Genetic Diversity in Field Populations of *Cochliobolus carbonum* on Corn in North Carolina

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ABSTRACT

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Mean frequencies of race 2 (round and oval lesions) and race 3 (long, linear lesions) among 314 isolates of *Cochliobolus carbonum* from corn leaves in 10 North Carolina fields were 0.80 and 0.20 in the western piedmont and 0.82 and 0.18 in eastern North Carolina. Even though races 2 and 3 occurred in the same fields, they were genetically distinct. There were clear distinctions in lesion types, which are polygenically inherited, and the frequencies of cycloheximide tolerance and ability to form pseudothecia differed significantly in the two races. Frequencies of race, mating type, ability to form pseudothecia and asci with ascospores, and tolerance of cycloheximide and carboxin varied considerably from field to field, even within short geographical distances. This suggests that inoculum dispersal and gene flow among populations is restricted in *C. carbonum*. Calculations of Nei's genetic distances between field populations based on frequencies of these polymorphic traits were not correlated with geographical distance between the fields, indicating that the traits are not good indicators of microevolutionary divergence between populations, probably because the traits are not selectively neutral.

Additional keywords: *Bipolaris zeicola*, *Helminthosporium carbonum*, maize, *Zea mays*.

Cochliobolus carbonum Nelson (anamorph *Bipolaris zeicola* (Stout) Shoemaker = *Helminthosporium carbonum* Ullstrup) is a common necrotrophic foliar pathogen of corn (*Zea mays* L.) and other grasses (16,17). Three races of *C. carbonum* pathogenic to corn have been described, and a fourth, race 0, which is avirulent to corn was reported recently (20). Race 1, which produces a host-specific toxin, rarely is found in the field because modern corn hybrids are resistant to the toxin (9). Race 2 induces small, round to oval lesions on corn leaves and is common nearly everywhere corn is grown, but race 2 rarely causes significant damage (3). Race 3, which induces long, linear lesions on most corn inbreds and hybrids, is the most prevalent race of *C. carbonum* on corn in the Appalachian Mountains from Georgia to Pennsylvania (9). It first was described in 1973 (15) although there is good evidence that race 3 had been common in the mountains of Virginia at least 20 years earlier (9).

Race 3 spread from the mountains and was first found in eastern North Carolina in 1974. By 1977 it had increased to 17% of the population of *C. carbonum* in fields there. The transition from populations that were predominantly race 3 to those predominantly race 2 occurred along a steep cline over a 7- to 20-km distance east from the Blue Ridge escarpment (11). In this region where both races were common, they were isolated frequently from the same corn leaf.

In spite of sharing a common habitat in North Carolina, race 2 and race 3 appeared to be genetically isolated in the 1970s. The distinctive lesion type of race 3 is polygenically inherited (2,8), but no isolates with intermediate lesion types were found in the 1976–1977 survey (11), indicating little or no hybridization between the races. In addition, identified genes for pseudothecial production and sensitivity to cycloheximide occurred at significantly higher frequencies in race 2 than in race 3 (9,11), which also suggests lack of genetic exchange between races.

The role of the sexual stage in the life cycle of *C. carbonum* and its role in maintaining genetic diversity in the fungus populations have not been determined. Fertile pseudothecia of *C. carbonum* can be induced readily in the laboratory, but pseudothecia have not been found in the field. No genetic barriers to the production of hybrids or exchange of genes between the races 2 and 3 have been detected in laboratory crosses. The lack of genetic recombination between race 2 and race 3 in the field may indicate that the sexual stage is rare or absent in nature or that races 2 and 3 only recently came into contact with each other. The previous studies (9,11) may have been made too early in the overlapping of geographical ranges of races 2 and 3 for significant genetic exchange to have taken place.

The objectives of the present study were to determine whether the frequency of race 3 continued to increase in eastern North

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Carolina after 1977 and whether the range of predominantly race 3 populations had expanded eastward from the Blue Ridge escarpment, compare frequencies of previously identified polymorphic traits within race 2 and race 3 populations to assess the possible extent of genetic exchange between the two races since 1977, and compare genetic variation among populations of *C. carbonum* in selected corn fields within and between geographical regions of North Carolina as an indication of gene flow between fields or regions.

MATERIALS AND METHODS

Sampling. Ten corn fields in North Carolina were sampled for foliar infections of *C. carbonum* in 1985. A single leaf per plant was sampled from plants at approximately 2-m intervals along three rows 5–10 m apart in the fields. Sampled leaves were taken without regard to symptoms. To allow the greatest probability of infection of sampled leaves by *C. carbonum* without excessive colonization by saprophytes, we selected the oldest leaves that had not yet senesced. Leaves were collected from 60 plants in fields 1–9 and from 42 plants in field 10.

