Isozyme Variation Within and Among Pathogenic Races of *Cochliobolus carbonum* on Corn in North Carolina

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ABSTRACT

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We used starch gel electrophoresis to investigate isozyme variation among 194 isolates of races 0, 2, and 3 of *Cochliobolus carbonum* collected in 1987 in two corn fields in North Carolina as well as six isolates of race 1 from previous collections in different states of the United States. Of eight enzyme systems studied in detail, only one, phosphogluconate dehydrogenase, was monomorphic. Diaphorase, malate dehydrogenase, glutamate oxaloacetate transaminase, and esterase were highly polymorphic in race 0, whereas isozyme variation in races 2 and 3 was very limited. Race 0 accordingly had a significantly higher average gene diversity than did race 2 or race 3. Genetic similarity between races 2 and 3 was very high. Their allele frequencies differed clearly at only two putative esterase loci. Races 2 and 3 were only distantly related to race 0, as demonstrated by cluster analysis and principal coordinates analysis. There was no apparent genetic variation among five race 1 isolates from North Carolina and South Carolina. Their common electrophoretic type occurred also in race 2 but not in race 0 or race 3, suggesting a common ancestry with race 2. Only a small proportion of race 2 and race 3 isolates or race 2 and race 0 isolates shared common electrophoretic types, which suggests that there is little, if any, genetic exchange between these races.

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

Cochliobolus carbonum R. R. Nelson (anamorph, *Bipolaris zeicola* (G. L. Stout) Shoemaker = *Helminthosporium carbonum* Ullstrup) is an ubiquitous foliar pathogen of corn (*Zea mays* L.) and other grass hosts in many temperate regions of the world (18,34). Several races in this fungus have been described on the basis of their lesion types on corn. The avirulent race 0, observed only in western North Carolina (43,44), causes flecks or minute necrotic lesions on corn. Race 1 produces a host-specific toxin and induces large lesions on susceptible corn genotypes but only small lesions on hybrids that are resistant to the toxin. Since the removal of susceptible corn lines from commercial production, race 1 rarely has been found in the United States (16). In our 1987 survey of two corn fields in North Carolina and one in Tennessee, we found no race 1 isolates among the 90 *C. carbonum* isolates that we tested on the susceptible inbred N31 (44). Race 2 induces small, round to oval lesions and is the most prevalent race in the United States, except in the Appalachian mountains where race 3 predominates (16,17,22,44). Race 3 induces long, linear lesions on corn and has been reported in Japan as a foliar pathogen of both corn (41) and rice (46). Recently, Dodd and Hooker (5) reported a fifth race that causes large, zonate lesions and early leaf death on certain corn genotypes but that is less virulent on inbred W64A.

Races 1, 2, and 3 have different genes for pathogenicity on corn. Race 0 has no known pathogenicity gene (45), but it may be pathogenic on wild grasses. Race 1 expresses a single gene for toxin production (33) and another independent gene or genes for lesion induction (26). The lesion type of race 2 is inherited as two major genes (45) that also may be shared by race 3. Crosses of race 2 × race 3 by Dalmacio et al (4) gave no indication of major gene segregation. Instead, evidence from these crosses and similar crosses by Leonard (14) indicated that the linear lesion type of race 3 is under polygenic control.

The races of *C. carbonum* in North Carolina appear to be genetically isolated because 1) gene frequencies of several phenotypic traits differ significantly between races (44), and 2) no isolates with lesion types intermediate between those of race 2 and race 3 have been observed in the field (16,17,22,44). However, little is known about the genetic relatedness and evolution of *C. carbonum* races. It was suggested that race 3 may be a biotype of race 2, but no evidence was presented (34). Using seven phenotypic markers, Welz and Leonard (44) found that some haplotypes (i.e., marker combinations) were in fact shared by races 2 and 3, while race 0 was relatively distinct from races 2 and 3. Jones and Dunkle (12) presented evidence from polymerase chain reaction amplifications with arbitrary and gene-specific primers that race 0 is genetically distinct from races 1, 2, 3, and 4 of *C. carbonum.*

Isozyme analysis has been a successful tool in studying the evolution of races or formae speciales in other fungal species (e.g., 1,3,7,13). In several of these studies, isozyme markers were particularly helpful, because no other genetic markers were available or because the only available markers, viz. virulence genes, were not selectively neutral. Similarly, the phenotypic traits in *C. carbonum,* such as mating type, fertility, fungicide tolerance, and colony morphology, may affect fitness (17) and, therefore, are not ideal markers for many population studies. Isozymes, on the other hand, have proved to be neutral with regard to fitness in nearly all organisms examined (20).

Recently, Simcox et al (35) reported isozyme polymorphisms in races 1, 2, and 3 of *C. carbonum* in a small collection of isolates from different geographical regions. As in the study by Simcox et al, one of our objectives was to find a set of useful isozyme markers for investigating genetic variation within and among races of *C. carbonum*. Our second objective was to use the isozyme markers to shed some light on the evolution of races of *C. carbonum*. For our study, we selected 194 isolates of races 0, 2, and 3 from our 1987 collection, which had already been characterized for seven other phenotypic traits (44), as well as six race 1 isolates from previous collections.

MATERIALS AND METHODS

Isolates. We tested 29 isolates of race 0, 90 of race 2, and 75 of race 3 that represent subsamples from a larger collection of *C. carbonum* isolates made in 1987 in two fields in North Carolina (44). We also tested six isolates of race 1, five of which were collected between 1965 and 1976 in two fields in North Carolina (four isolates) and one in South Carolina. The sixth race 1 isolate came from Michigan and was also collected before 1977. All isolates were stored as frozen conidial suspensions in 30% glycerol at −70 C.

Isozyme analysis. Isozymes were identified by starch gel electrophoresis. Samples were prepared by growing isolates on potato-lactose agar for about 14 days. When isolates produced a crop of conidia in amounts similar to that of a wild-type race 2 isolate ($>10^6$ conidia per plate), a single plate yielded enough spores for electrophoresis. If sporulation was sparse, conidia from several cultures were combined. The conidia were harvested and suspended in 25 ml of a phosphate buffer (0.01 M, pH 7.0, from a 0.1 M stock solution of 61 ml of 0.2 M disodium hydrogen phosphate, 39 ml of 0.2 M sodium dihydrogen phosphate, and 100 ml of distilled water) containing 2.5 ml of Tween 20 and 10 μl of ethanol. Flasks containing the conidial suspensions were placed on a revolving shaker run at three agitations per second for 5 min to wash off substances that might inhibit germination (24). The suspension was then centrifuged at 1,000 rpm for 4 min, the supernatant was discarded, and the conidia were resuspended in 25 ml of phosphate buffer. This suspension was shaken for 6 h to induce conidial germination and then centrifuged. The conidia were resuspended in a few drops of phosphate buffer and transferred to 2-ml reaction vials, which were stored at −80 C until further use. Usually fewer than 50% of the conidia germinated under the conditions described, but comparative tests indicated that their enzyme activity was higher than that of untreated conidia. Storage for several weeks did not reduce the enzyme activity of the samples.

