Genetic Diversity and Structure in Regional *Cercospora beticola* Populations from *Beta vulgaris* subsp. *vulgaris* Suggest Two Clusters of Separate Origin

Noel L. Knight,¹ Niloofar Vaghefi,¹ Julie R. Kikkert,² Melvin D. Bolton,³ Gary A. Secor,⁴ Viviana V. Rivera,⁴ Linda E. Hanson,⁵ Scot C. Nelson,⁶ and Sarah J. Pethybridge^{1,†}

¹ Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell AgriTech at the New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

² Cornell Vegetable Program, Cornell Cooperative Extension, Canandaigua, NY 14424

³ U.S. Department of Agriculture Agricultural Research Service (USDA ARS), Red River Valley Agricultural Research Center, Fargo, ND 58102

⁴ Department of Plant Pathology, North Dakota State University, Fargo, ND 58105

⁵ USDA ARS Sugar Beet and Bean Research Unit, Michigan State University, East Lansing, MI 48824

⁶ Department of Tropical Plant and Soil Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, HI 96822

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ABSTRACT

Cercospora leaf spot, caused by *Cercospora beticola*, is a highly destructive disease of *Beta vulgaris* subsp. *vulgaris* worldwide. *C. beticola* populations are usually characterized by high genetic diversity, but little is known of the relationships among populations from different production regions around the world. This information would be informative of population origin and potential pathways for pathogen movement. For the current study, the genetic diversity, differentiation, and relationships among 948 *C. beticola* isolates in 28 populations across eight geographic regions were investigated using 12 microsatellite markers. Genotypic diversity, as measured by Simpson's complement index, ranged from 0.18 to 1.00, while pairwise index of differentiation values ranged from 0.02 to 0.42, with the

greatest differentiation detected between two New York populations. In these populations, evidence for recent expansion was detected. Assessment of population structure identified two major clusters: the first associated with New York, and the second with Canada, Chile, Eurasia, Hawaii, Michigan, North Dakota, and one population from New York. Inferences of gene flow among these regions suggested that the source for one cluster likely is Eurasia, whereas the source for the other cluster is not known. These results suggest a shared origin of *C. beticola* populations across regions, except for part of New York, where population divergence has occurred. These findings support the hypothesis that dispersal of *C. beticola* occurs over long distances.

Population genetics studies have been widely utilized to assess global relationships of economically significant plant pathogens (Ali et al. 2014; Friesen et al. 2005; Linde et al. 2009; Sakalidis et al. 2013; Vaghefi et al. 2017a). Relationships among pathogen populations convey important information with implications for disease epidemiology. For example, hypotheses concerning sources of origin, changes in diversity, and patterns of dispersal can be developed by studying geographically or temporally distinct populations. This information may inform disease management strategies and control measures for the movement of plant materials (McDonald 2015; Milgroom and Fry 1997).

Cercospora beticola Sacc. causes the destructive foliar disease Cercospora leaf spot (CLS) on sugar beet and table beet (*Beta vulgaris* subsp. *vulgaris* L.) (Weiland and Koch 2004). The disease has been reported across Africa, Asia, Europe, North America, Oceania, and South America (Holtschulte 2000; Jacobsen and Franc 2009). In sugar beet, CLS may cause losses of up to 40%,

predominantly as a result of reductions in root weight and extractable sucrose (Franc 2010; Shane and Teng 1992; Smith and Martin 1978; Smith and Ruppel 1973). In table beet, CLS lesions may lead to consumer rejection in the fresh market, while the subsequent defoliation prevents harvesting by top-pulling machinery in broad-acre fields and may result in complete crop loss (Pethybridge et al. 2017).

Colonized plant debris and alternative hosts have traditionally been considered the primary sources of inoculum for CLS epidemics (Jacobsen and Franc 2009; McKay and Pool 1918; Pool and McKay 1916). Survival of *C. beticola* as pseudostromata in colonized plant residues may allow the fungus to perpetuate into subsequent growing seasons and is dependent on factors such as tillage, moisture, temperature, and soil microbiota (Khan et al. 2008). *C. beticola* has been detected in plant debris 9 months after incorporation into the soil, and up to 39 months after placement of plant debris on the soil surface (Khan et al. 2008; Solel 1970). The surviving pseudostromata produce conidia, which undergo localized dispersal through water splash or wind (Khan et al. 2008; Lawrence and Meredith 1970; Meredith 1967). Subsequent colonization of leaf tissues results in circular necrotic lesions and conidial production, with multiple infection cycles occurring during a growing season under favorable environmental conditions (Rathaiah 1977; Weiland and Koch 2004).

The dispersal and movement of *C. beticola* inoculum has been examined using direct detection methods (Lawrence and Meredith 1970; McKay and Pool 1918; Meredith 1967; Pool and McKay 1916) and indirect methods investigating the genetic relationships of pathogen populations (Groenewald et al. 2008; Knight et al. 2018; Vaghefi et al. 2017a, c). Recent genetic studies have provided evidence that long-range dispersal of *C. beticola* has occurred

†Corresponding author: S. J. Pethybridge; sjp277@cornell.edu

Current address of N. Vaghefi: Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia.

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(Groenewald et al. 2008; Knight et al. 2018; Vaghefi et al. 2017a, c). High levels of gene flow and low differentiation between European and Iranian populations of *C. beticola* were suggested to result from human-mediated movement of infested plant material (Groenewald et al. 2008). In contrast, a differentiated population from New Zealand was hypothesized to have originated from a founding event (Groenewald et al. 2008). Vaghefi et al. (2017a) described potential gene flow between populations of *C. beticola* from Europe and the United States, based on shared clonal lineages detected using genotyping-by-sequencing and genome-wide single nucleotide polymorphism analyses. Examination of *C. beticola* populations from New York indicated shared inoculum sources in broad-acre monoculture fields, while significant genetic differentiation between populations from mixed-cropping farms (three to five rows of various crops) was suggestive of disparate inoculum sources (Knight et al. 2018; Vaghefi et al. 2017c). In these studies, the role of isolated local inoculum sources was considered; however, inoculum introduced from a source outside the field of interest may have been the progenitor of the distinct genetic population.

Mating-type ratios of *C. beticola* populations within distinct regions and fields around the world have been reported to be in either equilibrium or disequilibrium (Bakhshi et al. 2011; Bolton et al. 2012; Groenewald et al. 2006b; Knight et al. 2018; Obuya et al. 2011; Vaghefi et al. 2017c). Along with reports of high genotypic diversity in *C. beticola* populations (Groenewald et al. 2007; Knight et al. 2018; Moretti et al. 2004, 2006; Turgay et al. 2010; Vaghefi et al. 2016, 2017c), these findings have been interpreted as evidence for a history of sexual recombination events (Groenewald et al. 2008; Vaghefi et al. 2017c). However, no physical identification of a sexual morph has been reported (Groenewald et al. 2006b). Furthermore, alleles at microsatellite loci in *C. beticola* populations from Hawaii and New York have been in linkage disequilibrium (Knight et al. 2018; Vaghefi et al. 2017c), which is not suggestive of random mating (Milgroom 1996). It is possible, and perhaps most likely, that a mixed mode of reproduction exists in *C. beticola* populations (Vaghefi et al. 2016).

Knowledge of the relationships among *C. beticola* populations from different geographic regions is critical for determining paths of gene flow and sources of origin. The significant differentiation of *C. beticola* populations reported in New York table beet fields (Knight et al. 2018; Vaghefi et al. 2017a, 2017c) highlights that knowledge of population origin would be useful for designing effective disease management strategies. Vaghefi et al. (2017a) provided the first indication of potential *C. beticola* gene flow from Europe to the United States using populations from Eurasia, Hawaii, Michigan, New York, and North Dakota; however, small population sizes from Michigan ($n = 4$) and North Dakota ($n = 12$) limited the power of the analysis. In order to expand the analysis of the global genetic structure of *C. beticola* and examine potential gene flow among geographic regions, 14 additional populations from Canada, Chile, Michigan, New York, and North Dakota were assessed, in conjunction with the previously genotyped populations from Vaghefi et al. (2017a), using microsatellite markers. The relationships between regions indicated by this assessment will aid in understanding the pathways of *C. beticola* dispersal.