Six of the sampled fields were in Wilkes County, which extends eastward from the Blue Ridge Mountains in northwestern North Carolina, and a seventh field was in Yadkin County, which borders the eastern edge of Wilkes County (Figs. 1 and 2) (10). Analysis of samples along transects through this region in 1976–1977 identified the region as a transition zone between predominantly race 3 populations of *C. carbonum* in the mountains and predominantly race 2 populations in the piedmont (11). Field 9 also was just east of the Blue Ridge Mountains in McDowell County southwest of Wilkes County. Fields 7 and 8 were in eastern North Carolina; field 8 was in Wake County at the eastern edge of the piedmont and field 7 was in Edgecombe County in the coastal plain. Elevations of the fields ranged from 38 m above sea level in the east to 428 m in western North Carolina (Table 1).

Each field was sampled once. Fields 1–6 were sampled on 16 July; fields 7, 8, and 9 were sampled on 24, 25, and 28 July, respectively; and field 10 was sampled on 8 August. Sampled leaves were dried in a plant press and kept at room temperature until used.

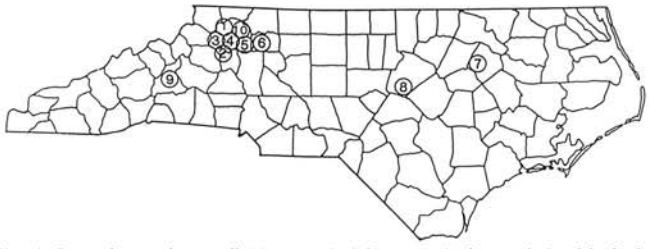

Fig. 1. Locations of corn fields sampled for populations of *Cochliobolus carbonum* in North Carolina in 1985.

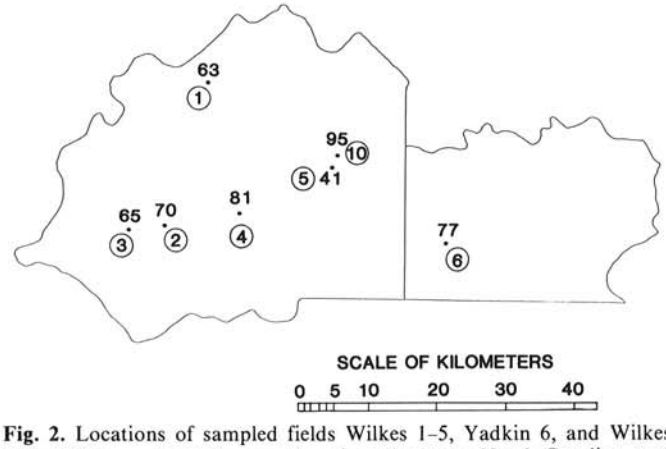

Fig. 2. Locations of sampled fields Wilkes 1–5, Yadkin 6, and Wilkes 10 in Wilkes and Yadkin counties of northwestern North Carolina, and incidences (%) of *Cochliobolus carbonum* in corn leaves sampled from those fields.

Isolation. Pieces of leaf blades up to 8 cm long were surface sterilized by immersion for 30 sec in 70% ethanol followed by 30 sec in 0.5% NaOCl, rinsed in tap water, and incubated on moist filter paper in petri dishes at 22–24 C with a 12-hr photoperiod under fluorescent lights. After 2–4 days of incubation, the leaf pieces were examined for conidiophores and conidia of *C. carbonum*. Conidia were picked from conidiophores with a sterile needle and transferred to potato-dextrose agar (PDA) with 10 g of dextrose/L. Resulting cultures, which originated from one to several conidia from a single conidiophore, were transferred once to ensure purity and stored as conidial suspensions in 30% glycerol frozen at −70 C. Usually, a single isolate of *C. carbonum* was kept per leaf sample. When two isolates were kept from the same leaf sample, the isolates were obtained from different surface-sterilized leaf pieces to ensure independence of the isolates.

Race identification. Pathogenic race identifications of isolates were based on lesion type on seedlings of Pioneer Brand 3369A hybrid corn grown and inoculated in the greenhouse. Inoculum was prepared by washing conidia from 7- to 10-day-old cultures on PDA and filtering the suspensions through four layers of cheesecloth. Conidial suspensions were sprayed onto 3- to 4-wk-old seedlings in the four- to six-leaf stage with an atomizer (DeVilbiss Co., Somerset, PA) attached to an air pump. Atomizers were rinsed with 70% ethanol between inoculations with different isolates. Inoculated plants were incubated overnight in a polyethylene moist chamber in the greenhouse. Lesion types were recorded 1 wk after inoculation.

