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Dominance of a single clonal lineage in the *Phytophthora infestans* population from northern Shaanxi, China revealed by genetic and phenotypic diversity analysis

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Late blight caused by *Phytophthora infestans* is the most serious disease of potato worldwide. To understand the *P. infestans* population structure in northern Shaanxi, an emerging potato production region in China, 125 single-lesion isolates were randomly collected from farmers' fields in 2009 and characterized phenotypically and genotypically. A mating type assay showed that 94 isolates were A1 mating type. Virulence determination of selected isolates on a set of differential potato lines containing *R1* to *R11*, respectively, showed the presence of two pathotypes, of which the pathotype lacking avirulence genes *Avr3*, *Avr4* and *Avr10* was dominant. Isolates lacking all avirulence factors *Avr1* to *Avr11* were detected but at lower frequency (13·6%). Analysis for mtDNA haplotype showed all 61 examined isolates were IIa. A total of seven multilocus genotypes were distinguished among 125 isolates, as determined with seven polymorphic microsatellite markers. The genotype SG-1 was dominant in the population with a frequency of 75·2% and was present throughout the region. Analysis of the phenotypic and genotypic structures of *P. infestans* populations indicated strict clonal reproduction of the pathogen and suggested that sexual reproduction probably does not occur. Potential implications for disease management are discussed.

Keywords: asexual reproduction, genetic diversity, microsatellites, *Phytophthora infestans*

Introduction

The oomycete *Phytophthora infestans*, the causal agent of late blight, is considered the most important biotic constraint to potato production worldwide (Fry, 2008) and is a major threat to food security in developing countries (Forbes, 2012). The most famous late blight epidemic occurred in the 1840s and led to the Irish potato famine, where one million people starved to death in Ireland (Ristaino *et al.*, 2001). As one of the four major global food crops, potato plays an important role in guaranteeing food security and helping to keep farmers from poverty worldwide (Haas *et al.*, 2009). Disease management strategies have been important for this crop, due to the importance of potato tubers as a basic source of food and starch. The primary line of control has been chemical, but breeding for resistant cultivars has also had a limited impact (Cooke *et al.*, 2011). Monitoring changes in a *P. infestans* population can facilitate adjustment of control strategies and lead to a better understanding of pathogen–host co-evolution and more successful disease control.

Phytophthora infestans is a diploid and heterothallic organism with two mating types designated A1 and A2. The patterns of reproduction of *P. infestans* include both asexual (via sporangia or zoospores) and sexual reproduction via oospores (Fry, 2008). The asexual cycle enables rapid population growth in susceptible host tissues, and is also the predominant reproduction mode worldwide. The asexual sporangia are formed on infected host tissue and can re-infect and cause symptoms on leaves, stems and tubers (Fry, 2008). Massive numbers of sporangia are produced from diseased tissues and are carried by wind and rain-splash to other host tissues where they germinate directly or release multiple mobile zoospores that infect, colonize and release new sporangia via host stomata (Fry, 2008). This process is so quick that whole potato fields can be transformed from slightly diseased to nearly completely destroyed within several days.

Long-distance transmission of *P. infestans*, more often through movement of seed potato tubers, has been inferred from shared genotypes found among European, Asian and Russian pathogen populations (Carlisle *et al.*, 2001; Elansky *et al.*, 2001). Additional migrations could increase the level of genetic variation in *P. infestans* populations by bringing compatible strains of the two different mating types together. The worldwide situation changed after the importation of large quantities of potato from Mexico and North America to Europe in 1976, done to offset a reduction in European yields caused by drought (Montarry *et al.*, 2010). It is generally speculated that this migration introduced the A2 mating type from Mexico to Europe, which has subsequently spread globally (Montarry *et al.*, 2010).

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Coexistence of both A1 and A2 mating types allows the possibility of sexual reproduction of *P. infestans*. The sexual oospores contribute greatly to the overwintering survival and to the nature of the population structure of *P. infestans* (Fry, 2008). For example, in the Toluca Valley of Mexico, oospores of *P. infestans* were commonly found in soil and A1 and A2 mating types were typically present in equal proportions (Goodwin *et al.*, 1992; Fernandez-Pavia *et al.*, 2004). These observations, coupled with the fact that the pathogen population was dramatically diverse, led to the conclusion that sexual reproduction was common and was an important component of the life history of *P. infestans* in that location.