MATERIALS AND METHODS

***C. beticola* populations.** For the purpose of this study, populations are described using three terms: sampling populations (a collection of isolates from one location and time point), regional populations (a group of sampling populations from a similar geographic region), and genetic groups (isolates grouped together with a genetic clustering method). Sampling populations of *C. beticola* were collected from CLS lesions on table and sugar beet leaves from a range of countries and regions (Table 1). Fungal isolations were performed as described in Ruppel et al. (1980) for

the Canadian and Michigan sampling populations and in Bolton et al. (2013) for the remaining sampling populations. Axenic cultures were established on clarified V8 agar [10% (vol/vol) clarified V8 broth, 15 g/liter agar] (Miller 1955; Secor and Rivera 2012). Dried mycelial plugs of axenic cultures were stored at -20°C .

Collection of CLS-affected leaves, and naming of sampling locations, in New York followed the convention presented in Vaghefi et al. (2016, 2017c) and Knight et al. (2018). Isolates from Hawaii were collected as described by Vaghefi et al. (2017c). The remaining sampling populations were provided from collections at the U.S. Department of Agriculture Agricultural Research Service (USDA ARS) Red River Valley Agricultural Research Center (North Dakota), the North Dakota State University Department of Plant Pathology (Chile and Eurasia), and the USDA ARS Sugar Beet and Bean Research Unit at Michigan State University (Canada and Michigan). These collections include eight novel sampling populations and 20 sampling populations previously assessed using genetic markers (Knight et al. 2018; Vaghefi et al. 2016, 2017a, b, c). Assessment, as described below, was performed with individual sampling populations or regional populations.

DNA extraction and genotyping. Fungal mycelia were harvested from 14-day-old cultures grown in 10% (vol/vol) clarified V8 broth at 25°C , and DNA was extracted as described by Vaghefi et al. (2016), except for populations from Michigan in 2016, Chile, and Canada. These were extracted using the Wizard Genomic DNA Extraction kit (Promega, Madison, WI), following the manufacturer's plant DNA extraction protocol.

C. beticola isolates were genotyped with 12 microsatellite markers (CbSSR1, CbSSR2, CbSSR3, CbSSR6 [Groenewald et al. 2007] and CbSSR20, CbSSR21, CbSSR22, CbSSR23, CbSSR24, CbSSR25, CbSSR26, and CbSSR27 [Vaghefi et al. 2017b]) as described previously (Vaghefi et al. 2017b). Alleles were scored using Microsatellite Plugin version 1.4.4 in Geneious version 10.2.3 (<https://www.geneious.com>; Kears et al. 2012). Genotyping was replicated for a random 15% of the samples and all isolates exhibiting null alleles. Error rates, defined as the number of allelic mismatches divided by the number of replicated alleles, were quantified for each locus.

Mating-type identification. Mating types of individual isolates were identified using the polymerase chain reaction (PCR) assays described by Vaghefi et al. (2017c). PCR products were visualized after electrophoresis on a 2 g/liter agarose gel in Tris-acetate-EDTA containing 0.001% (vol/vol) GelRed (Biotium Inc., Hayward, CA). Product size was determined against a 100-bp DNA ladder (Axygen Inc., Union City, CA).

Microsatellite data set refinement. Microsatellite data for each sampling or regional population was refined in the R version 3.4.1 (R Core Team 2017) package *poppr* version 2.4.1 (Kamvar et al. 2014, 2015). To reduce the confounding effect of missing data or genotyping errors on genotypic diversity, raw genotypes were contracted to multilocus genotypes (MLGs) with the *mlg.filter* function in *poppr*. The Bruvo's distance (Bruvo et al. 2004) threshold for contraction (0.0208333) was estimated using the *cutoff_predictor* function and applied to raw genotypes using the farthest neighbor algorithm. All subsequent analyses were conducted on the contracted data set. The contracted mMLGs were clone-corrected at the sampling and regional population levels.

Measures of genetic diversity. The number of MLGs, expected number of MLGs after rarefaction, MLG frequencies across populations, evenness, Nei's gene diversity (Nei 1978), and Simpson's complement index of genotypic diversity (Simpson 1949) (corrected for sample size) were estimated in *poppr*. The total number of alleles, number of private alleles, and unbiased haploid gene diversity (u_h ; also called Nei's gene diversity or expected heterozygosity [H_e] in diploids) were determined for each microsatellite locus using GenAlEx version 6.5 (Peakall and Smouse 2006, 2012). Allelic richness and private allelic richness were

estimated with rarefaction to the smallest population size in ADZE version 1.0 (Szpiech et al. 2008).

Tests of mating-type ratios, linkage equilibrium, and random mating. Sampling populations were assessed for mating-type ratios, multilocus linkage equilibrium, and random mating. Regional populations were not biologically relevant for these assessments as a result of geographic separation of their subpopulations.

Mating-type frequencies were assessed for significant deviations from a 1:1 ratio using the χ^2 goodness-of-fit test (Mangiafico 2015). This was performed for both clone-corrected and non-clone-corrected population data sets. χ^2 assessments were not performed when population sizes were <10 (the Manoa community garden [MCG] and Diamond Head community garden [DH] in Hawaii; New York Farm 1 in 2015 and 2016 [NY-Farm 1-2015 and NY-Farm 1-2016] and Farm 2 in 2016 [NY-Farm 2-2016], after clone-correction; and Denmark, England, Italy, Sweden, and Turkey, before and after clone-correction).

Multilocus linkage equilibrium was assessed for both clone-corrected and non-clone-corrected data sets of each population using the index of association (Brown et al. 1980) and standardized index of association (Agapow and Burt 2001), which were estimated for significance after 1,000 permutations in *poppr*.

The proportion of compatible pairs of loci (PrCP) (Estabrook and Landrum 1975) for each sampling population was estimated using MultiLocus version 1.3 (Agapow and Burt 2001), with statistical significance assessed using 999 replications of a randomly recombined data set in MultiLocus. Two loci are considered compatible (PrCP = 1) if all observed genotypes can be accounted for by mutations only in the absence of homoplasmy (reversals, parallelisms, or convergences) or recombination. Under the assumption that recurrent, parallel, and reverse mutations are rare, phylogenetic incompatibility (PrCP < 1) provides evidence of genetic exchange and may be interpreted as evidence for recombination (Milgroom 2015). The null hypothesis of random mating was rejected ($P < 0.05$) if fewer incompatible loci than

expected in a randomized population were observed (Bennett et al. 2005; Xu et al. 2009).

Population identity and differentiation. A dendrogram based on Nei's genetic distance was calculated using the unweighted pair group method with arithmetic mean (UPGMA) in *poppr* for the sampling and regional clone-corrected populations.

Genetic fixation, as measured by standardized Φ'_{PT} , was inferred in an analysis of molecular variance (AMOVA) framework in GenAlEx (Peakall and Smouse 2006, 2012). Standardized Φ'_{PT} , an analog of F_{ST} , also estimates pairwise population genetic differentiation.

The AMOVA and pairwise population differentiation analyses were performed on the eight regional populations using both non-clone-corrected and clone-corrected data sets, with significance tested using 999 permutations.

