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Genetic diversity of *Phytophthora capsici* recovered from Massachusetts between 1997 and 2014

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Abstract Phytophthora blight caused by *Phytophthora capsici* limits the production of cucurbits and peppers in the United States and is a growing threat to sustainable vegetable production in New England. Little is known about the genetic diversity of *P. capsici* in New England, and a total of 210 *P. capsici* isolates from 18 sites were genotyped using 46 single nucleotide polymorphism markers, revealing 85 unique and 34 repeated multi-locus genotypes. Both mating types were recovered from 7 of the 18 locations. Isolates with identical genotypes (clonal lineages) ranged from 2 to 16. Three clonal lineages were recovered from multiple sites within the same year, although none were recovered across multiple years. Bayesian clustering revealed individuals with a complex genetic cluster composition. This, coupled with a high outcrossing rate (mean $t = 0.87$) and no clear clustering in principal coordinates analysis, suggests outcrossing among the populations. Phylogenetic and genetic distance analysis indicate differentiation based on farm location and movement among farms may be infrequent. There was no obvious differentiation based on cucurbit, tomato or pepper hosts.

Keywords Oomycetes · SNP · Multi-locus genotyping · Bayesian clustering

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Introduction

Phytophthora blight caused by *Phytophthora capsici* is recognized as the most destructive disease of cucurbits (*Cucurbita* spp.) and peppers (*Capsicum* spp.) in the United States (Babadoost 2004; Hausbeck and Lamour 2004; McGrath 2001; Wick 1994; Zitter 1989). First described on pepper in the United States in 1922, *P. capsici* has a cosmopolitan distribution across the globe (Lamour 2013; Leonian 1922; Park et al. 2008). In the northeast US, the pathogen is responsible for significant loss on peppers, eggplant, tomatoes, pumpkins, melons and squash. Currently, the pathogen is a growing threat to sustainable vegetable production in New England and surrounding states (UMass Extension 2016).

Phytophthora capsici is soil-borne and, once introduced to a new location, can survive many years as dormant thick-walled oospores, even after crop rotation to non-hosts (Bowers et al. 1990; Lamour and Hausbeck 2003). In general, *P. capsici* requires the interaction of A1 and A2 mating types to produce oospores (Hausbeck and Lamour 2004), although there is evidence of apomixis (Hurtado-Gonzales and Lamour 2009) and homothallic oospore production (Islam et al. 2005). Both mating types are often present in the same field, and in near-equal ratios, at farms in Massachusetts and the surrounding region (New York, Pennsylvania and Connecticut) (Pan 1997; Hausbeck and Lamour 2004; Gobena et al. 2012a; Dunn et al. 2010). In

addition to oospores, some strains of *P. capsici* can produce asexual survival spores (chlamydospores) that have been reported from Illinois and could enable the persistence of highly virulent clonal lineages (Islam et al. 2005).

Population studies of *P. capsici* in the middle and eastern US indicate that clonal lineages generally do not overwinter (Dunn et al. 2010; Gobena et al. 2012a; Granke et al. 2012; Lamour and Hausbeck 2002; Quesada-Ocampo et al. 2011). The situation in North America is significantly different from populations in the Latin American countries of Peru and Argentina and in central China, where clonal lineages are widely dispersed and survive multiple years (Hulvey et al. 2011; Gobena et al. 2012b; Hurtado-Gonzalez et al. 2008). Our objective was to assess the genetic structure of *P. capsici* populations in New England (primarily Massachusetts) to better understand pathogen survival and spread.