For enzyme extraction, the frozen conidial suspensions were allowed to thaw and were then cooled over ice. A few grains of quartz sand were added, and the conidia were homogenized with a glass rod for a few seconds. Solutions were then centrifuged for 10 min (15,000 rpm, 4 C). The supernatant was absorbed onto paper chromatography wicks (7 × 3 mm), and the wicks were inserted into a sample slot 2 cm from the cathodic end of the starch gel (12% [w/v] starch, thickness 7 mm, solidified overnight). Gels were cooled on both sides (4 C).

After electrophoresis, each gel was cut into three slices. Three buffer systems were used (Table 1). Eight enzyme systems yielding high activity and well-separated bands were selected for routine work (Table 2): phosphogluconate dehydrogenase (PGD), NADH-diaphorase (DIA), α-esterase (EST), glucosephosphate isomerase (GPI, syn. PGI = phosphoglucose isomerase), aconitase (ACO), phosphoglucomutase (PGM), malate dehydrogenase (MDH), and glutamate oxaloacetate transaminase (GOT, syn. AAT = aspartate aminotransferase). Enzymes were stained according to the protocols of Harris and Hopkins (11) and Soltis et al (37). EST and GOT were stained in solution baths, whereas the agar overlay technique was applied with the other enzymes. In preliminary experiments, we also tested a number of additional enzyme systems that did not give satisfactory activity or band resolution (Table 2) with the three buffer systems used (Table 1). When the genetic basis of isozymes was clear from this or a related (45) study, enzyme alleles were coded according to the relative migration distance (R_f) from the origin near the cathode, with the most frequent allele representing, by definition, R_f = 100. When the genetic basis of isozyme banding patterns was not clear, each band was considered to be due to a different locus, and loci were designated tentatively with the prefix B for band followed by the migration distance (e.g., *Got-B62*).

Data analysis. All computations were done with the program package NTSYS-pc, Numerical Taxonomy and Multivariate Statistical Analysis System (31), unless stated otherwise.

The Dice index of similarity (36) was computed to qualitatively compare different electrophoretic types (ETs). The Dice index is computed as

$$F = \frac{2n_{ab}}{n_a + n_b},$$

in which F represents the proportion of alleles shared by two ETs (n_{ab}) among the sum of alleles that ETs a and b express (n_a and n_b).

We calculated Nei's standard genetic distance for comparisons between races of *C. carbonum* (25). It is defined as

$$d_{ab} = -\ln \frac{\sum_{i=1}^{k} x_{ia}x_{ib}}{\left(\sum_{i=1}^{k} x_{ia}^2 \sum_{i=1}^{k} x_{ib}^2\right)^{1/2}},$$

in which two taxonomic units, a and b, are compared over k loci, and x_{ia} and x_{ib} denote the relative frequency of the ith allele in units a and b.

The UPGMA (unweighted pair-group method with arithmetic averaging) clustering method (38) was used to group ETs on the basis of their qualitative similarity (Dice index) and to compare the relatedness of races by using the Nei's standard genetic distance measure, d. Results are presented as phenograms prepared by programs SAHN and TREEG in NTSYS-pc.

We also used principal coordinates analysis (9,10) to corroborate the result from the cluster analysis of ETs. The same similarity matrix used for cluster analysis of ETs (Dice index) was transformed to scalar product form by program DCENTER in NTSYS-

TABLE 1. Buffer composition and run parameters for starch gel electrophoresis of isozymes from isolates of *Cochliobolus carbonum*

Buffer system	Gel buffer	Tray buffer	Parameters	Target enzyme[a]
Tris-borate	0.015 M Tris pH 7.7 with citric acid	0.3 M boric acid 0.031 M NaOH pH 7.5 with 1 M NaOH	3 h 60 mA	PGD, DIA, EST
Histidine-citrate	9.02 M L-histidine-HCl pH 5.7 with 1 M NaOH	75 mM Na_3-citrate pH 5.7 with citric acid	3.5 h 100 mA	GPI, ACO
Tris-citrate	9 mM Tris 0.3 mM citric acid pH 7.0 with Tris	0.135 M Tris 0.045 M citric acid pH 7.0 with 1 M NaOH	3 h 100 mA	PGM, MDH, GOT

[a] PGD = phosphogluconate dehydrogenase; DIA = NADH-diaphorase; EST = α-esterase; GPI = glucosephosphate isomerase; ACO = aconitase; PGM = phosphoglucomutase; MDH = malate dehydrogenase; and GOT = glutamate oxaloacetate transaminase.

pc, and eigenvalues and eigenvectors were computed by program EIGEN in NTSYS-pc. These vectors are the principal coordinate axes. The first three principal coordinates explained a great part of the similarity among ETs, so they were depicted graphically with Proc G3D in SASGRAPH (32). With no missing values and with the Dice similarity index, results from a principal coordinates analysis are equal to those from a principal components analysis (31). The two techniques differ in that a principal components analysis is based on a covariance matrix of allele frequencies, whereas in a principal coordinates analysis, a genetic distance or similarity matrix of taxonomic units serves as the basis for the actual multivariate analysis. Consequently, computation of the principal coordinates analysis is more convenient when the number of allele frequencies is greater than the number of taxonomic units (9), a situation met frequently in restriction fragment length polymorphism and isozyme analyses.

