



Temporal Genetic Dynamics of an Experimental, Biparental Field Population of *Phytophthora capsici*

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Defining the contributions of dispersal, reproductive mode, and mating system to the population structure of a pathogenic organism is essential to estimating its evolutionary potential. After introduction of the devastating plant pathogen, *Phytophthora capsici*, into a grower's field, a lack of aerial spore dispersal restricts migration. Once established, coexistence of both mating types results in formation of overwintering recombinant oospores, engendering persistent pathogen populations. To mimic these conditions, in 2008, we inoculated a field with two *P. capsici* isolates of opposite mating type. We analyzed pathogenic isolates collected in 2009–2013 from this experimental population, using genome-wide single-nucleotide polymorphism markers. By tracking heterozygosity across years, we show that the population underwent a generational shift; transitioning from exclusively F₁ in 2009–2010, to multi-generational in 2011, and ultimately all inbred in 2012–2013. Survival of F₁ oospores, characterized by heterozygosity excess, coupled with a low rate of selfing, delayed declines in heterozygosity due to inbreeding and attainment of equilibrium genotypic frequencies. Large allele and haplotype frequency changes in specific genomic regions accompanied the generational shift, representing putative signatures of selection. Finally, we identified an approximately 1.6 Mb region associated with mating type determination, constituting the first detailed genomic analysis of a mating type region (MTR) in *Phytophthora*. Segregation patterns in the MTR exhibited tropes of sex-linkage, where maintenance of allele frequency differences between isolates of opposite mating type was associated with elevated heterozygosity despite inbreeding. Characterizing the trajectory of this experimental system provides key insights into the processes driving persistent, sexual pathogen populations.

Keywords: *Phytophthora*, population genetics, inbreeding, mating system, self-fertilization, mating type, bottleneck, plant pathogen

INTRODUCTION

Phytophthora capsici is the filamentous, soil-borne oomycete plant pathogen responsible for Phytophthora blight, a disease inflicting significant annual crops losses worldwide (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Granke et al., 2012; Lamour et al., 2012). Success of *P. capsici* is facilitated by its widespread ability to overcome fungicides (Lamour and Hausbeck, 2000), dearth of resistant cultivars (Granke et al., 2012), and large, diverse host range

(comprising >15 plant families), including widely grown, economically important vegetable crops in the *Cucurbitaceae*, *Solanaceae*, and *Fabaceae* plant families (Satour and Butler, 1967; Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). Extreme weather events often initiate new infestations by introducing inoculum into agricultural fields via flood waters (Dunn et al., 2010). Contaminated soil and infected plant material are commonly implicated in pathogen spread (Granke et al., 2012), however, *P. capsici* is not aerially dispersed (Granke et al., 2009).

Once introduced into a field, the explosive asexual cycle of *P. capsici* catalyzes the rapid escalation of disease within a growing season. When exposed to water saturated conditions, a single sporangium can release 20–40 zoospores, each capable of inciting root, crown, or fruit rot, the characteristic symptoms of Phytophthora blight (Hausbeck and Lamour, 2004). For sexual reproduction, the heterothallic *P. capsici* requires two mating types, classically referred to as A1 and A2 (Erwin and Ribeiro, 1996). Exposure to mating type specific hormones ($\alpha 1$ and $\alpha 2$) stimulates production of the gametangia, subsequent outcrossing, and formation of recombinant oospores (Ko, 1988). However, both mating types produce both male and female gametangia, and thus are capable of self-fertilization (Shattock, 1986; Ko, 1988), which is thought to occur at a lower rate relative to outcrossing in *P. capsici* (Uchida and Aragaki, 1980; Dunn et al., 2014).

While the asexual reproductive cycle directly inflicts crop damage, sexual reproduction confers several epidemiological advantages. First, unlike asexual propagules, oospores survive exposure to cold temperatures (Hausbeck and Lamour, 2004; Babadoost and Pavon, 2013). Thus, in regions with cold winter conditions, oospores are the primary source of overwintering inoculum (Bowers, 1990; Lamour and Hausbeck, 2003; Granke et al., 2012). Second, oospores remain in the soil for years regardless of host availability, enabling the persistence of the pathogen between growing seasons and rendering eradication unfeasible. In the spring, in the presence of susceptible hosts, germinating oospores, potentially formed in distinct years, initiate the repeating, asexual reproductive cycle (Hausbeck and Lamour, 2004; Granke et al., 2012).

Where both mating types coexist, sexual reproduction is associated with persistent pathogen populations, genetic diversity, and an approximate 1:1 ratio of A1:A2 mating types (Lamour and Hausbeck, 2001; Dunn et al., 2010). While asexual reproduction can increase the prevalence of a specific genotype within a sexually reproducing population, the inability of asexual propagules to survive cold winters (Hausbeck and Lamour, 2004; Babadoost and Pavon, 2013) implies that each year meiosis disrupts linkage between the particular combination of alleles observed within a clone (Kondrashov, 1988). As a consequence, sexual reproduction mediates the effects of clonal propagation on *P. capsici* population structure (Lamour and Hausbeck, 2001). Furthermore, in geographic regions where sexual reproduction occurs, genetic differentiation between field populations, even within close proximity, suggests that after an initial introduction limited gene flow occurs between fields (Lamour and Hausbeck, 2001; Dunn et al., 2010), consistent with a lack of aerial

dispersal (Granke et al., 2009). Rapid asexual proliferation upon colonization, in addition to formation of long-lived resting spores, likely contribute to population subdivision, consistent with the monopolization hypothesis (De Meester et al., 2002). In this framework, local adaptation by colonizing genotypes would further engender geographic patterns of differentiation (De Meester et al., 2002).

Given this infection scenario, i.e., an initial inoculation but no subsequent introductions, we would expect *P. capsici* populations to exhibit signatures of a bottleneck event: reductions in genetic diversity and an increase in inbreeding over time, proportional to the number of founding isolates (Kirkpatrick and Jarne, 2000). (We define inbreeding strictly as inter-mating between related isolates, and reserve selfing to refer to self-fertilization events.) In populations which undergo a so-called founder effect, inbreeding is expected to decrease mean population fitness over time due to the expression of recessive deleterious alleles, i.e., the genetic load, in the homozygous state (Charlesworth and Charlesworth, 1987; Hartl and Clark, 2007). A related phenomenon, inbreeding depression, i.e., the difference in fitness between selfed and outcrossed progeny in a population (Kirkpatrick and Jarne, 2000), is considered a major driver of obligate outcrossing, and may contribute to maintenance of self-incompatibility in hermaphroditic plant species (Charlesworth and Charlesworth, 1987). Charting the genetic trajectory of isolated populations of *P. capsici* in the context of these processes, is essential to understanding pathogen evolution in an agriculturally relevant scenario.

Thus, in 2008, to investigate the response of *P. capsici* to a severe bottleneck, we established a closed, biparental field population, by inoculating a research field once with two heterozygous strains of opposite mating types. In a preliminary study, we tracked the allele and genotypic frequencies of five microsatellite markers in the field population from 2009–2012 (Dunn et al., 2014). We demonstrated that sexual reproduction resulted in high genotypic diversity, a function of the proportion of unique isolates (Grünwald et al., 2003), in 2009–2011, with a reduction in genotypic diversity in 2012. However, five markers afforded limited statistical power to characterize population and individual level phenomena. While we observed an increase in the inbreeding coefficient (F_{IS}) in 2012 relative to prior years, pairwise F_{ST} values comparing isolates from 2009 through 2012 were not significantly different from zero. Thus, our initial study found minimal evidence for changes in allele frequency due to drift and/or selection.

Therefore, in the present study, we analyzed isolates collected in 2009–2013 from the *P. capsici* field population with genotyping-by-sequencing (GBS), a multiplexed reduced-representation sequencing technique, which simultaneously identifies and scores single nucleotide polymorphism (SNP) markers distributed throughout the genome (Elshire et al., 2011). The closed experimental field design excluded introduction of new alleles via migration, providing a unique opportunity to address the influence of inbreeding on population genetic phenomena in *P. capsici*. In high-density SNP genotyping isolates from the biparental field population, our goal was threefold: (1) Evaluate the effects of oospore survival on population structure;

(2) Quantify the genome-wide incidence of inbreeding with respect to mating type; and (3) Identify whether specific regions deviate from the rest of the genome in terms of changes in allele frequency.

RESULTS

GBS of the Experimental Biparental Isolates

We genotyped a total of 232 isolates collected from a closed, biparental field population of *P. capsici* from 2009–2013, with 35–55 isolates from each year (Supplementary Table S1). Each single-zoospore field isolate was collected from a plant which exhibited symptoms of Phytophthora blight, therefore we inherently selected for pathogenic isolates. Additionally, we genotyped 46 single-oospore progeny from an *in vitro* cross performed in the laboratory between the same founding parents. These *in vitro* isolates served as a reference for the field isolates, for which generation was *a priori* unknown. Three of the *in vitro* progeny were identified as putative selfs by Dunn et al. (2014), which was confirmed by our analysis (see ‘Selfing in the lab and field’), and are hereafter referred to as *in vitro* selfs to distinguish them from the *in vitro* F₁ progeny. The A1 (isolate: 0664-1) and A2 (isolate: 06180-4) founding parents were genotyped 14 and 11 times, respectively, to estimate laboratory and genotyping errors (Supplementary Table S2).

Out of the 401,035 unfiltered variant calls, initial site filters reduced the data set to 23,485 high-quality SNPs (Supplementary Figure S1), with an average SNP call rate (i.e., the percentage of individuals successfully genotyped at each SNP) of 95.93% (median of 97.64%). The 23,485 SNPs were equally distributed among 307 scaffolds (scaffold size and number of SNPs were highly correlated ($r^2 = 0.95$)), with an average SNP density of approximately 1 SNP every 2.5 kb. There was essentially no correlation between mean individual read depth and heterozygosity per SNP among all isolates ($r^2 = 0.009$, P -value = 0.10), indicating that heterozygous calling post-filtering was robust to differences in mean individual sequencing coverage (Supplementary Figure S2). Genotype files are available in VCF format (Supplementary Files S1, S2).