Mating type and fertility. Mating type (MAT) and fertility of each isolate were determined by pairing the field isolate with known albino tester isolates (7) of MAT-1 (A) and MAT-2 (a) of *C. carbonum*. Autoclaved 1-cm-diameter disks of senescent corn leaves were placed on modified Sachs agar (6) in petri dishes, and mycelial plugs from 5- to 7-day-old PDA cultures of the isolates to be paired were placed on opposite sides of the leaf disks. Mating plates were incubated in the dark at 20 C. Pseudothecia formed within 7 days in fertile combinations of opposite mating types. The albino tester isolates formed white pseudothecia; therefore the ability of the field isolates to form pseudothecia was determined by the presence or absence of wild-type, black pseudothecia. A single gene conditioning inability to form pseudothecia is common in populations of *C. carbonum* (9,11,14). After 20–25 days, 5–10 black pseudothecia from each mating were crushed and examined for asci and ascospores. Matings in which white but not black pseudothecia were produced

TABLE 1. Genetic diversity and proportion of race 3 in populations of *Cochliobolus carbonum* in 10 North Carolina corn fields sampled in 1985

Region and field	Elevation (m)	No. of isolates	Proportion of race 3	Shannon index of diversity[a]
West				
Wilkes 1	428	42	0.20	2.37 ± 0.15[b]
Wilkes 2	330	46	0.46	2.45 ± 0.11
Wilkes 3	390	33	0.06	2.17 ± 0.11
Wilkes 4	310	51	0.28	2.39 ± 0.09
Wilkes 5	360	23	0.04	1.87 ± 0.17
Yadkin 6	350	51	0.14	2.46 ± 0.10
Wilkes 10	296	61	0.20	2.34 ± 0.12
McDowell 9	370	6	0.00	1.56 ± 0.26
Mean			0.20[c]	2.20
East				
Edgecombe 7	35	39	0.32	2.34 ± 0.13
Wake 8	120	37	0.03	2.10 ± 0.12
Mean			0.18	2.21

[a]Based on frequencies of races 2 and 3 and five traits listed in Table 2.
[b]Standard error.
[c]Proportion of race 3 in McDowell field 9 was excluded from the mean because of the small number of isolates of *C. carbonum*.

or in which black pseudothecia failed to form asci or ascospores were repeated. Inability to form pseudothecia, asci, or ascospores was regarded as confirmed if none were formed in any of three serially replicated mating tests with pairings on two leaf disks per test.

Fungicide tolerance. Mycelial plugs (5 mm diameter) from the margins of 5- to 7-day-old cultures of each isolate on PDA were transferred to plates of PDA amended with either 2 μg/ml of cycloheximide (3-2-[3,5-dimethyl-2-oxocyclo-hexyl]-2-hydroxyethyl glutarimide) or 100 μg/ml of carboxin (5,6-dihydro-2-methyl-1,4-oxanthiin-3-carboxanilide). Control plugs were transferred to unamended PDA at the same time. Isolates were considered sensitive to the fungicide if radial growth after 5 days on fungicide-amended PDA was less than 20% of that on unamended PDA (9). Monogenic control of tolerance/susceptibility to the fungicides at these concentrations was demonstrated by MacKenzie et al (12) and Leonard (9).

Data analysis. The Gleason, Shannon, and Simpson indexes of genetic diversity (4) were calculated for populations from each field and for races 2 and 3 over all fields. The Gleason index reflects the number of distinct phenotypes detected in a sample and is calculated from r, the number of phenotypes detected in the sample, and N, the number of isolates in the sample, according to the following equation:

$$H_g = (r - 1)/\log_e N$$

The Shannon and Simpson indexes reflect not only the number of phenotypes detected but also the relative evenness of their frequencies in the sample (that is, lack of dominance of one or a few phenotypes). The Shannon index is calculated according to the following equation:

$$H_w = -\sum_i p_i \log_e p_i$$

in which p_i is the frequency of the ith phenotype. Standard errors of Shannon indexes were calculated as described by Poole (18). In the Simpson index:

$$H_s = 1 - \sum_i [n_i(n_i - 1)/N(N - 1)]$$

n_i is the number of isolates of the ith phenotype, and N is the sample size.