Over the past decade, several studies have focused on phenotypic and genotypic characterization of *P. infestans* populations in the main potato production areas in China, including mating type distribution, resistance to the fungicide metalaxyl, and pathotype determination as defined using *R*-gene differentials. Previous work carried out by Guo *et al.* (2009) identified a single clonal lineage in northern China using two SSR markers. Guo *et al.* (2010) reported that the most predominant RFLP genotype identified in their study was SIB-1. Li *et al.* (2013) identified three clonal lineages in western China using 10 SSRs. Unfortunately, so far, there is little information on the sexual reproduction of *P. infestans* populations in China (Guo *et al.*, 2010; Li *et al.*, 2013). A recent study of *P. infestans* populations in a potato germplasm nursery revealed the introduction of genetically complex 'new' *P. infestans* populations (Ma *et al.*, 2013). Additional studies have consistently shown that *P. infestans* populations in different regions in China have become increasingly diverse genetically and phenotypically, with greater numbers of pathotypes lacking avirulence factors found on the standard set of differentials (Guo *et al.*, 2009; Li *et al.*, 2013).

Northern Shaanxi is an emerging and important potato production region in China where the rainy season coincides with potato tuber initiation. Potato late blight poses a potential threat to sustainable development of potato production in the region. However, no information on the *P. infestans* population structure has been reported in Shaanxi province to date. This study took advantage of recently developed SSR markers (www.eucablight.org; Knapova & Gisi, 2002; Lees *et al.*, 2006; Li *et al.*, 2010) to test the hypothesis that the population structure of *P. infestans* in northern Shaanxi province is clonal. The specific objectives were to: (i) determine the occurrence of A1 and A2 mating types within the population; and (ii) explore any indications of sexual reproduction. Such information could contribute to development of late blight control measures.

Materials and methods

Sampling and purification of *P. infestans* isolates

The *P. infestans* isolates were collected from multiple sites at four distant sampling locations in the region. The sample sites in each location were separated from each other by distances of 10–40 km (Fig. 1). The 125 *P. infestans* isolates were obtained during the potato growing season in 2009, with different numbers sampled in each location (Table 1). Isolates were collected from Yuyang and Hengshan (representing fields of farm-saved seed), Jingbian (seed from the local agricultural research institute) and Dingbian (from a local potato seed company). Within a field, only one leaf per plant was sampled, with plants coming from five different points in the field. Samples were packed individually in paper bags and maintained in an icebox until isolation in the laboratory. Fresh potato tubers were used to induce the growth of *P. infestans*; mycelium was then transferred to RSA (rye sucrose agar) medium amended with antibiotics (ampicillin 100 μg mL^{-1}, rifampicin 20 μg mL^{-1}). The plates were incubated at 16°C in darkness for several days to allow mycelia to grow prior to use in the characterization assays (Goodwin *et al.*, 1992).

Mating type determination

The mating type of each unknown *P. infestans* isolate was determined by pairing on RSA medium (separated by 2 cm) with either a known A1 or A2 reference isolate (NL80029 and NL88133, respectively). The reference A1 and A2 strains were kindly provided by Dr Francine Govers (Wageningen University, the Netherlands). Oospore formation was examined at the hyphal interfaces after 10–14 days of incubation in the dark at 16°C. Isolates producing oospores with both known A1 and A2 were designated A1A2. These putative self-fertile isolates were confirmed by single zoospore isolation, which eliminated the possibility of mixed cultures (Smart *et al.*, 1998).

Pathotype determination

A detached leaf assay was used to assess the pathotype of 22 *P. infestans* isolates on a set of 11 potato near-isogenic differential lines, each possessing a single *R*-gene (*R1–R11*) from *Solanum demissum*. Differentials were kindly provided by Dr Liping Jin (Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China).

