Genetic Diversity and Pathogenic Variation of *Colletotrichum lindemuthianum* in the Three Centers of Diversity of Its Host, *Phaseolus vulgaris*

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ABSTRACT

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Population subdivision of *Colletotrichum lindemuthianum*, the causal agent of anthracnose, was studied in three regions located in three centers of diversity of its host, *Phaseolus vulgaris*. Random amplified polymorphic DNA (RAPD) markers, restriction endonuclease analysis of the amplified ribosomal internal transcribed spacer region, and virulence on a set of 12 cultivars were used to assess the genetic diversity of *C. lindemuthianum* strains isolated in Mexican, Ecuadorian, and Argentinean wild common bean populations. The three regions were signifi-

cantly differentiated for molecular markers. For these markers, Mexico was the most polymorphic and the most distant from Ecuador and Argentina. The majority of the RAPD alleles present in Ecuador and Argentina were found in Mexico, suggesting that Andean populations have been derived from the Mesoamerican center. Pathogenicity tests on a set of 12 cultivars showed that all but one of the Mexican strains were virulent exclusively on Mesoamerican cultivars. Argentinean strains were virulent preferentially on southern Andes cultivars, and the Ecuadorian strains, except for one strain, were avirulent on all cultivars. These results suggest an adaptation of strains on cultivars of the same geographic origin. Thus, based on molecular and virulence markers, *C. lindemuthianum* strains isolated from wild common bean populations were divided into three groups corresponding to host gene pools.

Colletotrichum lindemuthianum, the causal agent of anthracnose in common bean, is disseminated by splashing or infected material. Sexual forms of the pathogen have been observed a few times after attempted crosses in laboratories but never in nature (3). Thus, the genetic determinism of virulence has not been studied for this pathogen. However, identification of specific resistance genes in common bean has led to the hypothesis of a gene-for-gene relationship similar to the system described by Flor (10) for flax and flax rust. High pathogenic variability in *C. lindemuthianum* has been detected in many areas of Latin America, which is the area of origin of common bean (25).

Wild *Phaseolus vulgaris* populations are found in Latin America along a north-south distribution from northern Mexico to northwestern Argentina. The plants are grouped in three centers of diversity: Mesoamerican and southern and northern Andean. The Mesoamerican and southern Andean centers were defined based on morphological traits (7), phaseolin electrophoretic types (1,12), isozymes (20,30), restriction fragment length polymorphisms (31, 33), and incidence of genes for hybrid lethality (21). The Mesoamerican center of diversity extends from northern Mexico to Colombia, and the southern Andean center extends from southern Peru to northwestern Argentina. A parallel pattern of geographic differentiation between wild and cultivated common bean suggests the occurrence of at least two independent domestication events, one in the Mesoamerican center and the other in the southern Andean center (13,14). The northern Andean center, composed of the Ecuadorian and northern Peruvian wild common bean populations, has been differentiated from the two major gene pools by allozyme analysis (6).

Some authors have suggested that coevolution between common bean and its pathogens may have led to parallel gene pools (12,14). A recent study of *Phaeoisariopsis griseola*, another important bean pathogen, showed that isolates are divided into two molecular groups. One appears to consist of isolates from Mesoamerican beans; the other consists of isolates from southern Andean cultivars (17). The same hypothesis was suggested for *C. lindemuthianum*. However, the pathogen population structure has never been studied in the wild gene pools, and whether the emergence of two groups of strains was the result of selection by cultivars or preceded domestication events remains unknown. Moreover, the diversity of the pathogen in the northern Andean common bean populations has not been investigated.

Our objective was to study the population subdivision of *C. lindemuthianum* in wild common bean gene pools to determine whether regional adaptation exists between the pathogen and its host. *C. lindemuthianum* was isolated from the wild *P. vulgaris* populations of Mexico, Argentina, and Ecuador, located in the Mesoamerican and southern and northern Andean centers of diversity, respectively. Virulence and neutral molecular markers were used as tools for studying the genetic diversity of *C. lindemuthianum*. Virulence was determined on an international set of common bean differential cultivars (24). Restriction endonuclease analysis of the amplified internal transcribed spacer (ITS) region of the rDNA locus and random amplified polymorphic DNA (RAPD) techniques were used to generate molecular markers. The distribution of virulence and molecular markers among the three regions was analyzed.