Nei's (13) standard genetic distance was calculated between race 2 populations in each possible paired combination of fields sampled in 1985 and between race 2 and race 3 populations over all fields. Frequencies of race 2 and race 3 were not included in calculations of genetic distance between field populations because the difference in lesion type between the two races is inherited polygenically and because evidence from the survey indicated that little or no genetic interchange occurred between the two races. Nei's standard genetic distance is given by

$$D = -\log_e I$$

in which

$$I = J_{XY}/(J_X J_Y)^{1/2}$$

For dimorphic loci, the frequencies of the two alleles at the ith locus in populations X and Y can be written as p_{Xi} and $(1 - p_{Xi})$ in X and p_{Yi} and $(1 - p_{Yi})$ in Y. In this case with just two alleles per locus:

$$J_{XY} = (1/L) \sum_i [p_{Xi}p_{Yi} + (1 - p_{Xi})(1 - p_{Yi})]$$

$$J_X = (1/L) \sum_i [p_{Xi}^2 + (1 - p_{Xi})^2]$$

$$J_Y = (1/L) \sum_i [p_{Yi}^2 + (1 - p_{Yi})^2]$$

in which L is the number of loci.

We calculated a second measure of genetic distance among populations of race 2 because of evidence that reproduction by *C. carbonum* in the field is primarily asexual. Nei's standard genetic distance is an estimate of the mean number of net codon substitutions in the DNA of two randomly chosen genomes of different populations. It is most applicable to alleles of neutral fitness in randomly mating populations. In asexual populations, the alleles at different loci may be associated in clones that represent only a small proportion of the total number of possible genotypes. A pair of asexual populations with equal frequencies of each allele still could be genetically dissimilar if they do not share clones of identical genotypes. For instance, a population with 50% A,B and 50% a,b genotypes is not the same as a population with 50% A,b and 50% a,B genotypes even though their gene frequencies are identical.

Therefore, we adapted Nei's measure of minimum genetic distance to compare differences in frequencies of phenotypes within each population. Considering a single locus with multiple alleles, Nei's minimum genetic distance is given by

$$d = \Sigma (p_{Xi} - p_{Yi})^2/2$$

in which p_{Xi} and p_{Yi} are the frequencies of the ith allele at the locus in populations X and Y, respectively. As Nei (13) pointed out, d is equal to the square of Roger's distance, a measure often used in comparing phenotypic differences in numerical taxonomy. In our adaptation of this measure for asexual populations, each distinct phenotype is treated in this equation as a separate allele at a single locus; therefore p_{Xi} and p_{Yi} represent the frequencies of the ith phenotype in populations X and Y. Thus, populations are compared on the basis of the frequencies of the phenotypes that occur in the populations, not on the basis of the frequencies of individual alleles that make up those phenotypes.

Phenograms of genetic distance displaying relationships among populations were computed by the unweighted pair-group method with arithmetic mean (13). In this method genetic distances are calculated between all possible pairs of populations. The pair separated by the least genetic distance is identified. Then the mean genetic distance between that pair and every other population is computed. If one of the other populations is more closely related to the first pair than to any other population, it is added to the pair to make a cluster of three, and the process is repeated to compare each remaining population with the cluster, and so

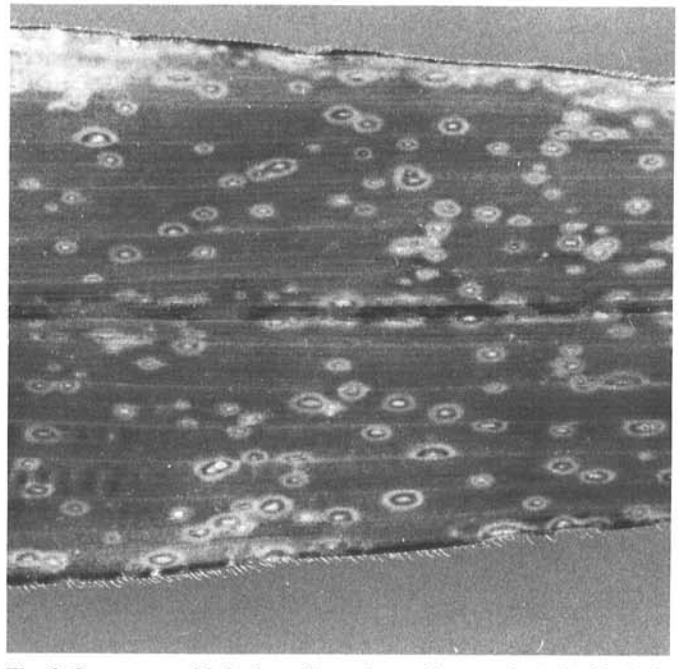

Fig. 3. Symptoms of infection of corn leaves by race 2 of *Cochliobolus carbonum*.

on. New clusters are formed when populations within the remaining group show closer relationships to each other than to any established cluster. When there is more than one cluster, the mean distance between all members of each cluster is computed to determine genetic distance between the clusters. In this way, a hierarchy of population clusters is derived, from the genetically most closely related clusters to the most distant clusters.