Materials and methods

Isolate collection, DNA extraction and mating type determination

Infected plant material (root, crown, stem and fruit) with typical symptoms of Phytophthora blight were collected from farms in the New England region from 1997 to 2014. Each farm was assigned a unique identifier as follows: AQ: Aqua Vita Road, BG: Bagdon Farm, CU2: CU-2, FPP: Foppoma, GD: Gardner, HF: Harvest Farm, KG: Kitchen Garden, MED: Medeiros, MRF: Marini Farm, MTF: Matusko's Farm, NBO: Next Barn Over, PTR: Plumtree Road, RLF: Riverland Farm, S: S1-S9, SS: Stone Soup, UMF: UMass Farm, UNK: Unkown, VRF: Verrill Farm. The collected material was transported to the laboratory for isolation. Small sections (≈ 1 cm^2) of infected tissue were plated onto PARP-V8 agar media (160 mL V8 juice, 3 g CaCO3, 25 ppm pimaricin, 100 ppm ampicillin, 25 ppm rifampicin, 25 ppm pentachloronitrobenzene and 840 mL deionized water) and the plates checked daily for mycelial growth. Mycelium was sub-cultured as hyphal-tips onto new plates and a unique identifier assigned.

For DNA extraction, plates with mycelia of 7 days growth were flooded with lysis buffer and mycelium was scraped from plates and transferred into 1.5-mL tubes. Genomic DNA was extracted using a modified chloroform/isoamyl fungal protocol (Hulvey et al. 2011). DNA quality was assessed with a Quawell Q3000 micro-volume spectrophotometer (Quawell Technology, San Jose, USA). Mating type was determined by growing isolates on PARP-V8 agar with known A1 and A2 isolates and observing the margin between the growing mycelium for the presence/absence of amphigynous oospores under a light microscope.

Targeted sequencing and genotyping

Genomic DNA samples with a concentration of at least 5 ng/μL and a 260/280 ratio of ~1.8 were submitted to Floodlight Genomics (Floodlight Genomics, Knoxville, USA) for targeted sequencing for 45 single nucleotide polymorphism (SNP) loci based on *P. capsici* LT1534 genome v.11.0 available from the Joint Genome Institute (http://genome.jgi-psf.org/) which were previously used in other *P. capsici* population studies (Castro-Rocha et al. 2016; Hu et al. 2013). A subset of the isolates was duplicated (blindly) and all samples were processed twice. Floodlight Genomics PCR amplifies targets (80–100 bp) in a multiplexed PCR reaction and then generates sample-specific sequences using a next-generation sequencing device (e.g., Ion Proton or Illumina MiSeq). The sample amplifications and targeted sequencing were carried out at no charge as part of the Floodlight Genomics Educational and Research Outreach Program.

The raw sample-specific sequence data were made available via an FTP site and processed using CLC Genomics Workbench v.8.0 (CLC bio, Aarhus, Denmark) to map reads to a reduced representation reference genome composed solely of the genetic target sequences. The mapping parameters were set at 90% length and 90% similarity. After mapping, the genotypes were assigned for target sites with at least 20× coverage. Sites with <15% alternate allele were considered homozygous and >15% and <85% were considered heterozygous.

Data analysis

Genetic data were analyzed using GENALEX 6.5 to identify identical multi-locus genotypes (Peakall and Smouse 2006). Isolates with identical genotypes are considered members of the same mitotically-derived asexual clonal lineage (CL) and were assigned identifiers (CL-1, CL-2, etc.) and duplicate genotypes were removed before conducting further analysis. Genotypes were grouped into populations based on the farm from which the samples were collected. Bayesian algorithms were used to determine the most probable number of genetic clusters (K) among the populations using Structure 2.3.4, and to determine population outcrossing rate (t) and inbreeding coefficient (F) with the program BORICE using the default parameters and assuming isolates from the same farm belonged to the same population (Pritchard et al. 2000; Koelling et al. 2012). Structure analysis was conducted with the parameters set to no prior population information using an admixture model with allele frequencies correlated. Isolate collection site and hosts data were included in the structure analysis. The alpha value was set to 1 and simulations from K = 1 to K = 50 for 20 iterations with 200,000 burn-in cycles and 200,000 Markov Chain Monte Carlo runs were computed. Structure results were tested with Structure Harvester to select

the most probable K (Earl and vonHoldt 2012). A condensed neighbor-joining tree of the multi-locus genotypes was determined using Mega 6.06 and using 1000 Bootstrap repetitions with the maximum composite likelihood method at default parameters and a cut-off value equal to 50% (Saitou and Nei 1987; Tamura et al. 2004, 2013). Neighbor net and parsimony splits phylogenetic networks were constructed from the multi-locus genotypes with SplitsTree4 at default parameters (Huson and Bryant 2006). GENALEX 6.5 was used to determine pairwise population fixation index (F_{ST}) values, Nei's genetic distances, Shannon diversity estimate and principal coordinate analysis (PCoA) based on site of collection. GENALEX was also used to calculate the allele frequencies for each marker when all unique multi-locus genotypes were combined and to test for deviations from Hardy–Weinberg equilibrium at 0.05, 0.01 and 0.001 levels of significance.