MATERIALS AND METHODS

Fungus collection and isolation. Isolates were obtained from infected wild common beans collected from three regions located

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in the three centers of diversity. Sampling was done in 1992 in Argentina, in 1993 in Ecuador, and in 1994 in Mexico (Table 1).

Isolations were made from infected pods. Tissue excised from disease lesions was surface-sterilized in 1% (wt/vol) CHCl_3 solu-

tion for 1 min, wet in distilled water with 40 g of streptomycin per liter for 1 min, dried with sterile filter paper, and plated on potato dextrose agar. Plates were incubated at 20°C in the dark until acervuli with conidia were observed. One single-conidial isolate

TABLE 1. Strain, geographic distribution, pathotype number, random amplified polymorphic DNA (RAPD) haplotype, and internal transcribed spacer (ITS) group of 128 wild *Colletotrichum lindemuthianum* strains

Region Strain	State	Location	Pathotype no. ^a	RAPD ^b haplotype	ITS group
Mexico					
M1	Morelos	Huitzilac	v1	1	II
M2	Morelos	Huitzilac	v20	2	I
M3	Morelos	Huitzilac	v19	3	II
M4	Morelos	Huitzilac	v20	4	I
M5	Morelos	Huitzilac	v24	5	II
M6	Morelos	Huitzilac	v19	6	I
M7	Morelos	Tepozteco	v1	7	II
M8	Morelos	Tepozteco	v21	8	II
M9	Morelos	Tepozteco	v19	9	II
M10	Morelos	Tepozteco	v22	10	II
M11	Morelos	Tepozteco	v21	11	II
M12	Morelos	Tepozteco	v23	12	II
M13	Morelos	Tepozteco	v19	13	I
M14	Morelos	Tlalnepantla	v19	14	II
M15	Morelos	Tlalnepantla	v1	15	I
M16	Michoacan	Zirahuato	v23	16	I
M17	Michoacan	Zirahuato	v23	17	I
M18	Michoacan	La cortina	v17	18	I
M19	Guerrero	Arcelia	v24	19	II
M20	Jalisco	Cajititlan	v25	20	II
M21	Jalisco	Cajititlan	v19	20	II
M22	Jalisco	Cajititlan	v19	21	II
M23	Jalisco	Cajititlan	v25	22	II
M24	Jalisco	Cajititlan	v23	23	II
M25	Jalisco	Cajititlan	v19	24	II
M26	Jalisco	Cajititlan	v1	25	I
M27	Jalisco	Cajititlan	v16	26	I
M28	Jalisco	Cajititlan	v19	27	II
M29	Jalisco	Cajititlan	v1	28	I
M30	Jalisco	Cajititlan	v1	29	I
M31	Jalisco	Tala	v25	30	I
M32	Jalisco	Tala	v25	31	I
M33	Jalisco	Tala	v23	32	I
M34	Jalisco	Tala	v23	33	I
M35	Jalisco	Balnearo	v18	34	I
M36	Jalisco	Balnearo	v19	35	I
M37	Jalisco	Balnearo	v1	36	I
M38	Jalisco	Balnearo	v18	37	I
M39	Jalisco	El Tule	v17	38	I
M40	Jalisco	El Tule	v1	39	I
M41	Jalisco	El Tule	v1	40	I
M42	Jalisco	El Tule	v1	41	I
M43	Jalisco	El Tule	v1	42	I
M44	Jalisco	Rio de Los Sanchez	v1	43	I
M45	Jalisco	Rio de Los Sanchez	v23	44	I
M46	Jalisco	Rio de Los Sanchez	v23	38	I
M47	Jalisco	Rio de Los Sanchez	v19	45	I
M48	Jalisco	Rio de Los Sanchez	v1	46	I
M49	Jalisco	Santa Maria	v21	47	I
M50	Jalisco	Santa Maria	v19	48	I
Ecuador					
E1	Chimborazo	Pallatanga	v1	49	II
E2	Chimborazo	Pallatanga	v1	50	II
E3	Chimborazo	Pallatanga	v1	49	II
E4	Chimborazo	Pallatanga	v1	49	II
E5	Chimborazo	Pallatanga	v1	49	II
E6	Chimborazo	Pallatanga	v1	49	II
E7	Chimborazo	Pallatanga	v1	49	II
E8	Chimborazo	Pallatanga	v1	49	II
E9	Chimborazo	Pallatanga	v1	49	II
E10	Chimborazo	Pallatanga	v1	50	II
E11	Chimborazo	Pallatanga	v1	49	II

(Continued on next page)

^a Twenty-five pathotypes were identified on twelve differentials: v1 to v25.