To test for nonrandomness in associations of specific traits within race 2 and race 3 populations, two-way contingency tables were prepared listing the four combinations of the two forms of the first trait (for example, MAT-1 and MAT-2) with the two forms of the second trait (for example, Psu+ [ability to form pseudothecia] and Psu−) as described by Alexander et al (1). Observed numbers of phenotypes in each category were compared with the numbers expected from random assortment of the traits as determined by their frequencies in the population. The *G*-test, a log likelihood test of independence, was used to determine the statistical significance of deviations of associations from expected values (19).

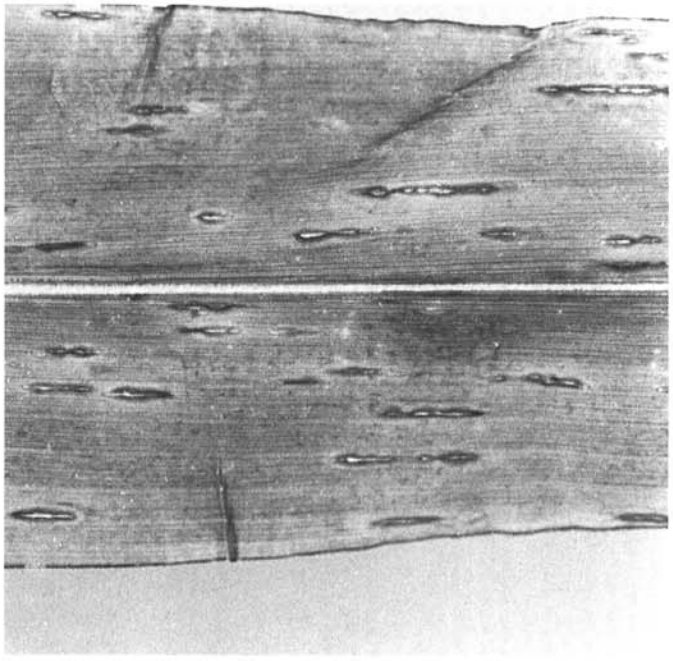

Fig. 4. Symptoms of infection of corn leaves by race 3 of *Cochliobolus carbonum*.

RESULTS

Incidence of *C. carbonum* was high in sampled leaves from all fields except field 9 (11%) in McDowell County. *C. carbonum* was isolated from 41 to 95% of the leaves collected from fields in Wilkes and Yadkin counties (Fig. 2), from 62% in Wake County, and from 66% in Edgecombe County. All isolates of *C. carbonum* induced lesions typical of either race 2 or race 3 (Figs. 3 and 4); no other races or intermediate types were found.

Frequencies of race 3 varied from 0 to 0.46 in the sampled fields (Table 1). The mean frequency of race 3 in fields of western North Carolina did not differ significantly from that in eastern fields, and there was no correlation between frequency of race 3 and altitude of the sampled fields. No evidence of an east-west cline in frequency of race 3 was found in the Wilkes County-Yadkin County area.

Frequencies of other polymorphic traits varied considerably among fields and between races 2 and 3 (Table 2). With the exception of mating type and ability to form asci and ascospores, the frequencies of traits in race 2 were not related to the frequencies of the same traits in race 3 in the same field. Over the six fields with at least 10% race 3, the frequency of mating type ($R^2 = 0.68$, $P < 0.05$) and ability to form asci and ascospores ($R^2 = 0.87$, $P < 0.05$) were significantly correlated between race 2 and race 3 populations in the same fields. Cycloheximide tolerance and carboxin tolerance were the least variable traits within races. The mean frequency of cycloheximide tolerance was significantly lower in race 2 than in race 3, whereas mean frequency of the ability to form pseudothecia was significantly greater in race 2 than in race 3.

Of the three diversity indices calculated, only the Shannon index values are presented in Table 1; over all fields the Shannon indices were significantly correlated with the Simpson indices ($R^2 = 0.66$, $P < 0.05$) and highly significantly correlated with the Gleason indices ($R^2 = 0.88$, $P < 0.001$). None of the indices of diversity was significantly correlated with sample size in these comparisons. Although the amounts of genetic diversity varied among fields, there was no broad geographical pattern to the variation. On average, there was little difference between genetic diversity within the fields in western North Carolina (Shannon index = 2.20) and that within fields in eastern North Carolina (Shannon index = 2.21). Over all fields, race 2 (Shannon index = 2.35, Gleason index = 2.79, Simpson index = 0.88) was more genetically variable than race 3 (Shannon index = 1.69, Gleason index = 2.30, Simpson index = 0.76). The difference in Shannon indices for races 2 and 3 was highly significant ($P < 0.001$); Poole (18) does not give methods for comparing the statistical significance of Gleason and Simpson indices.