Population dynamics. The program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) was used to assess whether *C. beticola* populations had experienced significant deviations from population mutation-drift equilibrium as a result of recent founder events, population bottlenecks, or population expansions. Populations that have experienced a bottleneck have greater heterozygosity (H) than the heterozygosity expected at mutation-drift equilibrium (H_{EQ}) ($H > H_{EQ}$), whereas populations that have experienced a recent population expansion will have lower H than expected under H_{EQ} ($H < H_{EQ}$), because the average number of alleles increases faster than the H until H_{EQ} is attained (Nei et al. 1975). Each clone-corrected sampling and regional population was examined under the infinite allele model (IAM), stepwise mutation model (SMM), and two-phase model (TPM), considered the most appropriate for microsatellite data (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Piry et al. 1999). The TPM was defined as 95% of mutations conforming to an SMM and a variance among multiple steps of 12 (Piry et al. 1999). The sign test and Wilcoxon's signed-rank test with 1,000 iterations were used to determine

TABLE 1. Sampling locations and origins of *Cercospora beticola* populations and the number of isolates genotyped

Continent	Country	State/province	County/area	Field	Year	Host	Isolates (n)	Reference ^a
North America	USA	Hawaii	Honolulu	DH ^b	2015	Table beet	43	Vaghefi et al. 2017c
North America	USA	Hawaii	Honolulu	MCG ^b	2015	Table beet	19	Vaghefi et al. 2017c
North America	USA	Michigan	Ingham	MSU Farm ^b	2011	Sugar beet	11	Vaghefi et al. 2017a
North America	USA	Michigan	Tuscola	Garden	2016	Table beet	30	—
North America	USA	Michigan	Tuscola	SVREC ^b	2016	Table beet	20	—
North America	USA	Michigan	Tuscola	Garden	2016	Sugar beet	23	—
North America	USA	Michigan	Tuscola	SVREC	2016	Sugar beet	26	—
North America	USA	North Dakota	Cass	USDA ^b Fargo	2014	Sugar beet	20	Vaghefi et al. 2017a
North America	USA	North Dakota	Richland	Wahpeton	2016	Sugar beet	24	—
North America	USA	Minnesota	Clay	Sabin	2016	Sugar beet	67	—
North America	USA	New York	Genesee	Field 1	2014	Table beet	33	Vaghefi et al. 2017b
North America	USA	New York	Genesee	Field 2	2014	Table beet	43	Vaghefi et al. 2017b
North America	USA	New York	Genesee	Field 2	2016	Table beet	38	Knight et al. 2018
North America	USA	New York	Genesee	Field 3	2015	Table beet	69	Vaghefi et al. 2017c
North America	USA	New York	Genesee	Field 4	2015	Table beet	54	Vaghefi et al. 2017c
North America	USA	New York	Livingston	Field 5	2015	Table beet	72	Vaghefi et al. 2017c
North America	USA	New York	Schuyler	Farm 1	2015	Table beet	44	Vaghefi et al. 2017c
North America	USA	New York	Schuyler	Farm 1	2016	Table beet	43	Knight et al. 2018
North America	USA	New York	Ontario	Farm 2	2015	Table beet	127	Vaghefi et al. 2017c
North America	USA	New York	Ontario	Farm 2	2016	Table beet	31	Knight et al. 2018
North America	Canada	Ontario	Lambton and Kent	—	2016	Sugar beet	32	—
South America	Chile	—	Chillan	—	2015	Sugar beet	28	—
Europe	Denmark	—	Nykobing	—	2011	Sugar beet	9	Vaghefi et al. 2017a
Europe	England	—	Suffolk	—	2011	Sugar beet	9	Vaghefi et al. 2017a
Europe	Germany	Lower Saxony	Northeim	—	2011	Sugar beet	10	Vaghefi et al. 2017a
Europe	Italy	—	Emilia-Romagna	—	2007 to 2011	Sugar beet	9	Vaghefi et al. 2017a
Europe	Sweden	—	Kronoberg	—	2011	Sugar beet	8	Vaghefi et al. 2017a
Asia	Turkey	—	Central Anatolia	—	2014	Sugar beet	6	Vaghefi et al. 2017a
Total							948	

^a References describing the original collection of the sampling populations. A dash indicates a population that was unique to the current study.

^b DH = Diamond Head community garden, MCG = Manoa community garden, MSU = Michigan State University Crops Research Farm, SVREC = Saginaw Valley Research and Extension Center, and USDA = U.S. Department of Agriculture.

whether a population exhibited a significant number of loci with heterozygosity deficiency/excess.

Bidirectional migration between the eight regional populations was estimated using Bayesian inference in the program Migrate-n version 3.6.11 (Beerli 1998, 2009; Beerli and Felsenstein 1999, 2001). Migrate-n estimated values for mutation-scaled effective immigration rates ($M = m/\mu$, where m is the migration rate and μ is the mutation rate per generation and locus) and values for the mutation-scaled effective population size ($\theta = xN_e\mu$, where x is the inheritance scalar and N_e is the population size). The mutation-scaled immigration rate reports the effect of immigration versus mutation as factors for introducing variation into a population. The second allele in the input file was coded as missing. The Brownian motion data model, assuming stepwise mutation, was applied and mutation rates for the 12 loci were allowed to vary and were estimated relative to each other from the data. Parameters were estimated from one long chain with sampling of 10,000 steps with a

50-step sampling increment. The chain was replicated 100 times, with static heating at temperatures of 1.0, 1.5, 3.0, and 1,000,000, and Metropolis-Hastings sampling with the first 10,000 steps discarded as burn-in. This resulted in 50,000,000 sampled parameter values. The posterior distribution over all loci was visualized as a unimodal plot and used to assess convergence of chains and estimates of posterior probabilities of the estimated parameters. The eight-regional population analysis in Migrate-n was run using parallel nodes with a message-passing interface on the BioHPC resources at the Cornell University Institute of Biotechnology in Ithaca, New York.

Population structure. A minimum spanning network illustrating the frequency distribution and the genetic relatedness among MLGs of the regional populations was created in *poppr*. The genetic distance among MLGs was calculated based on Bruvo's distance (Bruvo et al. 2004) using the *bruvo.msn* function. The minimum spanning network was viewed using the *msn()* tool.

TABLE 2. Statistics and indices of genetic diversity for sampling and regional *Cercospora beticola* populations

Population	n^a	MLG ^b	eMLG ^c	E5 ^d	CF ^e	$1 - \lambda^f$	H _E ^g	Ra ^h	Prare ⁱ
Hawaii ^j	62	9	6.63	0.55	0.85	0.65	0.32	1.32	0.29
HI-MCG	19	4	3.65	0.72	0.79	0.63	0.37	1.37	0.09
HI-DH	43	5	2.56	0.49	0.88	0.33	0.18	1.18	0.09
Michigan ^j	110	60	20.78	0.54	0.45	0.96	0.50	1.50	0.18
MI-MSU Farm	11	6	6.00	0.73	0.45	0.80	0.40	1.40	0.05
MI-Tb Garden	30	19	9.07	0.83	0.37	0.96	0.48	1.48	0.03
MI-Tb SVREC	20	10	5.95	0.48	0.50	0.71	0.33	1.33	0.04
MI-Sb Garden	23	18	9.79	0.90	0.22	0.98	0.51	1.51	0.03
MI-Sb SVREC	26	19	9.06	0.74	0.27	0.95	0.48	1.48	0.05
North Dakota ^j	111	48	14.50	0.24	0.57	0.76	0.42	1.42	0.21
ND-Fargo	20	16	9.48	0.82	0.20	0.96	0.64	1.64	0.14
ND-Wahpeton	24	6	3.29	0.43	0.75	0.38	0.23	1.23	0.03
Minnesota									
MN-Sabin	67	27	6.04	0.31	0.60	0.73	0.38	1.38	0.05
New York ^j	427	162	22.30	0.46	0.62	0.98	0.44	1.44	0.33
NY-Farm 1-2015	44	7	3.40	0.58	0.84	0.55	0.35	1.35	0.16
NY-Farm 1-2016	43	4	1.96	0.42	0.91	0.18	0.05	1.05	0.01
NY-Farm 2-2016	31	9	4.47	0.45	0.71	0.58	0.32	1.32	0.12
NY-Field 1	33	23	9.14	0.69	0.30	0.95	0.46	1.46	0.10
NY-Field 2-2014	43	30	9.76	0.81	0.30	0.97	0.43	1.43	0.12
NY-Field 2-2016	38	32	10.47	0.92	0.16	0.99	0.39	1.39	0.10
NY-Field 3	69	39	9.96	0.87	0.43	0.98	0.40	1.40	0.09
NY-Field 4	54	30	9.38	0.78	0.44	0.97	0.45	1.45	0.05
NY-Field 5	72	19	7.36	0.72	0.74	0.90	0.36	1.36	0.04
NY-Farm 2-2015 ^k	127	38	8.55/16.39	0.66	0.70	0.94	0.36	1.36	0.10/0.12
Canada ^k	32	20	9.38/18.54	0.89	0.38	0.97	0.50	1.50	0.07/0.13
Chile ^k	28	25	10.47/25	0.92	0.11	0.99	0.49	1.49	0.06/0.12
Eurasia ^j	51	38	22.75	0.78	0.25	0.98	0.50	1.50	0.14
Denmark	9	6	–	0.74	0.33	0.83	0.34	–	–
England	9	4	–	0.75	0.56	0.69	0.38	–	–
Germany	10	10	–	1.00	0.00	1.00	0.54	–	–
Italy	9	9	–	1.00	0.00	1.00	0.47	–	–
Sweden	8	3	–	0.89	0.63	0.68	0.20	–	–
Turkey	6	6	–	1.00	0.00	1.00	0.52	–	–
Total ^l	948	378	10.37	0.43	0.60	0.99	0.61	–	–

