservation from differences in allele frequencies that gene flow is restricted to some degree among subpopulations of *C. parasitica* in eastern North America. However, ascribing further causation or estimating current levels of gene flow with any accuracy is not possible. The interpretations are subjective because it is not possible to determine with certainty whether long-distance gene flow is rare in this species in North America or whether populations are approaching equilibrium. Unfortunately, making inferences about evolutionary processes from population genetic data often relies on assumptions that cannot be independently tested (e.g., long-distance gene flow and equilibrium). Caution is needed in future studies, or in interpreting data from published studies, on gene flow in plant pathogens because, in most cases, current levels of gene flow cannot be distinguished from historical effects, as demonstrated in *C. parasitica* in eastern North America.

LITERATURE CITED

1. Anagnostakis, S. L. 1987. Chestnut blight: The classical problem of an introduced pathogen. Mycologia 79:23-37.
2. Anagnostakis, S. L. 1988. *Cryphonectria parasitica:* Cause of chestnut blight. Adv. Plant Pathol. 6:123-136.
3. Anagnostakis, S. L. 1994. Chestnuts and the introduction of chestnut blight. Annu. Rep. North. Nut Growers Assoc. 83:39-42.
4. Anagnostakis, S. L., and Kranz, J. 1987. Population dynamics of *Cryphonectria parasitica* in a mixed-hardwood forest in Connecticut. Phytopathology 77:751-754.
5. Boeger, J. M., Chen, R. S., and McDonald, B. A. 1993. Gene flow between geographic populations of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. Phytopathology 83:1148-1154.
6. Ennos, R. A., and Swales, K. W. 1991. Genetic variability and popula-

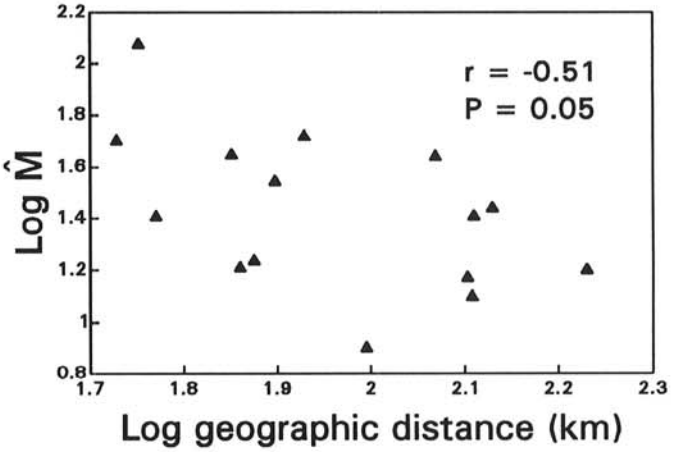

Fig. 3. The relationship between the estimated average number of migrants per generation between each pair of subpopulations, $\hat{M}$, and geographic distance for *Crumenulopsis sororia,* a fungal pathogen of Scots pine, in Scotland. These data were taken from Ennos and Swales (6) and are shown here to illustrate the concept of isolation by distance (34) in a native pathogen. The correlation of $\log(\hat{M})$ and log(geographic distance) is significant ($P = 0.05$).

