Investigating the Genetic Structure of *Phytophthora capsici* Populations

L. M. Quesada-Ocampo, L. L. Granke, M. R. Mercier, J. Olsen, and M. K. Hausbeck

Department of Plant Pathology, Michigan State University, East Lansing 48824.

Accepted for publication 7 April 2011.

ABSTRACT

Quesada-Ocampo, L. M., Granke, L. L., Mercier, M. R., Olsen, J., and Hausbeck, M. K. 2011. Investigating the genetic structure of *Phytophthora capsici* populations. *Phytopathology* 101:1061-1073.

Phytophthora capsici Leonian is a destructive soilborne pathogen that infects economically important solanaceous, cucurbitaceous, fabaceous, and other crops in the United States and worldwide. The objective of this study was to investigate the genetic structure of 255 *P. capsici* isolates assigned to predefined host, geographical, mefenoxam-sensitivity, and mating-type categories. Isolates from six continents, 21 countries, 19 U.S. states, and 26 host species were genotyped for four mitochondrial and six nuclear loci. Bayesian clustering revealed some population structure by host, geographic origin, and mefenoxam sensitivity, with some clusters

occurring more or less frequently in particular categories. Bayesian clustering, split networks, and statistical parsimony genealogies also detected the presence of non-*P. capsici* individuals in our sample corresponding to *P. tropicalis* ($n = 9$) and isolates of a distinct cluster closely related to *P. capsici* and *P. tropicalis* ($n = 10$). Our findings of genetic structuring in *P. capsici* populations highlight the importance of including isolates from all detected clusters that represent the genetic variation in *P. capsici* for development of diagnostic tools, fungicides, and host resistance. The population structure detected will also impact the design and interpretation of association studies in *P. capsici*. This study provides an initial map of global population structure of *P. capsici* but continued genotyping of isolates will be necessary to expand our knowledge of genetic variation in this important plant pathogen.

Phytophthora capsici Leonian is a destructive soilborne pathogen that is distributed worldwide. It can infect a broad range of hosts, including economically important solanaceous, cucurbitaceous and fabaceous crops in the United States (16,21,23,66). *P. capsici* also causes disease on tropical hosts such as cacao (*Theobroma cacao*), rubber (*Hevea brasiliensis*), macadamia (*Macadamia integrifolia*), papaya (*Carica papaya*), black pepper (*Piper nigrum*) (11), and rocoto pepper (*Capsicum pubescens*) (36). Additional hosts continue to be identified (23,65,66). *Phytophthora capsici* is a diploid oomycete that is heterothallic (11). Hence, sexual reproduction occurs when the A1 and A2 mating types (MTs) come together to produce oospores (43). It is common in vegetable-producing regions in the United States for both MTs to be present (32,42,69). The success of crop rotation as a management strategy is limited by the long-term survival of oospores in the soil (45) and the number and diversity of susceptible hosts (11).

Developing resistant cultivars for economically important hosts has been challenging due to the diversity of pathogen populations and the existence of different physiological races within *P. capsici* in the United States (56,81). A detailed knowledge of the population structure and distribution of genetic variation of *P. capsici* is needed to understand the genetic composition of isolates that occur in particular hosts or regions to develop and intelligently deploy host resistance. Characterizing *P. capsici* populations for fungicide resistance is also important to establish whether a product will provide effective chemical control against local isolates. Chemical control of *P. capsici* with mefenoxam, a commonly used fungicide, may not protect susceptible crops from

resistant pathogen populations, which have been documented throughout the United States (42,43,46,59).

Populations of *P. capsici* have been previously studied within states in the United States (18,44,79,82) and in countries where this pathogen causes significant losses (50,73). Such investigations have provided information about local genetic diversity of isolates and the importance of recombination in maintaining genetic variability (43,44). Other studies have analyzed *P. capsici* intraspecific data using traditional phylogenetic methods and have established a correlation between phylogenetic cluster and host type, where isolates from woody perennials group together and separate from isolates obtained from vegetable crops (7,10). Previous experiments have also used *P. capsici* intra- and inter-specific data to determine whether *P. capsici* and *P. tropicalis*, a sibling species, truly constitute separate species (7,10,53,88) but there is still controversy regarding the evolutionary relationships of these pathogens (7). Studies including worldwide samples to examine the global population structure and distribution of genetic variation in *P. capsici* by geography and host are still lacking.

Most studies of plant pathogen diversity are based on sampling from predefined populations, referred to as “categories” in this article. These categories are usually defined by host, geography, or physiology and may not reflect underlying genetic relationships. Bayesian clustering can be applied to assign individuals in a sample to subpopulations, referred to as “clusters” in this article, based on their distinct allele frequencies (63). This method has been successfully used to visualize overall patterns of genetic structure in several species (5,13,14,17,40,71,78). Nonetheless, Bayesian clustering has not been as extensively used to detect population structure in plant pathogens and explore the distribution of isolates in different regions or hosts, which may have implications for disease management. In this study, we used Bayesian clustering and other methods to investigate the genetic structure of global *P. capsici* populations. Specifically, we sought to (i) establish whether we could detect population structure in the

Corresponding author: M. K. Hausbeck; E-mail address: hausbec1@msu.edu

*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains three supplementary figures and five supplementary tables.

doi:10.1094/PHYTO-11-10-0325

© 2011 The American Phytopathological Society

sample; (ii) determine whether the predefined grouping of *P. capsici* isolates in host, geography, mefenoxam-sensitivity, and mating-type categories showed direct correspondence with inferred genetic clusters; and (iii) examine the distribution of isolates belonging to each cluster within the predefined categories.

MATERIALS AND METHODS

Isolate selection, maintenance, and phenotypic characterization. In total, 255 *P. capsici* isolates originating from diverse hosts throughout the world were obtained from colleagues or selected from the culture collection maintained in the laboratory of Dr. Hausbeck at Michigan State University (MSU) (Supplementary Table 1). Type cultures for *P. capsici* 13691 (American Type Culture Collection [ATCC] 64531, Italy 1927) and 13692 (ATCC 15399, New Mexico, deposited 1965) and *P. tropicalis* 13602 (ATCC 76651, Hawaii 2001) were included in the analysis. Actively growing, single-spore cultures were obtained from long-term stock cultures as previously described (65). Agar plugs from actively growing cultures of each isolate were transferred to 50-ml centrifuge tubes containing 30 ml of unclarified V8 juice broth (UCV8B; 3 g of CaCO₃, 160 ml of unfiltered V8 juice, and 840 ml of distilled water), which were maintained at room temperature (21 ± 2°C) under constant fluorescent lighting and shaking (0.03 × g) to obtain agar-free tissue for DNA extraction. Isolates were characterized according to MT and sensitivity to mefenoxam as previously described (42). The departure of MT ratios from the expected ratio of 1:1 (A1:A2) was investigated using a binomial test with exact *P* values to account for small sample sizes (PROC FREQ, SAS statistical package version 9.1; SAS Institute, Inc., Cary, NC). *P. capsici* was confirmed using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (83).

DNA extraction. Contents of UCV8B centrifuge tubes with actively growing mycelia were vacuum filtered through one layer of Whatman grade 1 filter paper. Tissue remaining on the filter paper was transferred to a mortar using a toothpick and ground with a pestle in liquid nitrogen. All implements were previously sterilized. Genomic DNA was extracted from approximately 1 g of ground tissue using the DNeasy Plant Minikit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was quantified using the NanoDrop ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE). DNA integrity was analyzed by electrophoresis in 2% (wt/vol) agarose gel in 0.5× Tris-borate-EDTA buffer (52), stained with ethidium bromide (5 µg/ml) for visualization.

Primer design, DNA amplification, and sequencing. Genomic regions to be used as a source of single-nucleotide polymorphism (SNP) markers in *P. capsici* were identified in GenBank (Supplementary Table 2). Selected regions were previously used in oomycete coalescence, genetic diversity, and phylogenetic studies (6,10,25,58). *P. capsici* sequences from GenBank were input into Primer3 (72) for primer design using default settings. Four regions of the mitochondrial genome (Cox 1, Cox 2, Nad 1, and Nad 5) and six nuclear genes (β-tubulin, EF-1α, Enolase, HSP90, Tig A, and Ura 3) were amplified by polymerase chain reaction (PCR). PCR was performed in a total volume of 25 µl and contained 1 µl of 5-ng/µl DNA, 5 µl of 5× PCR reaction buffer (Invitrogen, Carlsbad, CA), 1.25 µl of 25 µM MgCl₂ (Invitrogen), 0.5 µl of 10 µM dNTP mix (Invitrogen), 1 µl of each 10 µM primer (MSU Macromolecular Structure Facility, East Lansing), 0.7 µl of Platinum Taq DNA polymerase (Invitrogen), and 14.6 µl of sterile water. The PCR was performed in a programmable Eppendorf mastercycler ep systems thermal cycler (Eppendorf, Westbury, NY) starting with 3 min of denaturation at 94°C; followed by 45 cycles at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s; with a final extension step of 10 min at 72°C. PCR products were

analyzed by electrophoresis in 2% (wt/vol) agarose gel in 0.5× Tris-borate-EDTA buffer (52), stained with ethidium bromide (5 µg/ml) for visualization, and compared with a 100-bp ladder (Invitrogen). Controls with no *P. capsici* DNA were included.