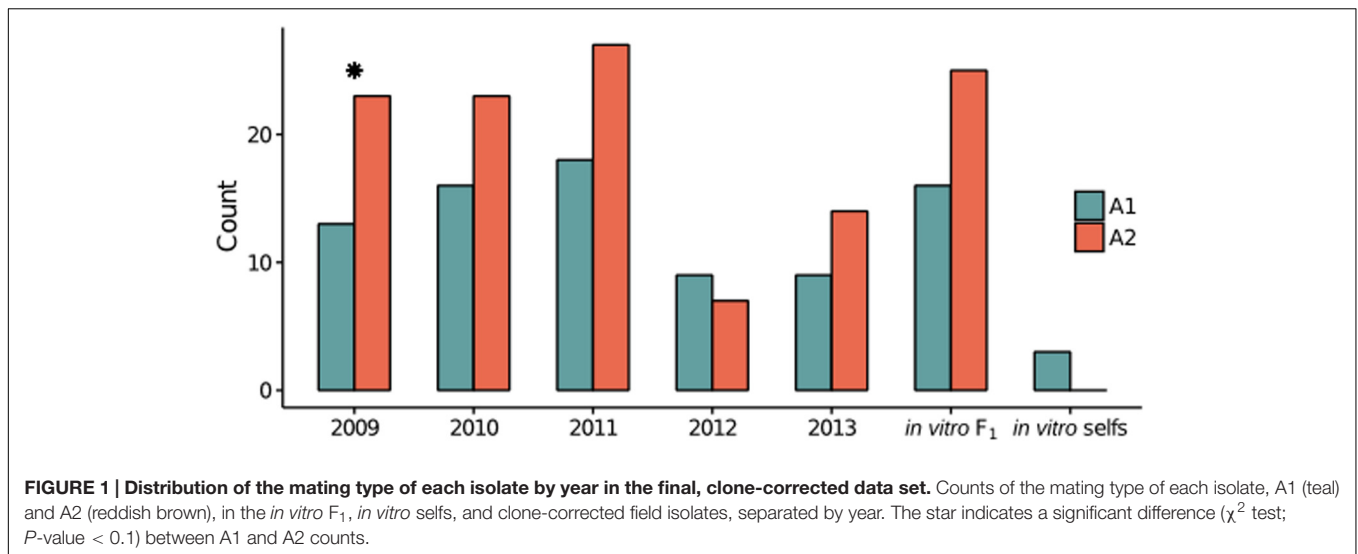
To assess SNP genotyping accuracy, we compared biological and technical replicates of the parental isolates. Replicates of the A1 parent ($n = 14$) and A2 parent ($n = 11$), representing 4–5 distinct serial cultures, shared on average 98.30% ($s = 0.45\%$) and 98.17% ($s = 0.51\%$) alleles identity-by-state (IBS), respectively (Supplementary Table S2). This corresponded to 3.60% ($s = 0.86\%$) discordant sites on average among non-missing genotypes between replicates. Lower average discordance ($\bar{x} = 2.86\%$, $s = 0.33\%$) between only replicates of the same parental culture ($n = 54$ pairwise comparisons) suggested variation associated with distinct culture time points. Therefore, our overall genotyping error rate, inclusive of variation in mycelial and DNA extractions, but not different culture time points, was approximately 3%. Among technical replicates [same DNA sample ($n = 4$) sequenced 3–4 times] the error rate was on average 2.95%, indicating that most of the genotype discrepancies

were attributed to sequencing and genotyping errors rather than distinct mycelial harvests. When we excluded heterozygous calls in each pairwise comparison ($n = 21$) of the technical replicates, less than 0.0001% sites were discordant, indicating that heterozygote genotype discrepancies drove genotyping errors. As in the total data set, the association between individual sequencing coverage and heterozygosity was negligible in both sets of parental replicates (Supplementary Figure S2).

Phytophthora capsici reproduces asexually, therefore, it was theoretically possible to sample the same genotype from the field multiple times within a year. To remove the bias imparted on population genetic analyses by including clones, we retained only one isolate for each identified unique genotype (Milgroom, 1996). Pairwise identity-by-state (IBS) between replicates of the A1 and A2 parental isolates were compared to establish a maximum genetic similarity threshold to define clones (see Materials and Methods), akin to (Rogstad et al., 2002; Meirmans and van Tienderen, 2004). Applying this threshold, we identified 160 unique field isolates out of the initial 232 field isolates (Supplementary Table S1). Two *in vitro* isolates and one field isolate were identified as outliers with respect to deviation from the expected 1:1 ratio of allele depths at heterozygous sites ($n = 2$) or heterozygosity ($n = 1$), and subsequently removed (Supplementary Figure S3). Previous studies have shown that deviation from a 1:1 ratio of allele depths at heterozygous sites, the expectation for diploid individuals, is correlated with ploidy variation (Rosenblum et al., 2013; Yoshida et al., 2013; Li et al., 2015), therefore the two allele depth ratio outliers provide preliminary evidence for ploidy variation in *P. capsici*. After outlier removal, the final data set consisted of 159 field isolates, 41 *in vitro* F₁, and three *in vitro* selfs.

Clones did not appear in multiple years, consistent with the inability of asexual propagules to survive the winter (Hausbeck and Lamour, 2004; Babadoost and Pavon, 2013). After clone-correction, the A2 mating type was more represented in the field (A1:A2 = 65:94; χ^2 test, P -value = 0.02), a phenomenon also observed in the *in vitro* F₁ (A1:A2 = 16:25; χ^2 test, P -value = 0.16; **Figure 1**). The only exception was 2012, which may be explained by a smaller sample size in this year, artificially compounded by loss of several unique isolates (based on microsatellite profiles (Dunn et al., 2014) in culture prior to this study). We observed lower genotypic diversity in 2012–2013 (Supplementary Table S1), consistent with Dunn et al. (2014).

To reduce oversampling of specific genomic regions, which can disproportionately influence population genetic inference (Price et al., 2006; Abdellaoui et al., 2013), without making assumptions about linkage disequilibrium (LD), we randomly selected one SNP within a given, non-overlapping 1 kb window. Final quality filters and including only SNPs in scaffolds containing at least 300 kb ($n = 63$) resulted in a data set of 6,916 SNPs (Supplementary Figure S1). Bimodal heterozygosity and minor allele frequency (MAF) distributions in this reduced SNP set were consistent with distributions in the unpruned data set (Supplementary Figure S4). The pruned data set had a median SNP call rate of 98.01% and median site depth of 18.61 (i.e., average number of reads per individual per SNP).



The median sample call rate (i.e., percentage of SNPs genotyped in each sample) was 97.77%, and the median sample depth (i.e., average number of reads per SNP per individual) was 20.36. Among technical replicates ($n = 4$) the error rate was on average 1.52%. We utilized the pruned data set for all subsequent analyses.

Population Differentiation Increases With Year

To broadly define genetic relationships between the *in vitro* and field isolates relative to the founding parents, we analyzed the field, *in vitro* and parents (represented by consensus parental genotypes, see Materials and Methods) jointly, with principal component analysis (PCA). The PCA exhibited the expected biparental population structure, in that the majority of isolates clustered in between the parental isolates along the major axis of variation, principal component (PC) 1 (**Figure 2A**). Most 2009–2011 isolates clustered with the *in vitro* F₁, whereas, many 2012–2013 isolates were dispersed along both axes, suggesting differentiation associated with year.

To explore structure exclusively within the field population, we performed PCA on only the field isolates. Along PC1, isolates from 2012 to 2013 were differentiated from prior year isolates (**Figure 2B**). Whereas, PC2 described differentiation within and between years.

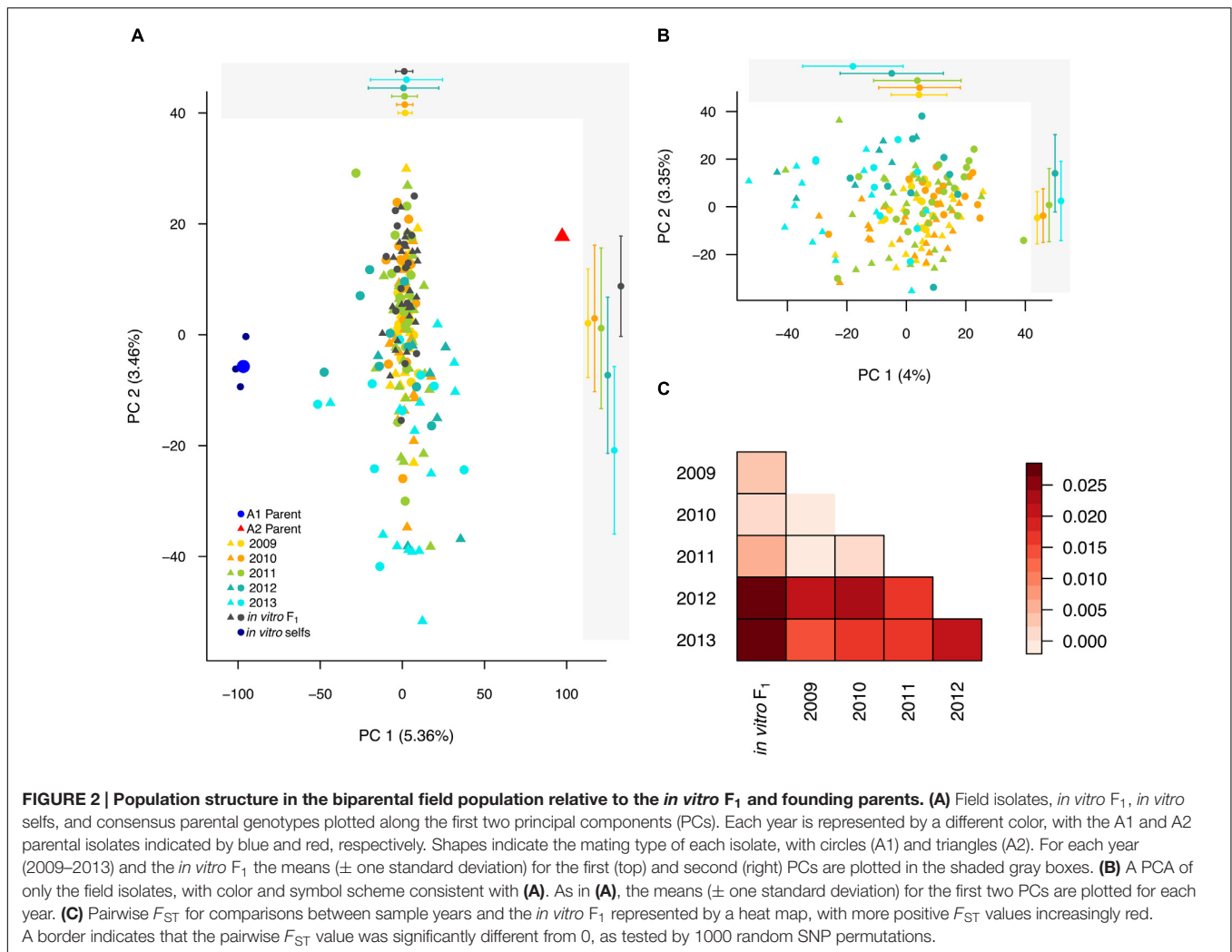
To assess the variance in allele frequencies between years, we estimated pairwise F_{ST} (Weir and Cockerham, 1984) between years, where each year was defined as a distinct population. All pairwise comparisons were significantly greater than zero, excluding pairwise F_{ST} between 2009 and 2010. Small F_{ST} estimates for comparisons between 2009, 2010, 2011 and the *in vitro* F₁ indicated minimal variation in allele frequencies between these years. The greatest differences were observed between years 2012 and 2013 compared to 2009, 2010 and the *in vitro* F₁ populations (**Figure 2C**), consistent with the PCA results. In addition, years 2012 and 2013 were also significantly differentiated from each other ($F_{ST} = 0.027$).