tion structure in the canker pathogen *Crumenulopsis sororia*. Mycol. Res. 95:521-525.
7. Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C., and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81:1330-1336.
8. Fulbright, D. W., Weidlich, W. H., Haufler, K. Z., Thomas, C. S., and Paul, C. P. 1983. Chestnut blight and recovering American chestnut trees in Michigan. Can. J. Bot. 61:3164-3171.
9. Garrod, S. W., Fulbright, D. W., and Ravenscroft, A. V. 1985. Dissemination of virulent and hypovirulent forms of a marked strain of *Endothia parasitica* in Michigan. Phytopathology 75:533-538.
10. Goodwin, S. B., Saghai-Maroof, M. A., Allard, R. W., and Webster, R. K. 1993. Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. Mycol. Res. 97:49-58.
11. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
12. Gordon, T. R., Okamoto, D., and Milgroom, M. G. 1992. The structure and interrelationship of fungal populations in native and cultivated soils. Mol. Ecol. 1:241-249.
13. Griffin, G. J. 1986. Chestnut blight and its control. Hortic. Rev. 8:291-336.
14. Griffin, G. J. 1989. Incidence of chestnut blight and survival of American chestnut in forest clearcut and neighboring understory sites. Plant Dis. 73:123-127.
15. Hebard, F. V. 1982. Biology of virulent and hypovirulent *Endothia parasitica* on American chestnut (*Castanea dentata*). Ph.D. diss. Virginia Polytechnic Institute, Blacksburg.
16. Karl, S. A., and Avise, J. C. 1992. Balancing selection at allozyme loci in oysters: Implications from nuclear RFLPs. Science 256:100-102.
17. Kohli, Y., Brunner, L. J., Yoell, H., Milgroom, M. G., Anderson, J. B., Morrall, R. A., and Kohn, L. M. 1995. Clonal dispersal and spatial mixing in populations of a plant pathogenic fungus. Mol. Ecol. 4:69-77.
18. Leuchtmann, A., and Clay, K. 1989. Isozyme variation in the fungus *Atkinsonella hypoxylon* within and among populations of its host grasses. Can. J. Bot. 67:2600-2607.
19. Leung, H., and Williams, P. H. 1986. Enzyme polymorphism and genetic differentiation among geographic isolates of the rice blast fungus. Phytopathology 76:778-783.
20. Liu, Y.-C., and Milgroom, M. G. 1992. Analysis of genetic diversity within vegetative compatibility groups of *Cryphonectria parasitica* using DNA fingerprinting. (Abstr.) Phytopathology 82:245.
21. McDermott, J. M., and McDonald, B. A. 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31:353-373.
22. McDonald, B. A., and Martinez, J. P. 1990. DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathology 80:1368-1373.
23. Merkel, H. W. 1905. A deadly fungus on the American chestnut. Pages 97-102 in: N.Y. Zool. Soc. Annu. Rep., 10th.
24. Michna, A. F., II. 1988. Comparison of vegetative compatibility and sporulation of *Endothia parasitica* from selected sites in Michigan and West Virginia. M.S. thesis. West Virginia University, Morgantown.
25. Milgroom, M. G., Lipari, S. E., Ennos, R. A., and Liu, Y.-C. 1993. Estimation of the outcrossing rate in the chestnut blight fungus, *Cryphonectria parasitica*. Heredity 70:385-392.
26. Milgroom, M. G., Lipari, S. E., and Powell, W. A. 1992. DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics 131:297-306.
27. Milgroom, M. G., Lipari, S. E., and Wang, K. 1992. Comparison of genetic diversity in the chestnut blight fungus, *Cryphonectria* (*Endothia*) *parasitica* from China and the US. Mycol. Res. 96:1114-1120.
28. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.
29. Nei, M., and Chesser, R. K. 1983. Estimation of fixation indices and gene diversities. Ann. Hum. Genet. 47:253-259.
30. Peever, T. L., and Milgroom, M. G. 1994. Genetic structure of *Pyrenophora teres* populations determined with RAPD markers. Can. J. Bot. 72:915-923.
31. Prey, A., Hall, D., and Cummings Carlson, J. 1988. Forest pest conditions in Wisconsin. Wis. Dep. Nat. Res. Bur. For. Annu. Rep. 1987.
32. Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.
33. Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787-792.
34. Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264-279.
35. Swofford, D. L., and Selander, R. B. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-283.
36. Wang, K., and Milgroom, M. G. 1994. Genetic diversity in populations of *Cryphonectria parasitica* in eastern China. (Abstr.) Phytopathology 84:1127.
37. Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-156.
38. Wright, S. 1943. Isolation by distance. Genetics 28:114-138.
39. Zambino, P. J., and Harrington, T. C. 1989. Isozyme variation within and among host-specialized varieties of *Leptographium wageneri*. Mycologia 81:122-133.