PCR products were purified using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA) following the manufacturer's instructions. Cycle sequencing reactions (1 µl of purified PCR product, 3 µl of primer, and 8 µl of sterile water) were done twice directly from the clean PCR products at the MSU Research Technology Support Facility (East Lansing) using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). A subset of samples with heterozygote positions (40%) determined from sequence analysis were resolved into haplotypes by cloning the corresponding PCR product using the pGEM-T Easy Vector System (Promega, San Luis Obispo, CA) and Subcloning Efficiency DH5α Competent Cells (Invitrogen) following the manufacturer's instructions. Cloned PCR products were purified using the QIAquick PCR purification kit (Qiagen) following the manufacturer's instructions, and amplified and sequenced as described above. Obtained haplotypes were confirmed by using PHASE (74) as implemented in DnaSPv5 (49) by running simulations with 5,000 iterations. In all cases, the inferred haplotype with cloning and direct sequencing data matched the haplotypes obtained using PHASE. Haplotypes of remaining samples with heterozygote positions were inferred with PHASE.

Sequence analysis. Manual editing of base calls and sequence alignment were performed using Lasergene SeqMan Pro version 8.0 (DNASTAR Inc., Madison, WI). The alignment was exported as a FASTA file and imported into MacClade (51) to produce a NEXUS file required for subsequent analyses. The two haplotypes within sequences containing heterozygote sites were inferred by haplotype subtraction (25) according to data from direct and cloned PCR product sequencing or by using PHASE as described above. Sequences from mitochondrial and nuclear loci were analyzed individually and collapsed into unique haplotypes using DnaSPv5. Haplotypes found for each gene were deposited in GenBank under accession numbers HQ388837 to HQ389193. Haplotype sequences were compared with sequence data publicly available and sequences from type cultures included in our study by using nucleotide BLAST (1,2) to determine that data corresponded to the *P. capsici* target genes. BLAST analyses indicated that some *P. capsici* isolates corresponded to *P. tropicalis* and to another group of isolates, with some genes showing high similarity to *P. capsici* and others to *P. tropicalis*. These isolates are referred to in this article as intermediate. Two separate data sets were created for subsequent analysis to account for the presence of isolates that did not have high genetic similarity to *P. capsici* in all sequenced genes: *P. capsici* sensu stricto (SS), which included isolates with all gene sequences having high similarity to only *P. capsici*, and *P. capsici* sensu lato (SL), which included all isolates. Base substitutions in SS were classified as phylogenetically informative or uninformative, transitions or transversions, and synonymous or nonsynonymous substitutions using DnaSPv5.

DNA sequence variability, neutrality tests, recombination, and genealogies. Polymorphism, neutrality, and recombination analyses were performed for each gene and all SS isolates using the program DnaSPv5. Polymorphism estimates were also calculated for SS isolates grouped in geographic (hemisphere, continent, country, U.S. state, and United States or not), host (vegetable crop or not, host family, and host species), mefenoxam resistance, and MT categories. Polymorphism analysis was also performed for SL isolates grouped in a species (*P. capsici* or not) category. Previous work used five individuals as the minimum needed for population analyses in *P. ramorum* (28). We included only categories with at least eight individuals in SS for analyses. Sequence diversity estimates and statistics, including the number of segregating sites (*s*) and haplotypes (*h*), haplotype diversity

(Hd) (54), Tajima's π (76), the average number of pairwise nucleotide differences (k) (76), and Watterson's theta (θ_w) per sequence (84), were calculated. Sequence variation in each gene for all SS isolates was tested for deviations from neutrality by using Fu and Li's D and F and Tajima's D (19,77). The recombination parameter (R) per gene (34) and minimum number of recombination events (RM) (35) were also estimated for each gene and all SS isolates. The split network method implemented in SplitsTree v. 4b06 (37) was used to visualize incompatibilities in SL haplotypes by generating a NeighborNet network based on uncorrected P distances. Network support was assessed by running 10,000 bootstrap replicates. Statistical-parsimony genealogies for haplotypes in SL were generated using TCS v. 1.13 (8).

Population subdivision analysis. Population subdivision was assessed with the model-based Bayesian clustering algorithm implemented in Structure 2.3X (63). The values for burnin, chain replication, and lambda were set at 300,000, 100,000, and 1, respectively, based on results obtained in preliminary analyses. The optimal number of populations (K) was determined by comparing posterior distribution likelihoods among three independent runs of $K = 1$ to 40 using the established parameters. Data included all loci individually coded by haplotype and were analyzed under the admixture model with correlated allele frequencies and without previous population information. Population structure figures with prior population information, obtained from the defined geographic, host, mefenoxam-sensitivity, and MT categories for SS and species category for SL and sorted by Q, were generated using the Population Sorting Tool (PST), a graphic editing program created in R (67) to visualize the distribution of clusters in predefined categories (J. J. Morrice, *unpublished data*). Genetic differentiation indexes (Wright's F_{ST}) were calculated for SS data grouped by geographic, host, mefenoxam-sensitivity, and MT categories and for SL for a species category using DnaSPv5. Statistical significance was determined for each index by running 5,000 permutations ($\alpha = 0.05$).

RESULTS

Haplotype sequences for each gene were compared with publicly available *P. capsici* sequences in GenBank and sequences from type cultures included in our study through BLAST (data not shown). Nineteen isolates that were not as similar to *P. capsici* as expected were found. Some isolates ($n = 9$) showed higher similarity to *P. tropicalis* than to *P. capsici* across all genes analyzed. Interestingly, other isolates ($n = 10$) showed higher similarity to *P. tropicalis* than *P. capsici* in some genes but had higher similarity to *P. capsici* than to *P. tropicalis* in others (intermediate isolates). *P. tropicalis* and intermediate isolates were included in the SL ($n = 255$) data set and excluded from SS ($n = 236$) for further analyses.

All genes were polymorphic but nuclear genes were more variable than mitochondrial genes, as indicated by polymorphism analysis (Supplementary Table 3) and diversity estimates (Supplementary Table 4). The total number of segregating sites detected for all SS genes was 120 out of 4,170 bp analyzed. In general, all nuclear genes presented moderate to high values of R and RM. Fu and Li's D and F indicated a significant deviation from neutrality in Nad5, and Fu and Li's D showed a deviation in TigA (Supplementary Table 4). Unique haplotypes occurred in all genes and their frequency range was between 20% (Nad1) and 40% (TigA) (Supplementary Table 5). Of the 255 isolates, 220 unique multilocus genotypes were detected. Each of the 9 *P. tropicalis* and 10 intermediate isolates possessed a unique multilocus genotype.

The SS structure analysis detected some population structure by host, geography, and mefenoxam sensitivity, where some clusters occurred more frequently in certain categories than others. However, the analysis showed no direct correspondence between

groupings of *P. capsici* isolates in the predefined categories (host, geography, MT, and mefenoxam sensitivity), and inferred genetic clusters. A clear peak in likelihoods in SS was observed when the number of clusters was set to nine ($K = 8$, $\ln P = -6001$; $K = 9$, $\ln P = -5926$; $K = 10$, $\ln P = -6028$). Individual ancestry coefficients were highly consistent across replicate runs. The bar plots indicated that some isolates are highly admixed, while others belong mostly to one particular cluster (Fig. 1). Isolates belonging to clusters one and seven presented fewer admixtures than individuals from other clusters. The type culture of *P. capsici* from New Mexico had membership predominantly in cluster three and partial membership in clusters four and eight, which was similar to cluster membership for current New Mexican samples. The type culture from Italy (year 1927) had membership mostly in cluster six and partial membership in clusters two and four, which was similar to cluster membership for current Italian samples.

Population structure was detected when data were organized using host categories as prior population information (Fig. 1A to C). Isolates from vegetable hosts contained representatives from all clusters but isolates belonging to cluster one occurred more frequently in nonvegetable than in vegetable hosts (Fig. 1A). F_{ST} estimates indicated moderate differentiation between isolates from vegetable and nonvegetable hosts (Table 1). Diversity estimates for isolates from nonvegetable hosts were higher than from vegetable hosts (Table 2). Cucurbitaceous hosts presented individuals from all clusters, except for cluster two that was only sampled from solanaceous hosts (Fig. 1B) and was mostly associated to *C. annuum*, *C. pubescens*, and *Solanum lycopersicon* (Fig. 1C). Solanaceous hosts presented members from all clusters. The family *Fabaceae* had isolates from clusters four, five, seven, and eight. F_{ST} values indicated low differentiation among isolates from hosts in the *Fabaceae*, *Cucurbitaceae*, and *Solanaceae* families (Table 1). The highest and lowest diversity estimates of isolates grouped by host family were observed for isolates from the *Solanaceae* and *Cucurbitaceae* families, respectively (Table 2). The pattern of cluster occurrence among cucurbit species was more similar than among solanaceous species. *C. annuum* contained members from all clusters except for clusters one and seven, while *S. melongena* included several isolates belonging mostly to cluster seven. F_{ST} estimates detected low differentiation among isolates from cucurbitaceous species but moderate to high differentiation between isolates from cucurbitaceous and solanaceous species and among isolates from solanaceous species (Table 1). The highest and lowest diversity estimates of isolates grouped by host species were observed for isolates from *C. annuum* and *C. sativus*, respectively (Table 2).

When geography categories were used as prior population information in the cluster analysis, some population structure was revealed (Fig. 2). Isolates from the Northern hemisphere were represented in all clusters while isolates from the Southern hemisphere contained less cluster diversity (Fig. 2A); this may be due to a greater sampling in the Northern versus Southern hemisphere. F_{ST} estimates indicated moderate differentiation between isolates from the Northern and Southern hemispheres (Table 3). Diversity estimates for isolates from the Northern hemisphere were higher than those obtained for isolates from the Southern hemisphere (Table 4). Clusters four and eight were sampled from all continents. North America contained isolates from all clusters and isolates belonging to cluster five were only found in this continent (Fig. 2B). Isolates belonging to cluster two were present only in North and South America. Individuals from cluster six were primarily sampled in Europe. Members from cluster three were only found in North America and Asia, and both continents had the highest diversity of cluster occurrence. High genetic differentiation was detected between isolates from Europe and from all other continents according to F_{ST} values (Table 3). Moderate to high genetic differentiation occurred among isolates

from North America, South America, and Asia. The highest and lowest diversity estimates of isolates grouped by continent were observed in Europe and South America, respectively (Table 4).

