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Evidence for inbreeding and apomixis in close crosses of *Phytophthora capsici*

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A series of inbreeding crosses, recurrent backcrosses and successive sibling crosses were completed up to the sixth generation in the plant pathogen *Phytophthora capsici*, generating a total of 692 oospore-derived isolates. All of the crosses stemmed from an initial mating between two wild-type *P. capsici* isolates. The heterozygosity level, as measured through the inheritance of 20 amplified fragment length polymorphism (AFLP) markers, decreased incrementally with continued inbreeding and was reduced by ~60–75% by the second consecutive sibling cross. Of the eight crosses analysed, all but one cross produced a proportion of oospore-derived progeny that were identical to one or other parent, indicating that apomixis can play a role in *P. capsici* intraspecific crosses. There was no evidence of isolates emerging through selfing or generation of homothallic isolates. Overall, these results indicate that back and sibling crosses are possible and that the large reservoirs of naturally occurring genetic variation in *P. capsici* may be useful for developing inbred lines to characterize complex genetic traits in *Phytophthora*.

Keywords: complex genetic traits, oomycete, single nucleotide polymorphism, single oospore progeny

Introduction

The genus *Phytophthora* includes over 90 species and infects more than 1000 plant species worldwide (Erwin & Ribeiro, 1996; Kroon *et al*., 2004). Draft genome sequences of two oomycetes are now available (*P. sojae* and *P. ramorum*) (Tyler *et al*., 2006) and the genomes for the oomycetes *Hyaloperonospora parasitica*, *P. capsici* and *P. infestans* are currently being sequenced. Functional genetic analyses in *Phytophthora* are conducted by means of transformation, heterologous expression systems, gene silencing, and gene disruption via mutagenesis (Kamoun *et al*., 1998; van West *et al*., 1999; Vijn & Govers, 2003; Whisson *et al*., 2005; Lamour *et al*., 2006; Vleeshouwers *et al*., 2006). In general, these approaches focus on the effects of single genes in a few 'wild-type' background genotypes. However, there are few studies in which phenotypes have been investigated as complex genetic traits ruled by more than one locus (quantitative genetics). The majority of key developmental processes, such sporangiogenesis, zoosporogenesis, oosporogenesis and host colonization (pre- and post-invasion), are complex traits and these processes are most likely regulated by the orchestrated expression of multiple genes (Tani *et al*., 2004; Prakob & Judelson, 2007).

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In other organisms such as mice, fruit flies, roundworms, *Arabidopsis* and crops such as tomatoes, maize and beans, elucidating complex traits is greatly facilitated by the availability of mapping populations, recombinant inbred lines (RILs) and near isogenic lines (NILs) (Nuzhdin *et al*., 1997; van Swinderen *et al*., 1997; Johnson & Gepts, 1999; Saliba-Colombani *et al*., 2000; Limami *et al*., 2002; Loudet *et al*., 2002; Peirce *et al*., 2004). Researchers working with economically important hosts of *P. capsici* such as *Capsicum annuum*, *Cucurbita moschata* and *Solanum lycopersicum* have developed recombinant inbred lines to study genetic resistance against *P. capsici* (Bosland, 2007; Kabelka, 2007; Prince, 2007). A few of these economically important hosts are also being sequenced, thus increasing the options for using whole-genome techniques to investigate the plant–pathogen interaction. The development of similar inbred line resources for members of the genus *Phytophthora* could be useful in understanding key developmental stages and may aid in developing novel control strategies for these damaging pathogens. There are a number of factors that have slowed the development of genetic resources that rely on extensive crossing, including low oospore germination rates for some species (e.g. *P. infestans*), aberrant segregation ratios, difficulties in separating the sexual oospores from the surrounding parental asexual spores and mycelium, and a limited number of well-characterized nuclear markers (Judelson, 1996; Kamoun, 2003).