TABLE 2. Activity, resolution of bands, and morphism of enzyme systems in *Cochliobolus carbonum*

Activity and resolution[a]	Enzyme[b]	Morphism[c]
A+ R+	ACO (aconitase, EC 4.2.1.3)	P
	DIA (NADH-diaphorase, EC 1.6.2.2)	P+
	EST (α-esterase, EC 3.1.1.1)	P+
	GPI (glucosephosphate isomerase, EC 5.3.1.1.9)	P
	GOT (glutamate oxaloacetate transaminase, EC 2.6.1.1)	P+
	MDH (malate dehydrogenase EC 1.1.1.37)	P+
	PGD (phosphogluconate dehydrogenase, EC 1.1.1.44)	M
	PGM (phosphoglucomutase, EC 2.7.5.1)	P+
A± R+	ACP (acid phosphatase, EC 3.1.3.2)	
	LAP (leucine amino peptidase, EC 3.4.11)	
	XDH (xanthine dehydrogenase, EC 1.2.1.37)	
A+ R−	ADH (alcohol dehydrogenase, EC 1.1.1.1)	
	GD (glucose-6-phosphate dehydrogenase, EC 1.1.1.49)	
	SOD (superoxide dismutase, EC 1.15.1.1)	
A− R−	ALP (aluminum phosphatase, EC 3.1.3.1)	
	F-1,6-DP (fructose-1,6-diphosphatase, EC 3.1.3.11)	
	FH (fumarate hydratase, EC 4.2.1.2)	
	GDH (glucose dehydrogenase, EC 1.1.1.47)	
	GPT (glutamate-pyruvate transaminase, EC 2.6.1.2)	
	SDH (shikimate dehydrogenase, EC 1.1.1.25)	

[a] + = High; ± = inconsistent; and − = low.
[b] Three different buffer systems were tested with each enzyme system (Table 1). EC = Enzyme Commission number from Harris and Hopkinson (11).
[c] P and P+ indicate polymorphism, i.e., variant electromorphs occurred at frequencies of 1-4% (P) or ≥5% (P+); M indicates monomorphism, i.e., variant allele frequencies <1%. Determined only for enzymes with high activity and resolution.

Average gene diversity $\hat{H}$ was computed as

$$\hat{H} = \sum_{j=1}^{r} \frac{\hat{h}_j}{r},$$

in which $\hat{h}_j$ is the gene diversity at the jth locus and r is the number of loci sampled. An unbiased estimate of gene diversity in a selfing population, being equivalent to a haploid population, was given by Nei (25) as

$$\hat{h} = \frac{n(1-\sum x_i^2)}{n-1}$$

in which n is the number of isolates and x_i is the frequency of the ith allele at the locus considered. The sampling variance of $\hat{H}$ was obtained by

$$V(\hat{H}) = \frac{V(\hat{h})}{r},$$

in which $V(\hat{h})$, the variance of $\hat{h}$ is

$$V(\hat{h}) = \frac{\sum_{i=1}^{r} (\hat{h}_j - \hat{H})^2}{r-1}.$$

The standard error of $\hat{H}$ is the square root of the total variance $V(\hat{H})$. $\hat{H}$ values of different races were compared by a t test for independent observations as outlined by Nei (25).

To compare the richness of variation in ETs among races, i.e., multilocus isozyme genotypes, the Shannon index of diversity (29) was calculated from the equation

$$H' = -\Sigma p_i \ln p_i,$$

in which p_i is the proportion of the ith ET in a race. Standard errors and t tests to compare H' values of different samples were computed according to Poole (29).

RESULTS

Of the eight enzymes that performed well, five were moderately to highly polymorphic, two were weakly polymorphic, and only one was monomorphic (Table 2). We did not investigate whether the inconsistent staining of acid phosphatase (ACP), leucine amino peptidase (LAP), and xanthine dehydrogenase (XDH) reflected inconsistent sample treatment or genetic polymorphism (i.e., functional and null alleles).

The genetic interpretation of PGD, PGM, ACO, and DIA banding patterns was straightforward; they were controlled by single loci with two to three alleles each (Table 3). The patterns of MDH, GOT, and EST indicated a rather complex genetic basis. In interpreting these patterns, we assumed that our isolates

TABLE 3. Relative allele frequencies (%) among races of *Cochliobolus carbonum*

		Putative isozyme allele[b]																								
		Pgd	*Gpi*		*Pgm*		*Aco*			*Dia*			*Mdh1*			*Mdh2*			*Got-B*		*Est-B*					
Race	*n*[a]	*100*	*100*	*89*	*108*	*100*	*125*	*100*	*88*	*140*	*100*	*60*	*133*	*111*	*100*	*67*	*56*	*33*	*100*	*62*	*100*	*97*	*92*	*87*	*80*	*77*	*73*
0	29	100	100	0	91	9	0	96	4	0	32	68	28	0	72	0	100	0	100	100	67	33	13	88	100	21	17
1	6	100	100	0	0	100	0	100	0	0	100	0	0	0	100	0	0	100	100	17	100	100	83	0	100	0	83
2	90	100	99	1	2	98	0	100	0	0	99	1	0	0	100	0	2	98	100	93	100	94	99	6	100	0	99
3	75	100	100	0	1	99	1	99	0	1	99	0	0	1	99	1	0	99	100	100	99	7	100	95	99	0	99

[a] Number of isolates.
[b] Alleles are designated by locus name (e.g., *Mdh1* = malate dehydrogenase locus 1) and relative allozyme mobility.

were typical haploid monokaryons. This assumption is based on the following: Heterokaryon incompatibility severely limits the potential for heterokaryon formation by *Cochliobolus* spp. in nature (40,47). Conidia produced by forced heterokaryons on artificial media are nearly always monokaryotic (40). Our isolates were derived from single conidia (44). The phenotypes of our isolates were stable.

In vertebrates, MDH usually has a dimeric structure (11), but in all isolates of races 1 and 2 and in 74 of 75 isolates of race 3, MDH appeared to be tetrameric. These isolates expressed a pattern of five regularly spaced MDH bands (R_f = 100, 83, 67, 50, and 33) with darker staining in the intermediate migration distances (R_f = 83, 67, and 50). This is typical of a tetrameric enzyme forming two homotetramers, R_f = 100 and 33, and three heterotetramers, R_f = 83, 67, and 50 (6). This pattern may be explained by alleles at two MDH loci, *Mdh1 100* and *Mdh2 33* (Table 3). The deviant two-banded MDH patterns of all race 0 isolates (R_f = 100 and 56, or R_f = 133 and 56) and the three-banded pattern of a single race 3 isolate (R_f = 111, 89, and 67) suggest that there may be three alleles at each MDH locus and that only the allozymes of *Mdh1 100* and *Mdh2 33* can form tetramers. This would be unusual, but variation is known to occur in subunit assembly of oligomeric enzymes among different taxa (6).

No isolate had more than one of the "fast" MDH bands at R_f = 100, 133, or 111. This is consistent with a model of an MDH locus, *Mdh1,* with three alleles. In crosses of two race 0 isolates (MDH bands 100 and 56) with two race 2 isolates (MDH bands 100, 83, 67, 50, and 33), there was a clear single gene segregation for MDH bands at R_f = 56 and 33 (45), suggesting that these bands are due to alleles at a single locus. Thus, there is additional evidence for at least two alleles at a second MDH locus, *Mdh2.* Both parents in each of the race 0 × race 2 crosses shared the *Mdh1 100* allele, so no segregation was seen at the *Mdh1* locus (45).