F_{ST} values showed high genetic differentiation between isolates from Italy and from all other countries and between isolates from Mexico and Taiwan (Table 3). When isolates were grouped by country, isolates from Italy and Taiwan showed the highest and lowest diversity estimates, respectively (Table 4). The U.S. contained isolates from all clusters, except for cluster one, which was associated with Guatemala and Mexico (Fig. 2D). Isolates from clusters five, seven, and nine were mostly sampled in the U.S., while isolates from clusters one and six were primarily found outside the U.S. (non-U.S.) (Fig. 2C). F_{ST} estimates indicated moderate differentiation between isolates from the U.S. and non-U.S. regions (Table 3). Diversity estimates for non-U.S. isolates were higher than those obtained for U.S. isolates (Table 4). Michigan, North Carolina, New York, and Ohio; Arizona, New Mexico, and Texas; and California and Georgia presented similar patterns of cluster occurrence (Fig. 2E). California, South Carolina, and New Jersey presented more diversity of cluster occurrence than other states. Michigan and Massachusetts contained a high number of isolates from cluster three compared with other states. Tennessee only had isolates from cluster seven. F_{ST} esti-

mates indicated moderate to high differentiation between isolates from Michigan and from all other states and isolates from Tennessee and from all other states (Table 3). The highest and lowest diversity estimates of isolates grouped by U.S. state were observed in New Jersey and Tennessee, respectively (Table 4). South Carolina was the only state with members from cluster two, which was also found in South America. Isolates from cluster six that were mostly sampled in Europe were also found within the United States in California, Hawaii, and Florida. Individuals from clusters three, four, five, seven, eight, and nine were sampled across multiple states in the United States.

Some structure was identified in isolates intermediately sensitive and insensitive to mefenoxam (Supplementary Figure 1). The category sensitive to mefenoxam contained individuals from all clusters. The categories insensitive to mefenoxam and intermediately sensitive to mefenoxam did not contain isolates from cluster nine. F_{ST} values showed low to moderate genetic differentiation between sensitive and intermediately sensitive isolates and sensitive and insensitive isolates (Table 5). Genetic differentiation was very low between insensitive and intermediately sensitive isolates (Table 5). The highest diversity estimates were found for isolates sensitive to mefenoxam (Table 6). Diversity estimates for intermediately sensitive and insensitive isolates were

Fig. 1. Genetic structure of *Phytophthora capsici* isolates grouped by **A to C**, host and **D**, species categories. Categories for sensu stricto: **A**, host type; **B**, host family; and **C**, host species and, for sensu lato: **D**, species. Each isolate is represented by a thin bar, often partitioned into colored segments, each representing the individual's proportionate genetic membership in a given Kth cluster (sorted numerically from the bottom to the top of the bar [i.e., cluster 1 will always be on the bottom of the bar, cluster 9 at the top]). Cluster colors are indicated in D and correspond to dark red, cluster 1; purple, cluster 2; yellow, cluster 3; light green, cluster 4; dark blue, cluster 5; aqua, cluster 6; dark green, cluster 7; light blue, cluster 8; pink, cluster 9; gray, cluster 10; pink, cluster 11. A white space separates individual subcategories.

comparable with each other (Table 6). Isolates insensitive and intermediately sensitive to mefenoxam were mostly found in North America, except for one isolate from Taiwan that was insensitive to mefenoxam and one from Taiwan and two from Spain that were intermediately sensitive to the fungicide. Isolates sensitive to mefenoxam were collected from many different regions of the world and hosts (Supplementary Table 1). *P. capsici* isolates insensitive and intermediately sensitive to mefenoxam originated from hosts belonging to the *Cucurbitaceae*, *Fabaceae*, *Piperaceae*, *Solanaceae*, and *Sterculiaceae* families.

No evident population structure was detected when the data were analyzed using MT (Supplementary Figure 1) as prior population information. Both MTs contained individuals from all clusters. F_{ST} values indicated very low genetic differentiation between A1 and A2 isolates (Table 5). Diversity estimates for A1 isolates were higher than those obtained for A2 isolates (Table 6). MT distribution in the total sample (A1: 125, A2: 121 for the total sample, binomial test $P = 0.8484$) (Supplementary Table 1) for North America ($P = 0.6426$) and in countries including Brazil ($P = 1.0000$), Mexico ($P = 1.0000$), Taiwan ($P = 1.0000$), and the United States ($P = 0.7497$) was approximately 1:1. Countries such as Cameroon ($P = 0.2500$), Chile ($P = 0.2500$), China ($P = 0.500$), Guatemala ($P = 1.0000$), Indonesia ($P = 0.5000$), Japan ($P = 0.0625$), Korea ($P = 1.0000$), Norway ($P = 0.2500$), Peru

($P = 0.2500$), Spain ($P = 0.1250$), Thailand ($P = 1.0000$), Uruguay ($P = 0.2500$), and the former Yugoslavia ($P = 1.0000$) and continents (Africa [$P = 0.2500$]) with a lower number of sampled isolates ($n < 8$) showed a prevalence of one MT over the other but this relationship was not statistically significant. For France ($P = 0.0312$), the A1 MT was significantly more prevalent than the A2 MT, even with a sample size of six isolates. Other countries and continents with good sampling ($n > 8$) presented an increased number of A2 (South America [$P = 0.7539$] and Asia [$P = 0.2478$]) or A1 isolates (Europe [$P = 0.0125$] and Italy [$P = 0.1796$]) but the ratio did not significantly differ from the expected 1:1 ratio except in Europe. Isolates obtained from cucurbitaceous, fabaceous, piperaceous, proteaceous, and solanaceous hosts also presented a 1:1 distribution of MTs ($P > 0.2103$). Isolates originating from the family *Sterculiaceae* presented deviations from the 1:1 MT distribution ($P = 0.0312$), with the A1 MT predominating.

When the structure of the SL data was analyzed, 11 clusters ($K = 10$, $\ln P = -6787$; $K = 11$, $\ln P = -6580$; $K = 12$, $\ln P = -6659$) were detected, 2 more that were detected in SS (Fig. 1D). If each isolate was assigned to the K th genetic cluster to which the isolate predominantly belonged, then 10 isolates belonged to cluster 1, 13 isolates to cluster 2, 42 isolates to cluster 3, 32 isolates to cluster 4, 30 isolates to cluster 5, 22 isolates to cluster 6, 26

TABLE 1. Average genetic differentiation estimates of mitochondrial and nuclear genes for *Phytophthora capsici* sensu stricto with isolates grouped in predefined host categories^a

	F _{ST}																	
Category	Vegetable		Cucurbitaceae		Fabaceae		Capsicum annuum		Cucurbita maxima		Cucurbita moschata		Cucurbita pepo		Cucumis sativus		Solanum lycopersicon	
Host type																		
Nonvegetable	0.12*	0.18*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Host family																		
Fabaceae	...	...	0.01	0.03*	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Solanaceae	...	...	0.02	0.04*	0.04*	0.03*	...	...	...	...	...	...	...	...	...	...	...	...
Host species																		
Cucurbita maxima	...	...	...	...	...	...	0.05*	0.03*	...	...	...	...	...	...	...	...	...	...
C. moschata	...	...	...	...	...	...	0.10*	0.04*	0.02*	0.02*	...	...	...	...	...	...	...	...
C. pepo	...	...	...	...	...	...	0.06*	0.05*	0.00	0.03*	0.03*	0.02*	...	...	...	...	...	...
Cucumis sativus	...	...	...	...	...	...	0.10*	0.08*	0.00	0.05*	0.03*	0.03*	0.02	0.03*	...	...	...	...
S. lycopersicon	...	...	...	...	...	...	0.10*	0.05*	0.00	0.10*	0.03*	0.14*	0.02	0.13*	0.00	0.17*	...	...
S. melongena	...	...	...	...	...	...	0.08*	0.15*	0.00	0.18*	0.03*	0.20*	0.02	0.19*	0.00	0.28*	0.00	0.22*

^a Categories with fewer than eight isolates were excluded from analyses and are not shown. Reported values correspond to average values for mitochondrial (left column) and nuclear genes (right column); * = significant at 0.05.