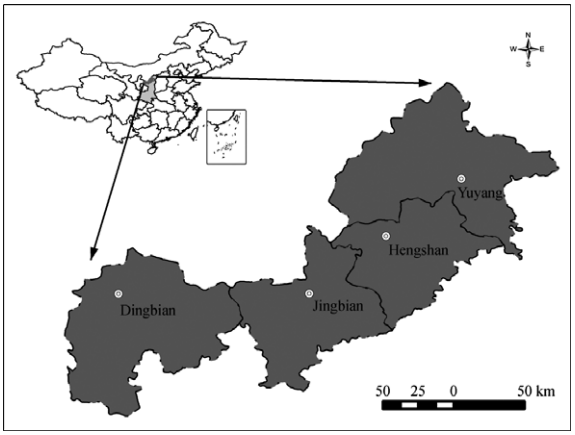

Figure 1 Sampling locations of *Phytophthora infestans*. Isolates from Yuyang and Hengshan were recovered from fields of farm-saved seed, isolates from Jingbian were from seed from the local agricultural research institute and isolates from Dingbian were from a local potato seed company.

Table 1 Mating type, mtDNA haplotype and genetic diversity of *Phytophthora infestans* in four subregions in northern Shaanxi province of China

	Population by sampling region				
Parameter	Yuyang	Hengshan	Jingbian	Dingbian	Total
N	45	39	37	4	125
MT	A1 (33)	A1 (31)	A1 (30)/A2 (2)	SF (1)	A1 (94)/A2 (2)/SF (1)
mtDNA	IIa (20)	IIa (17)	IIa (21)	IIa (3)	IIa (61)
NG	6	5	3	1	7
H	0·984	0·845	0·556	0·000	0·891
G	1·981	1·787	1·398	1·000	1·728
E_5	0·586	0·593	0·536	NA	0·507
A_O	2·3	2·1	2·0	1·7	2·6
H_O	0·679	0·711	0·670	0·714	0·695
H_E	0·368	0·378	0·369	0·408	0·369
F_{IS}	−0·845	−0·881	−0·816	−0·750	−0·883
$\bar{r}_d$	0·171[a]	0·276[a]	0·600[a]	NA	0·159[a]

N, no. of samples; MT, mating type; mtDNA, mitochondrial DNA haplotype; *NG*, number of multilocus genotypes; *H*, Shannon–Wiener index of multilocus genotype diversity; *G*, Stoddart and Taylor's genotypic diversity index; E_5, index of eveness; A_O, mean number of observed alleles per locus; H_O, observed heterozygosity; H_E, unbiased expected heterozygosity (Nei, 1973); F_{IS}, fixation index; $\bar{r}_d$, index of multilocus linkage disequilibrium; SF, self-fertile; NA, no further analysis due to the small number of *P. infestans* isolates in Dingbian.
[a]Significant linkage disequilibrium at $P < 0·01$.

Prior to the pathotype test, *P. infestans* isolates were cultured on RSA medium for two generations, each for 5–10 days, and then transferred to RSB (rye sucrose agar medium amended with beta-ditosterol) medium for 12 days at 16°C in darkness. The cultures were washed and rubbed with 5 mL distilled water to prepare sporangial suspensions. The suspension concentration was adjusted to 4×10^4 sporangia mL^{-1} under a microscope. The sporangial suspensions were chilled for 2 h at 4°C to promote release of motile zoospores. The zoospore release was monitored by microscopic examination before inoculation.

Leaflets of 6–10-week-old differential potato plants were placed abaxial face up in plastic trays with filter paper in the bottom saturated with distilled water. Each leaflet was drop-inoculated on the abaxial (lower) side with 15 μL sporangial/zoospore suspension adjusted to a concentration of about 4×10^4 sporangia mL^{-1}. A single leaflet was used as an experimental unit and six to eight replications were conducted per test. Inoculated leaflets were incubated in a growth chamber at 16°C in the dark for 12–24 h. The leaflets were then placed face up in the plastic trays, covered with plastic wrap and incubated in a climate chamber at 16–18°C with a 14 h light/10 h dark photoperiod. Disease symptoms were scored 4 days after inoculation while being observed for four consecutive days. The interaction was considered compatible when sporulation was clearly visible on at least half of the replicate leaflets (Flier *et al.*, 2007).