Inbreeding in the Field Population

To quantify changes in inbreeding in the closed, field population, we estimated the individual inbreeding coefficient (F) for each isolate, where F was defined as the proportion of heterozygous sites relative to expected heterozygosity in the population assuming random mating. While F does not directly measure identity-by-descent (IBD), it is highly correlated with IBD estimates in empirical and simulated data sets with relatively large numbers of markers (Kardos et al., 2015), particularly in highly subdivided, small populations (Balloux et al., 2004), such as the population under study. And, in a closed, biparental population, heterozygosity is proportional to the number of generations (Wright, 1921; Nei et al., 1975). Negative F estimates correspond to heterozygote excess relative to Hardy–Weinberg expectations for a reference population, defined here as the *in vitro* F₁. Positive F -values indicate heterozygote deficiency.

First, to establish expectations for a known F₁ population, we assessed the F distribution in the *in vitro* F₁, after removing the three self-fertilized isolates and two additional outliers, as described above. Importantly, because allele frequencies were estimated from the population under study, F is meaningful as a relative measure in the context of this system (Wang, 2014). The *in vitro* F₁, with a mean F of -0.366 , was more heterozygous than the founding parents [average F across replicates = -0.007 (A1) and -0.183 (A2); **Figure 3A**]. In contrast to the unimodal *in vitro* F₁, the field population had a bimodal F distribution, with one peak approximately centered at the *in vitro* F₁ mean, and a second peak centered at a less negative F -value. This second peak, representing a decline in heterozygosity relative to the *in vitro* F₁, indicated that inbreeding was occurring in the field population.

To dissect the bimodal shape of the field distribution, we analyzed F for each year separately. Both for 2009 and 2010, the distributions were unimodal with F means not significantly different from the *in vitro* F₁ mean (pairwise t -test; P -values = 1.0; **Figure 3B**). For years 2012 and 2013, distributions were also unimodal, but had F means significantly less negative



than the *in vitro* F₁ (P -values < 0.0001). Year 2011 had a bimodal F distribution.

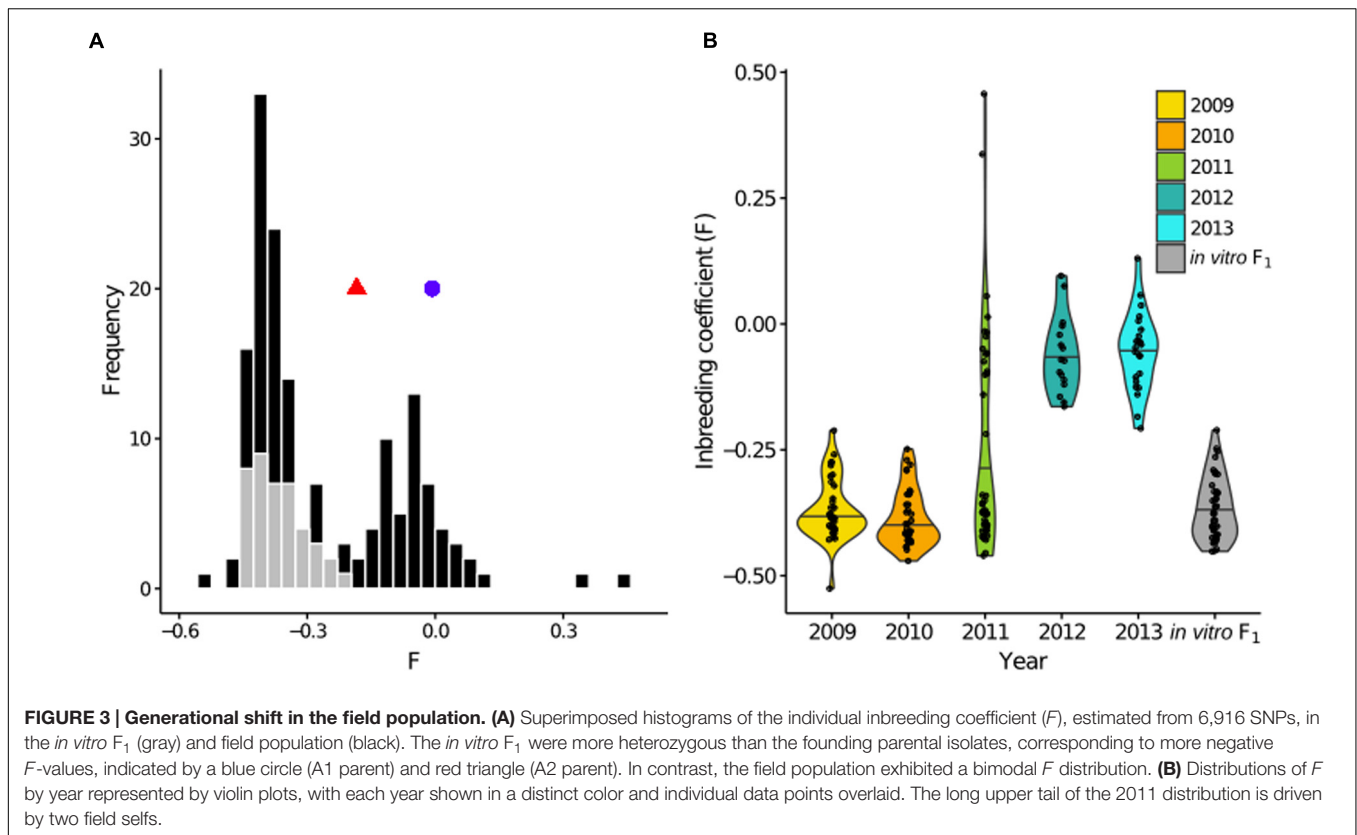
To interpret the effect of changes in inbreeding on genotypic and allele frequencies with time, we analyzed both SNP heterozygosity and MAF distributions for each year. In a biparental cross, clear expectations for these quantities in the F₁ generation makes them informative in distinguishing F₁ from inbred generations. Specifically, in the F₁ generation, sites should segregate with a MAF of either 0.25 (for a cross of $Aa \times AA$) or 0.5 (for $Aa \times Aa$ and $AA \times aa$), and population heterozygosity should be 50 or 100% at each SNP. In the F₂ generation, i.e., a population derived from a single generation of inbreeding, MAF should remain constant, whereas heterozygosity should decline. Our results showed that the *in vitro* F₁, 2009, and 2010 behaved in accordance with expectations for a predicted F₁; the heterozygosity distributions had peaks centered at approximately 50 and 100% (**Figure 4A**), and the MAF distributions had peaks at 0.25 and 0.5 (**Figure 4B**). In contrast, MAF and heterozygosity distributions in 2012 and 2013 were not consistent with F₁ expectations, in that we no longer observed obvious peaks (**Figure 4**). While genotypic frequency shifts in 2012 and

2013 indicated presence of inbreeding and deviation from F₁ expectations, changes in the MAF distribution also denoted that these were likely not canonical F₂ populations. Discrete generations are implicit in a F₂, therefore deviation from F₂ expectations may be attributed to violation of this assumption.

Finally, in 2011, both heterozygosity and MAF distributions were bimodal, as in an F₁, but with reduced heterozygosity and deviation in allele frequencies relative to the *in vitro* F₁ and prior years (**Figure 4**). These shifts in 2011 suggested coexistence of both F₁ and inbred isolates (i.e., non-F₁ isolates) in this year, consistent with the bimodal 2011 F distribution (**Figure 3B**).

Selfing in the Laboratory and Field

In addition to quantifying inbreeding (defined as inter-mating between related isolates), we also estimated the incidence of self-fertilization in the biparental, field population. The frequency at which *P. capsici* reproduces through self-fertilization in either field or lab conditions is unknown (Dunn et al., 2014). Given the limited prior evidence of selfing in *P. capsici*, we first confirmed that the three putative *in vitro* selfs were indeed the product of self-fertilization by the A1 parent, as hypothesized



by Dunn et al. (2014). To this end, we distinguished the *in vitro* selfs from the *in vitro* F_1 by four features: (1) Clustered with the A1 parent in PCA (dark blue circles in **Figure 2A**); (2) Alleles shared IBS disproportionately with the A1 versus A2 parent; (3) Heterozygosity approximately 50% of the A1 parent; and (4) Significantly higher inbreeding coefficients relative to the F_1 [>3 standard deviations (SD) from the mean; **Table 1**].

Having shown that generalized expectations for selfing applied to *P. capsici*, we utilized extreme heterozygote deficiency as an indicator of selfing in the field. As, in the field context, the first three aforementioned selfing features were inapplicable because the progenitor of a selfed isolate in the field was not *a priori* known. We observed that two of the 2011 field isolates were F outliers (>3 SD from the mean) with respect to the inbred field contingent distribution ($\bar{x} = -0.050$, $s = 0.12$). We classified these two A1 field isolates as field selfs (**Table 1**). Lack of disproportionate IBS of the field selfs with either founding parent denoted that these isolates were not the product of self-fertilization by either founding parent. Therefore, we observed selfing in the *in vitro* and field populations at frequencies of 3/46 (6.5%) and 2/159 (1.26%), respectively, denoting minimal incidence of selfing in both lab and field scenarios.

Classifying F_1 versus Inbred Isolates in the Field Using Mendelian Errors (MEs)

Based on the above results, we hypothesized that 2009–2010 were comprised of mainly F_1 , 2012–2013 inbred, and 2011 a mixture

of both F_1 and inbred isolates. However, we had heretofore not verified that each year was homogeneous with respect to F_1 and inbred composition. To quantify the number of F_1 isolates, we used the fact that the genotypes of the founding parents were known to calculate an additional individual summary statistic, the proportion of Mendelian errors (MEs). A ME is defined as a genotype inconsistent with the individual being an F_1 derived from specific parents (Purcell et al., 2007), here, the A1 and A2 founders. Commonly, MEs have been used to detect genotyping and experimental errors in SNP data sets where pedigree information is known (Purcell et al., 2007). The expectation is that a true F_1 individual should have very few MEs, a postulate we applied to assess whether each field isolate belonged to the F_1 generation.

Initial ME estimates revealed both randomly distributed and clustered ME-enriched SNPs (Supplementary Figure S6). In the Supplementary Text, we show that clustered ME-enriched SNPs corresponded to inferred mitotic loss of heterozygosity (LOH) events in the parental isolates in culture (see Supplementary Figures S6–S13 and Tables S3, S4). After removing all ME-enriched SNPs ($n = 848$), mean MEs per isolate for the *in vitro* F_1 and field F_1 subpopulations were 1.38 and 0.98%, below our estimated genotyping error rate of approximately 1.5%.