Seven different well-resolved esterase bands were expressed among the races of *C. carbonum* (Table 4, Fig. 1), but the majority of isolates expressed only five (Table 3). Additional faint bands occurred at relative migration distances outside the range considered for analysis (Fig. 1). The genetic basis of the observed EST patterns was difficult to infer from these data alone, but crosses between isolates of race 0 and race 2 (45) suggested the presence of a single locus relating to each band, although not all of the seven EST bands segregated in a clear 1:1 fashion. The patterns we observed among natural isolates are consistent with this interpretation. The observed patterns are not consistent with interlocus oligomer formation nor with allelism of genes for the EST bands at R_f = 100, 97, 87, 80, and 73. However, they do not exclude the possibility that the gene for the band at R_f = 77 might be allelic with a gene for a band at R_f = 100, 92, or 73. For consistency and simplicity, we assigned each band a tentative (indicated by B for band) locus designation (Table 3).

TABLE 4. Esterase phenotypes among field isolates of *Cochliobolus carbonum*

	Phenotype[b]							
R_f[a]	I	II	III	IV	V	VI	VIII	IX
100	–	–		–		–		
97	–		–		–	–	–	
92	–	–			–		–	
87		–	–	–			–	–
80	–	–	–	–	–	–		–
77			–					
73	–	–						–
Total[c]	93	73	5	13	3	1	1	4

[a] Relative migration distance.
[b] Additional esterase phenotypes were found among progeny of crosses between field isolates of *C. carbonum* (45).
[c] Number of times found.

Two GOT bands were expressed by most isolates (Table 3), but some had only a single band at R_f = 100. This band, GOT-B100, consistently stained more intensively when it occurred alone than when it occurred with band GOT-B62. The reason could be a duplication of the *Got* locus at R_f = 100 leading to stronger enzyme activity. Isolates with two (weaker) bands may indicate a mutation at one of the loci. The nonmutated wild-type allele at locus 1 could encode the "fast" band (R_f = 100) and the mutated allele at locus 2 the "slow" band (R_f = 62). Alternatively, one might suppose that the two bands are due to two independent loci, one monomorphic (R_f = 100) and the other polymorphic with one functional (R_f = 62) and one null allele. The latter model, however, does not take into account the consistent difference in staining intensity of the two bands. At any rate, there seem to be two GOT loci, which we tentatively named *Got-B100* and *Got-B62.*

GPI stains were difficult to score, because secondary bands frequently appeared on the gel about 10 min after the main band (R_f = 100) appeared. We followed the common practice of not regarding such bands as genetically distinct, although it appeared that some isolates produced these bands more intensively and reproducibly than others.

The isozyme data were used to compare the genetic relatedness of *C. carbonum* races and the level of genetic diversity within the races. Allele frequencies in races 1, 2, and 3 were highly correlated (Table 3), indicating a close genetic relation. Race 1 differed clearly from race 2 only in its much lower *Got-B62* frequency (Table 3) (but the sample size of race 1 was small). The only major difference between race 2 and race 3 was their contrasting frequencies of *Est-B97* and *Est-B87* (Table 3, Fig. 1). Race 0, on the other hand, was distinctly different from the other three races in most allele frequencies (Table 3).

The high degree of similarity between race 1 and race 2 is most evident from the list of ETs (Table 5). Of the six race 1 isolates analyzed, five represented ET9, which was the second most frequent type in race 2. These five isolates all came from North Carolina (four isolates) or South Carolina (one isolate), whereas the unique ET10 in race 1 belonged to one isolate from Michigan.

Races 2 and 3 shared similar phenotypes, but their shared phenotypes differed in frequency. ET12, the predominant type (93%) in race 3, ranked third at 4% frequency among race 2 isolates. ET11, the predominant type (82%) in race 2, ranked second at 5% among race 3 isolates (Table 5). Only one race 3 isolate had a type (ET16) not found among the race 2 isolates. In contrast, the only shared ET between race 0 and another race was ET6, which occurred once among race 0 isolates and once among race 2 isolates (Table 5).

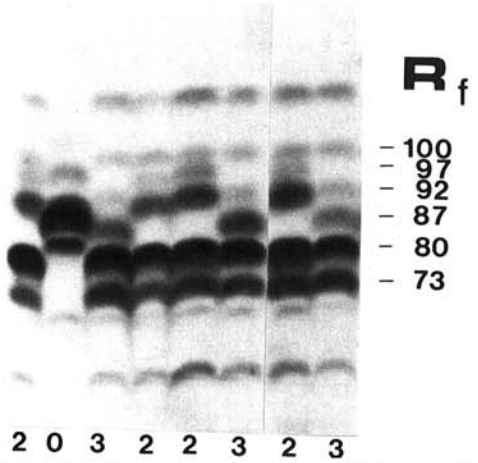

Fig. 1. α-Esterase banding patterns for one race 0, four race 2, and three race 3 isolates of *Cochliobolus carbonum.* Esterase bands with relative migration distance values of <73 or >100 were inconsistent and not included in the analysis.

TABLE 5. Frequencies of electrophoretic types among races of *Cochliobolus carbonum*

			Putative isozyme allele[c]																									
			Pgd	*Gpi*		*Pgm*		*Aco*			*Dia*			*Mdh1*			*Mdh2*			*Got-B*		*Est-B*						
Race	n[a]	ET[b]	*100*	*100*	*89*	*108*	*100*	*125*	*100*	*88*	*140*	*100*	*60*	*133*	*111*	*100*	*67*	*56*	*33*	*100*	*62*	*100*	*97*	*92*	*87*	*80*	*77*	*73*
0	14	1	1	1	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	1	0	0
	4	2	1	1	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	1	1	0
	4	3	1	1	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	1	0	1
	3	4	1	1	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	0	0
	1	5	1	1	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	1	1	0	1	0	1	1	1	0
	1	6	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	1	0	0
	1	7	1	1	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0	1	1	0	1	0	1	1	1	0
	1	8	1	1	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	1	1	1	0	0	1	1	0	0
1	5	9	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1
	1	10	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	0	0	1	0	0
2	74	11	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	0	1
	7	9	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	1	1	1	0	1	0	1
	4	12	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	0	1	1	1	0	1
	2	13	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	0	1
	1	6	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	1	1	0	0
	1	14	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	0	1	0	1
	1	15	1	0	1	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	0	1
3	70	12	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	0	1	1	1	0	1
	4	11	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	0	1
	1	16	1	1	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	0	1	1	1	0	0	0

[a] Number of isolates
[b] Electrophoretic type.
[c] Alleles are designated by locus name (e.g., *Mdh1* = malate dehydrogenase locus 1) and relative allozyme mobility.