DNA extraction, mitochondrial DNA haplotyping and SSR analysis

Phytophthora infestans isolates were grown for 10–12 days in darkness at 16°C in RSA broth. The mycelium was harvested, lyophilized and stored at −80°C. Genomic DNA of *P. infestans* was extracted using the protocol described by Goodwin *et al.* (1992) and stored in weak TE (10 mM Tris–HCl, 0·1 mM EDTA, pH 8·0) at −20°C until use.

Mitochondrial DNA haplotypes were determined by polymerase chain reaction (PCR) amplification of isolated total DNA using primer pairs designed for specific regions of the mitochondrial genome of *P. infestans*. PCR was performed in a PTC200 thermocycler (Bio-Rad). The amplified fragments were digested by *Msp*I and *Eco*RI enzymes, yielding restriction patterns by which the isolates could be classified into four mtDNA haplotypes: Ia, Ib, IIa and IIb (Griffith & Shaw, 1998).

A preliminary analysis of 20 isolates showed that seven out of 10 SSR loci were polymorphic and the selected seven SSR markers were used for subsequent genetic analysis. Markers used were Pi4B, Pi63, G11, D13, SSR4, SSR8 and SSR11 (Knapova & Gisi, 2002; Lees *et al.*, 2006; Li *et al.*, 2010). PCR amplification was performed in 20 μL containing 10 ng genomic DNA as described by Li *et al.* (2010) using the following conditions: an initial 2 min at 95°C, followed by 30 cycles of 20 s at 95°C, 25 s at 58°C (for Pi4B and Pi63), 60°C (for SSR4, SSR8 and SSR11), 50°C (for D13) or 56°C (for G11), and 60 s at 72°C, and a final extension step of 72°C for 5 min. PCR products were separated by electrophoresis in polyacrylamide nondenaturing gels. Gels were silver stained and dried using procedures as described (Bassam & Gresshoff, 2007). Briefly, the gel was washed twice with distilled water after electrophoresis, and stained for 10–15 min in 0·1% $AgNO_3$ solution. The stained gel was transferred into distilled water and washed twice followed by development in 1·5% NaOH solution containing 0·1% formaldehyde until visible DNA bands were revealed. Finally, the developed gel was washed twice with distilled water and photographed.

In order to analyse genetic relationships between *P. infestans* populations from northern Shaanxi and those from European countries and other regions (Table 2), PCR products of representative isolates with different SSR genotypes were cloned into pMD18-T vector and sequenced by GenScript (Nanjing, China).

Population genetic analysis

For genetic data analysis, *P. infestans* populations were defined according to geographic origin. To reveal genetic relationships among the detected *P. infestans* genotypes, an unrooted UPGMA tree based on shared allele distances was constructed using the PowerMarker software v. 3.25 (Liu & Muse, 2005). Phylogenetic trees were created by FigTree v. 1.3.1 (Rambaut & Drummond, 2010) using the distance matrix generated by PowerMarker and 1000 bootstrap replications.

Table 2 Origin, year of collection, mating type and MLG of *Phytophthora infestans* reference isolates used in this study

Isolate[a]	Origin	Year	Mating type	MLG
C1	Scotland	1996	A1	4-A1
C2	Scotland	1995	A1	8-A1
C3	Scotland	1997	A1	8-A1
C4	Scotland	1996	A1	5-A1
C6	USA	1998	A2	US-8
C7	Ecuador	1998	A1	EC-1
C10	Scotland	1997	A2	Misc
80029	Netherlands	1980	A1	Unknown
88133	Netherlands	1988	A1	Unknown
T30-4	Netherlands	1992	A1	Unknown

MLG, multilocus genotype.
[a]Genomic DNA of isolates C1–C10 were provided by Dr David Cooke (The James Hutton Institute, UK). The other three reference isolates (80029, 88133 and T30-4) were provided by Dr Francine Govers (Wageningen University, the Netherlands).

