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Population genetic analysis of *Phytophthora infestans* in northwestern China

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Late blight caused by *Phytophthora infestans* is the most devastating disease of potato worldwide. To understand the *P. infestans* population structure and dynamics in northwestern China, 959 single-lesion isolates were purified in three consecutive years (2009–2011) and were characterized for mating type, pathotype, mtDNA haplotype and molecular variation at eight SSR loci. The results showed that the distribution of mating types changed significantly over years, with self-fertile isolates dominant in 2010 and 2011. SSR genotyping distinguished 959 isolates into 151 genotypes, and association analysis indicated that *P. infestans* populations in 2010 and 2011 were strictly asexual while in 2009 they showed signs of sexual reproduction. Population analysis showed that the majority of genetic variation was within *P. infestans* populations. Isolates sharing identical SSR genotypes were detected in distant regions, indicating that migration of *P. infestans* could have occurred between regions. Pathogenicity assays on a set of potato differential lines containing *R1* to *R11* resistance genes detected four pathotypes from 74 selected isolates, with the pathotype virulent against all 11 *R* genes being dominant. Three mtDNA haplotypes (Ia, IIa, IIb) were detected with Ia being dominant among 507 isolates examined. Phylogenetic analysis indicated that *P. infestans* populations in northwestern China are distant from European lineages including 13-A2 (blue-13) at the time of this survey. The results have implications for the trade of healthy seed tubers as a means of managing late blight.

Keywords: clonal reproduction, genetic diversity, pathotype, *Phytophthora infestans*, SSR genotype

Introduction

Despite substantial advances in plant disease control strategies, the global food supply is still threatened by a multitude of pathogens and pests (Fry, 2008). Plant diseases can dramatically reduce crop yield and the impact of disease outbreaks is particularly acute in developing countries. Late blight, caused by the oomycete plant pathogen *Phytophthora infestans*, is an agriculturally important and devastating disease on potato worldwide. A detailed knowledge of the *P. infestans* population structure and dynamics is important for the optimization of late blight management strategies and breeding of disease-resistant cultivars (Forbes, 2012).

Many studies throughout the world have indicated that *P. infestans* populations outside central Mexico were dominated by the 'US-1' clonal lineage until the 1970s (Fry, 2008). The situation changed with the detection of the A2 mating type from several European countries in the early 1980s (Goodwin *et al.*, 1994; Garry *et al.*, 2005). Analysis of *P. infestans* populations in Europe indicated that the new population displaced the previous dominant population in only a few years and was further spread worldwide (Fry, 2008). Population displacement with increased fitness is a recurrent event (Vleeshouwers *et al.*, 2011). Recently, a new highly aggressive and metalaxyl-resistant A2 lineage of *P. infestans*, termed 13-A2 (also known as 'blue-13'), was reported in the Netherlands in 2004 (Cooke *et al.*, 2007, 2012). This lineage has now emerged in regions beyond Europe (Chowdappa *et al.*, 2013), where it poses a new major threat to potato and tomato production. Isolates of this lineage have a shorter latent period and spread rapidly, have overcome resistance of the potato cultivar Stirling, a cultivar used in organic potato production since the 1990s in England, and have become dominant in *P. infestans* populations, forming a potential threat to potato production (Cooke *et al.*, 2012).

Potato is an important crop to China, especially for farmers in mountainous areas. *Phytophthora infestans* has also been a major threat to sustainable potato production in China, where the earliest record of a serious loss caused by late blight was in the 1940s (Guo *et al.*, 2010). Previous research on a limited number of *P. infestans* isolates indicated that the most predominant RFLP genotype, SIB-1, was widely distributed in several provinces (Guo *et al.*, 2010). Li *et al.* (2013b) reported that the genotypes of *P. infestans* isolates in China were closely clustered according to the geographic origin. Tian *et al.* (2015) used seven SSR markers to conduct a systematic genetic analysis of the *P. infestans* population in

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Yulin, an emerging potato production region in northern Shaanxi of China, and identified a single clonal lineage. A recent study, focused on a potato germplasm nursery, revealed the introduction of genetically complex 'new' *P. infestans* populations (Ma *et al.*, 2013). These studies also showed that *P. infestans* populations in different regions have become increasingly diverse with more pathotypes detected on the standard set of differentials (Guo *et al.*, 2009; Ma *et al.*, 2013; Tian *et al.*, 2015).

The objectives of the present study were: (i) to investigate the extent of genetic and phenotypic diversity in *P. infestans* populations in northwestern China; (ii) to examine the distribution of genetic variation; (iii) to understand the population dynamics from 2009 to 2011; and (iv) to investigate the occurrence of sexual reproduction.