TABLE 2. Average diversity estimates of mitochondrial and nuclear genes for *Phytophthora capsici* sensu stricto (SS) with isolates grouped in predefined host categories

Category	Diversity estimates ^a									
	Isolates		Hd		π		θ_w		k	
	SL	SS	M	N	M	N	M	N	M	N
Host type										
Vegetable	226	224	0.16	0.8	0.001	0.006	0.79	2.4	0.27	2.88
Nonvegetable	29	12	0.34	0.72	0.001	0.006	0.77	2.5	0.5	2.88
Host family										
<i>Cucurbitaceae</i>	87	87	0.12	0.72	0.001	0.005	0	0	0.19	2.56
<i>Fabaceae</i>	13	12	0.12	0.76	0	0.006	0	0	0.16	2.76
<i>Solanaceae</i>	126	125	0.19	0.83	0.001	0.006	0	0	0.33	3.03
Host species										
<i>Capsicum annuum</i>	97	96	0.23	0.84	0.001	0.007	0.71	2.28	0.39	3.09
<i>Cucurbita maxima</i>	22	22	0.09	0.73	0.001	0.006	0.62	2.34	0.21	2.7
<i>C. moschata</i>	10	10	0.09	0.74	0	0.006	0.35	2.39	0.09	2.86
<i>C. pepo</i>	32	32	0.06	0.66	0	0.005	0.25	2.08	0.06	2.22
<i>Cucumis sativus</i>	12	12	0	0.66	0	0.005	0	1.74	0	2.17
<i>Solanum lycopersicon</i>	12	12	0	0.74	0	0.005	0	2.05	0	2.57
<i>S. melongena</i>	13	13	0.04	0.66	0	0.004	0.64	2.01	0.08	2.07

^a Categories with fewer than eight isolates were excluded from analyses and are not shown. Hd = haplotype diversity, π = Tajima's π , k = the average number of pairwise nucleotide differences, and θ_w = Watterson's theta. Reported values correspond to average values for mitochondrial (M, left column) and nuclear genes (N, right column).

isolates to cluster 7, 29 isolates to cluster 8, 18 isolates to cluster 9, 14 isolates to cluster 10, and 4 isolates to cluster 11. Fifteen isolates were not >0.1 (proportion membership) higher in one genetic cluster than another and could not be assigned predominant cluster membership. Individual ancestry coefficients were highly consistent across replicate runs, as was observed for SS. Isolates belonging to cluster 10 presented some admixture with SS clusters and isolates from cluster 11 showed no admixture. More admixture was observed in clusters generated from the SS group. When the data were analyzed using the categories *P. capsici*, intermediate, and *P. tropicalis* as prior population information, no genetic clustering directly corresponding to the categories was observed. However, some structure was observed in each category. The *P. capsici* category contained the same clusters detected in SS and no individuals from clusters 10 and 11. The intermediate group presented isolates from clusters 10 and 11 and some admixed individuals with partial membership in SS clusters and cluster 10. The *P. tropicalis* group only contained isolates from cluster 10 that showed very little admixture and included the type culture of *P. tropicalis*. Isolates from cluster 10 originated from Mexico, India, Australia, and Hawaii from tropical hosts and from Delaware from lima bean. Isolates from

cluster 11 presented no admixture and corresponded to intermediate isolates from Brazil and Michigan obtained from cacao and English Ivy (*Hedera helix*), respectively. Morphological characteristics of all isolates were consistent with the description of *P. capsici* included in the *Phytophthora* spp. key by Waterhouse (83).

The highest values of genetic differentiation observed in this study occurred between *P. capsici* and non-*P. capsici* isolates (Table 5). Diversity estimates for *P. capsici* isolates were lower than those obtained for non-*P. capsici* isolates (Table 6; Supplementary Table 4). The NeighborNet networks for genes HSP90, TgaA, EF-1 α , β -Tubulin, Ura3, and Cox1 showed three groups of haplotypes with moderate (>65) to high (>90) bootstrap support. The three groups presented several incompatibilities within each group but few or no incompatibilities among groups, depending on the gene (Supplementary Figure 2). These groups were not contradicted by the other genealogies but grouping of isolates had less resolution. Isolates belonging to the first group of haplotypes corresponded to *P. capsici* SS, while isolates belonging to the second and third groups of haplotypes included isolates from clusters 10 and 11, respectively. For *P. capsici* SS isolates, no distinctive groups of haplotypes with high bootstrap support were

Fig. 2. Genetic structure of *Phytophthora capsici* isolates grouped by predefined geographic categories. Categories for sensu stricto: **A**, hemisphere; **B**, continent; **C**, country group; **D**, country; and **E**, U.S. state. Geographic origin abbreviations: U.S., United States, and standard abbreviations for U.S. states. Each isolate is represented by a thin bar, often partitioned into colored segments, each representing the individual's proportionate genetic membership in a given *K*th cluster (sorted numerically from the bottom to the top of the bar [i.e., cluster 1 will always be on the bottom of the bar, cluster 9 at the top]). Cluster colors correspond to: dark red-one, cluster 1; purple, cluster 2; yellow, cluster 3; light green, cluster 4; dark blue, cluster 5; aqua, cluster 6; dark green, cluster 7; light blue, cluster 8; pink, cluster 9. A white space separates individual subcategories.

consistently observed in all or most networks. Networks overall indicated significant incompatibility within nuclear genes, and the highest number of incompatibilities was observed in Ura3. The mitochondrial genes presented a few incompatibilities, and Cox2 showed no networking. Statistical parsimony haplotype genealogies for SL presented a topology similar to the NeighborNet networks (Fig. 3; Supplementary Figure 3). *P. capsici* SS haplotypes appeared to be very closely related to each other, with only one or two missing haplotypes separating sampled haplotypes. Haplotypes from clusters 10 and 11 were more distantly related to

TABLE 3. Average genetic differentiation estimates of mitochondrial and nuclear genes for *Phytophthora capsici* sensu stricto with isolates grouped in predefined geographic categories

Category	F_{ST}^a												
	Hemi	Continent			Country			Group	U.S. state				
	Southern	Europe	N. Am.	S. Am.	Mexico	Taiwan	U.S.	Non-U.S.	MI	NJ	NY	SC	TN
Northern	0.04	...	...	...	...	...	...	...	...	...	...	...	...
Asia	0.05*	...	...	...	...	...	...	...	...	...	...	...	...
	...	0.16*	0.02*	0.07*	...	...	...	...	...	...	...	...	...
Europe	...	0.11*	0.04*	0.13*	...	...	...	...	...	...	...	...	...
	...	...	0.19*	0.27*	...	...	...	...	...	...	...	...	...
North America	...	...	0.09*	0.13*	...	...	...	...	...	...	...	...	...
	...	...	...	0.03*	...	...	...	...	...	...	...	...	...
Italy	...	...	...	0.08*	...	...	...	...	...	...	...	...	...
	...	...	...	...	0.30*	0.32*	0.34*	...	...	...	...	...	...
Mexico	...	...	...	...	0.11*	0.16*	0.12*	...	...	...	...	...	...
	...	...	...	...	...	0.03*	0.04*	...	...	...	...	...	...
Taiwan	...	...	...	...	...	0.15*	0.09*	...	...	...	...	...	...
	...	...	...	...	...	...	0.00	...	...	...	...	...	...
United States	...	...	...	...	...	...	0.08*	...	...	...	...	...	...
	...	...	...	...	...	...	...	0.06*	...	...	...	...	...
California	...	...	...	...	...	...	...	0.04*	...	...	...	...	...
	...	...	...	...	...	...	...	...	0.00	0.01	0.03*	0.02*	0.03*
Michigan	...	...	...	...	...	...	...	...	0.17*	0.08*	0.13*	0.11*	0.31*
	...	...	...	...	...	...	...	...	...	0.01	0.02*	0.01*	0.02*
New Jersey	...	...	...	...	...	...	...	...	...	0.16*	0.29*	0.19*	0.21*
	...	...	...	...	...	...	...	...	...	...	0.00	0.00	0.00
New York	...	...	...	...	...	...	...	...	...	...	0.10*	0.06*	0.20*
	...	...	...	...	...	...	...	...	...	...	...	0.01	0.00
South Carolina	...	...	...	...	...	...	...	...	...	...	...	0.15*	0.39*
	...	...	...	...	...	...	...	...	...	...	...	...	0.01
	...	...	...	...	...	...	...	...	...	...	...	...	0.24*

^a Abbreviations: Hemi = hemisphere, Group = country group, N. Am. = North America, S. Am. = South America, MI = Michigan, NJ = New Jersey, NY = New York, SC = South Carolina, and TN = Tennessee. Categories with fewer than eight isolates were excluded from analyses and are not shown. Reported values correspond to average values for mitochondrial (upper) and nuclear genes (lower); * = significant at 0.05.

TABLE 4. Diversity estimates of mitochondrial and nuclear genes for *Phytophthora capsici* sensu stricto (SS) with isolates grouped in predefined geographic categories

Category	Diversity estimates ^a									
	Isolates		Hd		π		θ_w		k	
	SL	SS	M	N	M	N	M	N	M	N
Hemisphere										
Northern	235	224	0.19	0.81	0.001	0.006	0.79	2.50	0.32	2.94
Southern	20	12	0.08	0.76	0.000	0.006	0.33	2.10	0.08	2.65
Continent										
Asia	28	27	0.21	0.77	0.001	0.006	0.65	2.45	0.40	2.66
Europe	28	28	0.36	0.83	0.002	0.006	0.51	2.32	0.60	2.88
North America	178	168	0.12	0.78	0.001	0.006	0.76	2.42	0.22	2.84
South America	13	10	0.00	0.74	0.000	0.005	0.00	1.97	0.00	2.57
Country										
Italy	14	14	0.28	0.83	0.001	0.006	0.78	2.36	0.52	2.73
Mexico	13	10	0.21	0.76	0.001	0.007	0.53	2.30	0.32	3.14
Taiwan	16	16	0.12	0.73	0.001	0.005	0.68	1.78	0.28	2.16
United States	164	157	0.11	0.77	0.001	0.006	0.71	2.34	0.19	2.77
Country group										
United States	164	157	0.11	0.77	0.001	0.006	0.71	2.34	0.19	2.77
Non-U.S.	91	79	0.32	0.85	0.001	0.007	0.71	2.51	0.50	3.12
U.S. state										
California	21	21	0.16	0.73	0.001	0.006	0.56	2.01	0.24	2.65
Michigan	35	34	0.08	0.59	0.000	0.004	0.73	1.60	0.17	1.96
New Jersey	8	8	0.12	0.79	0.001	0.006	0.58	2.36	0.19	2.93
New York	15	15	0.00	0.67	0.000	0.005	0.00	1.85	0.00	2.14
South Carolina	24	24	0.06	0.78	0.000	0.007	0.40	2.21	0.08	3.17
Tennessee	12	12	0.00	0.26	0.000	0.002	0.00	0.71	0.00	0.70

^a Categories with fewer than eight isolates were excluded from analyses and are not shown. Hd = haplotype diversity, π = Tajima's π , k = the average number of pairwise nucleotide differences, and θ_w = Watterson's theta. Reported values correspond to average values for mitochondrial (M, left column) and nuclear genes (N, right column).