A primary goal in the research programme of which this study is part is the development of genetic and genomic resources for the vegetable pathogen *P. capsici* (Lamour *et al*., 2007). *Phytophthora capsici* is unique among other heterothallic *Phytophthora* spp. because it regularly completes the sexual stage in natural populations in the USA (Lamour & Hausbeck, 2002). Populations are composed of genotypically diverse individuals of both mating types (Lamour & Hausbeck, 2000). The sexual stage appears to be under strong selection pressure in the USA because *P. capsici* does not produce thick-walled asexual chlamydospores and the primary hosts (cucurbits, tomatoes and peppers) are unavailable during the winter months. Consequently, USA populations carry a large amount of genetic variation and laboratory crosses are in many cases highly fecund, with a high percentage of viable oospores (Satour & Butler, 1968; Lamour & Hausbeck, 2000; Lamour & Hausbeck, 2002). In addition, *P. capsici* has a wide host range, including members of the Solanaceae, Cucurbitaceae and Fabaceae (Erwin & Ribeiro, 1996; Hausbeck & Lamour, 2004). The ability to easily complete crosses provides a unique opportunity for genetic investigation in the genus *Phytophthora*. Although high levels of genetic diversity are useful for mapping and population genetics, a high level of polymorphism can be a serious impediment to developing whole-genome resources. The assembly of highly heterozygous organisms like *P. capsici* presents serious challenges compared with inbred or single haplotype organisms (Vinson *et al*., 2005). The problems arise because it is difficult to determine if sequences derived from the two haplotypes are derived from the same location in the genome. Often this results in many additional contigs which are difficult to incorporate into a final consensus contiguous sequence.

The objectives of this study were twofold. The first objective was to generate a moderately inbred isolate to be used for the development of a genome sequence. The goal was to develop a representative isolate with fewer heterozygous sites than typical wild-type isolates, and this was accomplished via recurrent backcrossing. Backcrossing was employed instead of sib-crossing because initial attempts with sib-crossing failed. The second objective was to investigate inbreeding within *P. capsici* and to determine if the segregating populations may be useful for studying complex traits. Genetic data are presented from segregating AFLP and single-nucleotide polymorphism (SNP) markers as well as mating type for 692 oospore-derived isolates from eight inbreeding crosses. The implications and difficulties for the development of inbred *P. capsici* genetic resources are discussed.

Materials and methods

Isolates and crossing scheme

All of the crosses stem from an initial cross of *P. capsici* between two field isolates: LT51 (mating type A1), isolated from a cucumber fruit in Michigan in 1997, and LT263 (mating type A2), isolated from a pumpkin fruit in Tennessee during 2004 (cross named LT51 × LT263). Both isolates were fully pathogenic on cucumber and jalapeno fruits. The crossing scheme is outlined in Fig. 1. To generate oospores the parental isolates were plated approximately 2 cm apart on UCV8 juice agar amended with PARP (100 mg pimaricin, 100 mg ampicillin, 30 mg rifampicin and 100 mg pentachloronitrobenzene L^{-1}). Plates were wrapped with parafilm and kept in the dark at room temperature for at least 2 months before the oospores were separated from the parental material and stimulated to germinate (described below). The first backcross (BC_1F_1) was between isolates LT1021 (A1, F_1 progeny) and LT263 (A2) (cross LT1021 × LT263). A second recurrent backcross (BC_2F_1) was then set up between isolates LT1422 (BC_1F_1 derived progeny, A1) and LT263 (A2) (LT1422 × LT263). A first full-sibling cross (SC_1F_1) was then initiated with offspring from cross LT1422 × LT263 using isolates LT1503 (A1) and LT1530 (A2) (LT1503 × LT1530). Two different full-sibling crosses ($SC_{2\text{-}1}F_1$ and $SC_{2\text{-}2}F_1$) were established using progeny isolates from cross LT1503 × LT1530. The second sibling crosses, LT2209 × LT2222 and LT2211 × LT2222, were germinated 2, 3 and 5 months following mating. Recombinant progeny derived from cross LT2209 × LT2222 were used for generating a third sib-cross: LT3382 × LT3394. A diagram of the crosses is presented in Fig. 1.

Oospore germination and mating type

Mycelium was scraped from the surface area between the two parental inoculum plugs using a sterile spatula and the material transferred into 20 mL sterile distilled water in a 50-mL Falcon tube. The mycelium was then thoroughly disrupted by blending with a Tissue Tearor homogenizer (Fisher Scientific Inc.) for at least 1 min at 30 000 r.p.m. The wand was continuously moved up and down until no visible chunks remained. The homogenized solution was then passively filtered through a single layer of sterile Kimwipe (Kimberly Clark) into a clean 50-mL Falcon tube. The volume was adjusted to 18 mL with sterile water and amended with filter-sterilized crude lysing enzyme from *Trichoderma harziarum* (Sigma) to a final concentration of 1 mg mL^{-1} and a final volume of 20 mL. The 50-mL tubes were incubated overnight (16–20 h) at room temperature under laboratory lighting with gentle agitation using a Minilab Roller shaker (Labnet Int.) at 25 r.p.m. Following the overnight incubation, an aliquot of the oospore preparation was transferred to 60-mm diam. Petri dishes and observed under a light microscope for residual viable mycelium, sporangia or zoospore contamination. Oospores were quantified (number per μL) using a haemacytometer and the oospore preparation was diluted with amended filtered V8 broth to a final concentration of 1 oospore per 50 μL and dispensed into 384-well plates using an Apricot 96-channel pipette model PP-550DS (Apricot Designs, Inc.) to a volume of 50 μL per well. Plates were incubated at room temperature for 3–7 days and colonies from single-oospore