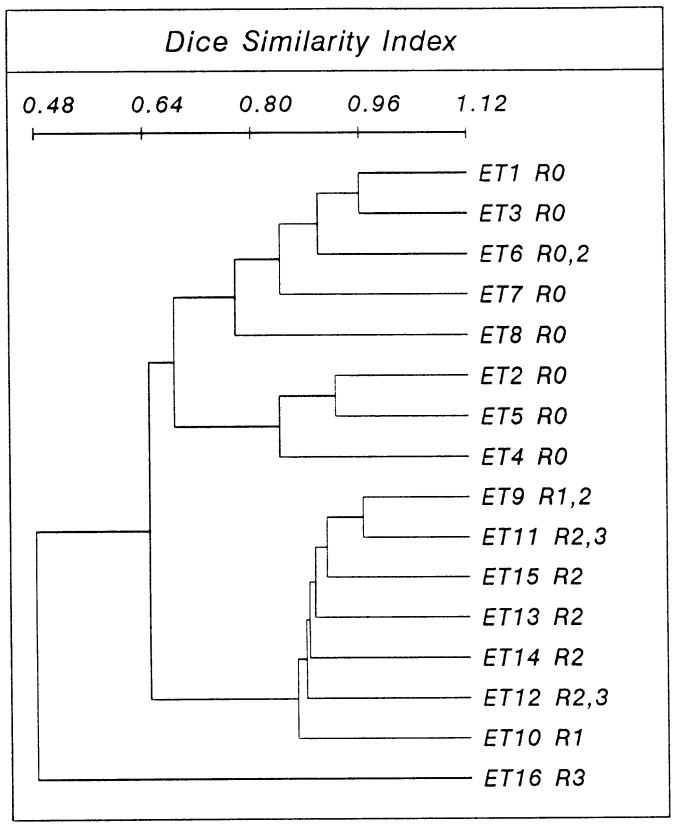

Fig. 2. Phenogram of similarity among 16 electrophoretic types (ETs) of races 0, 1, 2, and 3 of *Cochliobolus carbonum*. Some ETs occur in more than one race. The Dice index of similarity is based on the proportion of shared bands between ETs without regard to frequency of the ETs in the population. The phenogram was constructed by the unweighted pair-group method with arithmetic averaging.

Further insight into the relatedness of ETs is provided by the cluster analysis of the Dice indices of similarity among ETs (Fig. 2). The majority of ETs among races 1, 2, and 3 were very closely related and fell into a single cluster. Exceptions to this were ET16 of race 3, differing in a number of isozyme alleles from the rest of race 3 isolates (Table 5), and ET6, being shared by race 0 and race 2.

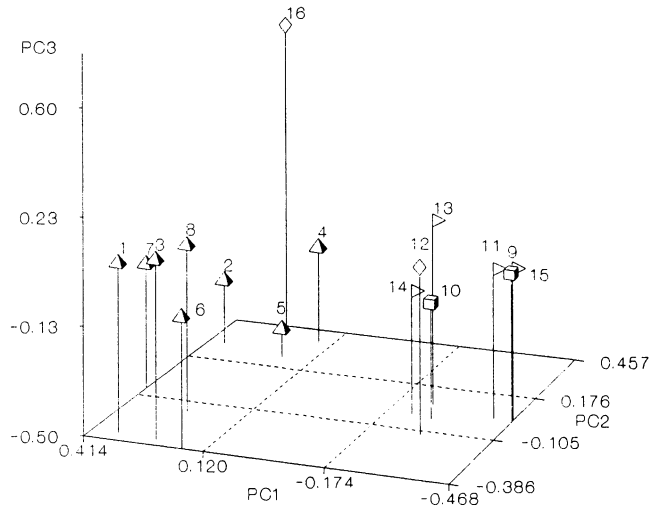

Fig. 3. First three principal coordinates (PCs) of electrophoretic types (ET) (1–16, Table 5) of *Cochliobolus carbonum* extracted from a PC analysis of a rectangular matrix of Dice similarity values. Pyramids = race 0; cubes = race 1; flags = race 2; and diamonds = race 3. When the same ET occurred in two different races (four ETs, Table 5, Fig. 2), only the race with the higher relative frequency of that ET is symbolized. The proportion of similarity explained by each PC decreases with PC number.

Results of the principal coordinates analysis regarding the similarity of ETs corroborated this picture (Fig. 3). The typical ETs of races 1, 2, and 3 were well separated from those of race 0. Figure 3 presents the first three principal coordinates that contributed 36%, 20%, and 15% to the genetic similarity, respectively. ET16 is an outlier, but because of the minor importance of coordinate 3, it appears to be more similar to ET2, ET4, and ET5 of race 0 than to any other ET of races 1, 2, or 3 (Fig. 3).

The phenogram of the four *C. carbonum* races as constructed with Nei's standard distance measure (Fig. 4) is a condensed representation of their apparent genetic relatedness on the basis

of isozyme data. Races 1 and 2 appear to be most closely related in a cluster that includes race 3. The distance of this cluster from race 0 is comparatively large.

A general impression of the level of genetic diversity within the four races can be gained by inspecting their allele frequencies. Race 0 had more alleles at intermediate frequencies than were found in the other races, indicating a higher level of diversity. On the basis of the more stringent definition of polymorphism (less frequent allele[s] ≥ 0.05), nine of 16 putative isozyme loci were polymorphic in race 0, in contrast to only three polymorphic loci in race 1, three in race 2, and two in race 3 (Tables 3 and 6).

Nei's unbiased estimate of average gene diversity ($\hat{H}$) also shows that race 0 was significantly more diverse than either race 2 or race 3 (Table 6). The t test as suggested by Nei (25) demonstrated a significant difference between $\hat{H}$ of race 0 and race 2 ($t = 3.368$, $P < 0.05$) but not between race 2 and race 3 ($t = 0.559$, $P > 0.05$). The same ranking in diversity was obtained by the Shannon index of diversity (H') calculated from the proportions of ETs found in each race (Table 6). Not only was the difference between the H' values of race 0 and race 2 highly significant ($t = 6.443$, $P < 0.001$), but the difference between race 2 and race 3 was significant as well ($t = 2.881$, $P < 0.01$).

DISCUSSION

Of the 20 enzyme systems that we evaluated, eight showed high activity and gave consistent band resolution under our conditions. Seven of these eight (all but PGD) were polymorphic at the 1% level. This high ratio of polymorphism may not be representative of the genome of *C. carbonum,* because 1) we preselected enzyme systems known to be polymorphic in other pathogenic fungi (1,2,19,21), and 2) we refined the protocols for detecting those enzymes that showed signs of polymorphism in preliminary experiments. These same qualifications, however, probably apply to most organisms studied electrophoretically (25) and, thus, do not diminish the validity of our intraspecific comparisons.