Results

A total of 210 *P. capsici* isolates were recovered from 18 sites (16 in Massachusetts and 2 on Long Island, New York) and genotyped for 45 polymorphic SNP loci (Tables 1, 2; Supplementary Fig. 1). All samples were genotyped in duplicate and showed identical multi-locus genotypes. A total of 86 genotypes were unique and 34 were repeated (clonal lineages) (Table 3). The number of duplicate genotypes ranged from 2 to 16 and most members of a clonal lineage were confined to single locations (Table 3). Both the A1 and A2 mating types were recovered from 7 of the 18 locations (Table 3). When combining all isolates, the allele frequencies for the clone-corrected set of data averaged 0.23, and 60% of the markers did not differ from the expectations of Hardy–Weinberg equilibrium (Table 2). Bayesian clustering analysis determined K = 38 (Delta K = 1214.23) as the most probable number of genetic clusters among the unique genotypes (Supplementary Fig. 2). The Bayesian clustering bar graph shows most populations have individuals with unique genetic clusters (Fig. 1). However, within those same populations appear individuals with a complex genetic cluster composition, typical for populations with outcrossing and intermixing. A high population outcrossing rate (mean t = 0.87) with a correspondingly low inbreeding coefficient (mean F = 0.07) also indicates that there is likely outcrossing among the populations. Phylogenetic analysis, either tree or networks (Fig. 2; Supplementary Figs. 3, 4, 5), grouped most isolates of the same farm closely together, indicating some degree of population differentiation, but this may be due to the relatively small number of isolates analyzed from individual locations. Clustering, from a host perspective, shows three groups for squash, three groups for pepper, two groups for pumpkin, one group for tomato and one group for melon' however, clustering of the same host at different locations do not group

Table 1 Summary data for locations in Massachusetts and Long Island, New York

Year	State	Town/Region	Farm
2014	Massachusetts	Hadley	Next Barn Over
	Massachusetts		Stone Soup
	Massachusetts	Sunderland	Kitchen Garden
2008	Massachusetts	Concord	Verrill Farm
	Massachusetts	Hadley	Aqua Vita Rd.
	Massachusetts		Plumtree Road
	Massachusetts	Sunderland	Riverland Farm
2007	Massachusetts	Sunderland	Bagdon Farm
	Massachusetts	Whately	Harvest Farm
2006	Massachusetts	Montague	Gardner
	Massachusetts	South Deerfield	UMass Farm
2002	Massachusetts	Northbridge	Foppoma
	Massachusetts	Swansea	Medeiros
1997	New York	Long Island	CU2
	New York	Long Island	S1-S10
N/A	Massachusetts	Hadley	Matusko's Farm
	Massachusetts	Hatfield	Unknown
	Massachusetts	Ipswich	Marini Farm

together. Pairwise population F_{ST} values and Nei genetic distances indicate different degrees of differentiation between the populations (Supplementary Tables 1, 2). The values obtained from both metrics correspond to the phylogenetic analysis, which suggests that sufficient differentiation has occurred among the populations to distinguish them during phylogenetic reconstruction. Shannon's diversity estimate indicates a lower diversity among populations (sH = 1.126) than within populations (sH = 1.548), which suggests inbreeding within farm populations is higher than outcrossing among farms (Supplementary Table 3). The PCoA supports the notion that, even though the populations have enough differentiation to be independently clustered one from another, some individuals appear intermediate between the clusters of multiple populations suggesting outcrossing among farms (Supplementary Fig. 5).