Materials and methods

Phytophthora infestans isolates

During three consecutive years (2009–2011), 959 *P. infestans* isolates were obtained from single lesion samples, during the early stage of late blight development, from 12 commercial potato sites in two of the most important potato production regions (Gansu and Ningxia) in northwestern China (Fig. 1). Within a field, one leaflet per plant, from five different points in the field, was sampled when approximately 10% of the leaf area was affected by blight. Samples were packed individually in paper bags and maintained in an icebox until isolation in the laboratory. Isolation was performed as described by Ma *et al.* (2013).

Mating type determination

The mating type of each *P. infestans* isolate was determined following the procedure described by Tian *et al.* (2015). The reference A1 (NL80029) and A2 (NL88133) strains were kindly provided by Professor F. Govers (Wageningen University, Netherlands).

Pathotype determination

A detached leaf assay was used to assess the pathotype of 74 randomly selected but different SSR genotype-based *P. infestans* isolates (see below) on a set of potato differential lines each possessing one of the *R1* to *R11* race-specific resistance genes derived from *Solanum demissum*, following a procedure described earlier (Flier *et al.*, 2007) with minor modification (Tian *et al.*, 2015).

DNA extraction and genetic analysis

Phytophthora infestans isolates were grown for about 2 weeks in darkness at 16°C in rye–sucrose broth. The mycelium was harvested, lyophilized and stored at −80°C. Genomic DNA of *P. infestans* was extracted as described by Griffith & Shaw (1998) and stored in TE (10 mM Tris-HCl, 0·1 mM EDTA, pH 8·0) at −20°C until use.

The mtDNA haplotype was determined according to the method described by Tian *et al.* (2015). Genotyping using SSR markers was performed according to the method described by Li *et al.* (2010). Markers used included Pi02, Pi63 and D13 (Lees *et al.*, 2006; www.eucablight.org), Pi4B and G11 (Knapova & Gisi, 2002), and SSR4, SSR8 and SSR11 (Li *et al.*, 2010). PCR products were separated by electrophoresis in

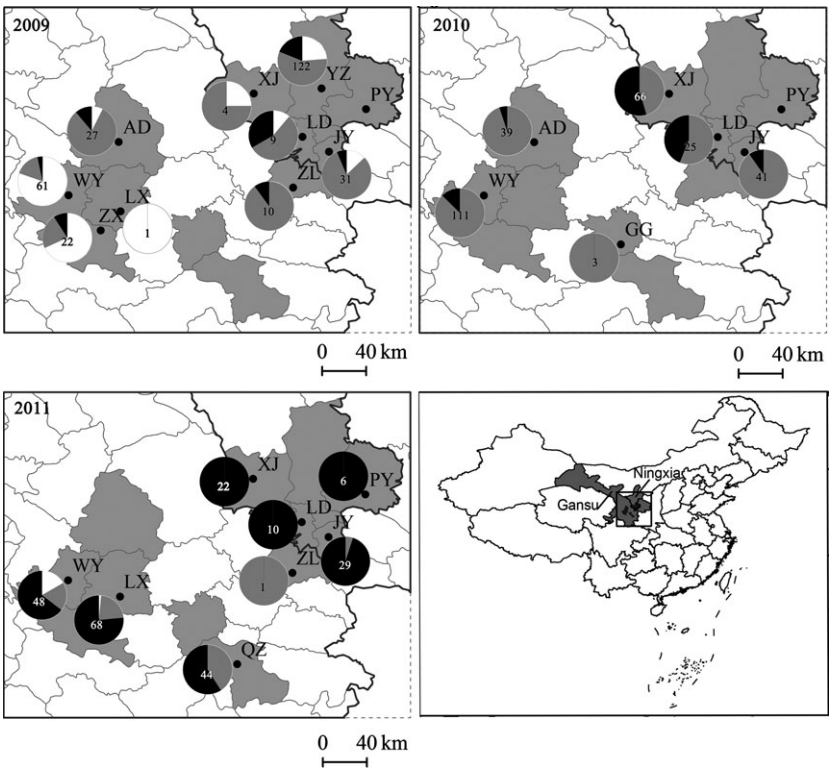

Figure 1 Sampling sites and mating type distribution of *Phytophthora infestans* isolates in northwestern China from 2009 to 2011. AD, Anding; WY, Weiyuan; LX, Longxi; ZX, Zhangxian; ZL; Zhuanglang; QZ, Qinzhou; GG, Gangu; XJ, Xiji; LD, Longde; JY, Jingyuan; YZ, Yuanzhou; PY, Pengyang. In the circle charts, the total numbers of isolates at each sampling site are indicated and the relative proportions of A1, A2 and self-fertile isolates are indicated as white, grey and black sections, respectively.

polyacrylamide non-denaturating gels according to the procedure described by Tian *et al.* (2015).