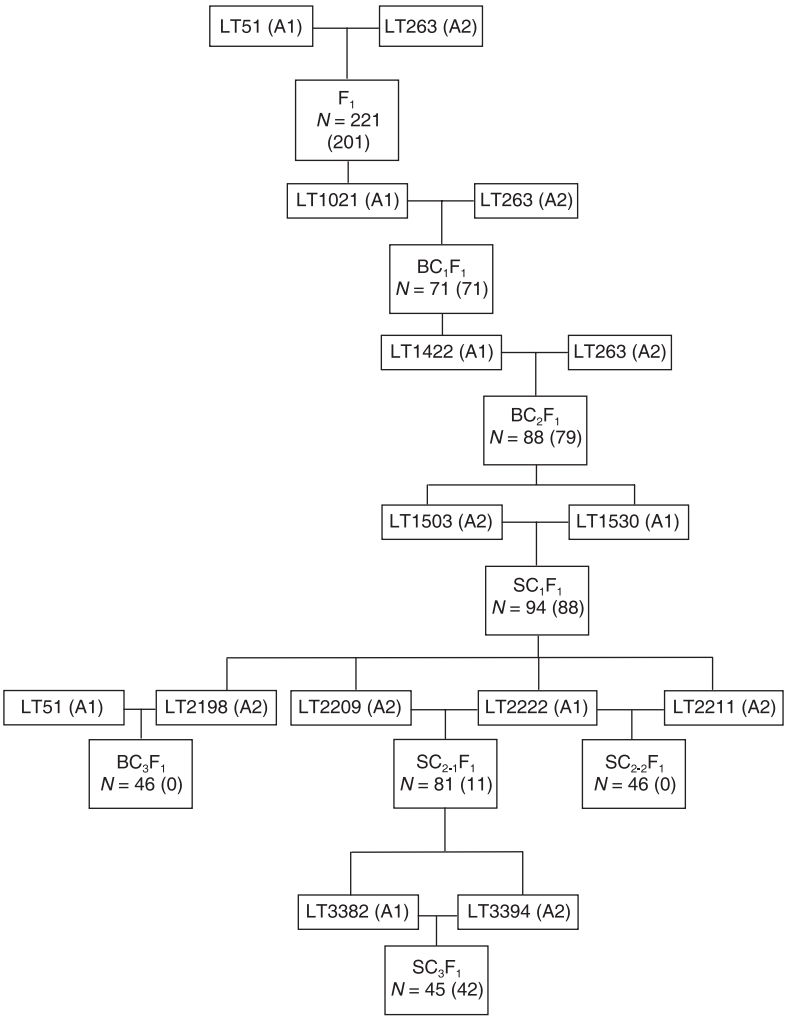

Figure 1 Overview of *Phytophthora capsici* crosses. *N* = total number of oospore-derived isolates, with number of genetically recombined progeny in parentheses. Isolate identifiers denoted by LT numbers. Tennessee (LT263) and Michigan (LT51) were the wild-type seminal isolates (cross LT51 × LT263).

germlings were picked and transferred to PARP-amended water agar media (15 g agar in 980 mL distilled water) in 60-mm plates. A single hyphal-tip was then subcultured after 2 days incubation at room temperature and each germinated oospore-derived isolate was assigned a unique identifier (LT number). For long-term storage, 7-mm plugs of agar with expanding mycelium were placed into 2-mL screw-top tubes containing 1 mL sterile distilled water and three hemp seeds and stored at room temperature.

To determine mating type of *P. capsici* isolates, plugs of actively expanding mycelium were placed at the centre of V8 juice agar plates approximately 2 cm away from 'tester' isolates CBS121656 (mating type A1) or CBS121657 (mating type A2). Plates were wrapped with Parafilm and incubated in the dark at room temperature for at least 1 week, after which observations were made for the production of oospores at the interface using a light microscope. Isolates able to produce oospores when crossed against CBS121656 were determined as A2 mating type. Conversely, isolates that were able to form oospores when paired against CBS121657 were determined as A1 mating type. Isolates unable to produce oospores with either parent were considered sterile. All crosses against testers were done twice.