Discussion

Overall, our results indicate that the survival and spread of *P. capsici* in Massachusetts is like the Midwest and Northeastern US, where sexual reproduction is crucial for overwintering and the dispersal of asexual sporangia and swimming zoospores is limited to the same or nearby farms (Dunn et al. 2010; Gobena et al. 2012a; Granke et al. 2012; Lamour and Hausbeck 2002; Quesada-Ocampo et al. 2011). Evidence supporting sexual reproduction includes the finding of both the A1 and A2 mating types at multiple

Table 2 Summary data for SNP markers including primers, allele frequencies (all genotypes combined) and probability of HWE

SNP markers[a]	Forward	Reverse	A[b]	C	G	T	Prob[c]
1_1107473	CAGGTTGGTCTGCGAGGT	ATCAAGCCCAAGGCCATC	0.85	0.00	0.15	0.00	ns
1_1552376	TACTGCGCTGCTGGCTTC	ATCGTAGCGCTCCAGACG	0.00	0.25	0.75	0.00	***
10_399503	GATACCTTGGGCCAGGATG	GCTGCTATCCGTGCGAAG	0.60	0.00	0.40	0.00	***
104_62690	CTCCGAGGTGCCACTGAT	GGACGCAGCTTTTGACG	0.00	0.25	0.00	0.75	ns
107_36606	CCCCATTCCGGATTCAGT	GATCAGGAGCGCAAGAAGA	0.00	0.48	0.00	0.52	ns
11_419981	GGAAACTTGGTCCATGCAG	CATCCGAATCGCCAACTG	0.00	0.37	0.00	0.63	**
12_58279	CTGGAATCGCTTCTAGCTCT	CCACAGGGATTTCGTCCA	0.85	0.15	0.00	0.00	***
13_177577	GGCTACGTCCCCGAGTCT	GGCGTCGTCGAAGTGAAA	0.00	0.64	0.00	0.36	ns
14_963750	AGCATCGGAACCGAACTCT	TCCTGCAGTTCCCACCTC	0.00	0.00	0.69	0.31	***
15_528446	GCAGCGCGATGTACACAA	CCAGGGTCTCTGGCAATG	0.40	0.00	0.60	0.00	ns
16_146633	TCCGTGCCAAGCTCAGTT	TCTTCCCTCCCGGGTTAC	0.00	0.78	0.00	0.22	ns
17_164249	CGCTTCCTACCTGCAGAGAT	CGCTTGCTCGAAAGTGTG	0.00	0.63	0.00	0.37	ns
17_429356	CCTTCACTGCTCCCAAGC	TTCACCACTGGCGTCTCA	0.41	0.00	0.00	0.59	ns
19_634212	CCAGGGAATCCATTTCCAC	GCCTATGAAGTGCTGTCTGA	0.27	0.00	0.73	0.00	***
2_764299	CGAGCAGCACACCGTAGA	CTGGAAATCCGCATCGAC	0.71	0.00	0.00	0.29	***
21_850808	TGAACGTCGTGGCCTTCT	CAGCGAAGTCCGAGCTGT	0.48	0.00	0.52	0.00	**
22_58618	GGAGGCGAACCGAGAAGT	CAGCGTTTGCTGGTGAAG	0.59	0.00	0.41	0.00	ns