Population genetic analysis

For genetic analysis, *P. infestans* populations were defined according to geographic locations and sampling years. For example, the sampling sites in Zhuanglang of Gansu are close to Ningxia, so the obtained *P. infestans* isolates were analysed together with those collected from Ningxia.

In order to eliminate the bias imposed by the large asexual reproductive capacity and avoid redundancy in the collection, a clone-corrected data set was constructed by including only one representative isolate of each genotype (Chen & McDonald, 1996). To reveal genetic relationships among the detected *P. infestans* genotypes, an unrooted UPGMA tree, based on Bruvo's distance, was constructed using the clone-corrected data with the POPPR package in R v. 3.1.1 (Bruvo *et al.*, 2004; R Development Core Team, 2011; Kamvar *et al.*, 2014). Phylogenetic trees were visualized in FIGTREE v. 1.3.1 (Rambaut & Drummond, 2010).

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 & Taylor's index G and genotypic evenness was estimated with the index E_5 (Grünwald *et al.*, 2003). The parameters were calculated with POPPR. Gene diversity was estimated according to Nei (1973), the mean number of observed alleles (A_O), observed heterozygosity (H_O) and expected heterozygosity (H_E) were estimated using the clone-corrected data in 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 non-differentiation among populations was tested by comparing the observed θ (equivalent to Wright's F_{ST}) 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). Associations among SSR loci were tested by the index of multilocus linkage disequilibrium ($\bar{r}_d$) with POPPR. Analyses were conducted with clone-corrected data sets. 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).

Population genetic structure was analysed by conducting an analysis of molecular variance (AMOVA) on clone-corrected data using GENALEX v. 6.5 (Peakall & Smouse, 2006). Principle coordinate analysis (PCA) with the clone-corrected data set was based on the genetic distance metric of Bruvo *et al.* (2004) implemented in the R package POLYSAT v. 1.3.3 (Clark & Jasieniuk, 2011).

Results

Significant shift of the dominant mating types of *P. infestans*

A total of 959 single-lesion *P. infestans* isolates were purified in three consecutive years (2009–2011), comprising 418, 293 and 248 isolates for 2009, 2010 and 2011, respectively. Of 791 isolates examined for mating type, 14% were A1, 52% A2, and 34% self-fertile. The percentage of A2 isolates was 52% in 2009, 76% in 2010 and 20% in 2011. No A1 isolate was detected in 2010, while only a few were detected in 2011, at Weiyuan, Gansu. Seven sampled sites in 2009 and one in 2011 contained both A1 and A2 isolates. The distribution of mating types at each site in each year varied in the subpopulations (Fig. 1). A decline of A1 isolates in 2010 and 2011 was notable and a marked increase in A2 and self-fertile isolates was clear over the corresponding period (Fig. 1).

Analysis of genetic diversity in *P. infestans* populations

A total of 151 multilocus genotypes were identified from 959 examined *P. infestans* isolates (Fig. 2). Thirty-three alleles were detected at eight SSR loci, with the number of alleles per locus ranging from two (at locus SSR8) to seven (at locus Pi02). Up to 122 genotypes were identified in 2009, whereas only 22 and 23 were detected in 2010 and 2011, respectively. Among 151 multilocus genotypes, 94 were detected only once. Of the 94 unique genotypes, 76 were from 2009. The other 18 unique genotypes were detected in 2010 and 2011, nine in each year. Of the remaining 57 genotypes, 38 were detected for multiple isolates.

Up to 36 genotypes were shared across different years and regions. Genotypic diversity estimated with Stoddart & Taylor's index was low for *P. infestans* populations. Higher G values for genotypic diversity and higher values for the evenness index E_5 were observed in the two subpopulations in 2009 than those in 2010 or 2011 (Table 1).

Clonality in *P. infestans* populations

The observed heterozygosity (H_O) varied between 0·468 (Gansuin 2010) to 0·584 (Gansu and Ningxia, 2009–2011) and in all populations it was higher than the expected heterozygosity and gave a negative fixation index (except Gansu in 2009; Table 1). The highest unbiased expected heterozygosity ($H_E = 0{\cdot}530$) was observed for the 2009 population in Gansu, whereas the lowest ($H_E = 0{\cdot}281$) was observed in Ningxia in 2010. F_{IS} multilocus estimates were highly variable among *P. infestans* populations, ranging from −0·808 to 0·117. Although negative values for F_{IS} were observed among nearly all of the *P. infestans*, a significant excess in heterozygotes was detected in *P. infestans* populations of 2010 and 2011 (Table 1, $P < 0{\cdot}05$).