^b Forty RAPD polymorphic markers allowed identification of seventy-five haplotypes: 1 to 75.

per plant was saved for further study. We obtained 128 isolates: 50 from Mexico, 29 from Ecuador, and 49 from Argentina (Table 1).

DNA extraction. Mycelium was obtained as described by Fabre et al. (9). Genomic DNA was extracted from lyophilized myce-

lium according to the method of Rogers and Bendich (29) and stored in 0.1× TE (10 mM Tris-HCl, pH 8; 0.1 mM EDTA) buffer at −20°C.

Polymerase chain reaction (PCR) amplification with target primers and restriction endonuclease digestion. Two primers

TABLE 1. (Continued from preceeding page)

Region Strain	State	Location	Pathotype no. ^a	RAPD ^b haplotype	ITS group
E12	Chimborazo	Pallatanga	v2	51	II
E13	Chimborazo	Pallatanga	v1	49	II
E14	Chimborazo	Pallatanga	v1	52	II
E15	Chimborazo	Pallatanga	v1	49	II
E16	Chimborazo	Pallatanga	v1	50	II
E17	Chimborazo	Pallatanga	v1	53	II
E18	Chimborazo	Simbambe	v1	55	II
E19	Chimborazo	Simbambe	v1	52	II
E20	Chimborazo	Simbambe	v1	52	II
E21	Chimborazo	Simbambe	v1	54	II
E22	Chimborazo	Capzol	v1	56	II
E23	Chimborazo	Capzol	v1	50	II
E24	Azuay	Giron	v1	50	II
E25	Azuay	Giron	v1	53	II
E26	Azuay	Giron	v1	53	II
E27	Azuay	Giron	v1	49	II
E28	Loja	Macara	v1	50	II
E29	Loja	Macara	v1	49	II
Argentina					
A1	Tucuman	Raco	v14	49	II
A2	Tucuman	Raco	v13	49	II
A3	Tucuman	Raco	v6	58	II
A4	Tucuman	Raco	v1	49	II
A5	Tucuman	Raco	v6	59	II
A6	Tucuman	Raco	v7	60	II
A46	Tucuman	Tafi del Valle	v8	57	II
A47	Tucuman	Tafi del Valle	v13	49	II
A7	Tucuman	Trancas	v8	61	II
A8	Tucuman	Trancas	v4	50	II
A9	Tucuman	Trancas	v1	62	II
A10	Tucuman	Trancas	v8	63	II
A11	Tucuman	Trancas	v10	64	II
A12	Tucuman	Trancas	v7	65	II
A13	Tucuman	Trancas	v6	65	II
A14	Tucuman	Trancas	v7	65	II
A15	Tucuman	Trancas	v5	66	II
A16	Tucuman	Trancas	v1	49	II
A17	Tucuman	Trancas	v6	67	II
A18	Tucuman	Trancas	v11	49	II
A19	Tucuman	Trancas	v6	70	II
A20	Tucuman	Trancas	v3	68	II
A21	Tucuman	Alpachiri	v3	49	II
A22	Tucuman	Alpachiri	v6	49	II
A23	Tucuman	Alpachiri	v12	50	II
A24	Tucuman	Alpachiri	v5	55	II
A25	Tucuman	Alpachiri	v12	69	II
A26	Tucuman	Alpachiri	v13	70	II
A27	Tucuman	Alpachiri	v13	65	II
A28	Tucuman	Yunca Suma	v6	49	II
A48	Tucuman	Higuerillas	v6	71	II
A49	Tucuman	Higuerillas	v6	71	II
A29	Salta	Lesser	v9	55	II
A30	Salta	Yaconnes	v6	49	II
A31	Salta	Yaconnes	v4	49	II
A32	Salta	Yaconnes	v2	49	II
A33	Salta	Yaconnes	v5	49	II
A34	Salta	Yaconnes	v2	49	II
A35	Salta	Yaconnes	v7	70	II
A36	Salta	Yaconnes	v2	49	II
A37	Salta	Yaconnes	v4	72	II
A38	Jujuy	Yala	v2	73	II
A39	Jujuy	Yala	v2	49	II
A40	Jujuy	Yala	v15	74	II
A41	Jujuy	Yala	v2	65	II
A42	Jujuy	Yala	v5	49	II
A43	Jujuy	Yala	v2	49	II
A44	Jujuy	Lozano	v2	49	II
A45	Jujuy	Lozano	v8	75	II