TABLE 2. Frequencies of dimorphic traits in populations of race 2 of *Cochliobolus carbonum* isolated from corn leaves in 10 fields in North Carolina in 1985

	Frequency of indicated trait[a] in race 2 and 3									
	MAT-1		Psu+		Asc+[b]		CyhR		CrbR	
Field	R-2	R-3	R-2	R-3	R-2	R-3	R-2	R-3	R-2	R-3
Wilkes 1	0.24	0.38	0.36	0.38	0.83	0.67	0.74	0.88	0.97	0.88
Wilkes 2	0.68	0.67	0.56	0.24	0.29	0.17	0.50	0.86	1.00	1.00
Wilkes 3	0.62	···	0.65	···	0.75	···	0.56	···	1.00	···
Wilkes 4	0.59	0.57	0.78	0.29	0.79	0.75	0.68	0.93	0.97	1.00
Wilkes 5	0.33	···	0.50	···	0.45	···	0.71	···	1.00	···
Yadkin 6	0.43	0.57	0.41	0.00	0.71	···	0.66	0.86	0.95	1.00
Edgecombe 7	0.27	0.46	0.70	0.23	0.84	1.00	0.77	1.00	1.00	1.00
Wake 8	0.56	···	0.64	···	0.70	···	0.56	···	0.97	···
McDowell 9	0.67	···	0.33	···	0.50	···	0.83	···	0.83	···
Wilkes 10	0.35	0.33	0.71	0.17	0.89	1.00	0.67	1.00	0.96	0.92
Mean[c]	0.47	0.50	0.56	0.22	0.60	0.67	0.67	0.92	0.96	0.97
	NS		*		NS		*		NS	

[a] MAT-1 indicates mating type 1; Psu+ indicates the ability to form pseudothecia; Asc+ indicates the ability to form ascospores; CyhR indicates tolerance of cycloheximide; and CrbR indicates tolerance of carboxin.

[b] Frequency of ability to form ascospores applies only to those isolates able to form pseudothecia.

[c] Means of fields with greater than 10% frequency of race 3. * indicates that the means are significantly different at $P < 0.05$ (Student's *t*-test); NS indicates that no significant difference was detected.

The two most similar pairs of race 2 populations by the measure of Nei's standard genetic distance, Wilkes 3 with Wake 8 and Edgecombe 7 with Wilkes 10, each match an eastern with a western field (Fig. 5). The race 2 population in Wilkes 2, located a few kilometers from Wilkes 3 and Wilkes 4, was rated genetically distant from populations in those fields and from those in other fields in Wilkes County. In fact, the Nei's standard genetic distance between race 2 and race 3 over all fields (0.058) was less than the genetic distance between Wilkes 2 and McDowell 9 populations of race 2 and those of other fields. This distance between race 2 and race 3 was equal to the genetic distance between two clusters of race 2 populations from Wilkes 3, Wake 8, Wilkes 4, Edgecombe 7, and Wilkes 10 in one group and Wilkes 1, Yadkin 6, and Wilkes 5 in the other (Fig. 5).

A total of 17 of the 24 possible phenotypes was found in race 2 populations with a maximum of 13 in any single field. Calculating genetic distance from phenotype frequencies by the adaptation of Nei's minimum genetic distance for multiple alleles at a single locus yielded a phenogram very similar to that for Nei's standard genetic distance (Fig. 6). The genetic distance between race 2 and race 3 calculated by this method was 0.069, which is less than the distance between the two major clusters of race 2 populations and that of Wilkes 2 and McDowell 9 populations from the rest of the race 2 populations.

Of the nine possible associations between pairs of traits, four could not be tested for significance for race 2 isolates because the two-way contingency tables for these traits contained one or more cells with expected frequency of less than five (19). Four of the five pairs that could be tested showed significant deviations from frequencies expected from random combination of traits (Table 3). For race 3, only one of nine possible associations could be tested, and that did not show a significant deviation from random combination of traits.

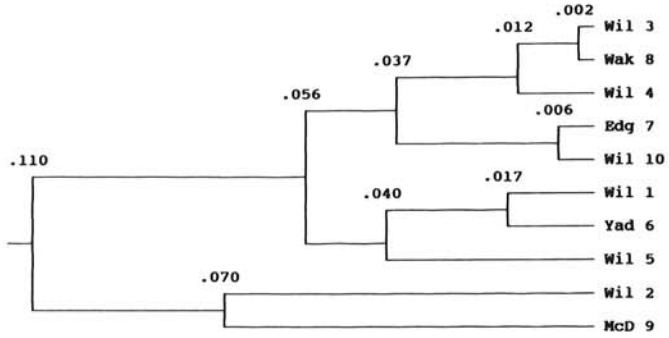

Fig. 5. Phenogram based on the unweighted pair-group method with arithmetic mean of Nei's standard genetic distance between populations of race 2 in 10 corn fields in North Carolina in 1985. Locations of fields are indicated in Table 1 and Figures 1 and 2. Values of Nei's standard genetic distance range from 0 for populations with identical gene frequencies over all loci to infinity for populations that share no alleles.