The multilocus linkage disequilibrium ($\bar{r}_d$) tests for *P. infestans* populations from two main regions in three consecutive years showed different results among *P. infestans* populations in 2009, 2010 and 2011 (Table 1). Tests on all *P. infestans* isolates collected in 2010 and 2011 rejected the null hypothesis of recombination ($P < 0{\cdot}01$) (data not shown). The same results were obtained after clone correction, which provided evidence for significant linkage disequilibria and supported the hypothesis of a clonally reproducing population. In contrast, the $\bar{r}_d$ values observed in *P. infestans* populations collected in 2009, for both full and clone-corrected data sets, indicated linkage equilibrium. Taken together, the results obtained from these analyses indicated that

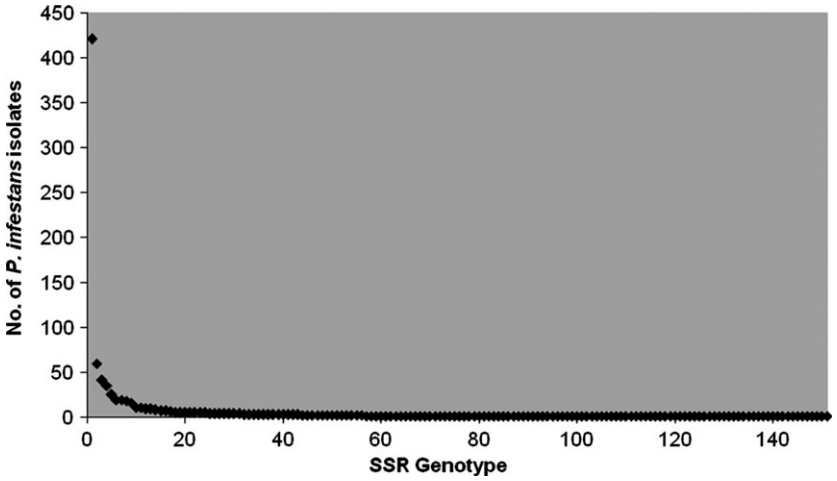

Figure 2 Number of isolates with different multilocus SSR genotypes detected in *Phytophthora infestans* populations in northwestern China, from 2009 to 2011. Each diamond indicates a unique SSR genotype that has been allocated a number from 1 to 151.

Table 1 Genetic diversity of *Phytophthora infestans* populations in northwestern China

	2009		2010		2011		2009–11		
Parameter	Gansu	Ningxia	Gansu	Ningxia	Gansu	Ningxia	Gansu	Ningxia	Gansu & Ningxia
N	176	242	157	136	189	59	522	437	959
NG	49	78	12	15	17	4	86	97	151
G^a	9·9	11·8	1·6	2·6	2·8	1·6	4·9	5·0	4·9
R^a	0·051	0·045	0·004	0·012	0·010	0·010	0·007	0·009	0·004
$E_5{}^a$	0·166	0·136	0·089	0·090	0·099	0·118	0·120	0·107	0·106
$A_O{}^a$	2·9	3·6	2·5	3·0	2·8	2·3	3·9	3·9	4·4
$H_E{}^a$	0·530	0·421	0·286	0·281	0·322	0·306	0·440	0·372	0·416
$H_O{}^a$	0·468	0·578	0·512	0·508	0·577	0·492	0·559	0·547	0·584
F_{IS}	0·117	−0·373*	−0·790*	−0·808*	−0·792*	−0·608*	−0·270*	−0·470*	−0·404*
$\bar{r}_d{}^a$	0·022	0·020	0·309*	0·269*	0·279*	0·315*	0·264*	0·257*	0·260*

N, number of *P. infestans* isolates; *NG*, number of multilocus genotypes; *G*, Stoddart & Taylor's genotypic diversity index; *R*, index of richness, $R = (G - 1)/(N - 1)$; E_5, index of eveness; A_O, mean number of observed alleles per locus; H_E, unbiased expected heterozygosity (Nei, 1973); H_O, observed heterozygosity; F_{IS}, fixation index where $F_{IS} = 1 - Ho/H_E$; $\bar{r}_d$, multilocus linkage disequilibrium.
[a]Tests were conducted with clone-corrected data sets.
*Multilocus linkage disequilibrium significant ($P < 0{\cdot}05$).

P. infestans populations in northwestern China were strictly clonally reproducing while *P. infestans* population in 2009 showed signs of linkage equilibrium.