22_655493	CAGGGTTCGGTTCCTGAAT	CCCGTCGTGTCGAAGCTA	0.07	0.00	0.93	0.00	ns
22_826381	CGGTCGCTCGACATCTTC	GCTCAACCTGCAGCGAAA	0.08	0.00	0.92	0.00	ns
23_49161	CACTGGGAGCTCCTTTGC	GTCGGACTGCTGGTGCTG	0.43	0.00	0.57	0.00	*
24_755911	GCCCATGATGTGAACACG	CAGTGCTGCAACCACCAG	0.56	0.00	0.44	0.00	ns
25_680731	GGGAGCTCGAGGACGATA	GCAGCGTCTCGAGGAATC	0.00	0.81	0.00	0.19	***
26_474538	GTCGGTCAGCGAACCATT	TACACAATCGCCGTGCTG	0.00	0.42	0.00	0.58	***
27_451724	TGCCCCTGAACAAGGCTA	GCCCGTGAACTTGGTGAA	0.00	0.68	0.00	0.32	ns
29_144322	GGTCGGCCTCGTAGGG	AAGCTCGCCAACCTGATG	1.00	0.00	0.00	0.00	ns
29_197194	CACCATGTAGGCGACACC	CCACGGCCACTGCTACTC	0.00	0.58	0.42	0.00	*
29_571713	TAGCGCTGCAGCATCTTC	CCTGGGCAACTCAATGCT	0.00	0.50	0.00	0.50	***
3_1579804	TCGTCCCGACTTGTCCTG	ATGACTCCAAGGGCAACG	0.24	0.00	0.76	0.00	ns
30_517298	TCACACGACGATTGACTGG	CCTGTCATCAGGGGCCTA	0.00	0.63	0.00	0.37	***
31_413194	GCGTTTGCGCTGGTTTTA	CGCTGCAGATGTCGCTTT	0.00	0.68	0.00	0.32	***
32_50202	GGGCGACACAGACAGCTC	AAGCTGCGTCCACTGAGG	0.74	0.26	0.00	0.00	**
32_87587	CAAGAGGGCGAAGCAGTG	CTTGTGCTGCTGGGTTCC	0.75	0.25	0.00	0.00	*
34_147979	ACCGCGTGCATCATTTTC	ATGTTCGGGCTGCAGAAG	0.02	0.98	0.00	0.00	ns
35_509383	CCACGATCTCGCCTTGAG	CCAAGAAGGACGTGAAAACG	0.00	0.35	0.00	0.65	ns
36_160455	GCGTACGTGATGCAGGAG	CGGAGTGCAGGAACCACT	0.39	0.00	0.00	0.61	*
37_296971	CGCAGATTCATGCTGGTG	GGCCTAGTTGTCCCAGCTC	0.41	0.00	0.59	0.00	*
38_502017	GCAGCTCGTTGTGTGCAG	CACAACTCCCGGATCGAC	0.69	0.00	0.31	0.00	ns
4_507540	CAAGCTCTTGCTCGAGTCC	ATCCCAGCAGAGCACCAG	0.00	0.25	0.00	0.75	***
41_363483	CCGTGAGGGTCTGCAGTT	CGCTGCACCTTCTTCTCC	0.36	0.00	0.64	0.00	***
5_118944	CCGTTCGACCATCGAAAT	CATCGGACGGCAATTCAT	0.00	0.84	0.16	0.00	**
71_157951	CGAGACCCGGAGTTCCTC	GGCGTGAAGCGACTGATG	0.78	0.00	0.22	0.00	ns
72_72952	GCTGTGCTTCAGCGCATA	TCAGGCGGACTTTGTGAAG	0.22	0.00	0.78	0.00	*
74_180705	CACATTTTCGGCCTGTGC	CATCGATTCGCTCCATCA	0.31	0.00	0.69	0.00	***
8_617199	AACGACGGCATCCACATC	GTCCTCCGGGTTCTCCAC	0.00	0.23	0.00	0.77	*
9_163520	GCGACGTTCTCGACGTG	GTGCGTGTTGTGCTCCTG	0.00	0.75	0.00	0.25	***