^a Population size before clone-correction.

^b Number of multilocus genotypes (MLGs) after clone-correction.

^c Expected number of MLGs (eMLGs) after rarefaction. Populations with <11 individuals were not included in the analysis. – indicates statistic was not calculated.

^d Population evenness defined as an estimation of the uniformity in distribution of the MLGs.

^e Clonal fraction (CF).

^f Simpson's complement index ($1 - \lambda$) defined as the probability that two genotypes randomly chosen from the population are different.

^g Nei's index of gene diversity (H_E) defined as the probability that two randomly chosen alleles are different.

^h Allelic richness (Ra) after rarefaction to the smallest sample size. Populations with <11 individuals were not included in the analysis. – indicates statistic was not calculated.

ⁱ Private allelic richness (Prare) is the number of private alleles after rarefaction to the smallest sample size. Populations with <11 individuals were not included in the analysis. – indicates statistic was not calculated.

^j Regional populations consisting of multiple sampling populations from each region (constituent populations listed below each regional population, excluding New York, which did not include NY-Farm 2-2015). HI = Hawaii, MCG = Manoa community garden, DH = Diamond Head community garden, MI = Michigan, MSU = Michigan State University Crops Research Farm, Tb = table beet, SVREC = Saginaw Valley Research and Extension Center, Sb = sugar beet, ND = North Dakota, MN = Minnesota, and NY = New York.

^k These populations were assessed as both sampling and regional populations. Values for eMLG and Prare indicate 'sampling/regional'-based rarefaction calculations.

^l Total represents all the individual isolates from each population grouped into one combined population.

Discriminant analysis of principal components (DAPC) (Jombart et al. 2010) among the eight regional clone-corrected populations was conducted in the R package *adeigenet* version 2.0.1 (Jombart 2008).

Population structure was further assessed without a priori population assumptions using four methods and clone-corrected data for isolates from 28 *C. beticola* sampling populations. A further DAPC was conducted in *adeigenet*, where the `find.clusters` function transformed the data using principal components analysis and identified the optimal number of clusters using the Bayesian information criterion (BIC). Subsequently, the variation among the clusters was summarized using discriminant analysis of the retained principal components. A model-based Bayesian clustering method was utilized in the program STRUCTURE version 2.3.4 (Pritchard et al. 2000). The parameters of the project consisted of the admixture model (assuming that individuals may have part of the genome from each of *K* genetic groups), the allele frequencies correlated model, and a burn-in period of 100,000 steps, followed by 1,000,000 Markov chain Monte Carlo steps. Assignment of MLGs to genetic groups was performed for *K* values ranging from 1 to 15, with 15 replicate runs (iterations) for each *K* value. The optimal *K* value was determined by calculating ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER version 0.6.94 (Earl and vonHoldt 2012). A graphical output was generated in CLUMPAK version 1.1 (Kopelman et al. 2015) using the 15 iterations for the optimal *K* value.

The fast maximum-likelihood genetic clustering method, *snapclust* (Beugin et al. 2018), in the R package *adeigenet*, was used as an alternative to STRUCTURE. This method relies on

maximizing the likelihood of a fixed number of panmictic genetic groups using a combined geometric approach and fast-likelihood optimization in the expectation-maximization algorithm (Beugin et al. 2018). The optimal number of clusters was chosen using the Akaike information criterion and the BIC.

A distance-based dendrogram constructed through UPGMA using Bruvo's distance (Bruvo et al. 2004) was plotted for isolates from all of the sampling populations using *poppr* with 1,000 bootstrap replicates, and branch support values >50% were retained.

RESULTS

***C. beticola* populations.** Sampling populations of *C. beticola* were obtained from symptomatic table beet and sugar beet leaves originating from a range of geographic areas (Table 1), broadly encompassing seven regions described as Canada, Chile, Eurasia, Hawaii, Michigan, New York, and North Dakota. MLGs and mating types were determined for 948 *C. beticola* isolates in total, with the greatest proportion of isolates (58%) originating from multiple fields in New York. Because of significant differentiation from the other New York populations ($\Phi'_{PT} = 0.42$; $P = 0.001$), the NY-Farm 2-2015 samples were analyzed as a separate regional population. The sampling population from Sabin, Minnesota originated close to the border with North Dakota and was combined into the regional North Dakota population for analysis. Sampling populations from Europe and Asia were combined into one Eurasian regional population after assessment of their genetic relationships (Supplementary Fig. S1) to allow regional comparisons. The genotyping

TABLE 3. Mating-type ratios (MAT), linkage equilibrium of alleles at microsatellite loci, and proportion of compatible pairs of loci for *Cercospora beticola* sampling populations