DNA isolation and AFLP profiles

Isolates were grown in V8 broth, the mycelium lyophilized, genomic DNA extracted, and amplified fragment length polymorphism (AFLP) profiles generated as described previously (Habera *et al*., 2004; Lamour & Finley, 2006). AFLP genotyping was performed using *Eco*RI and *Mse*I restriction endonucleases, adapters and primers as described by Vos *et al*. (1995). Pre-selective amplification was carried out using no selective nucleotides (Eco + 0/Mse + 0), but selective amplification was conducted with selective primer pair (Eco + CC/Mse + CA). Selective amplifications were diluted and labelled in a separate reaction, according to Habera *et al*. (2004). AFLP fragments were resolved and analysed on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter) following the manufacturer's protocols. AFLP profiles were generated twice for each isolate using independent DNA extractions for all the crosses in this study. AFLP markers were scored

Table 1 Primers and probe sequences used for *Phytophthora capsici* single-nucleotide polymorphism (SNP; bold and underlined) genotyping with Taqman assays

Locus name	Accession number	Primer sequence[a]	Internal reporter oligonucleotide (probe) VIC	Internal reporter oligonucleotide (probe) FAM
SNP12	EF566463	F_5′-CCCGCTAGAGATTCATTCATCCA-3′ R_5′-GCGGACCGTAATTCTGTGCATAA-3′	5′-CAGCCA**G**CAGACGC-3′	5′-ACAGCCA**A**CAGACGC-3′
SNP14	EF566464	F_5′-CGCTGTGTCGATAGTGGGAATG-3′ R_5′-TCTTCTCTCTGTGTTCGACTACCA-3′	5′-CTTGAGCT**A**ATTCTCT-3′	5′-TTGAGCT**C**ATTCTCT-3′
SNP18	EF566465	F_5′-GATGCGCCCCACAATGG-3′ R_5′-TTGGCCCACTGCGATGT-3′	5′-CAGCGCT**C**CACCACG-3′	5′-CAGCGCT**T**CACCACG-3′
SNP19	EF566466	F_5′-AGGGAAGCACCGCATTAGG-3′ R_5′-CGTGTCAATCTCCGTCACTAATAGA-3′	5′-CTCCCGAA**C**CGGATGA-3′	5′-CCCGAA**G**CGGATGA-3′
URA3	EF151190	F_5′-CGAAGGACAACGCGAACTTG-3′ R_5′-CTGCGTGACGCCATCAAC-3′	5′-TGCTGC**G**TCGACTG-3′	5′-TGCTGC**A**TCGACTG-3′

[a]F, forward; R, reverse.

for each isolate from the different crosses as one (1) for presence of the marker and zero (0) for absence. A binary matrix was constructed using only clearly resolved, replicated markers. A similarity analysis was carried out using NTSYSpc 2·11a (Exeter Software) and a dendrogram constructed by employing an unweighted pair-group method with arithmetic average (UPGMA) cluster analysis.

Loss of heterozygosity

Because of the limitations of AFLP markers (dominant markers), only a specific group of markers was considered for calculating the loss of heterozygosity. The segregation of the markers used was consistent with the following schemes: (i) polymorphic, but homozygous in each parent and not segregating in the offspring (case: AA × aa); (ii) polymorphic and heterozygous in one parent, with a 1:1 segregation ratio in the offspring (Aa × aa); and (iii) heterozygous in both parents and a 3:1 segregation ratio in the offspring (Aa × Aa). No attempts were made to determine if alleles were hetero- or homozygous based on the peak intensity of the fluorescent markers. Loss of heterozygosity was calculated directly by dividing the number of heterozygous alleles that had switched to a homozygous conformation in each of the inbreeding crosses by the total number of heterozygous alleles observed in the seminal cross LT51 × LT263.