Both genotypic and gene diversity are needed to estimate genetic diversity. Genotypic diversity was characterized by indices describing diversity, richness and evenness. Given the differences in sample sizes, genotypic richness was calculated using rarefaction curves. Genotypic diversity was quantified with Stoddart and Taylor's index G, and genotypic evenness was estimated with the index E_5 (Grünwald *et al.*, 2003). Gene diversity was estimated according to Nei (1973). The mean number of observed alleles per locus (A_O), observed heterozygosity (H_O) and expected heterozygosity (H_E) were estimated using the program POPGENE v. 1.31 (Yeh *et al.*, 1997). Fixation index (F_{IS}) was calculated for each population as $F_{IS} = 1 - H_O/H_E$. The hypothesis of nondifferentiation among populations was tested by comparing the observed θ value with the value calculated for data sets in which alleles were resampled without replacement (1000 randomizations) using MULTILOCUS v. 1.3 (Agapow & Burt, 2001). Confidence intervals for θ (equivalent to Wright's F_{ST}) were generated by bootstrapping over loci (1000 replications, 95% confidence level) (Grünwald *et al.*, 2003).

The occurrence of random sexual reproduction was also assessed by estimating the index of multilocus linkage disequilibrium ($\bar{r}_d$) with package POPPR (Kamvar *et al.*, 2014) in R v. 3.0.0 (R Development Core Team, 2011). The $\bar{r}_d$ statistic is much less dependent on the number of loci than the index of association (I_A). Departure from the null hypothesis (no linkage disequilibrium, i.e. $\bar{r}_d = 0$) was assessed by permuting alleles between individuals independently for each locus (1000 permutations).

To examine the distribution of genetic variation within and among populations, analysis of molecular variance (AMOVA) was performed using GENALEX v. 6.5 (Peakall & Smouse, 2006). The analyses used the standard data sets and 10 000 permutations to determine how the genetic diversity is partitioned within and among populations.

Results

Mating types in the *P. infestans* population

Of 97 *P. infestans* isolates examined for mating type, 94 were A1, two isolates from Jingbian were A2 and one putative self-fertile isolate was detected in Dingbian. The self-fertile isolate produced a few oospores in single culture but formed more oospores with the A1 isolate.

Pathotypes in *P. infestans* populations

Twenty-two *P. infestans* isolates were selected based on mating type and SSR genotype, and only two pathotypes were identified. The pathotype lacking *Avr3*, *Avr4* and *Avr10* was dominant, comprising 86·4% of the isolates. Three isolates lacked all avirulence factors *Avr1* to *Avr11*, of which two were collected from Jingbian and one from Dingbian. Virulence complexity measured as the average number of infected differential plants per isolate was 4·09.

Level of genetic diversity in *P. infestans* population

Analysis of 61 randomized *P. infestans* isolates showed that all were mtDNA haplotype IIa; thus all analyses discussed below refer to genomic diversity.

The genotype SG-1 was dominant and comprised 75·2% of the *P. infestans* population. Three genotypes (SG-5, 6, 7) were rare, each represented by a single isolate. Genotype SG-2 isolates were detected from all sampling locations except Dingbian and comprised 11·2% of the whole population. SG-3 isolates were collected only from Hengshan and Jingbian, and SG-4 isolates were collected only from Hengshan and Yuyang, and comprised 8·8 and 2·4% of the whole population, respectively.

A total of 17 alleles were detected over the seven SSR loci, with two to five alleles per locus. The mean number of alleles observed per locus in the single population ranged from 1·7 to 2·3 (Table 1). Several rare alleles were detected. Alleles 270 and 279 from locus Pi63 were detected in all *P. infestans* isolates (Table S1). The observed heterozygosity (H_O) varied between 0·670 and 0·714, higher than expected, and was given a negative fixation index. F_{IS} multilocus estimates were not highly variable among *P. infestans* populations, ranging from −0·881 to −0·750.

The Stoddart and Taylor's index showed a low level of genetic diversity in three of the subpopulations tested (Dingbian was not included due to small sample size; Table 1). The Weir and Cockerham's coefficient θ indicated very little genetic differentiation among the four subpopulations (Table 3). The $\bar{r}_d$ test for *P. infestans* populations showed no sign of sexual reproduction (Table 1). It provided evidence for significant linkage

Table 3 Estimation of Weir and Cockerham's coefficient of genetic differentiation θ among different origins of *Phytophthora infestans* populations

	Between subregions	
Populations	θ[a]	P[b]
Yuyang versus Hengshan	−0·0090	0·05
Yuyang versus Jingbian	−0·0109	0·31
Hengshan versus Jingbian	−0·0117	0·27

[a]θ values were generated by bootstrapping over loci (1000 replications) using TFPGA software.
[b]$P < 0·05$ indicates a significant differentiation among populations.

disequilibria and also supported the hypothesis of a clonally reproducing population.