P. capsici SS, with two or more missing haplotypes separating both clades, which was supported by all genealogies. All genealogies except TigA presented some *P. capsici* SS haplotypes that included one or two alleles of isolates from clusters 10 and 11. In some genealogies, several haplotypes corresponding to clusters 10 or 11 were grouped together and separated from each other and from *P. capsici* by at least one missing haplotype (Cox1, Cox2, Nad1, EF-1 α , and TigA). Some SS haplotypes were frequently sampled and found throughout the world on diverse hosts, while other haplotypes sampled at lower frequencies originated from specific states, countries, continents, and hosts (Supplementary Table 1).

DISCUSSION

P. capsici is an economically important plant pathogen with a wide geographic and host range, as reflected by the sampling in this study. To detect population structure and determine whether predefined categories based on host, geography, mefenoxam sensitivity, or MT reflect genetic relationships among *P. capsici* isolates, we sequenced 10 genes from 255 individuals to perform population structure analyses. Bayesian clustering of isolates identified 9 and 11 major genetically distinct clusters in SS and SL, respectively. Clustering analysis revealed some population structure by host, geographic origin, and mefenoxam sensitivity in SS and by species in SL, with some clusters occurring more or less frequently in particular categories. However, it showed no direct correspondence between any of the predefined categories and the number of inferred clusters. Pairwise F_{ST} comparisons and genetic diversity estimates among predefined host, geography, mefenoxam-sensitivity, MT, and species categories were consistent with the distribution of genetic variation detected by

Bayesian clustering and provided additional information on the genetic variability within and between categories. When isolates were grouped by host, differences in frequency of occurrence of certain clusters in particular hosts were observed. Isolates from cluster one occurred more frequently in non-vegetable hosts (cacao and black pepper) than in vegetable hosts. A previous study in *P. capsici* found temperate isolates from vegetable hosts (*Solanaceae*, *Cucurbitaceae*, and *Fabaceae* families) to be grouped together in the same clade, whereas tropical isolates appeared to have different genetic lineages (7), in agreement with our findings. Nonetheless, that study did not determine whether isolates corresponded only to *P. capsici* or whether *P. tropicalis* was present in their sample; therefore, it is not possible to determine whether *P. capsici* SS isolates were truly associated with tropical (nonvegetable) hosts or whether isolates from tropical hosts corresponded only to *P. tropicalis* and isolates assigned to cluster 11. Work by Donahoo and Lamour detected phylogenetic clustering of *P. capsici* SS isolates from tropical hosts (nonvegetable) in a separated but closely related clade from isolates from vegetable hosts (10), which further supports our findings of genetic differentiation between these categories. Tropical hosts of *P. capsici* have more woody tissue if compared with hosts from the *Cucurbitaceae*, *Solanaceae*, and *Fabaceae* families. In previous studies, it has been observed that *P. capsici* is difficult to recover from woody tissue of Fraser fir (65), oak (9), and various ornamentals (30). It is possible that isolates from different *P. capsici* clusters are not equally adapted to woody or herbaceous tissue, and this could result in isolates from a specific cluster such as cluster one occurring more frequently in woody hosts. A similar adaptation could be the cause of the almost exclusive association of cluster two with the *Solanaceae* family. Despite the large sampling in the *Cucur-*

TABLE 5. Average genetic differentiation estimates of mitochondrial and nuclear genes for *Phytophthora capsici* sensu stricto with isolates grouped in predefined mating-type and mefenoxam-sensitivity categories, and *P. capsici* sensu lato with isolates grouped by species category

Category	F_{ST}^a							
	Mating type		Mefenoxam sensitivity				Species	
	A1		S		IS		<i>P. capsici</i>	
Mating type								
A2	0.01	0.01	...	...	...	...	...	...
Mefenoxam sensitivity								
IS	...	...	0.00	0.05*	...	...	...	...
I	...	...	0.03*	0.03*	0.00	0.01	...	...
Species								
Not <i>P. capsici</i>	...	...	...	...	...	...	0.55*	0.54*

^a Categories with fewer than eight isolates were excluded from analyses and are not shown. For mefenoxam sensitivity coding, refer to Table 6. S, IS, and I indicate sensitive, intermediate sensitivity, and insensitive, respectively. Reported values correspond to average values for mitochondrial (left column) and nuclear genes (right column); * = significant at 0.05.

TABLE 6. Diversity estimates of mitochondrial and nuclear genes for *Phytophthora capsici* and sensu stricto (SS) with isolates grouped in predefined mating type and mefenoxam-sensitivity categories, and *P. capsici* sensu lato (SL) with isolates grouped by species category

Category	Diversity estimates ^a									
	Isolates		Hd		π		θ_w		k	
	SL	SS	M	N	M	N	M	N	M	N
Mating type										
A1	73	64	0.22	0.81	0.001	0.006	0.83	2.54	0.37	2.99
A2	76	66	0.15	0.78	0.001	0.007	0.69	2.31	0.23	3.18
Mefenoxam sensitivity										
S	218	201	0.20	0.81	0.001	0.006	0.76	2.51	0.33	2.93
IS	17	17	0.11	0.68	0.001	0.006	0.59	2.40	0.20	2.75
I	19	17	0.09	0.77	0.000	0.006	0.59	2.53	0.12	2.81
Species										
Not <i>P. capsici</i>	19	0	0.77	0.72	0.009	0.015	2.79	7.02	3.30	7.06

^a Categories with fewer than eight isolates were excluded from analyses and are not shown. Hd = haplotype diversity, π = Tajima's π , k = the average number of pairwise nucleotide differences, and θ_w = Watterson's theta. For mefenoxam sensitivity coding, refer to Table 6. S, IS, and I indicate sensitive, intermediate sensitivity, and insensitive, respectively. Reported values correspond to average values for mitochondrial (M, left column) and nuclear genes (N, right column).

bitaceae family ($n = 87$), no isolates belonging predominately to cluster two were found in hosts from this family. As with the woody hosts, it is possible that isolates from cluster two are not able to infect members of the *Cucurbitaceae* family as frequently as members of the *Solanaceae* family. Overall, isolates from solanaceous hosts appeared to be more diverse in our sampling but additional analyses with an equal sampling of isolates from each family would be necessary to confirm this observation.

Evidence of clustering should not be taken as evidence of physiological races or pathotypes because we did not associate virulence data with the genetic clusters in the current study. We associated occurrence of clusters in a host. Differences in virulence and pathogenicity have been reported between isolates occurring in the families *Solanaceae* and *Cucurbitaceae* (16,47,61,66,68) but the genetic basis of these differences is unknown. Experiments that associate virulence and pathogenicity with particular clusters are needed to determine whether cluster membership may be used to predict the occurrence of certain isolates in particular hosts and the virulence of the isolates. If such an association is found, cluster information could be applied to determine the most likely sources of virulent strains, which could be used for robust disease screenings in the search for resistant cultivars. The differences in cluster occurrence by host of origin found in this study have significant implications for the development of resistant cultivars. Breeding programs should include isolates that represent the genetic diversity of the pathogen in resistance screenings. Isolates obtained from hosts belonging to the *Cucurbitaceae* and *Solanaceae* families are commonly used in host resistance screenings (16,66) but this study suggests that inclusion of isolates from tropical hosts that show high genetic variation is necessary to capture the genetic diversity of *P. capsici*.

Several studies examining samples of *P. capsici* isolates from different geographic locations worldwide have been published; however, clear detection of distinct subgroups in *P. capsici* based on geographic origin through phylogenetic methods remains elusive (7,10,15,88). Some geographic structure was detected in our study. Some clusters occurred in all continents (four and eight), others were only present in select continents (two in North and South America) or were more frequently sampled in certain continents (six in Europe and three in North America and Asia) and countries (five, seven, and nine in the United States). North America and Asia presented a more diverse occurrence of clusters compared with other regions. The high diversity of *P. capsici* clusters found in the United States may challenge local breeding efforts. Breeding programs usually have a regional focus, where resistance for local pathogen populations is incorporated into host cultivars. Breeders working in areas with lower genetic diversity of the pathogen may have a higher chance of identifying cultivars resistant to local *P. capsici* populations (36). *P. capsici* populations with high levels of genetic variation are likely to adapt more rapidly to resistant hosts than populations with little genetic variation (36,41). In our study, regions including Cameroon, Chile, Peru, France, Italy, Norway, Spain, Uruguay, and Tennessee presented a predominant cluster. The absence of other clusters is probably due to our sampling, but clonal *P. capsici* populations have been identified in Argentina (24) and Peru (36) in more robust sampling efforts. Local population studies with intensive sampling are needed to characterize and determine the spatial genetic structure of *P. capsici* populations on a finer scale than examined in the present work. However, establishing the spatial distribution of the nine *P. capsici* clusters detected in this study provides an initial map of global population structure that will help in isolate selection for resistance screening and will guide future sampling to expand our knowledge of *P. capsici* population structure.