SNP genotyping

Five nuclear SNP markers were identified *in silico* from expressed sequence tags generated from mRNA of *P. capsici* isolate LT1534, an isolate derived from the second backcross generation. This isolate is being sequenced as part of the *P. capsici* genome sequencing project. Custom TaqMan® SNP Genotyping Assays were designed according to the manufacturer's instructions (Table 1). SNP assays were performed using 7·5 μL iQ™ Supermix (Bio-Rad Inc.), 0·325 μL Taqman® probe/primers allelic discrimination cocktail (40X), 2 μL DNA (~15–20 ηg) and 3·2 μL molecular-biology-grade H_2O. PCR reactions were carried out in 96-well plates (Bio-Rad) in triplicate for each isolate on an iQ5 real-time thermal cycler (Bio-Rad) using the following parameters: 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Each assay was standardized for optimal cycling PCR conditions and results were analysed with the accompanying iQ5 optical system software 1·0 (Bio-Rad) using the allelic discrimination option, adjusting parameters for Ct values and RFU (relative fluorescent units) according to the manufacturer's instructions. Table 1 provides the list of SNP markers used in this study.

Chi-squared analysis of the inheritance of SNP markers compared observed numbers to those expected under simple Mendelian inheritance (one degree of freedom for markers expected to be in a 1:1 ratio and two degrees of freedom for markers under a 1:2:1 ratio).

Results

Inheritance of molecular markers

A total of 692 oospore-derived isolates were germinated from eight different crosses (Fig. 1). The number of recombinant isolates identified through AFLP fingerprinting and/or SNP genotyping was 201, 71, 79, 88, 11 and 42 (N = 492) from the F_1, BC_1F_1, BC_2F_1, SC_1F_1, $SC_{2\text{-}1}F_1$ and SC_3F_1 crosses, respectively (Fig. 1). AFLP fingerprinting with the primer combination Eco + CC/Mse + CA revealed 40 clearly resolvable markers in the F_1 population. These markers were tracked for their presence/absence across the different offspring (BC_1F_1, BC_2F_1, SC_1F_1, and $SC_{2\text{-}1}F_1$ and SC_3F_1). Twenty of the 40 markers were determined to be heterozygous in the wild-type parents (LT51 and LT263) based on observations of their segregation in each of the recombinant progeny sets (Table 2). Based on these observations it was determined that the original heterozygosity was reduced by 10% in the BC_1F_1 progeny, 35% in BC_2F_1, 50–52% in SC_1F_1 and 60–75% in $SC_{2\text{-}1}F_1$ (Table 2).

AFLP markers were useful for identifying sexual progeny in the first four inbred generations (F_1, BC_1F_1,

Table 2 Summary table of *Phytophthora capsici* AFLP alleles(named by size) followed throughout the inbreeding process

	Cross				
Allele (bp)	F_1LT51 × LT263	BC_1F_1LT1021 × LT263	BC_2F_1LT1422 × LT263	SC_1F_1LT1503 × LT1530	$SC_{2\text{-}1}F_1$LT2209 × LT222
119	Aa × Aa	Aa × Aa	aa × Aa	aa × aa	aa × aa
125	aa × Aa	Aa × Aa	AA × Aa	Aa × Aa	A* × A*
202	aa × Aa	aa × Aa	aa × Aa	Aa × aa	Aa × aa
215	Aa × aa	Aa × aa	aa × aa	aa × aa	aa × aa
217	Aa × aa	Aa × aa	aa × aa	aa × aa	aa × aa
226	Aa × aa	aa × aa	aa × aa	aa × aa	aa × aa
233	Aa × Aa	aa × Aa	aa × Aa	Aa × Aa	aa × Aa
267	Aa × aa	Aa × aa	aa × aa	aa × aa	aa × aa
290	aa × Aa	Aa × Aa	AA × Aa	Aa × Aa	A* × A*
322	aa × Aa	aa × Aa	Aa × Aa	aa × Aa	aa × aa
363	Aa × aa	aa × aa	aa × aa	aa × aa	aa × aa
378	AA × Aa	Aa × Aa	AA × Aa	A* × A*	A* × A*
387	aa × Aa	Aa × Aa	aa × Aa	aa × Aa	aa × Aa
427	aa × Aa	Aa × Aa	AA × Aa	Aa × Aa	A* × A*
453	aa × Aa	aa × Aa	aa × Aa	Aa × aa	aa × aa
469	AA × aa	Aa × aa	aa × aa	aa × aa	aa × aa
492	aa × Aa	Aa × Aa	Aa × Aa	Aa × Aa	aa × Aa
595	Aa × aa	Aa × aa	aa × aa	aa × aa	aa × aa
614	aa × Aa	aa × Aa	aa × Aa	aa × aa	aa × aa
622	aa × Aa	aa × Aa	Aa × Aa	aa × aa	aa × aa
Fixed alleles (%)[a]	not calculated	10	35	50–52**	60–75**

*Allelic conformation ambiguous. **Calculated excluding ambiguous alleles.