(PN3, 5'CCGTTGGTGAACCAGCGGAGGGATC3', and PN10, 5'TCCGCTTATTGATATGCTTAAG3') located, respectively, in the 18S and 28S rDNA regions were used to amplify the two ITS (ITS 1 and 2) and the 5.8S subunit of the ribosomal genes. Amplification reactions were performed in a final volume of 100 µl, with 10 µl of 10× *Taq* DNA polymerase buffer (Appligène, Illkirch, France), 500 mM each dNTP (Pharmacia, Saclay, France), 50 pmol each primer, 100 ng of DNA, and 1 unit of *Taq* DNA polymerase (Appligène). Amplification was performed in a DNA thermal cycler 9600 (Perkin-Elmer Cetus, Norwalk, CT) programmed for 2 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. Twenty microliters of the amplified product was digested with 10 units of *Hae*III and *Msp*I. The digestion products were separated by electrophoresis in 3% Metaphor gel (FMC Bioproducts, Rockland, MD) containing 0.4 mg of ethidium bromide per ml in Tris-borate EDTA (TBE) buffer for 2 h at 2.5 V/cm. Experiments were performed twice.

PCR amplification with random primers. Four decamer oligonucleotides from Operon Technologies (Alameda, CA) (OPC-02, OPD-03, OPD-05, and OPD-08) chosen to provide reproducible polymorphisms among the Argentinean strains were used to test all the samples. Amplification reactions were performed in a final volume of 25 µl, with 2.5 µl of 10× amplification buffer (50 mM Tris-HCl, pH 9.0; 16 mM (NH₄)₂SO₄; 50 mM KCl; 7 mM MgCl₂; and 0.2 mg of bovine serum albumin per ml), 200 mM each dNTP

(Pharmacia), 10 pmol primer, 30 ng of DNA, and 1.5 units of *Taq* DNA polymerase (Appligène). Amplifications were conducted in a DNA thermal cycler 9600 (Perkin-Elmer) programmed for 2 min at 92°C, followed by 45 cycles of 30 s at 92°C, 1 min at 35°C, and 1 min at 72°C. Amplification products were separated by electrophoresis on 1.5% agarose gels containing 0.4 mg of ethidium bromide per ml in TBE buffer for 4 h at 2.5 V/cm. Control samples without DNA were used to check that no self-amplification or DNA contamination occurred. Reproducibility was tested by three independent replicates for each experiment.

Pathogenicity tests. The susceptible cv. La Victoire was used to test pathogenicity. Twelve differential cultivars proposed by Pastor-Corrales (24) were used for pathotype characterization. Of these, six differentials harbored single resistance genes (*Co-1*, -2, -3, -4, -5, and -6) and G2333 harbored at least two genes, *Co-5* and -7 (34). Four cultivars were of southern Andean origin and eight of Mesoamerican origin (Table 2). Ten plants of each differential were grown in randomized blocks at 23°C and 16 h of light. Conidia were harvested by flooding plates with distilled water, and the suspension was adjusted to 10⁶ spores per ml. Seven-day-old seedlings at the two-leaf stage were sprayed with the spore suspension and incubated at 19°C, 16 h of light, and high relative humidity (95%). Symptoms were scored 7 days after inoculation on a 5-point scale: 1 = plants without visible symptoms; 2 = plants showing brown, limited, necrotic lesions; 3 = plants with large lesions; 4 = plants with sporulating lesions; and 5 = dead plants. In-

TABLE 2. Virulence pathotypes for 128 strains of *Colletotrichum lindemuthianum* isolated from natural populations of *Phaseolus vulgaris* in Mexico, Ecuador, and Argentina