Population ^a	Non-clone-corrected			Clone-corrected			Non-clone-corrected		Clone-corrected		PrCP ^e
	MAT1-1	MAT1-2	χ^2 ^b	MAT1-1	MAT1-2	χ^2	$\bar{r}_d$ ^c	I_a ^d	$\bar{r}_d$	I_a	
HI-MCG	0	19	19.00*	0	4	— ^f	0.72*	6.48*	0.58*	5.16*	1.00*
HI-DH	13	30	6.72*	2	3	—	0.58*	5.73*	0.07	0.79	1.00*
MI-MSU Farm	9	2	4.45*	4	2	—	0.30*	3.02*	0.12*	1.19*	0.97
MI-Tb Garden	18	12	1.20	9	10	0.05	0.07*	0.77*	0.01	0.10	0.59*
MI-Tb SVREC	17	3	9.80*	7	6	0.08	0.47*	5.06*	0.23*	2.50*	0.94*
MI-Sb Garden	10	13	0.39	8	10	0.22	0.09*	0.80*	0.04*	0.36*	0.42
MI-Sb SVREC	18	8	3.85*	11	8	0.47	0.09*	0.90*	0.02	0.25	0.52*
ND-Fargo	11	9	0.20	10	6	1	0.11*	1.21*	0.03*	0.33*	0.35*
ND-Wahpeton	5	19	8.12*	5	1	—	0.61*	6.00*	0.06	0.54	0.86
MN-Sabin	17	50	16.25*	15	12	0.33	0.37*	3.86*	0.05*	0.54*	0.39
NY-Farm 1-2015	12	32	9.09*	3	4	—	0.80*	7.76*	0.44*	4.22*	1.00*
NY-Farm 1-2016	2	41	35.37*	2	2	—	0.73*	3.48*	0.40*	1.59*	1.00
NY-Farm 2-2015	60	67	0.39	16	22	0.95	0.10*	0.92*	0.09*	0.82*	0.48
NY-Farm 2-2016	27	4	17.07*	7	2	—	0.53*	5.08*	0.13*	1.25*	0.85*
NY-Field 1	18	15	0.27	15	8	2.13	0.07*	0.73*	0.06*	0.60*	0.53*
NY-Field 2-2014	35	8	16.95*	23	7	8.53*	0.06*	0.68*	0.02*	0.26*	0.59*
NY-Field 2-2016	21	17	0.42	18	14	0.5	0.06*	0.59*	0.05*	0.49*	0.47
NY-Field 3	45	24	6.39*	26	13	4.33*	0.04*	0.38*	0.03*	0.28*	0.53*
NY-Field 4	42	12	16.67*	22	8	6.53*	0.08*	0.77*	0.03*	0.32*	0.39*
NY-Field 5	54	18	18.00*	15	4	6.37*	0.10*	0.93*	0.05*	0.44*	0.82*
Canada	10	22	4.50*	6	14	3.2*	0.09*	0.89*	0.04*	0.44*	0.56*
Chile	11	17	1.29	11	14	0.36	0.04*	0.45*	0.03*	0.38*	0.14
Denmark	5	4	—	5	1	—	0.23*	2.04*	0.07	0.60	0.94
England	7	2	—	3	1	—	0.50*	4.95*	0.27*	2.41*	1.00
Germany	3	7	1.60	3	7	1.6	0.12*	1.08*	0.12*	1.08*	0.73*
Italy	2	7	—	2	7	—	0.08*	0.63*	0.08*	0.63*	0.80
Sweden	3	5	—	2	1	—	0.90*	2.70*	1.00	2.00	1.00
Turkey	4	2	—	4	2	—	−0.03	−0.30	−0.03	−0.30	0.92

^a HI = Hawaii, MCG = Manoa community garden, DH = Diamond Head community garden, MI = Michigan, MSU = Michigan State University Crops Research Farm, Tb = table beet, SVREC = Saginaw Valley Research and Extension Center, Sb = sugar beet, ND = North Dakota, MN = Minnesota, and NY = New York.

^b χ^2 value based on a 1:1 ratio and one degree of freedom. Mating-type ratios with significant deviation from 1:1 ($P < 0.05$) are indicated by an asterisk.

^c Standardized index of association ($\bar{r}_d$) scaled for the number of loci, with significant deviations from zero ($P < 0.05$) indicated by an asterisk.

^d Index of association (I_a), with significant deviations from zero ($P < 0.05$) indicated by an asterisk.

^e Proportion of compatible pairs of loci, with observations of significantly ($P < 0.05$) less incompatible loci than expected in a randomized population indicated by an asterisk.

^f Dashes indicate not calculated because of small sample sizes.

Fig. 1. Relationships among **A**, 28 sampling and **B**, eight regional clone-corrected *Cercospora beticola* populations. The dendrogram was constructed using the unweighted pair group method with arithmetic mean based on Nei's genetic distance. Numbers at branch points indicate the percent occurrence of the cluster to the right of the branch in 1,000 bootstrapped dendrograms. MN = Minnesota, ND = North Dakota, NY = New York, HI = Hawaii, DH = Diamond Head community garden, MCG = Manoa community garden, MI = Michigan, Tb = table beet, SVREC = Saginaw Valley Research and Extension Center, MSU = Michigan State University Crops Research Farm, and Sb = sugar beet.

error rate ranged from 0 to 0.01 across the sampling populations, with errors only observed for primer pair CbSSR3. Estimates of missing data (or null alleles) across the sampling populations ranged from 0.79 to 25.98% (Supplementary Fig. S2).

Genetic diversity. The 948 *C. beticola* isolates were composed of 406 MLGs prior to contraction and 378 MLGs after contraction using the mlg.filter function (Table 2). Clonality varied among sampling populations according to geographic origin. When assessed by region, Chile and Hawaii had the lowest (11%) and highest (85%) levels of clonality, respectively. Forty-one of the 378 MLGs (303 of the total 948 isolates) were shared among at least two of the sampling populations (Supplementary Fig. S3), while 20 of the 378 MLGs (135 of the total 948 isolates) were shared among at least two of the regional populations (Supplementary Fig. S4).

The number of alleles across all *C. beticola* isolates in this study ranged from 2 (CbSSR2) to 38 (CbSSR3), with an average of 9.25 alleles per locus (Supplementary Table S1). Unbiased haploid gene diversity per locus ranged from 0.12 (CbSSR1) to 0.84 (CbSSR3). The regional New York population exhibited 19 private alleles, with the remaining regional populations exhibiting zero (NY-Farm 2-2015) to six (North Dakota) private alleles. Noninformative or monomorphic loci were present in 19 sampling populations, with locus CbSSR1 monomorphic in 14 sampling populations.

Nei's index of gene diversity ranged from 0.05 (NY-Farm 1-2016) to 0.64 (Fargo, North Dakota) for sampling populations and from 0.32 (Hawaii) to 0.5 (Michigan) for regional populations (Table 2). Genotypic diversity, described by Simpson's complement index, ranged from 0.18 (NY-Farm 1-2016) to 1.00 (Germany, Italy, and Turkey) for sampling populations and from 0.65 (Hawaii) to 0.99 (Chile) for regional populations.

Mating-type ratios, linkage equilibrium, and random mating. For the non-clone-corrected data set, mating-type frequencies significantly deviated from a 1:1 ratio expected under random mating for 15 sampling populations (Table 3). After clone-correction, mating-type frequencies were significantly different from a 1:1 ratio for five sampling populations.

Significant linkage of alleles at microsatellite loci was detected in 27 of the non-clone-corrected sampling populations (excluding Turkey), rejecting the null hypothesis of random mating (Table 3). After clone-correction, 21 sampling populations exhibited significant linkage of alleles at microsatellite loci, rejecting the null hypothesis of random mating. The remaining seven sampling populations (Diamond Head community garden in Hawaii; the Table Beet Garden and the Sugar Beet Garden at the Saginaw Valley Research and Extension Center in Michigan; Wahpeton, North Dakota; and Denmark, Sweden, and Turkey) did not show significant linkage of alleles at microsatellite loci, accepting the null hypothesis of random mating in these populations. The population size was less than seven after clone-correction for five of these populations.

The proportion of incompatible pairs of loci for 15 sampling populations was significantly less ($P < 0.05$) than expected in a

randomly mating population (Table 3). The PrCP ranged from 0.35 to 1.00.

Population identity and differentiation. The UPGMA dendrogram, based on Nei's distance, demonstrated two major clusters of sampling populations: one including Canada, Michigan, and all but one sampling population from New York, and the other encompassing Chile, Eurasia, Hawaii, Minnesota, North Dakota, and NY-Farm 2-2015 (Fig. 1A). Assessment of regional populations clustered the New York population separately from all other regions (Fig. 1B). The clustering of the remaining regions indicated minor clusters of populations from NY-Farm 2-2015, Eurasia, North Dakota and Chile, Michigan and Canada, or Hawaii.

AMOVA for the eight regional clone-corrected populations indicated that 23% of the observed genetic diversity was partitioned among the populations and 77% was among individuals within populations ($\Phi'_{PT} = 0.23$; $P = 0.001$). The pairwise indices of differentiation quantified the genetic variation between regional populations (Table 4). Twenty-seven of the 28 regional population pairs had significant differentiation at the $P < 0.05$ level. Regional populations from Michigan and Canada were not significantly differentiated ($P = 0.055$). Significant pairwise Φ'_{PT} values ranged from 0.02 to 0.42, with the greatest differentiation between the New York and NY-Farm 2-2015 regional populations. The New York population consistently exhibited the greatest pairwise Φ'_{PT} values compared with each regional population (0.15 to 0.42).