Population genetic structure of *P. infestans*

No genetic differentiation was detected among populations in different sampling years (except population 2009 Gansu) nor among populations in different sampling regions in the same year (except two populations in 2009) when estimated with Weir & Cockerham (1984) coefficient of genetic differentiation θ (Table 2). The AMOVA of microsatellite genotype data revealed that 90% of the genetic variation was within populations, whereas the remaining (10%) was among populations ($P < 0{\cdot}01$) (Table 3).

Phylogeny analysis indicated two clusters in the *P. infestans* populations, and this was validated by PCA analysis (Fig. 3). Cluster I included 165 isolates and 54 multilocus SSR genotypes, with 120 isolates from Gansu and 32 from Ningxia in 2009. The other 13 isolates were one and five from Gansu in 2010 and 2011, respectively, and seven from Ningxia in 2011. The majority of isolates in this cluster were of mating type A1 and the mtDNA haplotype IIa. Of the 54 multilocus genotypes in this cluster, 38 were unique, each represented by a single isolate, from Gansu (16) and Ningxia (22) in 2009. The dominant genotype (SG-37) in Gansu in 2009 was in this cluster (Table S1; Fig. 4).

Cluster II consisted of 794 isolates and 97 multilocus SSR genotypes. This cluster contained isolates from all sampling regions and all years. The majority of A2 and self-fertile isolates of the Ia haplotype belonged to this cluster. The dominant genotype, SG-34, in the two consecutive years (2010 and 2011) was also in this cluster (Fig. 4). Among the 97 multilocus SSR genotypes, 55 unique genotypes were distributed across five populations, except Ningxia, in 2011. The dominant genotype

Table 2 Pairwise population differentiation estimated at eight SSR loci between populations of *Phytophthora infestans* in northwestern China

Population	2009 Gansu	2009 Ningxia	2010 Gansu	2010 Ningxia	2011 Gansu	2011 Ningxia
2009 Gansu		**	**	**	**	**
2009 Ningxia	0·3738		1	1	1	1
2010 Gansu	0·5607	0·1372		1	1	1
2010 Ningxia	0·5431	0·1400	0·0444		1	1
2011 Gansu	0·4776	0·1333	0·0835	0·0972		1
2011 Ningxia	0·4721	0·0993	0·0390	0·0733	0·0487	

Above diagonal, *P* values; below diagonal, θ values.
θ is Weir & Cockerham's coefficient of genetic differentiation and were generated by bootstrapping over loci (1000 replications). The observed θ value was compared with the value calculated for data sets in which alleles were resampled without replacement (1000 randomizations). Nei (1973) genetic unbiased distances among populations were calculated as implemented in TFPGA. The resulting distance matrix was used to construct a phenogram based on the UPGMA algorithm in TFPGA. Statistical support for phenogram branches was obtained using 1000 bootstrapped samples of the data set. An exact test using a Monte Carlo approach (10 batches, 2000 permutations per batch, and 1000 dememorization steps) was performed on pairwise combinations of populations using clone-corrected data sets.
$^{**}P < 0{\cdot}01$, significant differentiation among populations.

Table 3 Analysis of molecular variance of *Phytophthora infestans* populations on the basis of SSR data in northwestern China

Source of variation	d.f.	SS	Variation (%)
Among populations	1	5·091	10·0
Within populations	24	54·000	90·0**

d.f., degrees of freedom; SS, sum of squared observations; % variation, percentage of total variance.
P values are based on 1000 permutations; $^{**}P < 0{\cdot}01$.

(SG-34) in this cluster was detected in nearly all sampled regions, representing 53·0% of all isolates in this cluster. The genotype SG-34 comprised 14·6% of the isolates collected in 2009, 68·9 and 59·2% in 2010 and 2011, respectively. In addition, there were some regional differences of this genotype among sampling years. SG-34 was only detected at three sites in Ningxia (Jingyuan, Pengyang and Yuanzhou) in 2009, while it was detected at an additional six sites in Gansu (Anding, Weiyuan, Longxi, Zhuanglang, Gangu and Qinzhou) and two in Ningxia (Longde and Xiji) in the following years 2010 and 2011.

Broad-spectrum pathotype dominated *P. infestans* populations

Of 74 randomly selected but different SSR genotype-based isolates examined, only four pathotypes were distinguished, of which the pathotype virulent against all 11 *R* genes (*R1–R11*) was the most frequent and was widely distributed. Pathotypes virulent against *R3*, *R4*, *R6*, *R8*, *R9*, *R10* and *R11*, or virulent against *R3*, *R4*, *R6*, R8, *R10* and *R11*, were detected in isolates from Zhuanglang and Jingyuan in 2009, respectively. The pathotype virulent against *R3*, *R4* and *R10* was detected for four isolates from Yuanzhou, Ningxia. The results showed that the broad-spectrum pathotype virulent against all 11 *R* genes (*R1–R11*) was dominant in northwestern China.