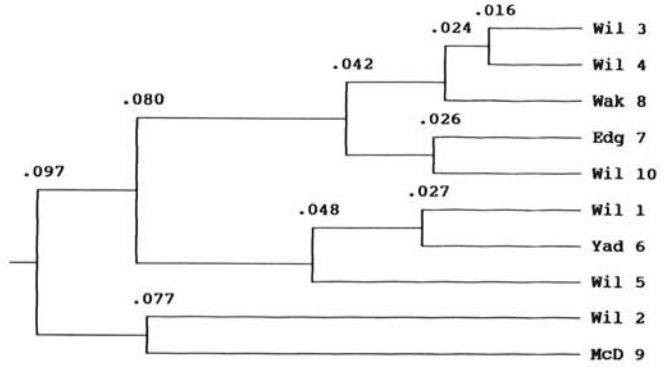

Fig. 6. Phenogram based on the unweighted pair-group method with arithmetic mean of an adaptation of Nei's minimum genetic distance adapted for asexual populations in which phenotypes are treated as the unit of variation to calculate genetic distance between populations of race 2 in 10 corn fields in North Carolina in 1985. Locations of fields are indicated in Table 1 and Figures 1 and 2. Values of the adapted minimum genetic distance range from 0 for populations with identical frequencies of each phenotype to 1.0 for populations that are monomorphic for different phenotypes.

DISCUSSION

In 1977 it appeared that race 3 of *C. carbonum* was increasing in frequency in eastern North Carolina and that the transition region (that is, between the predominantly race 3 populations of the Appalachian Mountains and the predominantly race 2 populations typical of lower elevations) might be moving eastward into the piedmont (11). The possibility that race 3 might eventually replace race 2 in the coastal plain where approximately 85% of the corn in North Carolina is grown caused concern because the larger lesions of race 3 make it a potentially more damaging foliar pathogen of corn. The 1985 survey established, however, that race 3 has not increased in frequency in North Carolina and that, in fact, its frequency in the western piedmont has declined since 1977. The reasons for this decline are not known.

In 1985, after coexisting with race 2 in eastern North Carolina corn fields for at least 11 yr, race 3 remained genetically distinct from race 2. Isolates with intermediate lesion types were not found, and gene frequency differences between the two races for ability to form pseudothecia and tolerance of cycloheximide were nearly as great in 1985 as in the earlier years (Table 4). The continuing presence of races 2 and 3 as genetically distinct populations in the same fields supports the conclusion that sexual reproduction by *C. carbonum* is rare or absent in these fields. This conclusion is supported further by the observed nonrandom association of genes in race 2. Four of the five pairs of traits that could be

TABLE 3. Association of phenotypic characters in isolates of race 2 and race 3 of *Cochliobolus carbonum* collected in North Carolina in 1985

Phenotype[a] combination	Number of isolates			
	Race 2		Race 3	
	Observed[b]	Expected[b]	Observed[b]	Expected[b]
MAT-1, Psu+	89	84 NS	10	9 NS
MAT-2, Psu−	75	70	31	30
MAT-1, Psu−	56	61	28	29
MAT-2, Psu+	92	97	8	9
MAT-1, Asc−	36	23 $P < 0.0001$		
MAT-2, Asc+	81	68		
MAT-1, Asc+	53	66		
MAT-2, Asc−	11	24		
MAT-1, CyhS	63	47 $P < 0.002$		
MAT-2, CyhR	123	110		
MAT-1, CyhR	82	95		
MAT-2, CyhS	44	52		
Psu+, CyhR	130	119 $P < 0.02$		
Psu−, CyhS	56	45		
Psu+, CyhS	51	62		
Psu−, CyhR	75	86		
Asc+, CyhR	111	96 $P < 0.001$		
Asc−, CyhS	28	13		
Asc+, CyhS	23	38		
Asc−, CyhR	19	34		

[a] MAT-1 indicates mating type 1; Psu+ indicates the ability to form pseudothecia; Asc+ indicates the ability to form ascospores; and CyhR indicates tolerance of cycloheximide.

[b] Observed number of isolates with indicated phenotype, and expected number of isolates with indicated phenotype if the traits were randomly combined. Significance levels for deviations of the four expected numbers from observed numbers for the phenotypic characters of the two indicated dimorphic loci are based on the *G*-test for nonrandomness in two-way contingency tables (19). NS indicates that no significant association was detected.

analyzed statistically showed significant deviations from phenotype frequencies expected in a randomly mating population. Genetic linkage could not account for the observed nonrandom association of MAT-1 with CyhS and MAT-2 with CyhR in race 2 because the genes for these traits have been shown to segregate independently (9).