Population dynamics. Assessment for potential recent population bottlenecks/founder events, or population expansion, detected significant deficiency in gene diversity in the regional New York population ($P < 0.001$) and the NY-Farm 2-2015 population ($P < 0.05$), assuming either the TPM or SMM (Table 5). This supports a recent population expansion following a bottleneck event. The regional Michigan and Chile populations exhibited significant excess ($P < 0.05$) in gene diversity under the IAM, indicating a recent bottleneck. Values for sampling populations are presented in Supplementary Table S2.

Migrate-n supported that migration varied among regional populations and directions (Table 6). All parameters had effective sample sizes $> 1 \times 10^5$ (the minimum value was 287,470). The measure of mutation-scaled effective population sizes reported values ranging from 0.85 (NY-Farm 2-2015) to 2.35 (Hawaii) for the eight regional clone-corrected *C. beticola* populations. The bidirectional mutation-scaled effective immigration rates for the paired *C. beticola* populations ranged from 0.83 (Hawaii to New York) to 8.83 (New York to Michigan). No single regional population was the common source for migration. The results produced posterior distributions in which the 95% credibility intervals included zero. Zero was consistently outside the 95% credibility interval for the immigration rates for which New York was the source. The highest rates of immigration occurred from New York to Canada, Hawaii, North Dakota, and Michigan (5.17 to 8.83), in contrast to the low immigration rates into the New York population (0.83 to 2.70). A high rate of immigration also occurred from Michigan to Canada (7.23).

TABLE 4. Pairwise Φ'_{PT} values (above diagonal) estimating differentiation of eight regional clone-corrected *Cercospora beticola* populations and probability values (below diagonal) estimated after 999 randomizations

Population	Eurasia	Chile	Canada	Hawaii	North Dakota	Michigan	New York	NY-F2-15 ^a
Eurasia	—	0.04	0.08	0.07	0.04	0.14	0.35	0.06
Chile	0.007	—	0.08	0.10	0.02	0.14	0.35	0.11
Canada	0.001	0.001	—	0.11	0.07	0.02	0.25	0.19
Hawaii	0.011	0.004	0.008	—	0.10	0.13	0.34	0.18
North Dakota	0.001	0.021	0.001	0.001	—	0.09	0.27	0.14
Michigan	0.001	0.001	0.055	0.001	0.001	—	0.15	0.24
New York	0.001	0.001	0.001	0.001	0.001	0.001	—	0.42
NY-F2-15	0.001	0.001	0.001	0.001	0.001	0.001	0.001	—

^a Population from New York-Farm 2-2015.

Population structure. A minimum spanning network depicted the relationships of MLGs within and between regional populations (Fig. 2). The regional New York population predominantly clustered separately from MLGs of other regions, which were intermixed.

DAPC using regional populations also supported a separate clustering of the New York population compared with other regional populations (Fig. 3). Regional populations from Eurasia, NY-Farm 2-2015, and Hawaii overlapped, while populations from Canada, Chile, Michigan, and North Dakota overlapped.

DAPC for the clone-corrected data set without a priori population assumptions demonstrated an initial reduction in BIC up to 36 clusters, followed by an increase with an increasing number of clusters (Supplementary Fig. S5). The plateau in BIC suggested that 10 to 18 clusters captured the most salient features of genetic group subdivision; however, the BIC exhibited the greatest decrease from one to two clusters. A *K* value of 2 represented a number of clusters that described the major variation present in the data set (Fig. 4). The isolate membership of cluster 1 (left cluster in Fig. 4, *n* = 224) was predominantly from the regional New York population (83%), whereas cluster 2 (right cluster in Fig. 4, *n* = 217) contained the majority of isolates from other regional populations (Table 7). Outputs for *K* values 3 to 10 are included in Supplementary Figure S6.

TABLE 5. Comparison of the observed gene diversity (H_E) with the expected gene diversity at mutation-drift equilibrium (H_{EQ}) calculated from the observed number of alleles under the infinite allele model (IAM), two-phase model (TPM), and stepwise mutation model (SMM) of eight regional clone-corrected *Cercospora beticola* populations

Population	Deficiency/excess ^a		
	IAM	TPM	SMM
Hawaii ^b	5/7	7/5	7/5
Michigan	2/10* [†]	7/5	7/5
North Dakota	4/8	6/6	7/5
New York	7/5	10/2** [†]	11/1** [†]
NY-F2-15 ^c	6/5	8/3* [†]	8/3* [†]
Canada	3/8	4/7	5/6
Chile	3/9*	6/6	6/6
Eurasia	5/7	6/6	6/6

^a Number of loci exhibiting deficiency/excess of gene diversity. A sign test was used to assess the significance of deviations in gene diversity from mutation-drift equilibrium for each mutation model (**P* < 0.05). A Wilcoxon one-tailed test was performed to assess significance of deficiency/excess under each model. **P* < 0.05 and ***P* < 0.01.

^b Population size of nine, below the recommended minimum population size of 10 for analysis.

^c Population from New York-Farm 2-2015.

The fast maximum-likelihood genetic clustering method implemented in *snappclust* (Fig. 5; Supplementary Fig. S7) and the model-based Bayesian method implemented in STRUCTURE (Fig. 5; Supplementary Fig. S8) both determined that two distinct clusters best described the data. Outputs for *K* values 3 to 10 are included for *snappclust* and STRUCTURE analyses in Supplementary Figure S9.

A UPGMA dendrogram based on Bruvo's distance grouped the clone-corrected *C. beticola* isolates into two major clusters with bootstrap support of 100 (*n* = 5 and *n* = 374, respectively) (*data not shown*). The large cluster divided into two subclusters reflecting the division of genetic groups by the a priori DAPC analysis. In the UPGMA dendrogram, minimum spanning network, and DAPC of regional populations, examples were present of isolates assigned to clusters that were predominantly composed of isolates from a different regional population (Figs. 2 and 3).

DISCUSSION

Assessment of *C. beticola* populations from Eurasia, South America, and North America has indicated the presence of two broad genetic groups: one from New York, and the other composed of isolates from Eurasia, South America, and North America. Evidence is provided for gene flow and migration among and within these groups over large distances. An indication of expansion in the New York population size suggested a recent introduction event into this region from a source of unknown origin. A single sampling population from one farm in New York clustered with Eurasian, South American, and North American populations, and separately from the remaining New York populations, which was suggestive of a separate inoculum introduction event into New York from a distant region.

The origin of the distinct group within *C. beticola* that predominantly consists of New York isolates is unclear. Similar differentiation was reported by Vaghefi et al. (2017a) using a subset of the populations from the current study. Evidence for a *C. beticola* genetic cluster separate to that from Europe was reported by Groenewald et al. (2008), where a population from New Zealand was significantly differentiated from each European and Iranian population. The differentiation of the New Zealand population reflects that reported for the New York population in the current study. Direct comparison of populations from these regions is required to determine whether they are genetically similar and may indicate either a similar founder population for both regions or further subdivision of *C. beticola* clusters.