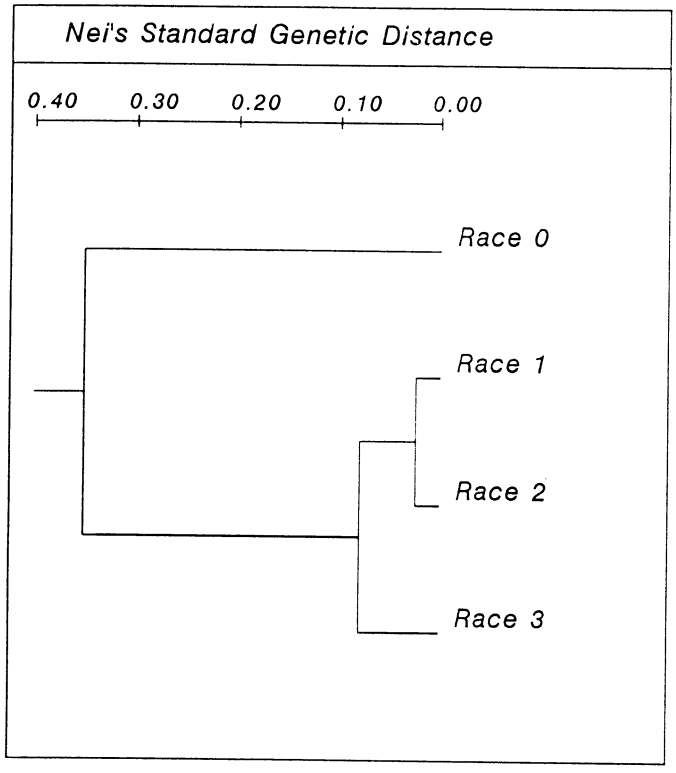

Fig. 4. Phenogram of Nei's standard genetic distance (25) between races 0, 1, 2, and 3 of *Cochliobolus carbonum* on the basis of isozyme allele frequencies. The phenogram was constructed by the unweighted pair-group method with arithmetic averaging.

Simcox et al (35) tested 20 isolates of *C. carbonum* for 36 enzyme systems and found polymorphisms for only five of the 19 that gave uniform staining and consistent resolution of bands. Three of the polymorphic systems identified by Simcox et al (i.e., GOT, EST, and GPI) were also polymorphic among our isolates. LAP, which gave inconsistent activity in our study, was polymorphic among the race 1 and race 3 isolates of Simcox et al (35), but none of their race 2 isolates showed LAP activity. Mannitol dehydrogenase (MADH) was polymorphic in their study but was not tested in ours. ACO, MDH, and PGM were polymorphic in our study but monomorphic among the isolates tested by Simcox et al (35). DIA also was polymorphic in our study but stained inconsistently for Simcox et al (35).

The greater number of polymorphic enzyme systems found in our study than in that of Simcox et al (35) can be attributed to our larger sample size (194 isolates) compared with that of Simcox et al (20 isolates). Even without the 29 isolates of the genetically diverse race 0, which was not tested by Simcox et al (35), our 90 race 2 and 75 race 3 isolates from two fields in the piedmont of North Carolina showed combined polymorphism for seven enzyme systems.

Nei (25) suggested that the proportion of polymorphic loci (P) is not an accurate measure of genetic diversity, but we found general agreement between the three diversity measures P, $\hat{H}$ (average gene diversity), and H' (Shannon index). The diversity of race 0 was dramatically greater than that of races 2 and 3, which exhibited only a small difference between their genetic diversities. The difference between race 2 and race 3 was not significant for $\hat{H}$, which is based on allele frequencies, but was significant for H', which is based on multilocus genotype frequencies. Thus, the Shannon index may be more sensitive than Nei's average gene diversity index as a measure of genetic diversity in fungi such as *C. carbonum,* in which reproduction is predominantly asexual. We also found a significantly greater Shannon index for race 2 than for race 3 in a previous study of genetic diversity in phenotypic markers such as mating capacity, fungicide tolerance, and colony morphology in the same populations of *C. carbonum* from Wilkes and Yadkin counties in North Carolina (44). With regard to these phenotypic markers, as with isozymes, race 0 was significantly more diverse than either race 2 or race 3.

The small sample of race 1 isolates tested in this study came from earlier collections made in the 1960s and 1970s (15,16), because we found no race 1 isolates in the 1987 survey (44). It is remarkable that all five race 1 isolates in this sample from North Carolina and South Carolina had identical isozyme patterns even though they were collected from different fields and in different years. The fact that all five also were of the same mating type (MAT-2 = mating type a) contrasts with frequencies of mating types in races 0, 2, and 3 near the presumed equilibrium of 50% (44). These data suggest that the small race 1 population in the Carolinas may be a clone. This could be an effect of random genetic drift, since the frequency of race 1 dropped below 1% in the *C. carbonum* population of North Carolina (16,44).

The sixth race 1 isolate came from Michigan and was collected before 1977. It also is mating type MAT-2 but has a unique isozyme profile. More isolates would be needed to determine whether this

TABLE 6. Unbiased estimates of average gene diversity ($\hat{H}$), proportion of polymorphic loci (P), and Shannon indices of diversity (H') among samples of *Cochliobolus carbonum* races from North Carolina

Race[a]	$\hat{H}$[b]	P	H'[c]
0	0.195 ± 0.047	0.563	1.592 ± 0.041
2	0.032 ± 0.011	0.188	0.730 ± 0.127
3	0.024 ± 0.011	0.125	0.277 ± 0.093

[a] Race 1 was not included because it was completely uniform in North Carolina.
[b] $\hat{H}$ ± standard error was computed from allele frequencies at 16 putative isozyme loci according to Nei (25). A locus is defined as polymorphic when the most frequent allele is present in ≤ 95% of the individuals.
[c] H' ± standard error was computed from proportions of electrophoretic types according to Poole (29).

difference resulted from genetic divergence between the southeastern and midwestern populations of race 1 in the United States. Our data and those of Simcox et al (35) do not allow a full comparison of isozyme patterns between their race 1 isolates from Urbana, Illinois, and our isolate from Michigan. The partial comparison that is possible does not rule out the possibility that these race 1 isolates are similar, although some bands detected in our study were not found in theirs and vice versa.