No. of strains representing each pathotype			Pathotype no.	Andean ^a cultivars				Mesoamerican ^a cultivars							
Argentina	Ecuador	Mexico		M.D.R.K. <i>Co-1</i> ^d	Per. Mar. ^b	Widusa	Kaboon	Michelite	Cornell <i>Co-2</i>	Mex. 222 ^c <i>Co-3</i>	PI 207262	TO <i>Co-4</i>	TU <i>Co-5</i>	AB 136 <i>Co-6</i>	G2333 <i>Co-5, -7</i>
3	28	13	v1	–	–	–	–	–	–	–	–	–	–	–	–
8	1		v2	V ^e	V	–	–	–	–	–	–	–	–	–	–
2			v3	–	–	–	–	V	–	–	–	–	–	–	–
3			v4	V	–	–	–	–	–	–	–	–	–	–	–
3			v5	V	–	–	–	V	–	–	–	–	–	–	–
10			v6	–	V	–	–	–	–	–	–	–	–	–	–
4			v7	–	V	–	–	V	–	–	–	–	–	–	–
5			v8	V	V	–	–	V	–	–	–	–	–	–	–
1			v9	–	V	–	V	–	–	–	–	–	–	–	–
1			v10	V	V	V	V	V	V	–	–	–	–	–	–
1			v11	–	–	–	–	V	–	–	V	–	–	–	–
2			v12	–	V	–	–	–	–	–	V	–	–	–	–
4			v13	–	V	–	–	V	–	–	V	–	–	–	–
1			v14	V	V	V	–	V	V	–	V	–	–	–	–
1			v15	V	–	–	–	–	–	–	–	V	–	–	–
		1	v16	–	–	–	–	–	–	V	–	–	–	–	–
		2	v17	–	–	–	–	–	V	V	–	–	–	–	–
		2	v18	–	–	–	–	–	–	–	V	–	–	–	–
		12	v19	–	–	–	–	–	–	–	–	V	–	–	–
		2	v20	–	–	–	–	V	–	–	–	V	–	–	–
		3	v21	–	–	–	–	–	V	–	–	V	–	–	–
		1	v22	–	V	–	V	–	V	–	–	V	–	–	–
		8	v23	–	–	–	–	–	–	V	–	V	–	–	–
		2	v24	–	–	–	–	V	–	V	–	V	–	–	–
		4	v25	–	–	–	–	–	V	V	–	V	–	–	–

^a Origin of differential cultivars.

^b Perry Marrow.

^c Mexico 222.

^d Resistance gene.

^e V = virulence and – = avirulence.

TABLE 3. Random amplified polymorphic DNA (RAPD) and virulence diversity of *Colletotrichum lindemuthianum* in Mexico, Ecuador, and Argentina

Region	Sample size	RAPD diversity				Virulence diversity			
		No. of markers	<i>H</i> ^a	No. of RAPD haplotypes	<i>H'</i> ^b	No. of virulence factors	<i>H</i>	No. of pathotypes	<i>H'</i>
Combined	128	40	0.173 (0.027)	75	3.36	9	0.186 (0.016)	25	2.36
Mexico	50	35	0.171 (0.023)	48	3.39	7	0.174 (0.004)	11	1.93
Ecuador	29	6	0.028 (0.014)	8	1.5	2	0.015 (0.000)	2	0.13
Argentina	49	17	0.043 (0.011)	22	1.97	8	0.205 (0.004)	15	2.29

^a *H* = Nei unbiased diversity index (23). Variance is indicated in parentheses.

^b *H'* = Corrected Shannon diversity index (16).

fection types 1 and 2 were classified as resistant, and infection types 3, 4, and 5 were classified as susceptible.

Data analysis. Each polymorphic RAPD band was considered a locus with two alleles: presence or absence of the amplified fragment. Pathogenicity on each cultivar of the differential set was considered one independent factor with two classes: virulent or avirulent. Intraregion diversity was determined for molecular markers or virulence factors by Nei's unbiased diversity index (23). Genetic differentiation for each RAPD locus or virulence factor among regions and random association among pairs of different markers within regions were tested by Fisher's exact tests with GENEPop software (28).

RAPD haplotypes and virulence pathotypes were used for multilocus analysis. A haplotype was defined as a combination of RAPD alleles at all polymorphic loci. A pathotype was identified as a combination of virulence factors of a strain on the set of 12 differentials. Haplotype or pathotype diversity was estimated within each region by a corrected Shannon diversity index (16,26).