Evidence for high immigration rates from New York to Canada, Hawaii, North Dakota, and Michigan further suggests the potential for gene flow within North America. Because the highest immigration rate occurred from New York to Michigan and these

TABLE 6. Mutation-scaled effective population size estimates (θ) and immigration rates (*M*) between pairs of eight regional clone-corrected *Cercospora beticola* populations

Source/recipient populations ^a	$\theta^{b,c}$	<i>M</i> ^{c,d}							
		Eurasia	Chile	Canada	Hawaii	North Dakota	Michigan	New York	NY-F2-15 ^e
Eurasia	1.15 (0–3.0)	–	3.10 (0.2–6.1)*	2.77 (0–5.8)	3.23 (0–6.5)	4.43 (1.1–7.9)*	2.50 (0–4.8)	1.70 (0–3.3)	3.17 (0.3–5.7)*
Chile	1.15 (0–2.9)	2.50 (0–4.9)	–	2.50 (0.1–4.7)*	3.17 (0–6.7)	2.30 (0–5.27)	2.10 (0–4.5)	1.57 (0–3.1)	2.57 (0.1–4.9)*
Canada	1.05 (0–2.8)	2.03 (0–4.5)	3.70 (0.3–6.9)*	–	2.50 (0–6.2)	2.23 (0–4.7)	3.03 (0.3–5.7)*	1.57 (0–3.1)	2.50 (0–5.1)
Hawaii	2.35 (0–10.1)	2.17 (0–4.3)	2.70 (0–5.7)	2.23 (0–5.6)	–	2.97 (0.3–5.7)*	1.30 (0–3.1)	0.83 (0–1.8)	2.17 (0–4.2)
North Dakota	1.85 (0–4.0)	5.10 (0–9.7)	2.90 (0–5.5)	4.77 (0.5–9.7)*	2.97 (0–6.5)	–	2.83 (0.2–5.3)*	1.57 (0–3.2)	2.17 (0–4.0)
Michigan	1.15 (0–3.1)	2.97 (0–6.0)	2.10 (0–4.3)	7.23 (1.6–14.0)*	2.83 (0–6.6)	1.97 (0–4.0)	–	2.70 (0.5–4.7)*	1.83 (0–4.1)
New York	1.65 (0–3.6)	3.90 (1.1–6.7)*	4.50 (0.5–8.6)*	6.43 (1.7–11.1)*	6.83 (2.6–13.4)*	5.17 (1.7–8.3)*	8.83 (4.6–15.6)*	–	3.23 (0.2–6.3)*
NY-F2-15	0.85 (0–2.4)	3.23 (0.6–5.8)*	4.30 (1.1–7.5)*	1.97 (0–4.3)	2.30 (0–4.9)	2.10 (0–2.4)	1.97 (0–3.9)	1.30 (0–2.7)	–

^a Populations treated as the source are listed vertically on the left, and receiving populations are horizontally across the top.

^b Median mutation-scaled effective population sizes.

^c Bayesian estimates of the migration parameters calculated using Migrate-n version 3.6.11 (Beerli 2009). The 95% confidence intervals for each parameter are provided by the 2.5 and 97.5% percentiles of the posterior distribution indicated in parentheses. *M* values with 95% confidence intervals that do not include zero are indicated by an asterisk.

^d Median mutation-scaled immigration rates (*M*), calculated as the immigration rate (*m*) divided by the mutation rate (μ) (*M* = *m*/ μ).

^e Population from New York-Farm 2-2015.

regions are closely situated, it can be postulated that the distinct regional population from New York may be spreading into the Michigan region. Similarly, the immigration rate from Michigan to Canada was high, which may be reflective of the close geographic proximity. However, the mechanism for this immigration is unclear, as harvested sugar beets are typically only shipped in the opposite direction. Alternatively, the inoculum source initiating the New York population may also be shared, along with other sources, with the Canada, Hawaii, Michigan, and North Dakota regions. The long-range dispersal of inoculum within North America requires further investigation.

The grouping of isolates in sampling populations from Canada, Chile, Eurasia, Hawaii, Michigan, NY-Farm 2-2015, and North Dakota suggests that there is ongoing gene flow among these regions. The strongest evidence for long-distance gene flow comes from the NY-Farm 2-2015 population, which clustered most closely with the Eurasian population. This New York population has been compared in several studies (Knight et al. 2018; Vaghefi et al. 2017a, c), where it similarly clustered separately from other New York populations, while being most similar to populations from

distant regions, including Europe, North Dakota, and Hawaii. Studies of *C. beticola* populations from Europe and Iran have reported high levels of gene flow across the region (Groenewald et al. 2008), which supports the results of this study where Eurasian populations clustered together. The European and Iranian populations were considered a panmictic population based on their genetic similarity, with human-mediated movement of inoculum suggested as the mechanism enabling population intermixing (Groenewald et al. 2008). While strong evidence for long-distance dispersal has been presented, there is potential that some introductions are short lived and may not survive into subsequent growing seasons (Knight et al. 2018).

The significant deficit of gene diversity in the regional New York population and the NY-Farm 2-2015 population is suggestive of a recent expansion in population size following a bottleneck effect or incorporation of rare alleles from genetically distinct populations (Cornuet and Luikart 1996; Luikart and Cornuet 1998). The indication of high immigration rates from the New York population to other North American populations also supports a recent expansion of this population. The populations from Michigan and

Fig. 2. Minimum spanning network of 378 *Cercospora beticola* multilocus genotypes (MLGs) based on Bruvo's measure of genetic distance from eight regional populations. Each node (circle) represents an MLG and node sizes are proportional to the frequency of the MLGs (inset of node sizes provides a reference for MLG frequency). Node colors represent population membership and are proportional to the pie size. Edge width and color are proportional to the genetic distance between MLGs.

Chile exhibited a significant excess in gene diversity under the IAM, suggesting a recent population bottleneck. The SMM and TPM are the most appropriate models for testing significant gene diversity excesses for microsatellite markers (Cornuet and Luikart 1996; Piry et al. 1999), which may explain the significant gene diversity excess under the IAM and not the SMM model. The BOTTLENECK program detects populations that have experienced a severe reduction in effective population size (N_e) within $0.2 N_e$ to $4.0 N_e$ generations ago (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Piry et al. 1999). Therefore, the bottleneck effects associated with the *C. beticola* populations are likely to be attributable to a recent introduction of inoculum into those regions.

The hypothesis of long-distance movement of *C. beticola* is supported by evidence of recent changes in population sizes and the suggestion of gene flow between geographically distant populations. The similarity of populations in the second major group implies a common origin, with dispersal of *C. beticola* likely occurring during historic movement of host plant materials. The center of origin for the *Beta* genus is considered to be the Middle East, with subsequent spread into the Mediterranean, culminating with sugar beet becoming a major crop in Europe in the early part of the nineteenth century (Cooke and Scott 1993). It is possible that the movement of *C. beticola* followed a similar pathway and, based on genetic similarity, further spread into distant regions, such as North America, on beet material originating from Europe (Groenewald et al. 2008). While gene flow between Eurasia and other regions, such as North America, has been reported (Groenewald et al. 2008; Vaghefi et al. 2017a), the timing of the supposed movement is unclear. In the current study, Eurasia was associated with immigration rates similar to other regional populations; however, the effect of timing of immigration events and population collection, the limited samples from the Eurasian population, and potential for *C. beticola* populations to genetically shift between years (Knight et al. 2018) may have obscured the hypothesized emigration of *C. beticola* from Europe. The effect of trade or exchange of beet or other host materials between regions, either

historically or recently, cannot be excluded as a method for dispersal of *C. beticola*. Further investigation is required to identify these pathways and then implement biosecurity regulations and improved detection strategies to reduce this source of inoculum.

Exchange of host plant materials, including seed, may also have differentially affected *C. beticola* population dynamics, as sugar beet, table beet, and other hosts may have moved along different trade routes (Harveson et al. 2009). Table beet seed production in Washington State accounts for 95% of U.S. production and 50% of global production (du Toit 2007). However, most seed companies have seed production in multiple countries or regions, such as New Zealand, South Africa, and parts of northern Europe (e.g., Denmark) (L. du Toit, *personal communication*). During the production process, table beet seed may move between several regions, resulting in convoluted trade pathways. Most commercial sugar beet seed production for the United States and Canada occurs in Oregon and Washington State (U.S. Department of Agriculture National Agricultural Statistics Service 2018). Exchange of sugar beet seed between production regions is limited, in part as a result of rules employed by the European Union and Organization for Economic Cooperation and Development (2018) seed schemes (Kockelmann and Meyer 2006). Movement of table and sugar beet crops is usually over a limited area, with production located around processing facilities (Pethybridge et al. 2018). Most of the populations evaluated in this study originated from sugar beet, with table beet only represented from Hawaii, Michigan, and New York. There is potential that populations from the two different crops became isolated and genetically diverged in the recent past, resulting in distinct groups such as that from New York. No evidence of adaptation of *C. beticola* for either of these hosts has been reported, and no genetic differentiation was detected between the Michigan populations from sugar beet and table beet in the current study. Populations of *C. beticola* originating from table beet grown in geographically distant regions around the world, and particularly the historical sources of table beet varieties grown in New York, would be ideal for future comparisons.