Akin to F , the proportion MEs per individual is a function of genotypic frequencies. Therefore, it was not surprising that year distributions of the ME statistic were consistent with F , with increased MEs in years 2012–2013 (**Figure 5A**). Because asexual propagules do not survive the winter (Hausbeck and Lamour,

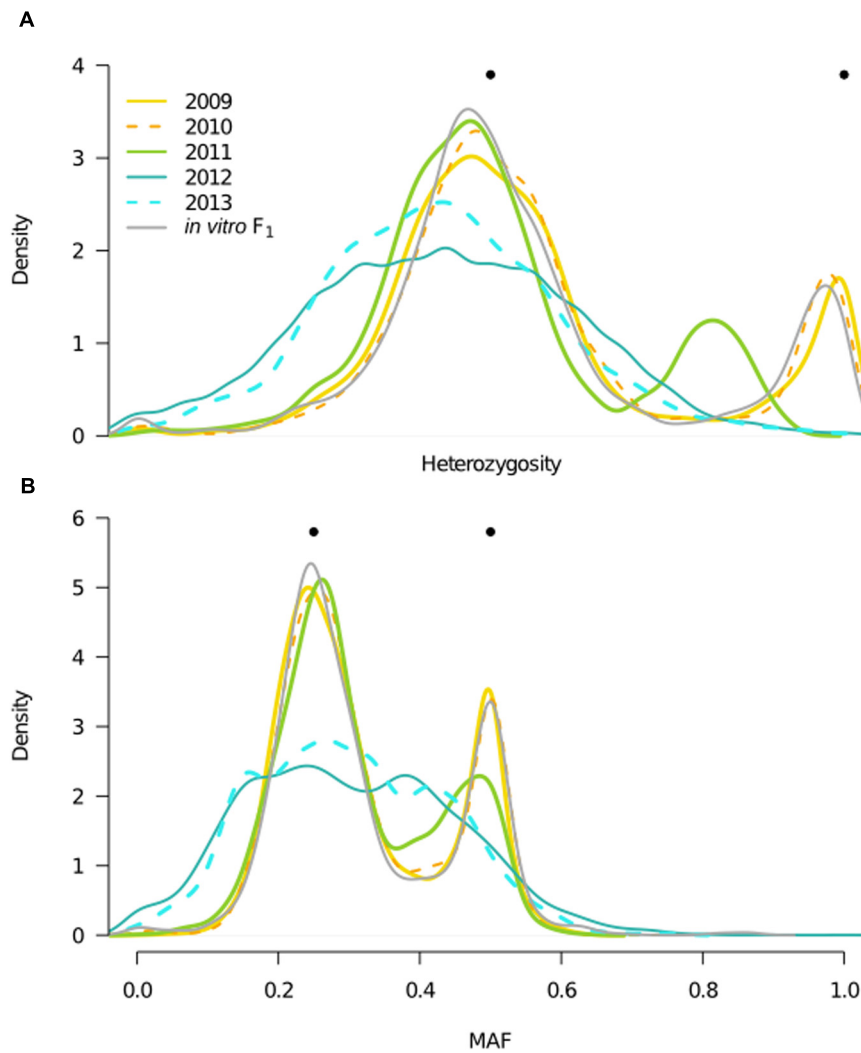
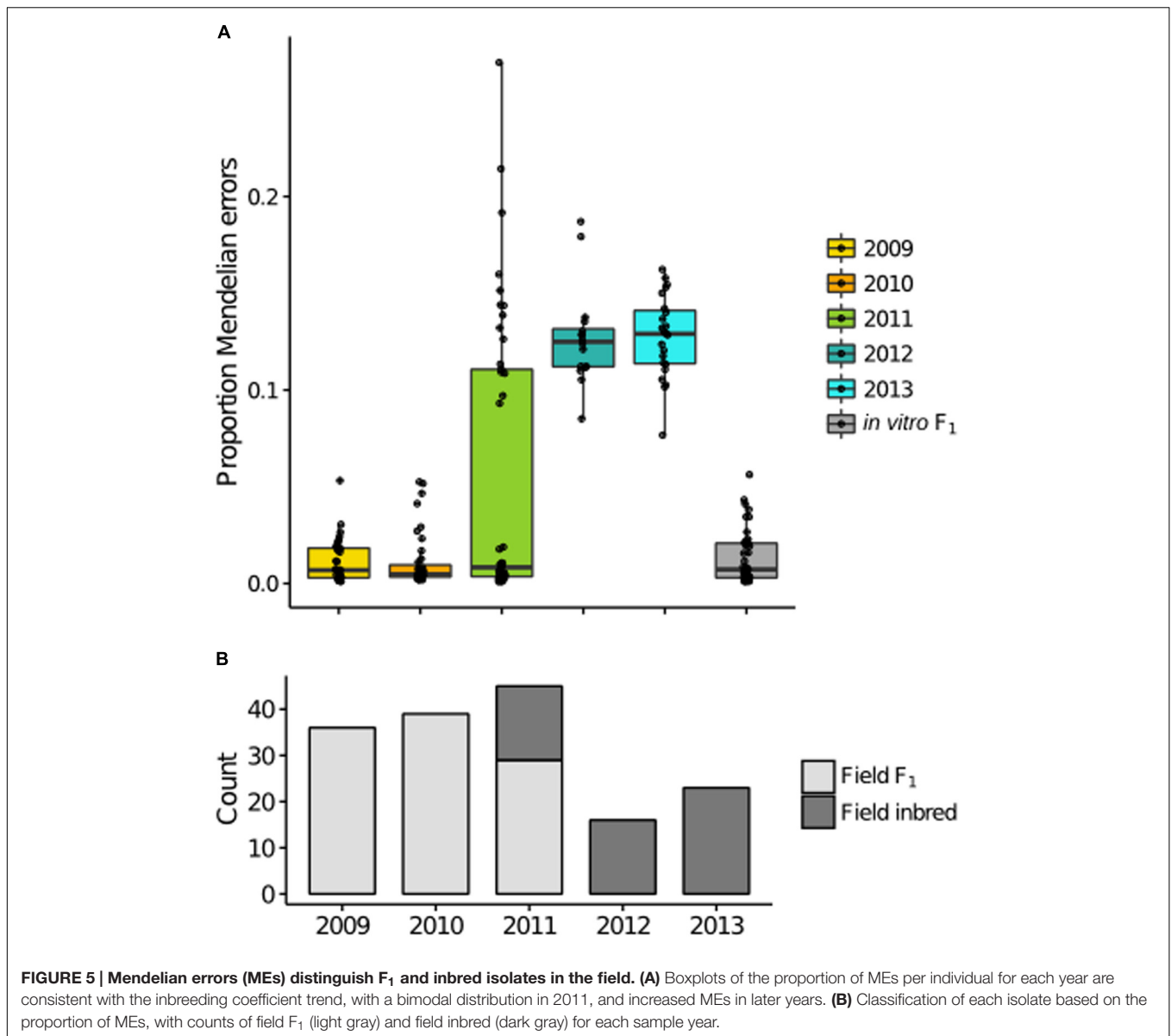


FIGURE 4 | Year heterozygosity and allele frequency (MAF) distributions. Filled, black circles indicate expectations for population heterozygosity and MAF in a theoretical F₁ population. **(A)** Distributions of the proportion of heterozygous individuals per SNP ($n = 6,916$) for each year and the *in vitro* F₁, represented by kernel density estimates, with color corresponding to year, and x-axis consistent with **(B)**. Bimodal distributions in the *in vitro* F₁ and years 2009–2010 are consistent with expectations for the F₁ generation, whereas unimodal distributions in 2012–2013 indicate presence of inbreeding. A shift in the bimodal distribution of 2011, indicates the mixed outbred and inbred composition of this year. **(B)** MAF distributions, where the minor allele is defined based on the frequency in the total field population, for each year and the *in vitro* F₁, with color designations the same as in **(A)**.

TABLE 1 | Selfed isolates in the *in vitro* and biparental field populations in terms of heterozygosity, Mendelian errors (MEs), and alleles shared identity-by-state (IBS) with either founding parent.

Statistic	Consensus parents		<i>In vitro</i> selfs			Putative field selfs	
	A1	A2	68_14	68_19	68_27	11PF_21A	11PF_26A
Individual heterozygosity ^a	0.41	0.48	0.25	0.21	0.22	0.22	0.27
F^b	-0.01	-0.18	0.38	0.48	0.46	0.46	0.34
MEs ^c	0.19	0.19	0.25	0.28	0.27	0.27	0.21
IBS with the A1 parent ^d	1.00	0.47	0.91	0.89	0.90	0.65	0.66
IBS with the A2 parent ^d	0.47	1.00	0.45	0.44	0.44	0.62	0.65

^aProportion of heterozygote, non-missing sites per individual. ^bIndividual inbreeding coefficient (F). ^cProportion of Mendelian errors (MEs) per individual (corrected for outlier SNPs), relative to the consensus parental genotypes. ^dIdentity-by-state (IBS) of each isolate with the A1 or A2 parental consensus genotype.



2004; Babadoost and Pavon, 2013), it can be assumed that all F₁ isolates in the field, in any year, were derived from oospores in the year of the initial field inoculation (2008). Applying a threshold of 5.58% MEs (3 SD from the *in vitro* F₁ mean) to characterize F₁ versus non-F₁, we observed exclusively F₁ in 2009–10, a mixture of F₁ and inbred isolates in 2011 (ratio of F₁ to inbred = 29:16) and all inbred isolates in 2012–2013 (Figure 5B). As such, F₁ dominated in 2009–2011, demonstrating that oospores were viable and pathogenic for at least three years.

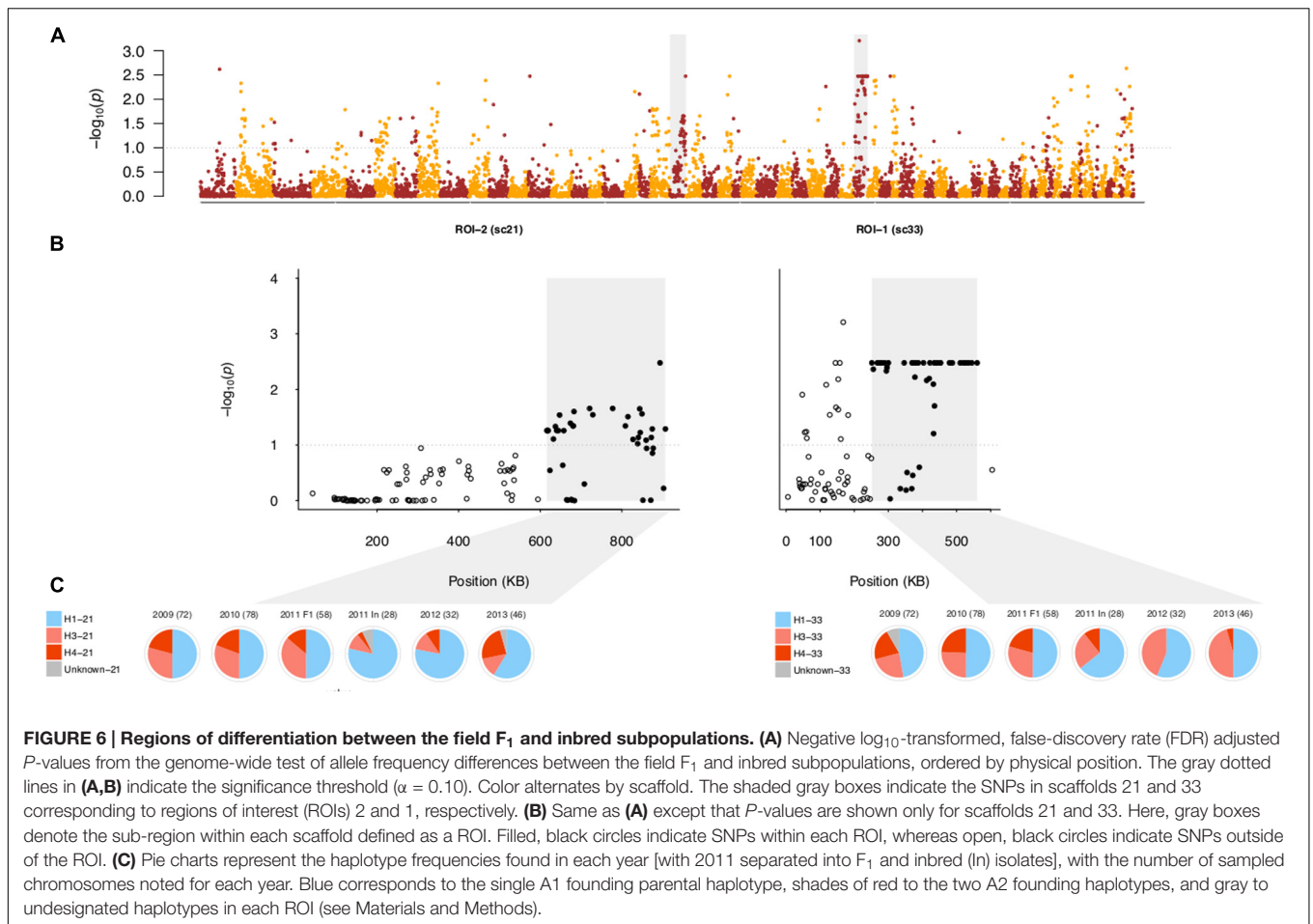
When the inbred isolates were removed from the 2011 data, the MAF distribution for 2011 was consistent with F₁ expectations (Supplementary Figure S5). Concurrent observation of both F₁ and inbred isolates in a single year (2011) provided direct evidence of overlapping generations in the field population, supporting overlapping generations as contributing