Analysis of molecular variance (AMOVA) (8) was used to estimate variance components for RAPD haplotypes and virulence pathotypes, partitioning the variation among individuals within and among regions. Genetic distance calculated as a Euclidean metric distance was computed between all pairs of isolates. The Euclidean metric distance was evaluated as the number of different alleles between two RAPD haplotypes and the number of different virulence factors between two pathotypes. Significance levels for variance component estimates were computed by nonparametric permutation procedures. The analyses were undertaken with the WINAMOVA program provided by L. Excoffier (Department of Anthropology and Ecology, University of Geneva, Geneva).

Distances between all pairs of isolates were evaluated on polymorphic and monomorphic RAPD products. The Jaccard dissimilarity index (18) was the distance index used. A dendrogram based on the distance values between all pairs of isolates was constructed by unweighted pair group method arithmetic (UPGMA) analysis. Distance matrices and cluster analysis were performed with the SAS computer program (release 6.03, SAS Institute, Cary, NC).

RESULTS

Genetic variability in the ITS region. Primers PN3 and PN10 amplified an ITS region of 600 bp for all strains. After digestion of the ITS region with *Hae*III and *Msp*I, two groups (I and II) similar to those found in a previous study (9) were distinguished. Group I has one additional *Msp*I and *Hae*III recognition site compared to group II. All of the Argentinean and Ecuadorian strains belonged to group II, whereas the Mexican population was composed of both groups I (32 strains) and II (18 strains) (Table 1).

RAPD analysis. The four primers used in this study revealed DNA polymorphism among the 128 strains of *C. lindemuthianum*

tested. A total of 40 polymorphic and three monomorphic bands were obtained. Within Mexico, 35 markers were polymorphic, whereas 17 were polymorphic in Argentina and 6 were polymorphic in Ecuador (Table 3). Regarding allele distribution, 21 private alleles were identified in Mexico, whereas only 4 were found in Argentina and 0 in Ecuador. Most of the alleles found in Argentina and Ecuador also were present in Mexico. Intraregion diversity estimated by Nei's unbiased index showed that the Mexican region had a diversity of 0.171, which is almost the total diversity, estimated at 0.173 (Table 3). Ecuador and Argentina appeared less polymorphic, with index values of 0.028 and 0.043, respectively. Analysis of region differentiation revealed that 22 loci significantly differentiated ($P < 0.05$) the Mexican region from the Andean regions. Two loci differentiated Ecuador from Argentina.

The 40 RAPD polymorphic markers allowed identification of 75 haplotypes among the three regions (Table 1). In Mexico, 48 haplotypes occurred, so almost every strain was represented by 1 haplotype. Among 49 and 29 strains in Argentina and Ecuador, 22 and 8 haplotypes were identified, respectively. To test whether the differences in the number of haplotypes could be due to unequal sample sizes, 20 combinations of 29 individuals randomly drawn from Argentina were used. An average number of 14 haplotypes was observed in Argentina using this procedure, with a minimum of 10 and a maximum of 18 haplotypes; thus, the number of haplotypes is higher in Argentina than in Ecuador. Haplotype 49 was the most frequent haplotype in Argentina (45%) and Ecuador (39%) (Table 1). This haplotype was widely distributed in the two regions and absent in Mexico. Intraregion diversity was estimated by the Shannon diversity index, which incorporated a measure of the number and evenness of haplotype frequencies. Results showed that the Mexican region was most polymorphic (Table 3).

AMOVA showed that 58.46% of the haplotype diversity was between regions. Statistical analysis revealed significant differences between the three regions. A more detailed analysis that computed pairwise differences between regions showed that the distance between the Mexican and Andean regions was higher than the distance between Ecuador and Argentina (Table 4).

This result was confirmed by phenetic analysis. A dendrogram was constructed by UPGMA analysis based on Jaccard distance between strains (data not shown). Two main clusters were identified among *C. lindemuthianum* isolates, with a distance of 0.46. All Mexican strains, except one (M19), belonged to one group. All Argentinean and Ecuadorian strains belonged to the second group.

Virulence variation. The 128 *C. lindemuthianum* strains were characterized for their virulence on the set of 12 differential cultivars. Virulence on nine differentials was observed and allowed identification of nine virulence factors, whereas three differentials were resistant to the 128 strains tested (Table 2). All strains presented the three avirulence genes that correspond to the resistance genes *Co*-5, -6, and -7, whereas resistance genes *Co*-1, -2, -3, and -4 were overcome by 23, 12, 17, and 33 of the 128 strains, respectively.