HWE test for deviations from Hardy-Weinberg equilibrium calculated using GENALEX

[a] Marker name indicates scaffold and position in the *P. capsici* reference genome (JGI, version 11)

[b] Allele frequencies for each SNP

[c] *ns* not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

locations, the overall high proportion of unique multi-locus genotypes and the limited survival and spread of clonal lineages. The importance of sexual oospores is not surprising as there are no hosts, such as rocoto (*C. pubescens*) in Peru, to serve as a "green bridge" through the fallow winter months (Hurtado-Gonzalez et al. 2008; Hulvey et al. 2011). This does not bode well for sustainable vegetable production in Massachusetts, because once *P. capsici* has been introduced to a new site, the thick-walled oospores can

persist for many years and planting susceptible hosts incurs the risk of total crop failure (Lamour and Hausbeck 2000).

Once the growing season is underway and oospores germinate and colonize host tissue, the rise in frequency of clonal lineages is common, as *P. capsici* produces massive numbers of deciduous sporangia on the surface of infected vegetable fruit (Granke et al. 2012). Although we identified multiple clonal lineages, they were generally limited to a single farm location and were not found across multiple years. This same

Table 3 *Phytophthora capsici* summary data including farm, year, host, genotypes, clonal lineages and mating types

Farm	Year(s)	Host(s)	Isolates	Repeated genotypes	Unique genotypes	A1/A2 mating types
Verrill Farm	2008	Pumpkin	19	CL-13 (2), CL-14 (2), CL-22 (3), CL-23 (6)	6	A1 (17), A2 (1), UNK (1)
Plumtree Road	2008	Squash	18	CL-1 (10), CL-25 (2), CL-34 (2)	4	A1 (6), A2 (12)
Aqua Vita Rd.	2008	Pumpkin	16	CL-6 (9), CL-7 (3)	4	A1 (1), A2 (15)
Matusko's Farm	UNK[a]	Squash	3	N/A[b]	3	A2 (2), UNK (1)
Stone Soup	2014	Pepper	13	CL-2 (2), CL-33 (2)	9	UNK (13)
Next Barn Over	2014	Squash	1	N/A	1	UNK (1)
Unkown	UNK	Pepper	15	CL-28 (3), CL-29 (6), CL-30 (3)	3	UNK (15)
Marini Farm	UNK	Pepper	16	CL-15 (2), CL-16 (2), CL-18 (2), CL-21 (2)	8	UNK (16)
S	1997	Squash	9	CL-19 (2), CL-20 (2)	5	A1 (8), A2 (1)
CU2	1997	Squash	5	CL-12 (2)	3	A2 (5)
Gardner	2006	Squash	8	CL-5 (1), CL-24 (1)	6	A1 (1), A2 (6), UNK (1)
Foppoma	2002	Squash	6	CL-31 (3)	3	A2 (6)
UMass Farm	2006	Squash	17	CL-3 (4), CL-5 (1), CL-8 (5)	7	A2 (17)
Bagdon Farm	2007	Squash	10	CL-9 (2), CL-10 (4)	4	A1 (7), UNK (3)
Riverland Farm	2008	Squash	18	CL-1 (6), CL-17 (9)	3	A2 (18)
Kitchen Garden	2014	Squash	1	N/A	1	UNK (1)
Medeiros	2002	Pepper, dquash	4	N/A	4	A1 (1), A2 (3)
Harvest Farm	2007, 2008	Melon, tomato	31	CL-4 (2), CL-11 (4), CL-24 (2), CL-26 (7), CL-27 (2), CL-32 (2)	12	A1 (5), A2 (26)

[a] *UNK* the data were not registered, not available or could not be determined

[b] *N/A* no repeated genotypes were found

trend has been recorded in New York and Michigan (Dunn et al. 2010; Gobena et al. 2012a; Lamour and Hausbeck 2001, 2003; Quesada-Ocampo et al. 2011), and suggests that *P. capsici* is not actively dispersed over long distances. The three clonal lineages found at multiple sites were recovered from farms no more than ~5 miles (8 km) apart.

An important question is how vegetable blight is spreading to new locations. One factor may be equipment sharing. Due to the small scale of most farming operations in New England, equipment is shared to reduce operating costs (Buckler 2012). In addition, a single operator may plow multiple fields, used for diverse crops, in the spring and fall. From 2002 to 2007, the average area of farmland in Massachusetts decreased from 85 to 67 acres (34 to 27 ha), with the decrease attributed, in

part, to a rising number of small farms in the state (USDA 2007). During that same 5-year period, market value of vegetable products increased from $36.4 to $58.9 million, driven by increasing interest in local and organic vegetables (USDA 2007). With an increasing number of small farms using shared equipment, the potential to spread soilborne pathogens like *P. capsici* is high. Irrigation water from above-ground sources can also be contaminated with *P. capsici* inoculum and must be considered (Gevens et al. 2007; Wang et al. 2009).