to deviation from F₂ expectations in the inbred 2012 and 2013 years.

In addition, the ME estimates allowed us to pool isolates from separate years to define subpopulations, the field F₁ ($n = 104$) and the field inbred ($n = 53$; excluding the field selfs), for subsequent analyses. As in the total field population, A2 isolates were overrepresented in both the field F₁ and inbred subpopulations (A1:A2 = 43:61 and 21:32, respectively).

Regions of Differentiation between Generations in the Field Population

The generational transition in the field population from F₁ to inbred was accompanied by changes in the MAF distribution (Supplementary Figure S5), implying the biased transmission of alleles to generations beyond the F₁. To identify which



SNPs drove this allele frequency shift, we performed a genome-wide Fisher's Exact test of allele frequency differences between the field F₁ and field inbred subpopulations. We collectively analyzed these two subpopulations, rather than compare allele frequencies between years, due to the presence of overlapping generations, which complicate interpretation of temporal dynamics (Jorde and Ryman, 1995). From this analysis, we observed several regions of differentiation between these subpopulations (Figure 6; see Supplementary Table S5 for coordinates).

First, we focused on the region with the most highly differentiated SNP, referred to as region of interest 1 (ROI-1; Figure 6B). Of the 94 SNPs spanned by ROI-1, 44% were among SNPs in the top 2% of loadings for PC1 in the field PCA, showing that this region was correlated with differentiation in the field population. To assess the relationship between allele frequency changes and parental haplotype frequencies, we locally phased all isolates using a deterministic approach (see Materials and Methods). Haplotypes in ROI-1 (H1, H3, and H4) were defined based on the sub-region (251,367–560,094 bp) which contained most of the significantly differentiated SNPs (44 out of 52 SNPs) and formed a LD block (Supplementary Figures S11, S14).

Segregation among the F₁ isolates in each year (2009–2011) followed the F₁ expectation of a 2:1:1 ratio of H1:H3:H4 haplotypes (χ^2 test; *P*-values = 0.91, 0.99, 0.65, respectively). In contrast, in 2011 (inbred isolates only), 2012, and 2013, we observed lower frequencies of H4 and higher frequencies of H3 relative to the field F₁ subpopulation (Figure 6C). The decline in H4 frequency from 22.12% in the field F₁ to 4.72% (and corresponding increases in H3 and H1) in the field inbred drove allele frequency changes in ROI-1 (Supplementary Table S6). Because the H4 sequence was most distinct from the other haplotypes, the reduction in H4 frequency, along with inbreeding, resulted in declines in heterozygosity in ROI-1. Consistent reductions in H4 frequency among inbred isolates in 2011–2013 compared to F₁ isolates in prior years, provided strong evidence for the influence of selection. However, absence of H4 in year 2012 is very likely an artifact of smaller sample size in this year.

We next focused on a region in scaffold 21, defined as ROI-2, with the highest density of significantly differentiated SNPs (67%; Figure 6B). In ROI-2, as in ROI-1, only three haplotypes segregated in the field population (Supplementary Table S7). While not significant (at $\alpha = 0.05$), segregation among the F₁ isolates in each year (2009–2011) deviated from the

F_1 expectation of a 2:1:1 ratio of H1:H3:H4 haplotypes (χ^2 test; P -values = 0.61, 0.35, and 0.05, respectively), primarily attributed to higher H3 versus H4 haplotype frequency in the field F_1 (χ^2 test; P -value < 0.01). A decline in frequency of the A2 parent haplotype, H3, by 19.47% and an increase in the A1 parent haplotype, H1, by 19.81% drove allele frequency changes (Figure 6C and Supplementary Table S7). While the frequency of H3 and H4 oscillated among inbred isolates in 2011–2013, the H1 haplotype frequency was consistently higher than in the field F_1 . In addition, we observed a high frequency of homozygous H1 genotypes (53%), whereas the H3 and H4 haplotypes were not observed in the homozygous state in the field inbred subpopulation, contrary to expectations (Supplementary Table S7).

To posteriorly assess the significance of changes in allele frequency in ROIs 1 and 2, we compared the median F_{ST} value for significantly differentiated SNPs in each of these regions to the genome-wide SNP F_{ST} distribution, where F_{ST} was defined as in Lewontin and Krakauer (1973). Assuming that drift acts equally throughout the genome and constant N_e , extreme deviations in F_{ST} provide evidence for selection (Lewontin and Krakauer, 1973). Median observed changes in allele frequency in ROIs 1 and 2 were in the 97th and 98th percentiles, respectively, relative to genome-wide F_{ST} , showing that allele frequency changes in these regions vastly exceeded the genome-wide average. As violation of these assumptions can lead to false-positive detection of regions under selection (Nei et al., 1975; Bonhomme et al., 2010), we interpret our results cautiously (see Discussion).

Heterozygosity Declines Are Slower in the Mating Type Region

To investigate whether the mating system was a direct driver of differentiation in the field population, we first identified mating type associated SNPs using a Fisher's exact test of allele frequency differences between isolates of opposite mating types in the field

F_1 ($n_{A1} = 43$ and $n_{A2} = 61$; Supplementary Figure S15). Most of the 184 significantly differentiated SNPs were in sub-regions of scaffolds 4 (37%) and 27 (43%), with additional differentiated SNPs in sub-regions of scaffolds 2, 34, and 40 (Figure 7 and Supplementary Table S8). All scaffolds containing significantly associated SNPs were in linkage group 10, consistent with a prior study (Lamour et al., 2012), and supporting presence of a single mating type determining region in *P. capsici*, as posited for *P. infestans* and *P. parasitica* (Fabritius and Judelson, 1997). SNPs in these five sub-regions comprised 20.29% of SNPs with elevated PC loadings (top 2%) in the PCA of only the field isolates, compared to 5.10% genome-wide, denoting that these SNPs were disproportionately correlated with differentiation in the field population.

At 98.30% of the $AA \times Aa$ SNPs associated with mating type in the field F_1 , the A2 parent was heterozygous (Aa) and the A1 parent was homozygous (AA). As such, heterozygosity in the field progeny at these SNPs was attributed to inheritance of the minor allele (a), descendent originally from the A2 parent. Therefore, segregation of the A2 but not A1 parental haplotypes was predominantly associated with mating type in the field F_1 .

We defined the mating type region (MTR) as consisting of genomic tracts encompassed by the minimum and maximum significant SNPs in scaffolds 4 and 27, which comprised 1.42 of the 1.64 Mb spanned by the five sub-regions, and contained 81% of the significantly differentiated SNPs. While we refer to a singular MTR, this was not intended to imply physical linkage between these two scaffolds. Based on the 293 SNPs in the MTR, the PCA of all isolates (*in vitro* and field; $n = 203$) showed incomplete differentiation according to mating type (Supplementary Figure S16).

To assess changes in heterozygosity in the MTR, we compared the heterozygosity distributions of the field F_1 ($n_{A1} = 43$ and $n_{A2} = 61$) and inbred ($n_{A1} = 21$ and $n_{A2} = 32$) isolates in the MTR to the respective genome-wide distributions (see Materials and Methods). Observed heterozygosity in the field F_1 in the

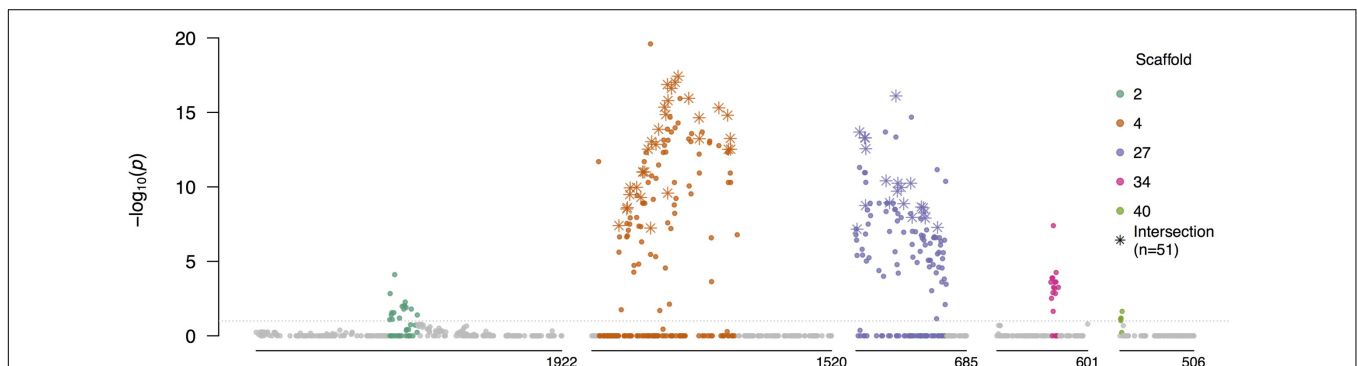


FIGURE 7 | Allele frequency differences between isolates of opposite mating types. Negative \log_{10} -transformed P -values, adjusted for multiple testing, from the Fisher's exact test of allele frequency differences between A1 and A2 isolates in the field F_1 , plotted against physical position, for scaffolds with significantly differentiated regions (see Supplementary Table S8 for coordinates). Colored SNPs were within the bounds of the minimum and maximum significant SNPs in each scaffold containing at least two significantly associated SNPs within 200 kb. Stars indicate the SNPs which were significant in tests of allele frequency differences between mating types in both the field F_1 and inbred subpopulations (Supplementary Text). All SNPs above the gray horizontal line were significant after the FDR correction ($\alpha = 0.1$).