The common electrophoretic type (ET9) of the race 1 isolates from North Carolina and South Carolina was shared by a significant part of the race 2 population (8%), suggesting a common ancestry of these races or genetic recombination between them. Race 1 and race 3 are known to differ not only in toxin production but also in polygenes for lesion type on corn lines insensitive to race 1 toxin. Therefore, it appears that race 1 is more closely related to race 2 than to race 3. The DNA region that encodes HC-toxin synthetase necessary for toxin production in race 1 has no apparent homology with DNA in either race 2 or race 3, but border sequences on either side of that region are shared by race 1 and race 2 (28). The origin of the gene or gene complex for toxin production in race 1 is not known.

Leonard (16) and Leonard and Leath (17) concluded that race 3 may have evolved more recently than race 2 on the basis of the following evidence: 1) race 3 shows less genetic variation than race 2; 2) race 3 was discovered more recently (27); and 3) race 3 has a narrower known geographic range than race 2. Our observation that all but one isolate of race 3 exhibited ETs found also in race 2 suggests that race 3 may have arisen from race 2. If race 3 arose from race 2, the completely different frequencies of two esterase alleles in race 2 and race 3 illustrate that the two race populations must have diverged genetically since the origin of race 3.

The absence of hybridization between races 2 and 3 in the field (14,16,17,22) may be a consequence of, rather than the reason for, their divergent evolution. However, the lack of hybridization between races 2 and 3 does not necessarily imply a total lack of sexual reproduction within these races. Welz and Leonard (44) found no significant gametic phase disequilibrium among phenotypic markers within race 2 or race 3. Some linkage disequilibrium might be expected in populations that completely lack sexual reproduction. The isozyme data are not very suitable for analysis of linkage disequilibria, because the isozyme allele frequencies in races 2 and 3 were mostly 0–5% or 95–100%. However, the presence of both mating types in each electrophoretic type observed more than twice in each race (H. G. Welz, *unpublished*) indicates that there was genetic variation within electrophoretic types of races 2 and 3.

Although Simcox et al (35) found isozyme analysis to be extremely useful in identifying specific races of *C. carbonum,* we found that a significant number of isolates could be misclassified if only isozyme data were used. For example, if we regarded ET11 as a race 2 phenotype and ET12 as a race 3 phenotype, we would have misclassified 5% of our race 3 isolates as race 2 and 4% of our race 2 isolates as race 3. This difference between our results and those of Simcox et al (35) may be attributed to differences in the sources of isolates. Seven of the nine race 2 isolates that Simcox et al (35) tested were from the Midwest, whereas eight of their nine race 3 isolates were from the southeast or the northeast areas of the United States. All 90 of our race 2 isolates and all 75 of our race 3 isolates came from the same two fields in North Carolina.

The avirulent race 0 of *C. carbonum* is phenotypically and genetically quite different from the other races in this fungus. This result had already emerged from the study of phenotypic markers (44) and was confirmed by our comparison of isozyme patterns as well as by Jones and Dunkle's (12) comparison of polymerase chain reaction amplification products from races 0, 1, 2, 3, and 4. Only one of our eight electrophoretic types of race 0, ET6, occurred also in another race, viz. race 2. It is interesting that the single race 0 isolate with ET6 had other typical race 0 alleles (*Pda*− for inability to sporulate on potato-dextrose agar and *CadR* for cadmium tolerance), whereas the single race 2 isolate with ET6 had the *Pda*+, *CadR* genotype (H. G. Welz, *unpublished*). Since cadmium tolerance is very rare (2%) in race 2 (16,44), this isolate might have resulted from hybridization between race 0 and race 2. It is also interesting that both ET6 isolates came from the same field.

The genetic diversity in race 0 was significantly greater than that in races 1, 2, or 3. This may indicate that the race 0 population in North Carolina is older than the race 1, 2, and 3 populations or that race 0 is subjected to less intense selection than are the other three races. The greater dependence of races 1, 2, and 3 on agricultural production of corn could cause greater fluctuations in their population sizes than occurs in race 0 populations. In *Magnaporthe grisea,* the rice blast fungus, Leung and Williams (19) found much greater variation among grass isolates than among isolates from rice. They interpreted this as niche-dependent variation being correlated with variation in the host population. We do not know the host range of race 0 of *C. carbonum,* but we have evidence of its low parasitic fitness on corn (42,44). Race 0 may be more of a saprophyte than a parasite and may be broadly adapted to survive under varying conditions of the soil or crop debris, or race 0 may persist as a pathogen of some weedy grass species in roadsides and corn fields.

Laboratory experiments with *Drosophila* have shown that allozyme heterozygosity was increased with environmental heterogeneity (23,30). Gillespie (8) showed in a model that polymorphism could be maintained mainly by spatial heterogeneity of the habitat. Spieth (39) extended Gillespie's theory to a model of "shifting multiple niches," in which the distribution of niches varies from one population to the next, causing different alleles and different levels of polymorphism to be favored in different populations. However, experimental evidence to support this theory is still lacking, mainly because selective effects of different allozyme alleles rarely could be proven (20). Furthermore, Lewontin (20) pointed out that, because of the typically large standard errors for average heterozygosity among allozyme loci, more than 100 loci must be analyzed in a species for reliable estimates of the correlations between ecological parameters and the level of heterozygosity. With molecular markers, it should be possible to reach that level of statistical power in pathogenic fungi. Jones and Dunkle's (12) recent study of polymerase chain reaction amplification products from races 0, 1, 2, 3, and 4 of *C. carbonum* provides a good start in this direction.

LITERATURE CITED

1. Bonde, M. R., Peterson, G. L., Dowler, W. M., and May, B. 1984. Isozyme analysis to differentiate species of *Peronosclerospora* causing downy mildews of maize. Phytopathology 74:1278-1283.
2. Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65:2067-2073.
3. Burdon, J. J., Marshall, D. R., Luig, N. H., and Gow, D. J. S. 1982. Isozyme studies on the origin and evolution of *Puccinia graminis* f. sp. *tritici* in Australia. Aust. J. Biol. Sci. 35:231-238.
4. Dalmacio, S. C., MacKenzie, D. R., and Nelson, R. R. 1979. Heritability of differences in virulence between races 2 and 3 of *Cochliobolus carbonum.* Philipp. Phytopathol. 15:47-50.
5. Dodd, J. L., and Hooker, A. L. 1990. Previously undescribed pathotype of *Bipolaris zeicola* on corn. Plant Dis. 74:530.
6. Ferguson, A. 1980. Biochemical Systematics and Evolution. Blackie, Glasgow, Scotland. p. 52.
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. Gillespie, J. 1974. Polymorphism in patchy environments. Am. Nat. 108:145-151.
9. Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325-338.
10. Gower, J. C. 1972. Measures of taxonomic distance and their analysis. Pages 1-24 in: The Assessment of Population Affinities in Man. J. S. Weiner and J. Huizinga, eds. Clarendon Press, Oxford.
11. Harris, H., and Hopkins, D. A. 1976. Handbook of Enzyme Electro-