P. capsici is water dispersed within fields and can be introduced into new fields through infected plant material or infested soil,

tools, and irrigation water (22,32). Wind dispersal is not an important mechanism for long-distance movement of *P. capsici* (29) and human activity likely plays a role in genetic structuring of populations. Migration of a few individuals per generation is sufficient to keep F_{ST} values at ≤ 0.1 (31). The observed Wright's F_{ST} values between several regions suggest that movement among locations is rare but historical information indicates it is possible. It has been suggested that *P. capsici* was introduced to the United States through contaminated pepper seed that entered New Mexico (1918) (48) and Florida (1932) (85) because some of the earliest reports of this pathogen in the United States come from those states (41). Because a type culture of *P. capsici* from New Mexico was included in this study and had membership mostly in cluster three and partial membership in clusters four and eight, we know those clusters were present in New Mexico in 1965. Present samples from New Mexico also had membership in those clusters, indicating that cluster variation is maintained within populations; however, information about the genetic diversity of those isolates is not available because our sample was smaller than eight isolates. Genetic diversity estimates for New Jersey, South Carolina, and California isolates were higher than for other states, which

A Cox1

B EF-1 α

Fig. 3. Haplotype genealogies of *Phytophthora capsici* sensu lato mitochondrial and nuclear genes. **A**, Mitochondrial gene: Cox1. **B**, Nuclear gene: EF-1 α . Other genes are shown in Supplementary Figure 3. Lines in the network represent possible paths of evolution. The haplotype with the highest probability to be ancestral is indicated by a square and other haplotypes are displayed as ovals. Empty dots represent missing or extant haplotypes. Bold ovals or squares indicate haplotypes that contain *P. capsici* sensu stricto isolates and isolates from clusters 10 or 11. Dashed ovals or squares indicate haplotypes that contain isolates from cluster 10. Double-line ovals or squares indicate haplotypes that contain isolates from cluster 11. Dotted-line ovals or squares indicate haplotypes that contain both cluster 10 and 11 isolates.

could be due to the longer presence of *P. capsici* in these states compared with Tennessee, New York, and Michigan. The type culture from Italy (year 1927) had membership mostly in cluster six and partial membership in clusters two and four, indicating that members of those clusters have been present in Italy since 1927, as reflected by the other Italian samples. Italian samples presented high levels of genetic variation that could be reflecting the long-term presence of *P. capsici* in Italy and subsequent diversification of isolates. Members of cluster six that were mostly sampled in Europe and, specifically, in Italy were also found in Hawaii, California, and Florida. It is possible that those U.S. isolates belonging to cluster six were introduced into the United States from Europe. Shared membership in a particular genetic cluster corresponds with shared ancestry in a particular population (k) (63). Our analysis provides a global framework to hypothesize about migration but evolutionary studies with appropriate markers are needed to determine migration pathways of *P. capsici*, as has been done with other pathogens (25,28). Such studies will provide valuable information for regulatory measures and may also identify the center of origin of *P. capsici*, which could guide samplings of resistant germplasm.

Some structure was detected in isolates grouped by mefenoxam sensitivity, where sensitive isolates contained members from all clusters but intermediately sensitive and insensitive isolates did not contain members from cluster nine. Isolates belonging predominantly to cluster nine ($n = 18$) were sampled in California and Georgia from pepper, tomato, and watermelon. Because we do not know the fungicide history for the fields from which these samples were collected, it is possible that mefenoxam was not used; it may be that our limited sampling simply did not include isolates that belong to cluster nine and are insensitive to mefenoxam. Isolates from Spain, Taiwan, Mexico, and the United States were found to be intermediately sensitive or insensitive to mefenoxam. Other studies have found isolates insensitive to mefenoxam in Italy (60) and the United States (18,32). Isolates sensitive to mefenoxam were sampled from more regions and hosts than isolates intermediately sensitive or insensitive to mefenoxam. Continued characterization of *P. capsici* populations for mefenoxam insensitivity will help determine whether mefenoxam is still an effective control method in a particular field or whether other products are needed.

No structure was detected when isolates were grouped by MT, and all clusters were sampled in A1 and A2 isolates. A previous study also found no correlation between restriction fragment length polymorphism lineages and MT in isolates from New Mexico, Europe, and Korea (38). Regions with lower sampling showed the prevalence of only one MT, whereas regions with higher sampling contained both A1 and A2 isolates. Because recombination via sexual reproduction generates high genetic variability in *P. capsici* that may result in isolates with increased virulence or fungicide resistance, it is important to know the distribution of MTs in different regions. If only one MT is present in a field, country, or state, efforts should be directed to avoid the introduction of the other MT so that the long-term efficacy of control methods such as host resistance and fungicides is not reduced.

High genetic diversity has been previously reported for *P. capsici* (39,44,73). Individuals with partial membership in several clusters, unique haplotypes (20 to 40%), and unique multi-locus genotypes (86%) reported here and in previous studies (70 to 90%) (43,44) are consistent with sexual reproduction in *P. capsici* (26), in contrast to the clear clonal structure that has been observed for other *Phytophthora* spp. such as *P. infestans* (27) and *P. ramorum* (64). Although admixture could be due to factors such as ancestral polymorphism, at least some admixture is likely a result of recombination, as indicated by recombination estimates for nuclear genes (75), incompatibilities observed in the NeighborNet networks, and extensive networking in statistical

parsimony genealogies. Although these findings are unsurprising in light of previous studies (42–44), the implications of these results for choosing the best method to analyze populations of *P. capsici* are significant. Traditional phylogenetic methods have been used to study intraspecific population data of *P. capsici* (7,10,88) even though recombination occurs frequently. Yet such methods are only appropriate under the assumption that recombination does not occur (62,86). If recombination occurs frequently in the plant pathogen of interest, methods such as Bayesian clustering, split networks, and statistical parsimony that allow for recombination events should be used (4,62). Bayesian clustering allows for the reconstruction of ancestral population structure and subsequent admixture in highly recombining species without subjective definitions of population groups or categories (86) and constitutes a useful tool to study population structure in *P. capsici*.

Characterization of genetic structure of *P. capsici* SS populations revealed moderate but significant population structure by host, geography, and mefenoxam sensitivity. Beyond the direct application of this knowledge in breeding efforts and development of fungicides, the detection of population structure in *P. capsici* has implications in using association genetics to identify the genetic basis of phenotypes of importance. Association studies have been performed to identify the genetic basis of disease in humans (20) and agronomic traits in plants (33). They have also been successfully used to determine the role of particular genes as virulence and avirulence determinants in plant pathogens such as *P. infestans* (3) and *Magnaporthe oryzae* (87). Association studies could help uncover the genetic bases of traits such as pathogenicity, virulence, or fungicide resistance in *P. capsici*. Nevertheless, even weak population structure obscures the discovery of functionally important variation using association genetics. Because the clusters identified are somewhat structured, stratification of *P. capsici* by geography, host, and mefenoxam sensitivity will be important to control for in association analysis of phenotypic traits of interest. Studies investigating the genetic structure of *P. capsici* populations by host or by mefenoxam sensitivity nested within geography will be key to select appropriate individuals for association studies in *P. capsici*.

Bayesian clustering, split decomposition networks, and statistical parsimony genealogies allowed for the detection of non-*P. capsici* isolates in our sample. Isolates from cluster 10 corresponded to *P. tropicalis* and isolates from cluster 11 corresponded to isolates from Brazil (cacao) and Michigan (English Ivy) that were closely related to *P. capsici* and *P. tropicalis* but formed a distinct group in our analyses. Oudemans and Coffey used phylogenetic methods to separate *P. tropicalis* isolates (CAP2) from *P. capsici* SS (CAP1) (57). Several studies have detected the separation of *P. capsici* and *P. tropicalis* but no evidence of admixture between them was previously found (10,53,57,88), because phylogenetic methods do not allow for the visualization of admixture. Clustering analysis in our study indicates that, even though *P. tropicalis* is found as a distinct cluster from *P. capsici*, some admixture has occurred between the species. The observed admixture could be due to ancestral polymorphism or recent recombination events, given that it is possible to obtain interspecific progeny in the lab (10); no hybrids have been characterized from nature. With more samples and several linked markers, it may be possible to detect introgression events or recent gene flow between the species and determine the existence of natural hybrids (12,55,80).

Isolates from cluster 11 appear separated from *P. capsici* and *P. tropicalis* in the networks and genealogies and show no admixture in the structure analysis. The lack of admixture in cluster 11 could be due to the few isolates we have in that cluster (three from Brazil and cacao and one from Michigan and English Ivy). With more samples, it is possible that admixture will be detected revealing that cluster 11 corresponds to *P. tropicalis* or *P. capsici*.

However, taking into consideration the genealogies and networks that support the separation of haplotypes in three distinct groups, it is also possible that cluster 11 corresponds to a distinct phylogenetic species. In their *P. capsici* study, Oudemans and Coffey also found a third distinct clade (CAP3) that contained isolates from Brazil and cacao (57) that likely correspond to cluster 11. Isolates from Brazil and cacao also formed a separate clade in other studies (7,10). Detection of isolates from cluster 11 seems to be possible by combining information from several markers. In the study by Donahoo and Lamour, these isolates appear on the same clade with a few *P. capsici* isolates in an internal transcribed spacer-based phylogeny but separation of such isolates was observed in other phylogenies based on amplified fragment length polymorphisms and nuclear genes in the same study (10). This is not surprising because haplotypes of cluster 11 showed high similarity to *P. capsici* in some genes, whereas other genomic regions were more similar to *P. tropicalis* according to BLAST. Because the structure analysis uses multilocus genotypes and allelic frequencies for clustering, we believe that it constitutes a valuable tool that could complement phylogenetic studies by providing information about population membership and admixture to aid in the delimitation of phylogenetic species in highly recombining pathogens. However, to truly define whether there are genetic barriers to mating among *P. capsici*, *P. tropicalis*, and isolates in cluster 11, comparisons of a wide range of inter- and intracluster crosses between isolates from diverse origins is needed.