Field populations of *C. carbonum* showed considerable variation in frequency of race 3 and in frequencies of other polymorphic traits, even when the fields were located close to one another. For instance, fields 2 and 3 in Wilkes County were less than 5 km apart, but the frequency of race 3 in Wilkes 2 was 46% and that in Wilkes 3 was only 6%. In the absence of evidence of strong selection to maintain different frequencies of genes in different corn fields, the variation from field to field suggests that inoculum dissemination is limited for this fungus and that local populations on corn are established more or less independently each year from local inoculum sources. Even small amounts of migration between populations would prevent significant genetic divergence of the populations through genetic drift (5).

An interesting feature of the 1976–1977 survey was that mating type frequencies differed between regions of North Carolina but were similar for race 2 and race 3 within regions. This similarity of mating type frequencies for races 2 and 3 was demonstrated within fields in the 1985 survey. The frequencies of MAT-1 in races 2 and 3 were significantly correlated over a range of 0.24 to 0.68 in the six fields in which comparisons could be made. Apparently, the variation in mating type frequencies is not accounted for solely by genetic drift because drift should occur independently in the two genetically isolated races. The evidence shows that sexual reproduction is not important in field populations of *C. carbonum*, and thus some other force of selection must prevent mating type frequencies from deviating too far from 0.5. That force seems to affect coexisting populations of race 2 and race 3 similarly even though they remain genetically dissimilar in other respects.

Based on the five polymorphic traits studied, race 2 is genetically more variable than race 3. This is consistent with the recent discovery of race 3 (15) and with the broad geographical range of race 2 compared with the restricted known range of race 3. In the 1972–1975 survey, Leonard (9) concluded that race 2 isolates from North Carolina were genetically more similar to race 2 isolates from the midwestern United States than to race 3 isolates from North Carolina.

Calculated values of genetic distance among field populations of race 2 were not correlated with geographical distances between the fields. This suggests that the traits studied are not selectively neutral. With the possible exception of the gene for carboxin tolerance, none of the genes controlling these traits had become fixed in the race 2 populations in any of the fields studied. Instead, the frequencies of the genes seem to fluctuate within limits about an equilibrium. For instance, mean frequencies of cycloheximide tolerance and the ability to form pseudothecia in race 2 have remained quite stable from 1972 to 1985 (Table 4). In fact, the frequency of cycloheximide tolerance has changed little since the mid-1960s when MacKenzie et al (12) found that 66% of their race 2 isolates were tolerant to cycloheximide. If gene frequencies for different traits fluctuate about equilibria and if there is little migration between field populations, the frequencies of traits in nearby fields at any given time could differ and those in distant fields could be similar depending on the direction and timing of the fluctuations. In that case, calculated genetic distances based on frequencies of these traits would not give a true measure of the microevolutionary divergence of geographically separated populations. Frequencies of allozyme or restriction fragment length polymorphism markers are better choices for comparisons of genetic distance because they are more likely to be selectively neutral.

TABLE 4. Comparison of frequencies of polymorphic traits in populations of *Cochliobolus carbonum* in North Carolina from 1972 to 1985

	Frequency of trait[b] in race 2 and race 3					
	Psu+		CyhR		CrbR	
Years[a]	Race 2	Race 3	Race 2	Race 3	Race 2	Race 3
1972–75	0.67	0.17	0.79	1.00	0.95	1.00
1976–77	0.50	0.11	0.75	0.99	0.99	0.99
1985	0.58	0.23	0.67	0.91	0.97	0.97

[a]Data for years 1972–75 are from Leonard (9) and for years 1976–77 are from Lodge and Leonard (11).

[b]Psu+ indicates the ability to form pseudothecia; CyhR indicates tolerance of cycloheximide; and CrbR indicates tolerance of carboxin.

In the 1985 survey, populations of *C. carbonum* were found to be locally self-contained. The fungus is well adapted for survival in crop debris; initial incidences of infection by *C. carbonum* in new corn crops are much greater than those of the more aggressive pathogens *C. heterostrophus* (Drechs.) Drechs. or *Exserohilum turcicum* (Pass.) Leonard & Suggs (10). Conversely, incidences of foliar infection by *C. carbonum* do not increase rapidly during the corn-growing season as those of *C. heterostrophus* and *E. turcicum* often do. This limits the potential for gene flow among populations of *C. carbonum* and permits random genetic drift and local variations in selection pressure to result in significant genetic variation across relatively small geographical distances.

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