[a]Percentage of homozygous alleles calculated based on total number of described alleles.

BC_2F_1 and SC_1F_1) as there were between 16 (F_1) and four (SC_1F_1) segregating markers (Table 2). However, AFLP profiles from advanced inbred crosses ($SC_{2\text{-}1}F_1$ and SC_3F_1) revealed that most of the markers were homozygous for one of the parents, making it difficult to accurately differentiate sexual recombinant progeny from parental genotypes. An UPGMA similarity tree illustrated the low level of genetic variability present at the higher levels of inbreeding, as ~90% of the identified markers were shared by the majority of isolates derived from the $SC_{2\text{-}1}F_1$ cross (Fig. 2).

As a result of the lack of microsatellite markers in *P. capsici*, SNP markers were developed from preliminary sequence data. Codominant SNP markers homozygous for different alleles were employed to overcome the AFLP limitations in those crosses where there were not enough AFLP markers to discriminate the parental from the recombinant genotype (Table 1). The SNP14 marker was homozygous in the parents from cross LT2209 × LT2222 (LT2209 = G/G and LT2222 = T/T) and recombinant progeny carried both alleles (G/T) (Table 3). Additionally, 60 and 10 oospore-derived isolates had identical SNP14 conformation to LT2209 and LT2222, respectively. Three additional SNP markers (SNP18, SNP19 and URA3) were also tested for their inheritance in this recombinant progeny. A comparison of the observed and expected ratios (1:1 or 1:2:1) from these three markers indicated that all followed a Mendelian segregation (Table 3). In the same way, identification of recombinant isolates from cross LT2211 × LT2222 was done using the SNP12 marker (LT2211 = G/G and LT2222 = A/A), but no recombinant progeny were recovered. All 46 oospore-derived isolates recovered had SNP12 in an allelic conformation identical to one of the parents (two isolates were identical to LT2222 and 44 identical to LT2211). Assessment of the URA3 SNP marker in F_1, BC_1F_1, BC_2F_1, SC_1F_1 and $SC_{2\text{-}1}F_1$ revealed significant correlation with Mendelian ratios, except for cross BC_1F_1 ($P < 0{\cdot}001$, Table 4).

Mating type

All of the F_1 isolates were able to produce oospores when mated with the opposite mating type (tester isolate). The number of sterile isolates throughout all the inbreeding crosses ranged between 0% and 12% (BC_1F_1 = 2·8%, BC_2F_1 = 2·8%, SC_1F_1 = 1·1%, $SC_{2\text{-}1}F_1$ = 0%, SC_3F_1 = 12%). The mating-type ratio (A1:A2) was 1:1 for F_1 (36:50), BC_1F_1 (31:38), $SC_{2\text{-}1}F_1$ (5:6) and SC_3F_1 (18:19) progenies, 1:2 for the BC_2F_1 progeny (26:42) and ~1:3 for SC_1F_1 (22:65). No self-fertile isolates were recovered from any of the crosses.

Apomictic progeny

Oospore-derived isolates with AFLP or SNP profiles identical to one or the other parent were recovered from all but one cross (LT1021 × LT263) (Fig. 1). The seminal cross LT51 × LT263 produced 20 out of 221 oospore progeny with AFLP genotypes identical to the A2 mating-type parent LT263. The first backcross, LT1021 × LT263, did not produce any parental clones, whereas from the