Isolates used in this study were obtained from colleagues around the world and from our *P. capsici* collection. Large numbers of isolates could not be obtained from several localities and most isolates come from common vegetable hosts. Isolates from tropical hosts were less represented, and no samples from wild hosts or weeds were obtained. Finding non-*P. capsici* isolates in our sample was unexpected; therefore, the number of isolates sampled for clusters 10 and 11 was low for a population-based study. Sparse sampling is common in plant pathogen worldwide population studies. One example is work done by Goss et al. in *P. ramorum* populations (28). Nonetheless, Bayesian clustering can yield valuable information about the population structure of plant pathogen populations. The number and type of markers used can affect the analysis. Loci included in our study were highly informative, as indicated by polymorphism, recombination, and diversity analysis. Nuclear loci were more diverse than mitochondrial loci in this study, as has been previously observed for *P. capsici* and *P. parasitica* (15). We were able to detect clustering in our samples with the 10 genes used. Previous studies have shown that, for clustering to be robust, <40 markers and as few as 7 markers (63) can be used if they are highly informative, but using at least 50 markers is preferred (70). Obviously, structure can only be detected in the samples being analyzed. More clusters likely exist in *P. capsici* because apparent structure may be underestimated when using sparse sampling and few markers (70). Future studies with uniform global sampling and hundreds of markers could determine whether additional genetic clusters are present in *P. capsici*. The approach used in this study could be applied to other plant pathogens, where using a large number of markers is not feasible due to high costs or scarce genomic resources available, given that the markers used are highly informative. Although sequencing 10 genes is likely necessary to classify isolates into the clusters identified in our study, ideally, a high-throughput and cost-effective set of >50 markers should be developed to more robustly classify isolates while minimizing the costs associated with genotyping isolates.

P. capsici is a diverse and highly recombining species of economic importance. Our findings of genetic structuring in *P. capsici* populations, coupled with observations of differences in virulence of particular isolates when inoculated in diverse hosts (16,21,65,66), highlights the importance of selecting isolates from

diverse hosts and geographic origin for breeding programs. When developing diagnostic tools, fungicides, and resistant host cultivars, representative isolates from all genetic clusters should be used to account for global genetic diversity. This investigation of genetic structure of *P. capsici* populations may be used to guide isolate selection to improve efficacy of breeding programs. Continued genotyping of *P. capsici* will be necessary to track the diversification of inferred clusters and to identify new ones.

ACKNOWLEDGMENTS

We thank the many colleagues listed in Supplementary Table 1 for providing isolates for this research; all the members of the Hausbeck lab for their valuable suggestions and help; H. Gutting, H. Sweet, A. Lebeis, K. Heslip, R. Heslip, M. Wood, and J. Passmore for technical assistance; J. J. Morrice for providing the program PST to generate population structure figures; and D. R. Matute and J. Smith for critical reading of the manuscript.

LITERATURE CITED

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.
- Armstrong, M. R., Whisson, S. C., Pritchard, L., Bos, J. I. B., Venter, E., Avrova, A. O., Rehmany, A. P., Böhme, U., Brooks, K., Cherevach, I., Hamlin, N., White, B., Fraser, A., Lord, A., Quail, M. A., Churcher, C., Hall, N., Berriman, M., Huang, S., Kamoun, S., Beynon, J. L., and Birch, P. R. J. 2005. An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *PNAS* 102:7766-7771.
- Awadalla, P. 2002. The evolutionary genomics of pathogen recombination. *Nat. Rev. Genet.* 4:50-60.
- Bergl, R. A., and Vigilant, L. 2007. Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). *Mol. Ecol.* 16:501-516.
- Blair, J. E., Coffey, M. D., Park, S.-Y., Geiser, D. M., and Kang, S. 2008. A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genet. Biol.* 45:266-277.
- Bowers, J. H., Martin, F. N., Tooley, P. W., and Luz, E. D. M. N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. *Phytopathology* 97:492-503.
- Clement, M., Posada, D., and Crandall, K. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657-1660.
- Davidson, J. M., Werres, S., Garbelotto, M., Hansen, E. M., and Rizzo, D. M. 2003. Sudden oak death and associated diseases caused by *Phytophthora ramorum*. *Plant Health Progress* doi: 10.1094/PHP-2003-0707-01-DG.
- Donahoo, R. S., and Lamour, K. H. 2008. Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* 100:911-920.
- Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora Diseases Worldwide*. American Phytopathological Society Press, St. Paul, MN.
- Falush, D., Stephens, M., and Pritchard, J. K. 2007. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Mol. Ecol. Notes* 7:574-578.
- Falush, D., Wirth, T., Linz, B., Pritchard, J. K., Stephens, M., Kidd, M., Blaser, M. J., Graham, D. Y., Vacher, S., Perez-Perez, G. I., Yamaoka, Y., Megraud, F., Otto, K., Reichard, U., Katzowitsch, E., Wang, X., Achtman, M., and Suerbaum, S. 2003. Traces of human migrations in *Helicobacter pylori* populations. *Science* 299:1582-1585.
- Fonseca, D. M., Keyghobadi, N., Malcolm, C. A., Mehmet, C., Schaffner, F., Mogi, M., Fleischer, R. C., and Wilkerson, R. C. 2004. Emerging vectors in the *Culex pipiens* complex. *Science* 303:1535-1538.
- Forster, H., Oudemans, P., and Coffey, M. D. 1989. Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. *Exp. Mycol.* 14:18-31.
- Foster, J. M., and Hausbeck, M. K. 2010. Resistance of pepper to *Phytophthora* crown, root, and fruit rot is affected by isolate virulence. *Plant Dis.* 94:24-30.
- Francois, O., Blum, M. G. B., Jakobsson, M., and Rosenberg, N. A. 2008. Demographic history of European populations of *Arabidopsis thaliana*. *PLoS Genet.* 4:e10000075.
- French-Monar, R. D., Jones, J. B., and Roberts, P. D. 2006. Charac-