MTR was not centered at a significantly greater mean than the field F_1 genome-wide distribution (one-sided Wilcoxon rank-sum test, all P -values > 0.87 ; Supplementary Figure S17). In contrast, observed heterozygosity in the field inbreds in the MTR was shifted toward a greater mean relative to the field inbred genome-wide distribution (all P -values < 0.005 ; Supplementary Figure S17). Therefore, in the field inbred subpopulation, heterozygosity declines were less appreciable in the MTR compared to the rest of the genome. In addition, we found that heterozygosity in the MTR was significantly higher than the rest of the genome for both the A1 and A2 isolates in the field inbred subpopulation (all P -values $< 10^{-4}$ and 0.003, respectively; Supplementary Figure S17), but not in the field F_1 (all P -values > 0.99 and 0.65, respectively; Supplementary Figure S17). Yet, heterozygosity in the MTR did not significantly exceed HWE expectations for A1 inbred isolates, as observed in the A2 inbred isolates, and both mating types in the field F_1 (Supplementary Figure S18). These results were replicated when the A2 inbred isolates were down-sampled to the A1 inbred sample size (data not shown).

To further dissect the genetic dynamics of mating type in the field population, we tracked the allele frequencies of markers that were heterozygous in one parent and homozygous in the other parent ($AA \times Aa$). These markers are particularly informative because the origin of the a allele can be unambiguously assigned to the heterozygous parent. Specifically, we calculated the frequency of the parental tagging allele (p_a) in the parents, the field F_1 , and the field inbreds at each of the $AA \times Aa$ SNPs in the MTR ($n_{AA \times Aa} = 206$), for each mating type separately.

For A2 tagging SNPs ($n = 98$), i.e., SNPs heterozygous in the A2 founding parent, we observed two classes of markers: those with p_a of approximately 0.5 in the A2 and 0.0 in the A1 field F_1 isolates, and the opposite scenario (**Figures 8A,B**; see Materials and Methods). In the first case ($n = 49$), differences in p_a between mating types were maintained in the field inbred subpopulation (**Figure 8A**). Whereas, in the second case ($n = 49$), the difference in p_a between mating types narrowed (**Figure 8B**). In contrast to the A2 parent tagging SNPs, markers heterozygous in the A1 parent and homozygous in the A2 parent ($n = 108$) predominantly followed a single pattern (**Figure 8C**). These markers were at approximately equal allele frequencies in both mating types in the field F_1 , but slightly diverged in frequency in the field inbred subpopulation. These three distinct segregation patterns were consistent with the association of presence/absence (P/A) of one of the A2 founding haplotypes in association with mating type.

While the structural basis of mating type determination in *P. capsici* is not known, observed segregation patterns in the MTR resemble those of an XY system, where P/A of the Y determines sex. Therefore, as frame of reference, we derived expectations for sex-linked loci in an XY system, i.e., loci conserved between both sex chromosomes (Clark, 1988; Allendorf et al., 1994), assuming that the A2 parent corresponded to the heterogametic sex. Using this model, expectations (blue and pink dotted lines in **Figure 8**) closely matched the observed p_a trajectories in all three cases (**Figures 8A–C**), further supporting the association of one of the A2 haplotypes with mating type determination.

DISCUSSION

To study the temporal genetic dynamics of *P. capsici* in response to a severe bottleneck, we SNP genotyped at high-density 232 isolates collected in years 2009–2013 from a closed, biparental field population founded in 2008, in Geneva, NY (Dunn et al., 2014). This experimental population mimics a potential infection scenario of a natural *P. capsici* epidemic, where a limited number of pathogen strains are thought to found a subsequently isolated population (Lamour et al., 2012; Dunn et al., 2014). Using filtered GBS data, we identified 159 unique field genotypes and obtained 6,916 high quality SNPs with high sequencing depth ($\sim 20X$ coverage), low missing data, and over 97% reproducibility of genotype calls with the implemented analysis pipeline, distributed throughout the genome. With these data, we assessed temporal heterozygosity and allele frequency changes in the biparental population, representing the only controlled, multi-year genomic field study of a plant pathogen to date.

With knowledge of the parental genotypes and assuming simple Mendelian inheritance, we developed a threshold to detect F_1 field isolates based on the incidence of MEs in the *in vitro* F_1 progeny. Our results showed that both field and *in vitro* F_1 progeny were characterized by individual heterozygosity in large excess of Hardy–Weinberg expectations, explained by the fact these isolates were descendent from only two parents. With small numbers of parents, the probability of allele frequency differences between opposite sexes (here, mating types) increases, consequently resulting in deviation from HWE among the progeny (Robertson, 1965; Pudovkin et al., 1996; Luikart and Cornuet, 1999; Balloux, 2004). In addition, discrepancies between the parental and progeny genotypes, i.e., MEs, provided evidence for mitotic LOH occurrence in lab culture. This finding indicates the need to account for mitotic LOH in genetic analyses of *P. capsici*, and denotes the limited utility of the parental genotypes for filtering markers used to characterize the progeny.

Over time, the field population underwent a generational shift, transitioning from F_1 in 2009–2010, to multi-generational in 2011, and ultimately all inbred in 2012–2013. Presence of exclusively F_1 in 2009 suggests that most of the oospores formed in the founding year (2008) were F_1 . As oospores require a dormancy period of approximately one month (Satour and Butler, 1968; Dunn et al., 2014), it is not surprising that there was insufficient time to produce multiple generations in the founding year. The presence of only F_1 and no inbred isolates in 2010, however, cannot be similarly explained. Rather, abundant sexual reproduction in the founding year, coupled with a lower rate in 2009, may have led to disproportionate presence of F_1 oospores (from 2008) surviving in the soil and germinating in 2010. Consequently, there would have been a low probability of sampling an inbred isolate in this year. Year 2011, where both inbred and F_1 isolates were observed in the field, signified a generational shift in the population. The absence of F_1 in the following years (2012–2013) is consistent with previous reports of oospore declines in viability over time (Bowers, 1990), and negligible oospore survival after four years in field conditions (Babadoost and Pavon, 2013). While we did not quantify disease incidence in the field,

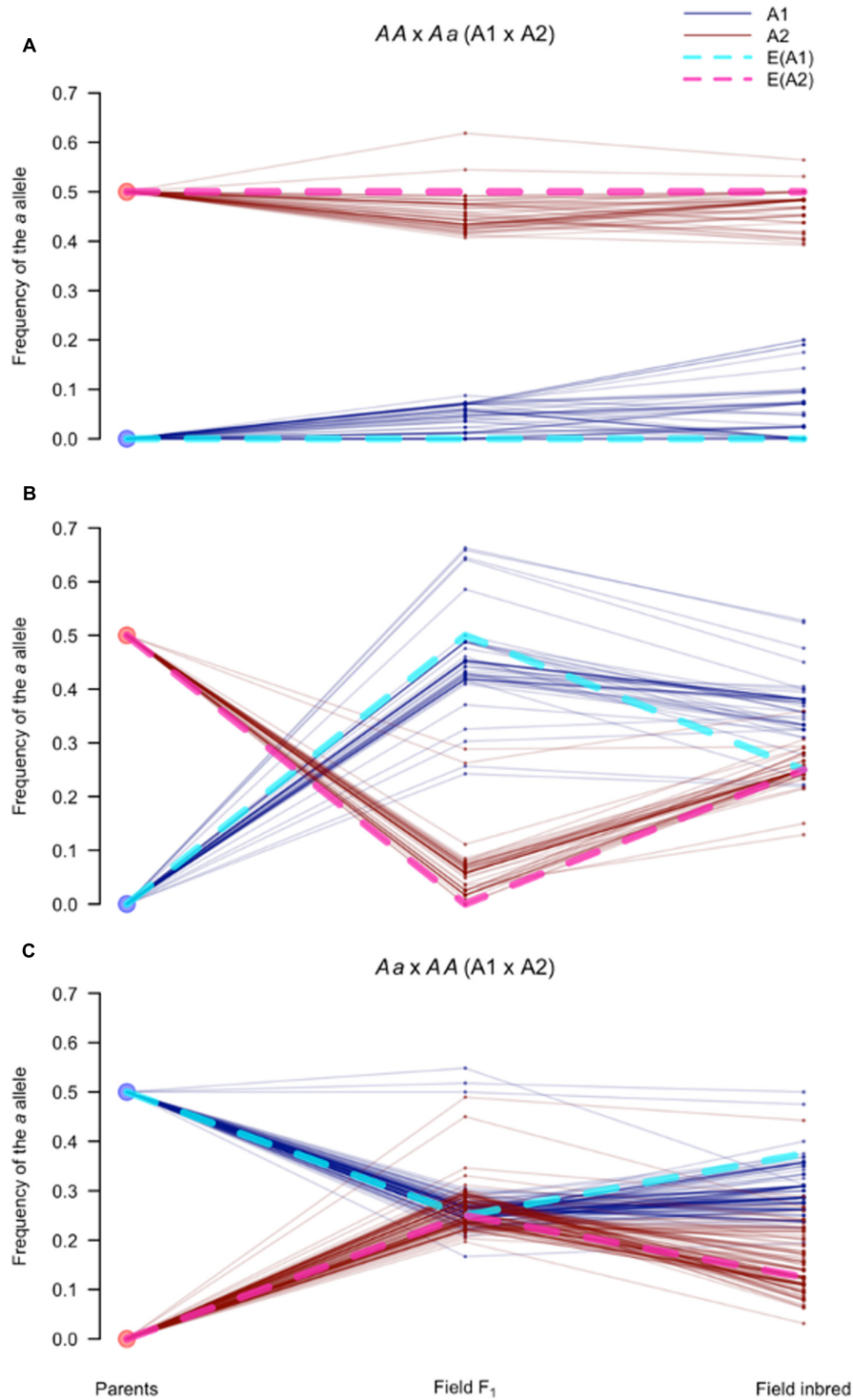


FIGURE 8 | Segregation of SNPs in the mating type region follow expectations for sex-linked loci. Frequency of the *a* allele (p_a), for *AA* × *Aa* and *Aa* × *AA* (*A1* × *A2*) markers in the mating type associated sub-regions of scaffolds 4 and 27, defined as the mating type region (MTR). Each parallel coordinate plot (A–C) tracks p_a at three time points (parents, field F_1 , and field inbred) in the *A1* (blue solid lines) and *A2* (red solid lines) isolates for: (A) *AA* × *Aa* markers ($n = 49$) with $p_a > 0.3$ in the *A2* and $p_a < 0.3$ in the *A1* field F_1 isolates; (B) Remaining *AA* × *Aa* markers ($n = 49$); and (C) All *Aa* × *AA* markers. Expectations for sex-linked loci, indicated by dotted lines, assuming the *A1* and *A2* mating types behave like the homogametic (light blue) and heterogametic (pink) sexes, respectively, when: (A) the *a* allele is in the *A2* determining haplotype (i.e., Y analog); (B) the *a* allele is in the non-*A2* determining haplotype (i.e., X analog); and (C) the *a* allele is in either of the non-*A2* determining haplotypes (i.e., X analogs).

observation of predominantly F_1 isolates in 2010–2011 suggests that highly productive years contributed disproportionately to population structure, in accordance with theoretical predictions for populations in which sexual propagules require a dormancy period, e.g., plant species with seed banks (Templeton and Levin, 1979; Nunney, 2002). As a consequence, heterozygosity did not immediately decline in the second year of the field population, similarly consistent with the delayed attainment of equilibrium genotypic frequencies attributed to seed bank dynamics (Templeton and Levin, 1979).