The analysis of molecular variance (AMOVA) of SSR genotype data revealed that the majority of the genetic variation (99%) was found within populations and only 1% genetic variation was observed among populations ($P < 0{\cdot}01$; Table 4).

Comparison of genetic relationships with some reference *P. infestans* lineages

The phylogenetic tree obtained from the shared SSR markers split the isolates into two main clades. One clade contained 10 reference isolates representing European lineages, such as US-8 and 8-A1, while all genotypes from northern Shaanxi made up the second clade (Fig. 2).

Table 4 Analysis of molecular variance (AMOVA) of *Phytophthora infestans* on the basis of SSR data in northern Shaanxi province, China

Source of variation	Degrees of freedom	Sum of squares	Variation (%)
Among population	3	1·012	1
Within population	121	33·692	99**
Total	124	34·704	

**$P < 0{\cdot}01$, based on 1000 permutations.

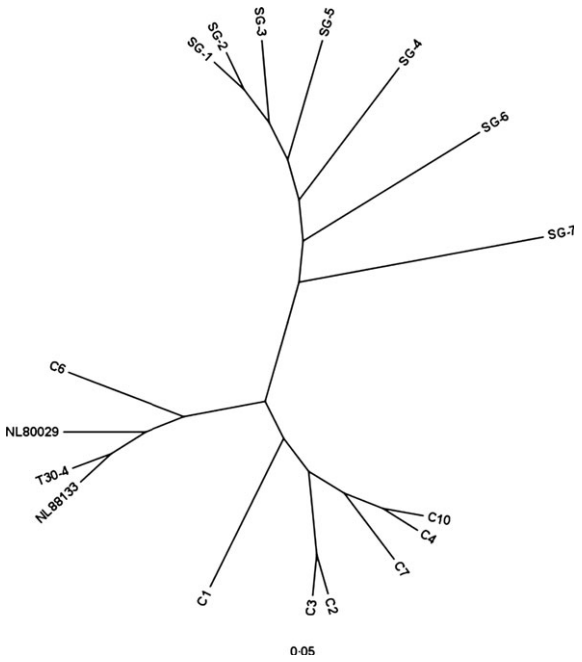

Figure 2 Phylogenetic analysis of Chinese and reference *Phytophthora infestans* isolates using the UPGMA method based on seven common SSR markers. *Phytophthora infestans* isolates collected in northern Shaanxi form a unique clonal lineage, being distant from foreign reference strains. Information on the 10 foreign reference strains is available in Table 1.

Discussion

This study revealed two important aspects of the *P. infestans* population in northern Shaanxi of China. First, it was found that the *P. infestans* population was clonal and was dominated by a single lineage; no sign of sexual reproduction was detected. Secondly, a low frequency of A2 isolates and isolates virulent to all 11 *S. demissum* resistance genes represented by the differential plants was found.

The *P. infestans* population structure deduced from both phenotypic and genotypic analyses in this study showed that the population was relatively low in genetic diversity and dominated by the A1 mating type. Guo *et al.* (2009) were able to differentiate 12 reference *P. infestans* isolates from four countries using two SSR markers, but found only one SSR genotype among the 48 isolates collected in northwestern China, even when the number of SSR markers was increased to nine. Therefore, the revealed low level of *P. infestans* diversity in the present study was not as a result of the markers chosen, and was unlikely to be a consequence of a lack of polymorphism detected by the SSR markers.

Only two pathotypes were detected in the selected isolates, with the pathotype lacking *Avr3*, *Avr4* and *Avr10* being dominant in the population. Thus the north Shaanxi population of *P. infestans* was found to have both low genotypic (SSR) and phenotypic (pathotype) diversity. This contrasts to recent independent studies finding high pathotype diversity in both Ecuador and Nicaragua. However, those locations differed in that genotypic diversity was high in Ecuador, but low in Nicaragua (Blandón-Diaz *et al.*, 2013). In the Ecuador study, Delgado *et al.* (2013) hypothesized that because most of the potato landraces are highly susceptible to late blight, the high pathotype diversity may be a result of the high mutation rates and there was little or no selection pressure (Delgado *et al.*, 2013). In contrast, the pathotype complexity observed in *P. infestans* isolates from Nicaragua could have arisen as a result of the selection pressure imposed by potato cultivars (Blandón-Diaz *et al.*, 2013). High pathotype diversity in a clonal lineage was also reported in northern China (Guo *et al.*, 2009). The finding in the present study of low pathotype diversity may be explained by the short history of potato cultivation in northern Shaanxi, and/or lack of selection pressure imposed by potato cultivars.