A role for inoculum introduction from outside the field of interest, particularly the involvement of seedborne inoculum in *C. beticola* dispersal, has been suggested in several studies (Knight et al. 2018; Moretti et al. 2006; Turgay et al. 2010; Vaghefi et al. 2017a, 2017c). In New York, infested seed was hypothesized to be associated with temporal genetic changes in *C. beticola* populations (Knight et al. 2018). However, direct evidence for seedborne inoculum, or infections originating from seed, is limited (Agarwal et al. 2006;

Fig. 3. Discriminant analysis of principal components of eight regional clone-corrected *Cercospora beticola* populations. The frequency distributions of the eigenvalues (insets) of the discriminant analysis (DA) and the principal component analysis (PCA) showed that the first two principal components adequately accounted for the genetic structure of the clusters.

Fig. 4. Discriminant analysis of principal components of a priori *Cercospora beticola* isolates partitioned into two clusters determined by *k*-means clustering. The two clusters consist of clone-corrected isolates from 28 *C. beticola* sampling populations, allowing retention of multilocus genotypes shared across populations. The frequency distributions of the principal component analysis (PCA; inset) showed that the first two principal components adequately accounted for the genetic structure of the clusters.

Byford and Gambogi 1985; McKay and Pool 1918; Vereijssen et al. 2004). A study assessing a range of host seed sources for *C. beticola* infestation is warranted.

Alternative hosts of *C. beticola* represent a further potential means of inoculum dispersal. *C. beticola* has been reported on a range of plant species, including *B. vulgaris* subsp. *vulgaris* (sugar beet, table beet, fodder beet, and Swiss chard) (McKay and Pool 1918; Vestal 1933), *Spinacia oleracea* (spinach) (Vestal 1933), *B. vulgaris* subsp. *maritima* (Jacobsen and Franc 2009), and *Carthamus tinctorius* (safflower) (Lartey et al. 2005), along with species in the genera *Amaranthus*, *Apium*, *Atriplex*, *Chenopodium*, *Chrysanthemum*, *Cycloloma*, *Limonium*, *Malva*, and *Plantago* (Groenewald et al. 2006a; Jacobsen and Franc 2009). Movement of these plant species, either as agricultural produce, ornamental plants (e.g., chard and chrysanthemums), or weeds, has the potential to disperse *C. beticola*. Genetic assessment of *C. beticola* populations collected from table beet and Swiss chard in Hawaii and New York reported no significant differentiation, indicating a potentially shared inoculum source, whereas isolates causing CLS on the weed *Chenopodium album* (common lambsquarters) within a table beet crop in Livingston County, New York, were identified as *C. chenopodii* (Vaghefi et al. 2017c). Further comparisons of *C. beticola* populations originating from various hosts, especially those in close proximity to each other, may inform potential pathways of dispersal and origin of inoculum.

Results from several analyses suggest potential recombination in several of the populations examined. The presence of high levels of genotypic diversity (Simpson's complement index >0.7 for 21 of the 28 sampling populations), along with both mating types in equilibrium (8 and 11 of the 28 sampling populations for non-clone-corrected and clone-corrected data sets, respectively), is an indication of potential sexual recombination (Groenewald et al. 2008; Milgroom 1996). High levels of genotypic diversity and equilibrium of mating-type ratios have frequently been reported for *C. beticola* populations (Groenewald et al. 2008; Knight et al. 2018; Vaghefi et al. 2016, 2017a, 2017c). Alleles at the microsatellite loci of seven sampling populations in the current study were in linkage equilibrium, with the remainder in linkage disequilibrium. Linkage equilibrium of alleles at different microsatellite loci is also suggestive of random mating (Milgroom 1996); however, microsatellite loci of *C. beticola* populations have frequently been found in disequilibrium (Knight et al. 2018; Vaghefi et al. 2017c). No consistent correlation was observed between linkage equilibrium and mating-type ratios of sampling populations to suggest either the

presence or absence of recombination. Although these results are not direct evidence for sexual recombination, the presence of both mating types in all but one sampling population, along with high levels of diversity, supports a possible mixed mode of reproduction for *C. beticola* (Bolton et al. 2012; Groenewald et al. 2006b; Knight et al. 2018; Vaghefi et al. 2016). Sexual recombination has potential implications for CLS disease management strategies, particularly in regard to fungicide resistance genes being combined into diverse genetic backgrounds in *C. beticola* populations (Abawi et al. 2014; Karaoglanidis et al. 2000; Vaghefi et al. 2016; Weiland and Koch 2004; Weiland et al. 2001), the possible emergence of new pathotypes that may affect the durability of any available partial host resistance (Miller et al. 1994; Weiland and Koch 2004), and long-distance dispersal of reproductive structures (Brown and Hovmøller 2002).

The genetic relationships and differentiation reported in the current study provide crucial information for understanding potential founding events of *C. beticola* populations and movement of inoculum in a global context. The identification of two major genetic clusters in globally diverse populations of *C. beticola* demonstrates two prominent epidemiological features: (i) the likely spread of the pathogen from Europe into the Americas via movement of beet and other host materials and (ii) the occurrence of a major divergence event that resulted in the New York genetic group. Investigating the pathways of movement may reveal both the historical spread of *C. beticola* as well as current methods of dispersal. Understanding these pathways will lead to improved

TABLE 7. Frequency of multilocus genotypes in eight regional clone-corrected *Cercospora beticola* populations based on two clusters determined by *k*-means clustering implemented in discriminant analysis of principal components

Population	Cluster 1	Cluster 2
Eurasia	0	38
Chile	2	23
Canada	4	16
Hawaii	0	9
North Dakota	5	43
Michigan	25	35
New York	155	7
NY-F2-15 ^a	1	37
Total	192	208

^a Population from New York-Farm 2-2015.

Fig. 5. Estimated *Cercospora beticola* genetic group structure as inferred by *snaphclust* (top) and *STRUCTURE* (bottom). Individuals from clone-corrected sampling populations are represented by a bar partitioned into *K* color segments (*K* = 2). Numbers designate sampling populations as follows: 1 = Denmark, 2 = England, 3 = Germany, 4 = Italy, 5 = Sweden, 6 = Turkey, 7 = Chile, 8 = Canada, 9 = HI-MCG, 10 = HI-DH, 11 = ND-Fargo, 12 = ND-Wahpeton, 13 = MN-Sabin, 14 = MI-MSU Farm, 15 = MI-Tb SVREC, 16 = MI-Tb Garden, 17 = MI-Sb Garden, 18 = MI-Sb SVREC, 19 = NY-Field 1, 20 = NY-Field 2-2014, 21 = NY-Field 2-2016, 22 = NY-Field 3, 23 = NY-Field 4, 24 = NY-Field 5, 25 = NY-Farm 1-2015, 26 = NY-Farm 1-2016, 27 = NY-Farm 2-2015, and 28 = NY-Farm 2-2016. HI = Hawaii, MCG = Manoa community garden, DH = Diamond Head community garden, ND = North Dakota, MN = Minnesota, MI = Michigan, MSU = Michigan State University Crops Research Farm, Tb = table beet, SVREC = Saginaw Valley Research and Extension Center, Sb = sugar beet, and NY = New York.

management strategies aimed at detecting the pathogen, limiting spread, and reducing the impact of CLS.

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