Approximate equilibrium genotypic frequencies were not observed in the field population until the fourth year (2012). Here, a single large increase in homozygosity in the total population was consistent with cycles of inbreeding beyond a theoretical F_2 resulting in less appreciable declines in heterozygosity relative to the prior generation (Wright, 1921). However, two excessively homozygous field isolates, identified as field selfs, significantly deviated from this trend. Given this low frequency of selfing, we conclude that *P. capsici* behaved essentially as an obligate outcrossing species in the biparental field population. Occurrence of selfing in *P. capsici* is consistent with a previous report of oospore induction when strains of opposite mating types were separated by a membrane (Uchida and Aragaki, 1980), but contradicts previous studies which found no evidence for self-fertilization under *in vitro* conditions (Hurtado-Gonzales and Lamour, 2009; Lamour et al., 2012). As a single generation of self-fertilization reduces heterozygosity by approximately 50% in the progeny, minimal incidence of selfing delayed heterozygosity declines in the field population, as described for hermaphroditic plant species (Balloux, 2004).

In addition, while we observed three A1 parental selfs among the 46 *in vitro* progeny, we did not observe selfs derived from either founding parent in the field, despite the larger field F_1 sample size. Field isolates were inherently selected for both viability and pathogenicity, as well as resilience to environmental factors, whereas, *in vitro* isolates were selected solely on viability in culture. Therefore, this result may reflect a fitness cost to self-fertilization, as observed in essentially all outcrossing species (Charlesworth and Charlesworth, 1987; Falconer and Mackay, 1996), manifest to a greater extent in the field versus laboratory conditions.

Given the potential fitness cost to self-fertilization in *P. capsici*, an increase in inbreeding may explain the allele frequency changes which accompanied the transition from an F_1 to inbred population, as inbreeding presents recessive deleterious alleles in the homozygous state, rendering them subject to selection (Kirkpatrick and Jarne, 2000; Charlesworth, 2003). Simultaneously, inbreeding indirectly influences allele frequencies by decreasing the effective population size (N_e) relative to the census population size, consequently amplifying the effects of genetic drift (Charlesworth, 2009). In addition to inbreeding, many other factors likely decreased N_e , thereby increasing the influence of genetic drift: imbalanced sex (here, mating type) ratios (Charlesworth, 2009); clonal reproduction (Balloux et al., 2003); variation in reproductive success (Hartl and Clark, 2007); small population sizes (Hartl and Clark, 2007) suggested by lower genotypic diversity in 2012–2013;

and overlapping generations (Felsenstein, 1971). Conversely, minimal differentiation between 2009 and 2011 denotes that oospore survival, in behaving like a seed bank, mitigates the aforementioned reductions in N_e by maintaining a reservoir of genetic variation in the soil (Templeton and Levin, 1979; Hairston and De Stasio, 1988; Nunney, 2002; Waples, 2006).

In contrast to the general trends described above, we characterized two regions (ROI-1 and ROI-2) that significantly deviated from the genome-wide distribution of allele frequency differences (median allele frequency change in the 97 percentile or greater) between the field F_1 and inbred subpopulations. In these two regions, which presented only three segregating haplotypes, in contrast to the expected four, for heterozygous parents, we associated allele frequency changes with haplotype frequency shifts. Genetic drift may still explain these results, as drift has a larger effect in regions of low variation, i.e., with higher effective inbreeding coefficients corresponding to lower local N_e (Charlesworth, 2003, 2009). However, extreme changes in allele frequency are also suggestive of natural selection (Lewontin and Krakauer, 1973; Galtier et al., 2000). Here, observation of corresponding haplotype frequency shifts is consistent with hitchhiking or background selection having a large effect in inbred populations (Charlesworth, 2003), particularly those which have undergone only a few generations since founding. Alternatively, mitotic LOH, a phenomenon reported in the present study (see Supplementary Text) and in numerous *Phytophthora* species (Chamnanpant et al., 2001; Grünwald et al., 2012; Lamour et al., 2012; Kasuga et al., 2016), may explain the observation of a disproportionate number of homozygous genotypes among inbred isolates in ROI-2. Evidence for mitotic LOH in numerous species, e.g., *Saccharomyces cerevisiae* (Magwene et al., 2011), *Candida albicans* (Forche et al., 2011), and the chytrid *Batrachochytrium dendrobatidis* (Rosenblum et al., 2013), supports the theoretical expectation that this process facilitates adaptation by interacting with selection to alter allele frequencies (Mandegar and Otto, 2007). Given the limited number of generations, we cannot unequivocally attribute these dramatic haplotype frequency shifts to selection. Furthermore, additional work is required to assess the role of these regions in pathogenicity and local adaptation.

While we observed a genome-wide increase in homozygosity in the field population due to inbreeding, reductions in heterozygosity in the identified mating type associated region were smaller relative to the genome for both A1 and A2 isolates. We show that this result is explained by persistent allele frequency differences between isolates of opposite mating types in the MTR. Maintenance of elevated heterozygosity in sex-linked regions has been attributed to differences in founding allele frequencies between sexes in several systems (Allendorf et al., 1994; Marshall et al., 2004; Waples, 2014). Further, AA × Aa SNPs associated with mating type in the field F_1 were predominantly heterozygous in the A2 parent, implying that one of the A2 founding haplotypes was associated with mating type determination. Consistent with this result, segregation patterns for SNPs in the MTR resembled the behavior of loci in the pseudoautosomal (conserved) regions of heteromorphic sex chromosomes (e.g., XY or ZW; Clark, 1988), where the A2 parent corresponded to the heterogametic,

male sex. These results suggest that in populations of *P. capsici* with few founders, heterozygosity in the MTR will be maintained despite inbreeding, proportional to LD between the mating type factor(s) and the rest of the genome. In addition, we did not detect significant temporal allele frequency changes in the MTR. This result suggests that frequency-dependent selection could play a role in maintaining polymorphism in this region (Fisher, 1930).

These findings, which represent the first detailed genomic analysis of mating type in a *Phytophthora* species, are consistent with the existing models of heterozygosity versus homozygosity at a single locus as determinant of mating type (Sansome, 1980; Fabritius and Judelson, 1997). However, our analysis does not demonstrate that heterozygosity *per se* confers the A2 mating type, nor does our analysis preclude the presence of heteromorphic mating type chromosomes in *P. capsici*. We applied stringent SNP filters to obtain a high-quality set of markers, likely discarding SNPs located in regions of structural variation (i.e., duplications, deletions, repeats). Indeed, early cytological work supports heterozygosity for a reciprocal translocation in association with mating type in *P. capsici* and numerous *Phytophthora* species, posited as a mechanism to suppress local recombination (Sansome, 1976). Given that chromosomal heteromorphism has arisen in diverse taxa as a consequence of suppressed recombination between sex-determining chromosomes (Bachtrog, 2013; Charlesworth, 2013), future studies will investigate the association of structural variation and recombination suppression with mating type determination in *P. capsici*. In addition, improved genomic resources will facilitate identification of the functional basis of mating type.

MATERIALS AND METHODS

Isolate and DNA Collection

In 2008, a restricted access research field at Cornell University's New York Agricultural Experiment Station in Geneva NY, with no prior history of *Phytophthora* blight, was inoculated once with two NY isolates of *P. capsici*, 0664-1 (A1) and 06180-4 (A2), of opposite mating types, as described in Dunn et al. (2014). From 2009 to 2013, the field was planted with susceptible crop species, and each year the pathogen was isolated from infected plant material, and cultured on PARPH medium (Dunn et al., 2014). Once in pure culture, a single zoospore isolate was obtained (Dunn et al., 2014), and species identity was confirmed with PCR using species specific primers, as previously described in Zhang et al. (2008) and Dunn et al. (2010).

Isolates collected in 2009–2012 were obtained from storage; isolates from 2013 were unique to this study and were collected from infected pumpkin plants (variety Howden Biggie). Single oospore progeny ($n = 46$) from an *in vitro* cross between the founding parents were obtained from storage (Dunn et al., 2014). To revive isolates from storage, several plugs from each storage tube were plated on PARPH media. After less than one week, actively growing cultures were transferred to new PARP or PARPH medium.

Mycelia were harvested for DNA extraction as previously described Dunn et al. (2010), except that sterile 10% clarified V8 (CV8) broth (Skidmore et al., 1984) was used instead of sterile potato dextrose broth. For each isolate, mycelia were grown in Petri plates containing CV8 broth for less than one week, vacuum filtered, and 90–110 mg of tissue were placed in 2 ml centrifuge tubes and stored at -80°C until DNA extraction. DNA was extracted using the DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions, except that mycelial tissue was ground using sterile ball bearings and a TissueLyser (Qiagen, Valencia, CA, USA) as previously described (Dunn et al., 2010).

Mating type was determined as previously described in Dunn et al. (2010). Briefly, each isolate was grown on separate unclarified V8 agar with known A1 and A2 isolates, respectively. After at least one week of growth, the plates were assessed microscopically for the presence of oospores. For each trial, the A1 and A2 tester isolates were grown in isolation and on the same plate as negative and positive controls, respectively. We obtained mating type designations for isolates from years 2009–2012 and the *in vitro* F₁ from Dunn et al. (2014).

Genotyping

All DNA samples were submitted to the Institute of Genomic Diversity at Cornell University for 96-plex GBS (Elshire et al., 2011). In brief, each sample was digested with *Ape*KI, followed by adapter ligation, and samples were pooled prior to 100 bp single-end sequencing with Illumina HiSeq 2000/2500 (Elshire et al., 2011). Sequence data for samples analyzed in this study were deposited at NCBI under BioProject PRJNA376558.

To validate experimental procedures, DNA samples from the parental isolates were included with each sequencing plate (except in one instance). The parental isolates were sequenced initially at a higher sequencing depth (12-plex). Genotypes were called for all isolates simultaneously using the TASSEL 3.0.173 pipeline (Glaubitz et al., 2014). This process involves aligning unique reads, trimmed to 64bp, to the reference genome (Lamour et al., 2012) and mitochondrial (courtesy of Martin, F., USDA-ARS) assemblies, and associating sequence reads with the corresponding individual by barcode identification to call SNPs (Glaubitz et al., 2014). The Burrows–Wheeler alignment (v.0.7.8) algorithm *bwa-aln* with default parameters (Li and Durbin, 2009) was used to align sequence tags to the reference genome (Lamour et al., 2012). To reduce downstream SNP artifacts due to poor sequencing alignment, reads with a mapping quality <30 were removed. Default parameters were otherwise used in TASSEL, with two exceptions: (1) Only sequence tags present >10 times were used to call SNPs; and (2) SNPs were output in variant call format (VCF), with up to four alleles retained per locus, using the *tbt2vcf* plugin. Genotypes were assigned and genotype likelihoods were calculated as described in (Hyma et al., 2015).