Another factor, difficult to assess due to the explosive epidemiology of *P. capsici*, is the potential for inoculum to be seedborne. Leonian states in the 1922 species description that *P. capsici* on pepper can be seedborne, and farmers in Tennessee (2004) and most recently, Maryland (2017), report
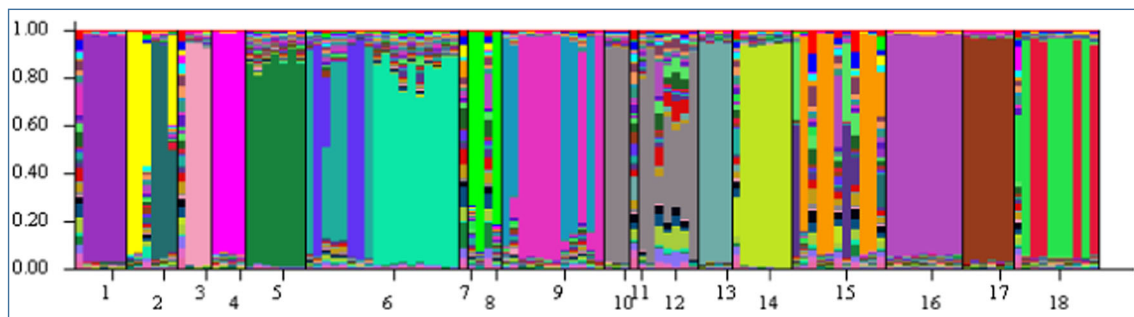

Fig. 1 Bayesian clustering of the unique multi-locus genotypes obtained from *P. capsici* isolates recovered from infected farms (*1* Aqua Vita Road, *2* Bagdon Farm, *3* CU-2, *4* Foppoma, *5* Gardner, *6* Harvest Farm, *7* Kitchen Garden, *8* Medeiros, *9* Marini Farm, *10* Matusko's Farm, *11* Next Barn Over, *12* Plumtree Road, *13* Riverland Farm, *14* S1–S9, *15* Stone Soup, *16* UMass Farm, *17* Unkown, *18* Verrill Farm)