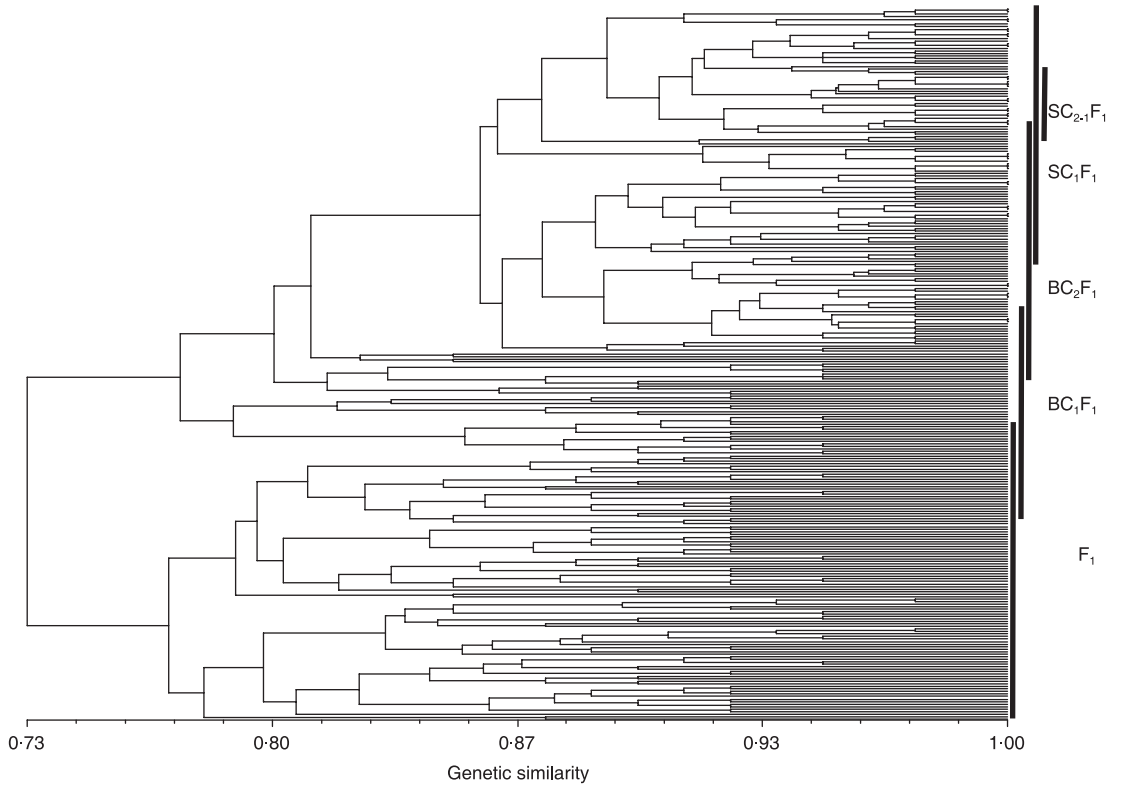

Figure 2 Unweighted pair-group method with arithmetic average (UPGMA) cluster analysis of *Phytophthora capsici* isolates from five crosses using 40 amplified fragment length polymorphism markers. Bars at the right represent the position of at least 85% of the isolates from each population. F_1 = filial one; BC_1F_1 = first backcross; BC_2F_1 = second backcross; SC_1F_1 = first sib-cross F_1; $SC_{2\text{-}1}F_1$ = second sib-cross F_1.

Table 3 Single nucleotide polymorphism (SNP) genotypes for *Phytophthora capsici* cross LT2209 × LT2222

Marker	SNP14	SNP18	SNP19	URA3
Allelic conformation	G/G[a] × T/T[b]	T/C × T/C	G/G × G/C	C/T × C/C
Expected conformation	GT	TT:2TC:CC	GG:GC	CC:CT
Number of isolates	11	1:8:2	8:3	6:5
χ^2	n.a.[c]	3·04	2·27	0·09
P[d]	n.a.	0·21	0·13	0·76

[a]An additional 60 isolates had identical SNP genotypes.
[b]An additional 10 isolates had identical SNP genotypes.
[c]n.a., non-applicable.
[d]Probability of observed ratio occurring by chance under null hypothesis of 1:1 (SNP19 and URA3 with 1 d.f.) or 1:2:1 (SNP18 with 2 d.f.) segregation.

second backcross, LT1422 × LT263, 10% of the isolates (nine out of the 88 oospore progeny) had AFLP genotypes identical to the LT263 parent isolate (A2 mating type). The first sibling cross LT1503 × LT1530 resulted in six oospore-derived isolates identical to LT1503 (A2 mating type). The majority of the isolates recovered from the second sibling crosses (LT2209 × LT2222 and LT2211 × LT2222) were identical to the A2 mating-type parent, with 73% of isolates recovered from LT2209 × LT2222 and 95% of those recovered from LT2211 × LT2222 identical to the A2 parent type. No recombinant isolates were recovered from LT2211 × LT2222. SNP genotyping using marker SNP14 revealed a single recombinant progeny, 10 isolates (59%) identical to the A2 mating-type parent (LT2209), and six isolates identical to LT2222 (data not shown). Likewise, oospores germinated from cross LT2211 × LT2222 did not produce any recombinant isolates and the same pattern was observed: a large number of isolates (28 out of 34 oospore progeny recovered) were identical to the A2 mating-type parent (LT2211) and only six isolates were identical to LT2222 (A1) (data not shown).