Individual and SNP Quality Control

Individuals with more than 40% missing data were removed from the analysis. To mitigate heterozygote undercalling due to low sequence coverage, genotypes with read depth <5 were set to missing using a custom python script (available upon request).

Subsequently, we utilized VCFtools version 1.14 (Danecek et al., 2011) to retain SNPs which met the following criteria: (1) Genomic; (2) <20% missing data; (3) Mean read depth ≥ 10 ; (4) Mean read depth < 50; (5) Bi-allelic; and (6) Minor allele frequency (MAF) ≥ 0.05 , where MAF was defined based on the population allele frequency. We removed SNPs with high read depth (> 50) as the likelihood of both heterozygote miscalling and misalignment increases at excessive read depths. Additionally, indels were removed.

To remove isolates with likely ploidy variation, we assessed allele depth ratios for each isolate, where the allele depth ratio was defined as the ratio of the major allele to the total allele depth at a heterozygous locus (Rosenblum et al., 2013; Yoshida et al., 2013; Li et al., 2015). Allele depths were extracted from the VCF file, using a custom python script (available upon request) to analyze the distribution of allele depth ratios for each individual across all SNPs.

Post clone-correction (see below) and outlier removal, SNPs were further filtered as follows. SNPs with heterozygosity rates >90% among all isolates (clone-corrected and parental replicates) were removed and/or average allele depth ratios <0.2 or >0.8. Only SNPs within scaffolds containing more than 300 kb of sequence, covering ~ 48 Mb ($\sim 75\%$ of the sequenced genome), were retained. We defined the minor allele as the least frequent allele in the clone-corrected field population.

Multiple sequencing runs of the parental isolates were used to define consensus genotypes for each parent using the majority rule. Sites where $\geq 50\%$ of calls were missing or where disparate genotype calls were equally frequent were set to missing.

All filtering and analyses, if not otherwise specified, were performed in R version 3.2.3 (R Core Team, 2015) using custom scripts (available upon request).

Identifying a Clone-Correction Threshold

To establish a maximum similarity threshold to define unique genotypes, the genetic similarity of all sequencing runs of the parental isolates were compared. Similarity was defined as IBS, the proportion of alleles shared between two isolates at non-missing SNPs. Parental replicates represented both biological (different mycelial harvests and/or independent cultures) and technical replicates (same DNA sample), thereby capturing variation associated with culture transfers, mycelial harvests, DNA extractions and sequencing runs (Supplementary Table S2). Based on the variation between parental replicates, individuals more than 95% similar to each other were considered clones, and one randomly selected individual from each clonal group was retained in the clone-corrected data set.

Population Structure

Principal component analysis was performed on a scaled and centered genotype matrix in the R package *pcaMethods* (Stacklies et al., 2007), using the *nipalsPCA* method to account for the small amount of missing data (method = 'nipals', center = TRUE, scale = 'uv'). This method was used for all PCAs performed. To estimate pairwise differentiation between years, we used Weir and Cockerham's (1984) F_{ST} measure (Weir and Cockerham, 1984), which weights allele frequency and variance estimates by

population size, implemented in the R package *StAMMP* with the *stampFST* function (Pembleton et al., 2013). We performed 1000 permutations of the SNP set to assess if F_{ST} estimates were significantly greater than zero (Weir and Cockerham, 1984; Pembleton et al., 2013).

Measures of Inbreeding

We used the canonical method-of-moments estimator of the individual inbreeding coefficient, $F = 1 - H_o/H_e$, where H_o is the observed individual heterozygosity, and H_e is the expected heterozygosity given allele frequencies in a reference population assumed to be at HWE (Purcell et al., 2007; Keller et al., 2011). In the absence of drift and segregation distortion, allele frequencies in an F_1 population should be equal to those in the parental generation. Therefore, we utilized allele frequencies in the *in vitro* F_1 to define expected heterozygosity, as the *in vitro* F_1 provided a more robust estimate of allele frequencies compared to the parental genotypes. For each isolate, F was calculated with respect to non-missing genotypes only. To compare average F between years, a pairwise *t*-test was implemented in R with *pairwise.t.test* (pool.sd = FALSE, paired = FALSE, p.adjust.method = 'bonferroni').

Heterozygosity was defined as the number of isolates with a heterozygous genotype at each SNP divided by the total number of non-missing genotype calls. Minor allele frequency (MAF) was defined as the number of minor alleles, where the minor allele was defined as the allele with the lowest frequency in the field population, present at each SNP divided by the total number of non-missing chromosomes (number of non-missing genotype calls multiplied by two). Heterozygosity and MAF distributions for each year and the *in vitro* F_1 were graphically assessed using the *density* function in R.

For each individual, we calculated the proportion of MEs, defined as the ratio of MEs to the total number of non-missing tested sites, analogous to the PLINK implementation (-mendel; Purcell et al., 2007). An ME was defined as a genotype inconsistent with the individual being an F_1 derived from the two founding parental isolates (Purcell et al., 2007). An isolate with a proportion MEs exceeding the *in vitro* F_1 mean by 3 SD was classified as field inbred and otherwise as field F_1 .

Genome Scan for Allele Frequency Differentiation

To detect regions of differentiation between the *in vitro* F_1 , field F_1 , and inbred isolates, we performed a Fisher's exact test of allele counts at each SNP for all pairwise comparisons between subpopulations, using the *fisher.test* function in R. *P*-values were adjusted for multiple testing using the Benjamini and Hochberg (1995) procedure, implemented with the *p.adjust* function, at a false discovery rate (FDR) of 10% (Wright, 1992; Benjamini and Hochberg, 1995). Significant SNPs were retained in further analyses only if another SNP within 200 kb also surpassed the significance threshold.

We compared the F_{ST} distribution of significantly differentiated SNPs within regions of interest (ROIs) to the genome-wide F_{ST} distribution according to Lewontin and

Krakauer (1973). Here, F_{ST} was defined as, $F_{ST} = \frac{(\Delta p)^2}{p_0(1-p_0)}$, where p_0 is the frequency of the minor allele in the field F_1 , and Δp is the difference in allele frequency between the field F_1 and field inbred subpopulations.

Haploview (Barrett et al., 2005) was used to estimate pairwise LD (r^2) between SNPs in scaffolds containing ROIs.

Haplotyping

As the population was established by two parental strains, assuming no mutation, all isolates were by definition combinations of the founding parental haplotypes. Therefore, we took a deterministic approach to phasing, akin to utilizing trio information to phase parental genotypes (Browning and Browning, 2011). Haplotyping in ROIs was further facilitated by the fact that either or both parents were homozygous, with the homozygous genotype assumed to represent a founding parental haplotype. We showed that this was a valid assumption by analyzing early replicates of the parental genotypes that exhibited the “ancestral” heterozygous genotype in a specific region (Supplementary Figure S13) and by comparison to homozygous genotypes of selfed isolates (data not shown). We used the homozygous parental stretches (haplotypes) to deduce the other haplotypes from consensus genotypes for the expected genotypic classes. Progeny membership in a genotypic class was defined by k -means clustering using the *kmeans* function in R (centers = 8, n.iter = 1000, nstart = 100). To further refine clusters and remove recombinant isolates, we calculated local pairwise relatedness, defined as IBS, between isolates within a cluster, and removed isolates that shared on average less than 90% IBS with the respective cluster members. Next, we defined the consensus genotype based on the refined clusters utilizing the majority rule (see “Identifying a Clone-Correction Threshold”), and heterozygous genotypes within haplotypes were set to missing.

To determine the haplotype composition of each isolate, the three identified haplotypes in a region of interest were used to construct reference genotypes for all possible haplotype combinations (e.g., H1/H2, H1/H1). Then, the genotypic discordance (i.e., the number of mismatched genotypes) between each isolate genotype and reference genotype were calculated. The most similar reference genotype was assigned if genotypic discordance was less than 25%. Otherwise, the isolate genotype was deemed “Unknown.”

To create phase diagrams, haplotype tagging SNPs (SNPs which unambiguously distinguished a specific haplotype) were identified at SNPs where all haplotypes had no missing data. Individual genotypes were then classified for homozygosity or heterozygosity at each haplotype tagging SNP.

Identifying Mating Type Associated SNPs

We performed a Fisher’s exact test of allele frequency differences between isolates of opposite mating types in the field F_1 . Multiple test correction was performed as above (see ‘Genome Scan for Allele Frequency Differentiation’).

Heterozygosity in the Mating Type Region (MTR)

To test differences between the heterozygote frequency distribution in the MTR relative to the rest of the genome, we compared the heterozygosity of genome-wide SNPs sampled in equal proportions of marker types (e.g., $AA \times Aa$) to the MTR using the *sample* function in R without replacement (replace = FALSE), excluding SNPs not polymorphic or with missing data in the parental isolates. The identified ME-enriched SNPs were excluded (see Supplementary Text). To account for an unequal ratio of A1:A2 mating type isolates, in each test, the A2s were down-sampled (without replacement) to equate with the A1 sample size in the respective subpopulation. We used the *wilcox.test* function in R to perform a one-sided, unpaired Wilcoxon rank sum test (alternative = ‘less’, paired = FALSE), repeated for 100 random SNP samples. Additionally, heterozygosity distributions of the A1 and A2 isolates in each subpopulation were compared to the respective genome-wide distribution, with SNP but not isolate down-sampling.

Heterozygote excess was tested at each locus in the A1 and A2 isolates for each subpopulation, using the function *HWEexact* in the R package HardyWeinberg (Wigginton et al., 2005; Graffelman, 2015). This amounts to a one-sided test of HWE where heterozygote excess is the only evidence of deviation from HWE. We controlled for multiple testing as above.

Allele Frequency Changes in the MTR

In the MTR, the frequency of the parental tagging allele (p_a) at SNPs heterozygous in one parent and homozygous in the other, was calculated for A1 and A2 isolates separately in the parental generation, the field F_1 and the field inbreds, excluding missing genotypes. The A2 tagging SNPs were separated into two categories based on p_a with respect to mating type in the field F_1 . The first case consisted of SNPs with $p_a \geq 0.3$ in the A2 isolates and $p_a \leq 0.3$ in the A1 isolates, and the second case consisted of the remaining SNPs. Expectations for p_a in theoretical F_1 and F_2 populations, for the three cases where the a alleles is in the haplotype background of the: (1) Y in the male sex; (2) X in the male sex; and (3) X in the female sex, were derived based on the formulas in Clark (1988) and Allendorf et al. (1994).

AUTHOR CONTRIBUTIONS

CS and MC conceived the experimental design; MC, CS, EG, and MG conceptualized the analysis; MC performed the experiments and analyses; MC and EG wrote the manuscript; CS and MG revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fgene.2017.00026/full#supplementary-material>

FILE S1 | VCF file containing all sequenced clone-corrected isolates

(**n = 23,485 SNPs**). This file includes isolates that were subsequently removed as outliers (short ids: 13PF_29A, 68_28, 68_40). The folder also contains a file with the mating type designation for each isolate.

FILE S2 | VCF file containing all sequenced non-clone-corrected isolates

(**n = 23,485 SNPs**). This file includes isolates that were subsequently removed as outliers (short ids: 13PF_29A, 68_28, 68_40). The folder also contains a file with the mating type designation for each isolate.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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