Fig. 2 Phylogenetic analysis of unique multi-locus genotypes obtained from *P. capsici* isolates. Evolutionary history inferred using the neighbor-joining method computed with MEGA6 (optimal tree sum of branch length = 9.16365606). (*AQ* Aqua Vita Road, *BG* Bagdon Farm, *CU2* CU-2, *FPP* Foppoma, *GD* Gardner, *HF* Harvest Farm, *KG* Kitchen Garden, *MED* Medeiros, *MRF* Marini Farm, *MTF* Matusko's Farm, *NBO* Next Barn Over, *PTR* Plumtree Road, *RLF* Riverland Farm, *S* S1–S9, *SS* Stone Soup, *UMF* UMass Farm, *UNK* Unkown, *VRF* Verrill Farm)
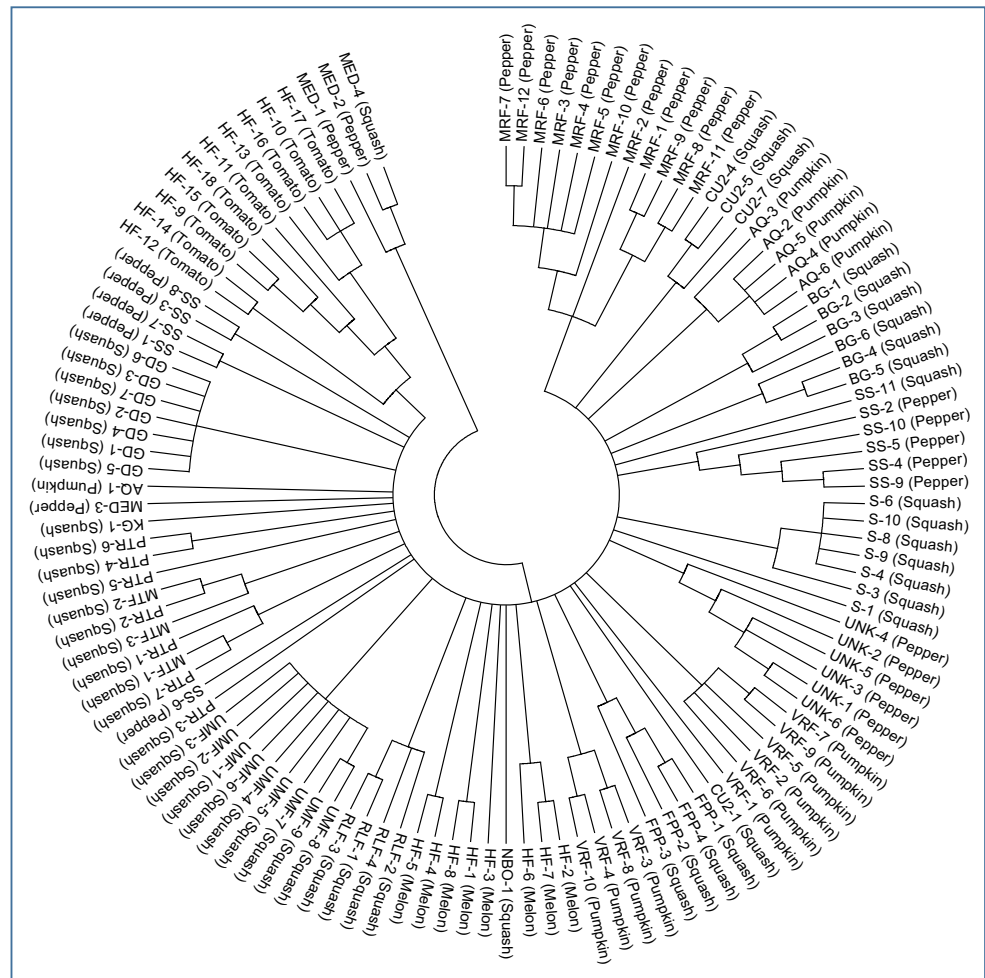
to K. Lamour (quite emphatically) that *P. capsici* entered their farm through a single lot of pepper (TN, 2004) or melon (MA, 2017) seed. In both scenarios, the blight started at specific (previously un-infested) locations in the field where a new variety was planted. In the case of pepper in Tennessee, the farmer identified the single pepper plant that showed initial wilt symptoms (located on well-drained, elevated ground) and described the subsequent movement down the row and, eventually, throughout his entire field, which included multiple varieties of pepper, tomato and squash hosts. Like the findings here, the isolates recovered from this epidemic showed no genetic structuring based on host (K. Lamour, unreported data).

The obvious goal in New England is to halt the movement of not just *P. capsici*, but of both mating types of *P. capsici* into a new location. Based on two recent findings, preventing the movement of both mating types in *P. capsici* may be more challenging than previously recognized. First, a multi-year study in New York characterizing the genetic diversity and inbreeding of a controlled bi-parental field population found a specific mating type region (MTR) of the *P. capsici* genome (previously identified via genetic mapping) that maintains a

high level of heterozygosity for A2 mating types; despite obvious inbreeding across the rest of the genome (Carlson et al. 2017; Lamour et al. 2012). Second, recent studies of vegetable blight in Taiwan and taro leaf blight (*P. colocasiae*) describe a novel dimension of plasticity for the Phytophthora genome: intra-genomic variations in ploidy (Barchenger et al. 2017; Shrestha et al. 2017). It appears that the *P. capsici* genome expands and contracts on a local (chromosome) scale during asexual clonal reproduction. Historical Taiwanese isolates were primarily of the A1 mating type and diploid across the 18 linkage groups of the *P. capsici* genome (Barchenger et al. 2017). In recent years, a shift occurred across the island and the 2016 populations were almost exclusively the A2 mating type. The change in mating type is likely related to increased ploidy across the MTR, and ploidy varied from diploid to triploid to tetraploid (and higher in *P. colocasiae*) within individual genomes.

The importance of these recent findings to New England (and elsewhere) is that *P. capsici*, due to expansion and contraction of the genome across different chromosomes (particularly the MTR) during clonal reproduction, may be able to generate an opposite mating type when only a single mating

type has been introduced to a new site. More work in this area is needed, as an epidemic with both mating types will produce long-lived, thick-walled oospore and, in most cases, lead to the loss of the use of a field for vegetable production, indefinitely.

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