TABLE 4. Φ -statistic values between pairs of regions estimated on random amplified polymorphic DNA (RAPD) haplotype or pathotype diversity of *Colletotrichum lindemuthianum* strains

Statistic Region	Mexico	Ecuador
Φ (P^a) estimated on RAPD		
haplotype diversity		
Ecuador	0.588 (0.000)	—
Argentina	0.616 (0.000)	0.056 (0.001)
Φ (P) estimated on pathotype		
diversity		
Ecuador	0.343 (0.000)	—
Argentina	0.432 (0.000)	0.395 (0.000)

^a Probability of having a larger Φ -statistic value than the observed value by chance alone is indicated in parentheses. Φ statistics were tested by 1,000 random permutations of individuals across regions without regard to their original region (analysis of molecular variance [AMOVA] software).

TABLE 5. Number of significant associations between pairs of random amplified polymorphic DNA (RAPD), internal transcribed spacer (ITS), and virulence markers within *Colletotrichum lindemuthianum* in three regions

Region	Marker	No. of significant ^a /no. of calculated combinations		
		ITS ^b	RAPD	Virulence
Mexico	RAPD	6/35 (17%)	43/595 (7%)	
	Virulence	1/7 (14%)	12/245 (5%)	1/21 (5%)
Ecuador	RAPD	—	1/15 (6%)	
	Virulence	—	0	1/1 (100%)
Argentina	RAPD	—	4/136 (3%)	
	Virulence	—	2/136 (1.5%)	1/28 (3.5%)

^a $P < 0.05$.

^b Dash indicates no possible calculation.

In Mexico, only one strain was virulent on cultivars of southern Andean origin (Table 2). In Argentina, the most frequent virulence was on the two Andean cultivars, Perry Marrow (71% of the strains) and M.D.R.K (44.9% of the strains); this virulence significantly differentiated ($P < 0.05$) Argentina from the two other regions. In Mexico, the most frequent virulence on Mesoamerican cultivars was on Cornell, Mexico 222, and TO, which significantly differentiated ($P < 0.05$) the Mexican from the other two regions.

A total of 25 pathotypes was identified among the 128 *C. lindemuthianum* strains tested (Table 2). The pathotype that was "avirulent on all differentials" (pathotype v1) was the only common pathotype in the three regions (Table 1). In Ecuador, this pathotype was the most frequent one, whereas in Mexico and Argentina no pathotype was predominant. Pathotype v1 was widely distributed in Mexico and Argentina. Ten pathotypes were specific to Mexico and thirteen to Argentina (Table 2). The diversity in each region, estimated by the corrected Shannon diversity index, showed that Ecuador was much less polymorphic than the two other regions (Table 3).

Variance component analysis showed that 41.5% of the pathotype diversity was between the regions, and the three regions were significantly differentiated ($P < 0.05$). The F values of differentiation between pairs of regions ranged from 0.343 between Mexico and Ecuador to 0.432 between Mexico and Argentina (Table 4).

Random association between ITS, RAPD, and virulence markers. The number of significant associations ($P < 0.05$) between RAPD and virulence markers ranged from 1 of 15 (6%) calculated combinations in Ecuador to 43 of 595 (7%) calculated combinations in Mexico (Table 5). Combinations between virulence markers within regions showed that one combination was detected in nonrandomly expected frequencies in each region (Table 5). This combination was different in each region, and the virulence factors involved were cvs. Widusa/Kaboon in Argentina, Perry Marrow/M.D.R.K. in Ecuador, and Perry Marrow/Kaboon in Mexico. Most combinations between RAPD and virulence markers were randomly associated in the three populations. Associations between ITS, RAPD loci, and virulence factors could be evaluated only in Mexico; results showed that most markers were randomly associated (Table 5). Only one combination of ITS type and virulence markers (virulence on differential TO) was found in nonrandomly expected frequencies; this combination involved isolates from different locations.

DISCUSSION

RAPD markers and the restriction patterns of the ITS region were used to characterize *C. lindemuthianum* isolates from Mexico, Argentina, and Ecuador. The highest level of molecular diversity was observed within the Mexican region. Many alleles were found only in Mexico, whereas the majority of the alleles present in Ecuador and Argentina also were found in Mexico. These results suggest that the Andean strains arose from the Mexican region. The genetic distance between the two Andean regions was lower than the genetic distance between the Mexican and Andean regions. Moreover, both Andean regions had the same predominant RAPD haplotype. Taken together, these results led to the hypothesis that founder events originating from Mexico gave rise to the Argentinean and Ecuadorian strains. A founder event also may explain the clear differentiation between the Mexican and Andean regions, which was relatively high compared to levels of differentiation reported for other fungal populations (2,15,22). The introduction of a limited number of strains from Mexico to the Andes favors genetic drift as an explanation, and differentiation as has been proposed by Peever and Milgroom (27) for *Pyrenophora teres*.