phoresis in Human Genetics. North-Holland, Amsterdam.
12. Jones, M. J., and Dunkle, L. D. 1993. Analysis of *Cochliobolus carbonum* races by PCR amplification with arbitrary and gene-specific primers. Phytopathology 83:366-370.
13. Koch, G., and Köhler, W. 1990. Isozyme variation and genetic distances of *Erysiphe graminis* DC. *formae speciales.* J. Phytopathol. 129:89-101.
14. Leonard, K. J. 1974. Genetic isolation of races 2 and 3 of *Cochliobolus carbonum* in North Carolina. (Abstr.) Proc. Am. Phytopathol. Soc. 1:94.
15. Leonard, K. J. 1976. Isolation of race 0 of *Bipolaris maydis* from corn ears harvested in North Carolina in 1965. Plant Dis. Rep. 60:245-249.
16. Leonard, K. J. 1978. Polymorphisms for lesion type, fungicide tolerance, and mating capacity in *Cochliobolus carbonum* isolates pathogenic to corn. Can. J. Bot. 56:1809-1815.
17. Leonard, K. J., and Leath, S. 1990. Genetic diversity in field populations of *Cochliobolus carbonum* on corn in North Carolina. Phytopathology 80:1154-1159.
18. Leonard, K. J., Thakur, R. P., and Leath, S. 1988. Incidence of *Bipolaris* and *Exserohilum* species in corn leaves in North Carolina. Plant Dis. 72:1034-1038.
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. Lewontin, R. C. 1991. Twenty-five years ago in GENETICS. Electrophoresis in the development of evolutionary genetics: Milestone or millstone? Genetics 128:657-662.
21. Linde, D. C., Groth, J. V., and Roelfs, A. P. 1990. The genetic basis of isozyme variation in the bean rust fungus (*Uromyces appendiculatus*). J. Hered. 81:134-138.
22. Lodge, D. J., and Leonard, K. J. 1984. A cline and other patterns of genetic variation in *Cochliobolus carbonum* pathogenic to corn in North Carolina. Can. J. Bot. 62:995-1005.
23. MacDonald, J. F., and Ayala, F. J. 1974. Genetic response to environmental heterogeneity. Nature 250:572-574.
24. Maheshwari, R., and Sussman, A. S. 1970. Respiratory changes during germination of urediospores of *Puccinia graminis* f. sp. *tritici.* Phytopathology 60:1357-1364.
25. Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
26. Nelson, R. R. 1970. Genes for pathogenicity in *Cochliobolus carbonum.* Phytopathology 60:1335-1337.
27. Nelson, R. R., Blanco, M., Dalmacio, S., and Shain Moore, B. 1973. A new race of *Helminthosporium carbonum* on corn. Plant Dis. Rep. 57:822-823.
28. Panaccione, D. G., Scott-Craig, J. S., Pocard, J.-A., and Walton, J. D. 1992. A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. Proc. Natl. Acad. Sci. USA 89:6590-6594.
29. Poole, R. W. 1974. An Introduction to Quantitative Ecology. McGraw-Hill, New York. p. 392.
30. Powell, J. R. 1971. Genetic polymorphisms in varied environments. Science 174:1035-1036.
31. Rohlf, F. J. 1989. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, version 1.50. Exeter, Setauket, NY.
32. SAS Institute. 1988. SAS User's Guide: Statistics. Release 6.03 ed. SAS Institute, Cary, NC.
33. Scheffer, R. P., Nelson, R. R., and Ullstrup, A. J. 1967. Inheritance of toxin production and pathogenicity in *Cochliobolus carbonum* and *Cochliobolus victoriae.* Phytopathology 57:1288-1291.
34. Shurtleff, M. C., ed. 1980. Compendium of Corn Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN.
35. Simcox, K. D., Nickrent, D., and Pedersen, W. L. 1992. Comparison of isozyme polymorphism in races of *Cochliobolus carbonum.* Phytopathology 82:621-624.
36. Sokal, R. R., and Sneath, P. H. A. 1963. Principles of Numerical Taxonomy. Freeman, San Francisco.
37. Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. 73:9-27.
38. Sneath, P. H. A., and Sokal, R. R. 1973. Numerical Taxonomy. Freeman, San Francisco.
39. Spieth, P. T. 1975. Population genetics of allozyme variation in *Neurospora intermedia.* Genetics 80:785-805.
40. Tinline, R. D. 1988. *Cochliobolus sativus,* a pathogen of wide host range. Pages 113-122 in: Advances in Plant Pathology. Vol. 6, Genetics of Plant Pathogenic Fungi. G. S. Sidhu, ed. Academic Press, London.
41. Tsukiboshi, T., Sato, T., and Kimigafukuro, T. 1986. Helminthosporium leaf spot on corn caused by *Bipolaris zeicola* in Japan. Ann. Phytopathol. Soc. Jpn. 52:492-495.
42. Welz, H. G., Leath, S., and Leonard, K. J. 1993. Sporulation by races 0, 2, and 3 of *Cochliobolus carbonum* on synthetic medium and sterilized corn leaves. Plant Dis. 77:1153-1157.
43. Welz, G., and Leonard, K. J. 1988. Genetic variation in field populations of races 0, 2, and 3 of *Bipolaris zeicola* in 1987. (Abstr.) Phytopathology 78:1574.
44. Welz, H. G., and Leonard, K. J. 1993. Phenotypic variation and parasitic fitness of *Cochliobolus carbonum* on corn in North Carolina. Phytopathology 83:593-601.
45. Welz, H. G., and Leonard, K. J. 1994. Genetic analysis of two race 0 × race 2 crosses in *Cochliobolus carbonum.* Phytopathology 84:83-91.
46. Xiao, J. Z., Tsuge, T., Doke, N., Nakatsuka, S., Tsuda, M., and Nishimura, S. 1991. Rice-specific toxins produced by *Bipolaris zeicola,* race 3; evidence for role as pathogenicity factors for rice and maize plants. Physiol. Mol. Plant Pathol. 38:67-82.
47. Yoder, O. C. 1988. *Cochliobolus heterostrophus,* cause of southern corn leaf blight. Pages 93-112 in: Advances in Plant Pathology. Vol. 6, Genetics of Plant Pathogenic Fungi. G. S. Sidhu, ed. Academic Press, London.