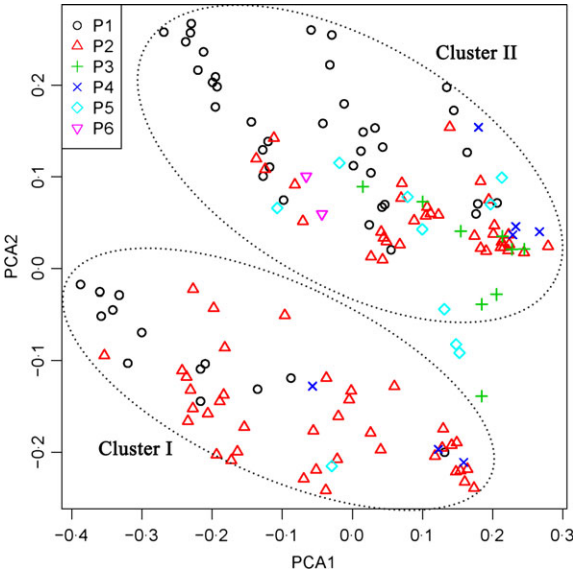

Figure 3 Principal coordinate analysis (PCA) of *Phytophthora infestans* populations in northwestern China. The analysis was performed on the 151 *P. infestans* multilocus genotypes defined with eight SSR markers, followed by a non-hierarchical classification. Each point represents a genotype. P1–P6 represent six *P. infestans* populations: P1, 2009 Gansu; P2, 2009 Ningxia; P3, 2010 Gansu; P4, 2010 Ningxia; P5, 2011 Gansu; P6, 2011 Ningxia.

Low level of mtDNA haplotype diversity in *P. infestans* populations

Three mtDNA haplotypes (Ia, IIa and IIb) were detected from the 507 *P. infestans* isolates examined. Compared to the isolates of 2009, the frequency of haplotype Ia increased sharply and became dominant with frequencies of 98·8 and 96·0% in 2010 and 2011, respectively (Table 4), indicating dramatic changes of *P. infestans* populations between potato growing seasons. Haplotype IIb was detected for a single isolate from Jingyuan, Ningxia in 2009.

Phylogenetic analysis of *P. infestans*

To examine the genetic relationship between *P. infestans* in northwestern China and the European epidemic clonal lineage 13-A2, a phylogenetic tree was constructed based on seven common SSR loci (Pi4B, Pi63, G11, D13, SSR4, SSR8 and SSR11) by the UPGMA method using shared allele distance. The result showed that the isolates were grouped into two clearly different clusters (Fig. 4),