Concerning the origin of the host species, two hypotheses have been proposed. Either wild *P. vulgaris* originated from Mesoamer-

ica (5), or the ancestral type is represented by the Ecuadorian/northern Peruvian populations (19). However, it seems unlikely that the *C. lindemuthianum* population from Ecuador is the ancestral population. If such was the case, we would have to suppose that most of the diversity of the Ecuadorian pathogen population was transferred to Mesoamerica and the southern Andes. Rather, it is still possible that the *C. lindemuthianum* origin was the Mesoamerican area, whereas the center of origin of *P. vulgaris* was located in the northern Andean region. Studies on *Phytophthora infestans* diversity showed that the center of origin of the pathogen was located in Mexico, which is not the center of origin of the cultivated host (11).

The three populations were differentiated by molecular markers as expected for a pathogen with limited means of dissemination such as *C. lindemuthianum* (32). The presence of 22 private alleles in Mexico and 4 in Argentina, and the absence of identical haplotypes in the Mexican and Andean regions provided further evidence of limited gene flow between these regions in the recent past. Results were less clear concerning migration between Ecuador and Argentina. The most frequent RAPD haplotype in Ecuador was also the most frequent in Argentina, which may suggest some exchange between these two regions. However, this haplotype may correspond to a genotype introduced from the Mesoamerican area to the Andean region.

Most combinations between RAPD, ITS, and virulence markers were randomly associated in the three regions. The low percentage of nonrandom associations agreed with the situation found in fungal species that reproduce sexually (27). The sexual form of *C. lindemuthianum* has been described in vitro, but experiments were not reproducible due to the low viability of the ascospores (3). It is possible that sexual or parasexual reproduction leading to recombination between loci occurs in natural populations.

Concerning pathotype diversity, the Ecuadorian region was different from the others because of the frequency of pathotype v1 (avirulent on all the differentials used), which was represented by all but one strain. Compared to its distribution range in Mexico and the southern Andes, wild common bean has a relatively narrow distribution in Ecuador. The few wild bean populations found in Ecuador might represent relics of a more abundant ancestral population (6). The reduction of plant population size could have been followed by a reduction in its pathogen population size, favoring genetic drift. The high frequency of pathotype v1 also could suggest that the common bean differentials did not allow an appropriate description of virulence diversity in Ecuador. Actually, the differential set did not contain any cultivar of northern Andean origin.

The three pathogen regions were highly differentiated for virulence factors. Moreover, Ecuador and Argentina were much more different for virulence factors than for RAPD markers. These results suggest that reciprocal selection pressures exerted between host and pathogen greatly influence pathogen diversity. All Mexican strains, except one, were virulent exclusively on Mesoamerican cultivars. Although Argentinean strains were able to infect cultivars of Andean and Mesoamerican origin, virulence on Andean cultivars was most frequent. These results suggest an adaptation of the Mexican and Argentinean strains to cultivars of the same geographic origin. The virulence factors of the Argentinean pathogen on the Mesoamerican cultivars may have been introduced during a founder event from Mesoamerica to Argentina. The maintenance of this virulence could be explained by the presence of the corresponding resistance genes in the wild host population. However, Cattan-Toupance et al. (4) reported that resistance genes *Co-2*, *-3*, *-4*, and *-5* are absent from the Argentinean wild common bean populations.

The three pathogen regions studied in this paper were differentiated for molecular and virulence markers. This supports the hypothesis that coevolution led to differentiated groups in the pathogen comparable to what was observed for *P. vulgaris* gene

pools (13). Some preliminary evidence for two molecular groups of pathogens, related to the Mesoamerican or southern Andean cultivated gene pools has been suggested for *P. griseola* (17). Our results indicate that pathogen groups appeared in natural populations and suggest that divergence resulted from founder events and host selection pressure. The hypothesis of differentiation in virulence among the three pathogen populations as a result of reciprocal selection between hosts and pathogens in the wild will be investigated further.

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