- terization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. *Plant Dis.* 90:345-350.
19. Fu, Y. X., and Li, W. H. 1993. Statistical tests of neutrality of mutations. *Genetics* 133:693-709.
 20. Garcia-Barcelo, M.-M., Tang, C. S.-m., Ngan, E. S.-w., Lui, V. C.-h., Chen, Y., So, M.-t., Leon, T. Y.-y., Miao, X.-p., Shum, C. K.-y., Liu, F.-q., Yeung, M.-y., Yuan, G., Guo, W.-h., Liu, L., Sun, X.-b., Huang, L.-m., Tou, J.-f., Song, Y.-q., Chan, D., Cheung, K. M. C., Wong, K. K.-y., Cherny, S. S., Sham, P.-c., and Tam, P. K.-h. 2009. Genome-wide association study identifies NRG1 as a susceptibility locus for Hirschsprung's disease. *PNAS* 106:2694-2699.
 21. Gevens, A. J., Ando, K., Lamour, K. H., Grumet, R., and Hausbeck, M. K. 2006. A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. *Plant Dis.* 90:1276-1282.
 22. Gevens, A. J., Donahoo, R. S., Lamour, K. H., and Hausbeck, M. K. 2007. Characterization of *Phytophthora capsici* from Michigan surface irrigation water. *Phytopathology* 97:421-428.
 23. Gevens, A. J., Donahoo, R. S., Lamour, K., and Hausbeck, M. K. 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap beans in Michigan. *Plant Dis.* 92:201-209.
 24. Gobena, D. J., Roig, J., Hulvey, J., and Lamour, K. 2010. Genetic diversity of the vegetable pathogen *Phytophthora capsici* in Argentina. (Abstr.) *Phytopathology* 100:S41.
 25. Gomez-Alpizar, L., Carbone, I., and Ristaino, J. B. 2007. An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *PNAS* 104:3306-3311.
 26. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. *Phytopathology* 87:462-473.
 27. Goodwin, S. B., Cohen, B. A., and Fry, W. E. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *PNAS* 91:11591-11595.
 28. Goss, E. M., Larsen, M., Chastagner, G. A., Givens, D. R., and Grunwald, N. J. 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoS Pathogens* 5:e10000583.
 29. Granke, L. L., Windstam, S. T., Hoch, H. C., Smart, C. D., and Hausbeck, M. K. 2009. Dispersal and movement mechanisms of *Phytophthora capsici* sporangia. *Phytopathology* 99:1258-1264.
 30. Hahn, R., and Werres, S. 1997. Development of a dot immunobinding assay to detect *Phytophthora* spp. in naturally dark-rooted woody plants. *Ann. Appl. Biol.* 130:453-466.
 31. Hartl, D. L., and Clark, A. G. 1997. Principles of Population Genetics. Sinauer Associates, Inc., Sunderland, MA.
 32. Hausbeck, M. K., and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: research progress and management challenges. *Plant Dis.* 88:1292-1303.
 33. Huang, X., Wei, X., Sang, T., Zhao, Q., Feng, Q., Zhao, Y., Li, C., Zhu, C., Lu, T., Zhang, Z., Li, M., Fan, D., Guo, Y., Wang, A., Wang, L., Deng, L., Li, W., Lu, Y., Weng, Q., Liu, K., Huang, T., Zhou, T., Jing, Y., Li, W., Lin, Z., Buckler, E. S., Qian, Q., Zhang, Q.-F., Li, J., and Han, B. 2010. Genome-wide association studies of 14 agronomic traits in rice landraces. *Nat. Genet.* 42:961-967.
 34. Hudson, R. R. 1987. Estimating the recombination parameter of a finite population model without selection. *Genet. Res.* 50:245-250.
 35. Hudson, R. R., and Kaplan, N. L. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics* 111:147-164.
 36. Hurtado-González, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R., and Lamour, K. 2008. Survival and spread of *Phytophthora capsici* in coastal Peru. *Phytopathology* 98:688-694.
 37. Huson, D. H., and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* 23:254-267.
 38. Hwang, B. K., Arthur, W. A., and Heitefuss, R. 1991. Restriction fragment length polymorphisms of mitochondrial DNA among *Phytophthora capsici* isolates from pepper (*Capsicum annuum*). *Syst. Appl. Microbiol.* 14:111-116.
 39. Jackson, K., Yin, J., Csinos, A., Scherm, H., and Ji, P. 2010. Diversity of *Phytophthora capsici* from vegetable crops in Georgia. (Abstr.) *Phytopathology* 100:S55.
 40. Keller, S. R., Olson, M. S., Silim, S., Schroeder, W., and Tiffin, P. 2010. Genomic diversity, population structure, and migration following rapid range expansion in the Balsam Poplar, *Populus balsamifera*. *Mol. Ecol.* 19:1212-1226.
 41. Lamour, K. 2009. *Phytophthora capsici*: Sex, Selection, and the Wealth of Variation. John Wiley & Sons, Inc., New York.
 42. Lamour, K. H., and Hausbeck, M. K. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. *Phytopathology* 90:396-400.
 43. Lamour, K. H., and Hausbeck, M. K. 2001. The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. *Phytopathology* 91:553-557.
 44. Lamour, K. H., and Hausbeck, M. K. 2001. Investigating the spatio-temporal genetic structure of *Phytophthora capsici* in Michigan. *Phytopathology* 91:973-980.
 45. Lamour, K. H., and Hausbeck, M. K. 2003. Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. *Plant Dis.* 87:841-845.
 46. Lamour, K. H., and Hausbeck, M. K. 2003. Susceptibility of mefenoxam-treated cucurbits to isolates of *Phytophthora capsici* sensitive and insensitive to mefenoxam. *Plant Dis.* 87:920-922.
 47. Lee, B. K., Kim, B. S., Chang, S. W., and Hwang, B. K. 2001. Aggressiveness to pumpkin cultivars of isolates of *Phytophthora capsici* from pumpkin and pepper. *Plant Dis.* 85:497-500.
 48. Leonian, L. H. 1922. Stem and fruit blight of chilies caused by *Phytophthora capsici* sp. *Phytopathology* 12:401-408.
 49. Librado, P., and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451-1452.
 50. Luz, E. D. M. N., Cerqueira, A. O., Faleiro, F. G., Dantas Neto, A., Matsuoka, K., and Marques, J. R. B. 2003. Diversidade genética de isolados de *Phytophthora capsici* de diferentes hospedeiros com base em marcadores RAPD patogenicidade e morfologia. *Fitopatol. Bras.* 28:559-564.
 51. Maddison, W. P., and Maddison, D. R. 2002. MacClade: Analysis of Phylogeny and Character Evolution. 4.06 OSX. Sinauer Associates, Sunderland, MA.
 52. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 53. Mchau, G. R. A., and Coffey, M. D. 1995. Evidence for the existence of two subpopulations in *Phytophthora capsici* and a redescription of the species. *Mycol. Res.* 99:89-102.
 54. Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
 55. Nielsen, E. E., Hansen, M. M., Ruzzante, D. E., Meldrup, D., and Grønkjær, P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Mol. Ecol.* 12:1497-1508.
 56. Oelke, L. M., and Bosland, P. W. 2003. Differentiation of race specific resistance to *Phytophthora* root rot and foliar blight in *Capsicum annuum*. *J. Am. Soc. Hortic. Sci.* 128:213-218.
 57. Oudemans, P., and Coffey, M. D. 1991. A revised systematics of twelve papillate *Phytophthora* species based on isozyme analysis. *Mycol. Res.* 95:1025-1046.
 58. Park, J., Park, B., Veeraghavan, N., Jung, K., Lee, Y.-H., Blair, J. E., Geiser, D. M., Isard, S., Mansfield, M. A., Nikolaeva, E., Park, S.-Y., Russo, J., Kim, S., H., Greene, M., Ivors, K. L., Balci, Y., Peiman, M., Erwin, D. C., Coffey, M. D., Rossman, A., Farr, D., Cline, E., Grunwald, N. J., Luster, D. G., Schrandt, J., Martin, F., Ribeiro, O. K., Makalowska, I., and Kang, S. 2008. *Phytophthora* database: A forensic database supporting the identification and monitoring of *Phytophthora*. *Plant Dis.* 92:966-972.
 59. Parra, G., and Ristaino, J. B. 2001. Resistance to mefenoxam and metalaxyl among field isolates of *Phytophthora capsici* causing *Phytophthora* blight of bell pepper. *Plant Dis.* 85:1069-1075.
 60. Pennisi, A. M., Agosteo, G. E., Cacciola, S. O., Pane, A., and Faedda, R. 1998. Insensitivity to metalaxyl among isolates of *Phytophthora capsici* causing root rot and crown rot of pepper in Southern Italy. *Plant Dis.* 82:1283.
 61. Polach, F. J., and Webster, R. K. 1972. Identification of strains and inheritance of pathogenicity in *Phytophthora capsici*. *Phytopathology* 62:20-26.
 62. Posada, D., and Crandall, K. A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends Ecol. Evol.* 16:37-45.
 63. Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotypic data. *Genetics* 155:945-959.
 64. Prospero, S., Hansen, E. M., Grunwald, N. J., and Winton, L. M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. *Mol. Ecol.* 16:2958-2973.
 65. Quesada-Ocampo, L. M., Fulbright, D. W., and Hausbeck, M. K. 2009. Susceptibility of Fraser fir to *Phytophthora capsici*. *Plant Dis.* 93:135-141.
 66. Quesada-Ocampo, L. M., and Hausbeck, M. K. 2010. Resistance in tomato and wild relatives to crown and root rot caused by *Phytophthora capsici*. *Phytopathology* 100:619-627.
 67. R-Development-Core-Team. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
 68. Ristaino, J. B. 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* 80:1253-1259.

69. Ristaino, J. B., and Johnston, S. A. 1999. Ecologically based approaches to management of *Phytophthora* blight on bell pepper. *Plant Dis.* 83:1080-1089.
70. Rosenberg, N. A., Mahajan, S., Ramachandran, S., Zhao, C., Pritchard, J. K., and Feldman, M. W. 2005. Clines, clusters, and the effect of study design on the inference of human population structure. *PLoS Genet.* 1:e70.
71. Rosenberg, N. A., Pritchard, J. K., Weber, J. L., Cann, H. M., Kidd, K. K., Zhivotovsky, L. A., and Feldman, M. W. 2002. The genetic structure of human populations. *Science* 298:2381-2385.
72. Rozen, S., and Skaletsky, H. J. 2000. Primer3 on the WWW for General Users and for Biologist Programmers. Humana Press, Totowa, NJ.
73. Silvar, C., Merino, F., and Díaz, J. 2006. Diversity of *Phytophthora capsici* in Northwest Spain: analysis of virulence, metalaxyl response, and molecular characterization. *Plant Dis.* 90:1135-1142.
74. Stephens, M., Smith, N. J., and Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68:978-989.
75. Stumpf, M. P. H., and McVean, G. A. T. 2003. Estimating recombination rates from population-genetic data. *Nat. Rev. Genet.* 4:959-967.
76. Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105:437-460.
77. Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA Polymorphism. *Genetics* 123:585-595.
78. Tatarenkov, A., Gao, H., Mackiewicz, M., Taylor, D. S., Turner, B. J., and Avise, J. C. 2007. Strong population structure despite evidence of recent migration in a selfing hermaphroditic vertebrate, the mangrove killifish (*Kryptolebias marmoratus*). *Mol. Ecol.* 16:2701-2711.
79. Tian, D., and Babadoost, M. 2003. Genetic variation among isolates of *Phytophthora capsici* from Illinois. (Abstr.) *Phytopathology* 93:S84.
80. Vähä, J.-P., and Primmer, C. R. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Mol. Ecol.* 15:63-72.
81. Walker, S. J., and Bosland, P. W. 1999. Inheritance of *Phytophthora* root rot and foliar blight resistance in pepper. *J. Am. Soc. Hortic. Sci.* 124:14-18.
82. Wang, Z., Langston, D. B., Csinos, A. S., Gitaitis, R. D., Walcott, R. R., and Ji, P. 2009. Development of an improved isolation approach and simple sequence repeat markers to characterize *Phytophthora capsici* populations in irrigation ponds in southern Georgia. *Appl. Environ. Microbiol.* 75:5467-5473.
83. Waterhouse, G. M. 1963. Key to the Species of *Phytophthora* de Bary. Commonwealth Mycological Society, Kew, Surrey, UK.
84. Watterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.* 7:256-276.
85. Weber, G. F. 1932. Blight of peppers in Florida caused by *Phytophthora capsici*. *Phytopathology* 22:775-780.
86. Wilson, D. J., Falush, D., and McVean, G. 2005. Germs, genomes and genealogies. *Trends Ecol. Evol.* 20:39-45.
87. Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., Win, J., Kamoun, S., and Terauchi, R. 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell* 21:1573-1591.
88. Zhang, Z. G., Zhang, J. Y., Zheng, X. B., Yang, Y. W., and Ko, W. H. 2004. Molecular distinctions between *Phytophthora capsici* and *Ph. tropicalis* based on ITS sequences of ribosomal DNA. *J. Phytopathol.* 152:358-364.