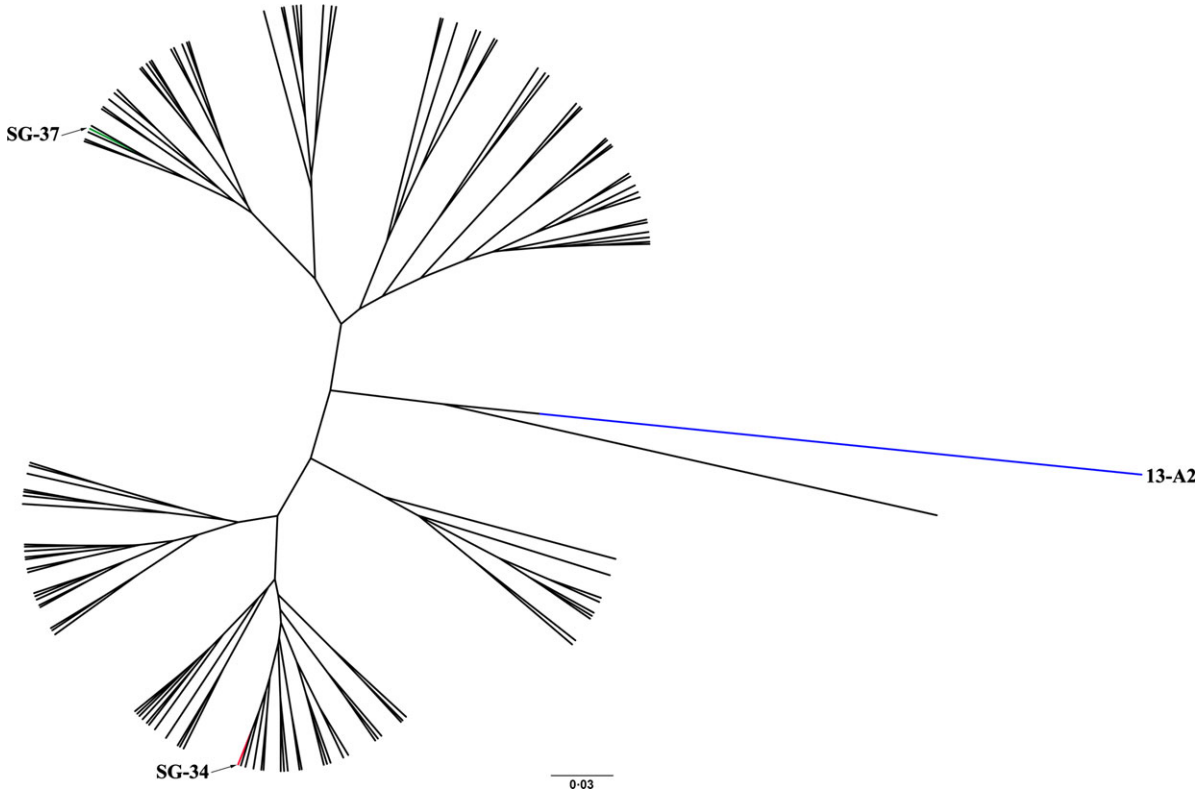

Figure 4 Phylogenetic analysis of *Phytophthora infestans* from northwestern China and the 13-A2 lineage. The analysis was carried out using Bruvo's distances based on seven common SSR markers (Pi4B, Pi63, SSR4, SSR8, SSR11, G11 and D13). The tree contains 151 clone-corrected isolates from northwestern China and five reference isolates of the 13-A2 lineage. The common SSR genotypes SG-34 and SG-37 are indicated. The SSR data of the reference isolates are described in Li *et al.* (2013a).

with one consisting of all Chinese isolates and the other of the reference isolates of 13-A2 lineage.

Discussion

A better understanding of *P. infestans* population dynamics would contribute to effective long-term disease management strategies. Populations of *P. infestans* in China have undergone genetic changes over the years, as shown by samples collected before 1999 containing fewer A2 isolates than those reported recently (Gotoh *et al.*, 2005). Moreover, reports from other laboratories have shown that A2 isolates were not detected in areas where they had been reported earlier (Guo *et al.*, 2009; Li *et al.*, 2013b). The present intensive survey of the mating type distribution of *P. infestans* in northwestern China has shown that isolates of the A1 mating type were widely distributed in 2009. Nonetheless, a notable shift took place in 2010, when no A1 was detected and A2 became predominant in the populations. The observed shift in mating type distribution in this study, together with the dramatic shift of mtDNA haplotypes, indicated that migration and selection at the end of the growing season might have strongly influenced population structure and diversity in regional populations.

Table 4 mtDNA haplotypes of *Phytophthora infestans* isolates in northwestern China during 2009–2011

		2009			2010			2011		
Region	Site	Ia	IIa	IIb	Ia	IIa	IIb	Ia	IIa	IIb
Gansu	Anding	3	1	0	26	0	0	–	–	–
	Weiyuan	8	41	0	93	0	0	34	8	0
	Longxi	0	0	0	–	–	–	48	0	0
	Zhangxian	2	2	0	–	–	–	–	–	–
	Gangu	–	–	–	2	0	0	–	–	–
	Qinzhou	–	–	–	–	–	–	54	0	0
	Zhuanglang	1	0	0	–	–	–	0	0	0
Total		14	44	0	121	0	0	136	8	0
Ningxia	Jingyuan	9	6	1	15	0	0	18	0	0
	Longde	7	1	0	5	0	0	10	0	0
	Yuanzhou	47	14	0	–	–	–	–	–	–
	Xiji	2	2	0	17	2	0	23	0	0
	Pengyang	0	0	0	–	–	–	5	0	0
Total		65	23	1	37	2	0	56	0	0

The results revealed that the genetic diversity of *P. infestans* populations in both 2010 and in 2011 was lower than that in 2009 in northwestern China. For the 2010 and 2011 populations, the results showed strong indication of an asexual propagation of clonal lineages. This

was supported by the genotypic diversity in the *P. infestans* populations being low, with most isolates belonging to one clonal lineage. In a typical sexually reproducing *P. infestans* population, like that in central Mexico, a high level of genotypic diversity is notable (Grünwald *et al.*, 2003). In addition, in the present study, most isolates from 2010 and 2011 were of the same mating type (self-fertile isolates could undergo sexual reproduction although they are of A2 background). Furthermore, the significant negative F_{IS} and higher level of linkage disequilibrium within *P. infestans* populations strongly indicated that these two populations were strictly clonal, although the frequency of self-fertile isolates increased.