Nonetheless, it is still possible that the less diverse northern Shaanxi *P. infestans* population may have derived from the more complex population found in northern China by Guo *et al.* (2009). Those authors found that isolates from the potato variety Zihuabai growing in Inner Mongolia were mating type A1 and mtDNA haplotype IIa. Thus, the Shaanxi population may represent a subset of the Inner Mongolia population which migrated south on infected seed tubers. In China, potato breeding has been a major focus in Inner Mongolia for over 30 years, using breeding materials from Russia, South America and other countries. The introduced

potato lines were subsequently distributed within China (Guo *et al.*, 2010). From the late 1950s to the 1990s, several hundred potato breeding lines, including both local and lines introduced from overseas, were collected and planted at the Wumeng Agricultural Institute, Inner Mongolia (Guo *et al.*, 2010). The experimental plots of the Wumeng Agricultural Institute were one of the few places where the A2 mating type and IIa haplotype isolates were first detected in China. When these observations are taken together, it is probable that the pathogen was introduced by infected potato seed tubers on one of the breeding lines imported from outside China.

The dominant multilocus genotype and the other six genotypes from northern Shaanxi that were identified in this study formed an independent branch genetically distant to the dominant clonal lineage spreading in commercial potato fields in Gansu and Ningxia provinces, northwest China (Y. Tian, unpublished results). However, there was nearly no genetic differentiation among four subpopulations of different sources of potato seed tubers in northern Shaanxi, as validated by Weir and Cockerham's coefficient of genetic differentiation θ. The AMOVA of SSR genotype data further revealed that genetic variation among subpopulations was minimal. The only observed variations were at two loci (Pi4B and SSR4). The limited variation observed in the *P. infestans* population has probably occurred through mutation. Taken together, the results indicate that there was no genetic relationship between *P. infestans* multilocus genotype and geographic origin in northern Shaanxi.

In this study, the low frequency of A2 isolates might be due to their low level of aggressiveness and fitness in *P. infestans* populations compared to the A1 isolates. Temporal fluctuations in A2 frequency have been observed in some European countries (Lehtinen *et al.*, 2007). Unfortunately, there have been no previous reports of the mating type distribution in northern Shaanxi for comparison.

The negative F_{IS} values, which indicate an excess of heterozygotes relative to random mating, could result either from recent migration events or from asexual reproduction (Montarry *et al.*, 2010). The significant multilocus F_{IS} and the higher level of linkage disequilibrium within each subpopulation indicated that the populations were strictly clonal. The high proportion of repeated multilocus genotypes also provided strong evidence for clonal reproduction. The test of multilocus linkage disequilibrium ($\bar{r}_d$) indicated rejection of the null hypothesis of recombination within each population, further indicating that *P. infestans* populations in northern Shaanxi were strictly undergoing asexual reproduction. In countries where sexual reproduction is thought to occur, such as some Nordic European countries, high levels of genotypic diversity have been described (Brurberg *et al.*, 2011).

Populations of *P. infestans* are dynamic and displacement of genotypes and continued monitoring of the population genetic structure over wider areas of the country, including mating types, pathotypes and fungicide sensitivity will be useful in the future to both aid late blight potato breeding programmes and improve disease management. In this study, the results revealed that the pathogen survives as clonal lineages, and thus probably overwinters on potato seed tubers. This suggests the importance of measures associated with pathogen-free planting materials in disease control (Yuen & Andersson, 2013). Strict regulation and quarantine measures for seed potato production are therefore useful to the sustainable production of potato in northern Shaanxi.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Allele sizes at seven microsatellite loci of the seven genotypes of *Phytophthora infestans* detected in northern Shaanxi of China.