In addition, of the 46 oospore-derived isolates recovered from the cross LT2198 × LT51, none appeared to be products of sexual recombination; 42 isolates had identical fingerprint to LT51 (A1 mating type) and four isolates had identical AFLP genotypes to the LT2198 parent (A2 mating type).

Discussion

The wild-type isolates used as parents in this study are typical of *P. capsici* found on vegetables in the USA; they readily infect common cucurbit and solanaceous vegetable hosts, grow rapidly at room temperature on V8 media, produce sexual and asexual spores abundantly, and are

Table 4 Summary data for inheritance of the URA3 single nucleotide polymorphism (SNP) in a series of *Phytophthora capsici* inbreeding crosses

	Cross				
	F_1	BC_1F_1	BC_2F_1	SC_1F_1	$SC_{2\text{-}1}F_1$
SNP marker	LT51 × LT263	LT1021 × LT263	LT1422 × LT263	LT1503 × LT1530	LT2209 × LT222
URA3 parental conformation	C/C × C/T	C/C × C/T	C/C × C/T	C/T × C/C	C/T × C/C
Progeny ratio	44:48	20:51	32:48	47:41	5:6
χ^{2a}	0·17	13·53	3·2	0·41	0·09
P^b	0·68	< 0·001	0·07	0·52	0·76

[a]χ^2 value for testing 1:1 segregation (1 d.f.).
[b]Probability of observed ratio occurring by chance under null hypothesis of 1:1 segregation.

highly fecund (Satour & Butler, 1968; Lamour & Hausbeck, 2002). Population studies indicate that individual *P. capsici* isolates carry significant genetic variation (Lamour & Hausbeck, 2002). The close crosses here showed a significant decrease in the overall heterozygosity for genetic markers consistent with the expected effect of inbreeding. This proved useful in allowing selection of an isolate (dubbed LT1534) for whole-genome sequencing that was estimated to have 30% reduced heterozygosity compared to the wild-type parental isolates. In addition to the reduction in heterozygosity, the crosses reported here may be useful for testing hypotheses based on findings within the genome sequence.

A phenomenon that should be considered when generating more advanced inbreeding crosses is the recovery of apomictic, or clonally derived, progeny from oospores. Neither of the parental isolates produces thick-walled asexual chlamydospores and it is unlikely that the results were caused by asexual material contaminating the oospore preparations. Meticulous visual observations of the overnight enzyme-treated oospore solution never revealed any visible hyphal fragments, sporangia or germinating zoospores. Also, there was no evidence that any of the oospore progeny were produced via selfing, as was previously reported for *P. infestans* (Shattock *et al*., 1985, 1986a,b). For selfing, heterozygous AFLP markers in the parental fingerprint type should be lost by recombination. In addition, the products of selfing are likely to be quite different from the parents because of the significant inbreeding. In all of the crosses presented here the apomictic oospore progeny were phenotypically and genotypically identical to one of the parent types. Aberrant segregation ratios have been reported as a limiting factor in *P. infestans* genetic analyses (Judelson, 1996; Fabritius *et al*., 1997). Previous investigations in crosses of *P. infestans* (backcrosses and sib-crosses) yielded distorted ratios for allozyme markers (Spielman *et al*., 1990). It is possible that non-recombinant oospores (apomicts) may have contributed to the distorted ratios (Judelson *et al*., 1995; Carter *et al*., 1999).

Although both parental *P. capsici* isolates contributed apomictic oospore progeny, the A2 mating-type parent contributed the majority of oospore-derived isolates. *Phytophthora* isolates are thought to vary for 'maleness' and 'femaleness' based on whether they contribute more to the production of antheridia or oogonia (Erwin & Ribeiro, 1996; Judelson, 1997) and this may have played a role in the results of this investigation.

Finally, the results suggest that developing inbred lines in the oomycete *P. capsici* is possible and may be helpful for studying genetic factors underlying complex traits. Currently, a limiting factor is the availability of codominant markers. AFLP analysis provided sufficient segregating markers early in the inbreeding process, but by the second sibling cross the number of segregating AFLP markers among the siblings was very low and it became difficult to accurately genotype individual progeny. One of the goals of the current *P. capsici* genome project is to catalogue codominant markers, single nucleotide polymorphism (SNP) and simple sequence repeat (SSR), and to develop a genetic marker resource. The availability of a database of codominant markers should provide the necessary tools to fully develop and exploit inbreeding resources for *P. capsici*.

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