The results for the 2009 *P. infestans* population were in contrast to those of 2010 and 2011; both mating types were detected and, more importantly, the results of multilocus linkage disequilibrium ($\bar{r}_d$) analyses performed without multicopies were consistent with the null hypothesis of recombination. Among 94 unique genotypes detected in the populations, as many as 76 unique genotypes were detected in 2009. These may have arisen by sexual recombination, but were unable to survive into the next season. Meanwhile, the existence of a high proportion of unique genotypes was probably the cause of genetic differentiation between the Gansu population in 2009 and other populations (Table 2). Apart from the presence of both mating types, many factors, such as temperature and rainfall, are involved in the natural occurrence of sexual recombination (Romero-Montes *et al.*, 2008; Brurberg *et al.*, 2011; Yuen & Andersson, 2013). However, no oospores were detected microscopically from hundreds of samples (mainly diseased leaves, examined after heat treatment with 3% NaOH). It is possible that sexual reproduction took place in 2008 or earlier, and a number of the offspring survived clonally. However, previous characterization of *P. infestans* in northwestern China before 2008 indicated dominance of the A1 mating type, which suggests that there was no sexual reproduction (Akino *et al.*, 2004; Guo *et al.*, 2009). It is also possible that the weather conditions temporarily favoured limited sexual reproduction in *P. infestans* populations in 2009. While the presence of sexual reproduction in 2009 populations is not certain, the results from analysis of subsequent *P. infestans* populations of 2010 and 2011 indicate their strict asexual reproduction. The present results indicate that clonal reproduction in *P. infestans* is widespread and sexual reproduction is not favoured under natural conditions in northwestern China.

It seems clear that there were migrations among *P. infestans* populations in the 3 years, particularly in 2010 and 2011, as shown by most isolates, sampled in different years and regions, sharing identical multilocus SSR genotypes, with the majority of isolates belonging to the dominant genotype. The detection of identical multilocus genotypes in distant subregions is direct evidence of long distance migration of clonal lineages, most probably via seed tubers. In northwestern China, the widespread occurrence of these common genotypes, and their persistence at the same or distant sites for several years, strongly indicates that they were asexually reproducing clones, which survived within tubers over the winter period in seed stores; this is consistent with potato cultivation practice in the region, where growers use a combination of home-saved and imported seed. Thus, effective management of late blight in the region should involve quality control of seed tubers leading to the planting of pathogen-free seed tubers, as well as fungicide treatment of seed tubers.

The phylogenetic analysis in this study has shown that *P. infestans* isolates in China are genetically distant from European lineages, including the recently identified 13-A2 lineage in the UK. In the present investigation, no isolates with genotype 13-A2 were detected in *P. infestans* populations in northwestern China, although its presence was reported in Sichuan and Yunnan provinces, southwestern China (Li *et al.*, 2013b). Although both the Chinese isolates and the European 13-A2 isolates are of A2 mating type and have mtDNA haplotype Ia, they have different SSR fingerprints. Most importantly, most isolates in the 13-A2 lineage are heterozygous at three loci (Pi4B, D13 and G11) and homozygous at locus SSR11 (Li *et al.*, 2013a), whereas the clonal lineages determined in this study are homozygous and heterozygous, respectively at these loci.

Changes in *P. infestans* population structure were also shown in the increasing frequency of self-fertile isolates in populations from 2009 to 2011. The existence of self-fertile *P. infestans* isolates in China has been mentioned by several researchers from as early as 1965, in which one isolate, from among 84 isolates collected across the country, was identified to be homothallic, confirmed by single sporangium or single zoospore isolation. (Huang, 2002). A recent study carried out by Han *et al.* (2013) detected A1 mating type and self-fertile isolates in Gansu in 2007, in which 15 out of 85 isolates were self-fertile. In addition, self-fertile isolates have been identified in many other countries, including Japan, Canada, the UK and the USA (Smart *et al.*, 1998). Smart *et al.* (2000) confirmed that the self-fertile isolates were neither the result of contamination, heterokaryon formation, long-term *in vitro* culturing, nor a result of apomixis. Further studies showed that environmental and genetic factors probably contribute to self-fertility in *P. infestans* (Smart *et al.*, 2000). However, little is known about the underlying genetic mechanisms of self-fertility in *P. infestans* under natural conditions and their roles in initiating plant infections. Given that a relatively high proportion of isolates detected in northwestern China were self-fertile, further research into the characteristics of these isolates is required in future studies.

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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 eight SSR loci analysed for the 151 multilocus SSR genotypes of *Phytophthora infestans* detected in northwestern China from 2009 to 2011.