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Population structure and host range of the potato late blight pathogen *Phytophthora infestans* in Peru spanning two decades

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The genetic diversity of the late blight pathogen *Phytophthora infestans* infecting cultivated potato and alternative hosts growing in the vicinity of fields in the main potato-growing areas of the Peruvian Andes was characterized using collections from 1997–2013 as reference. The Peruvian *P. infestans* population, including previously collected and current isolates, consists of four clonal lineages (EC-1, US-1, PE-3 and PE-7) that belong to the A1 mating type and have been present in the country for decades. The first report of US-1 was in isolates collected between 1982 and 1986; meanwhile, EC-1 and PE-3 appeared for the first time in isolates from 1992 and PE-7 was found in 1997. The pathogen has a very broad host range among the solanaceous plants infecting cultivated potato, tomato, pear melon and several wild species. The solanaceous species growing in the vicinity of the potato fields sampled were identified and surveyed for late blight-like symptoms. *Phytophthora infestans* was isolated from nine wild species, including three new host species: *Solanum zahlbruckneri*, *Solanum grandidentatum* and *Iochroma grandiflorum*. There was no clear host specialization, but geographical substructuring was found as well as changes in the pathogen populations at the regional level. The clonal lineage EC-1, which is mostly resistant to metalaxyl, has complex virulence and contains a high level of subclonal variation, continues to dominate the population. Some multilocus genotypes of the EC-1 lineage were sampled in high frequencies and were found among the previously collected and new samples.

Keywords: alternative hosts, genetic structure, late blight, pathogen, potato

Introduction

Late blight, caused by the oomycete *Phytophthora infestans*, is the most important potato disease in Peru (Pérez *et al.*, 2015) and worldwide (Haverkort *et al.*, 2008). Many potato varieties that were initially released as resistant to late blight have succumbed to the disease due to changes in the pathogen populations (Fry, 2008; Forbes, 2012). In Peru, in the traditional Andean farming context, native landrace potatoes that are mostly susceptible to late blight are cultivated in high mountain areas, where the disease is not frequently present. Recently, this has changed and late blight is currently found infecting potatoes at higher elevations than before. Severe yield losses, ranging from 50% to 100% were reported in native potatoes at 4090 m a.s.l. in 2010 in Peru (Pérez *et al.*, 2015), posing a risk to potato biodiversity in areas that were previously free from the disease. Late blight is controlled by frequent application of fungicides (Cooke *et al.*, 2011), although some strains are resistant to the fungicide metalaxyl (Pérez *et al.*, 2001).

Phytophthora infestans has a broad host range within the Solanaceae family including cultivated potato, tomato and pear melon, whilst another closely related pathogen, *P. andina*, infects tree tomato (Forbes *et al.*, 2016). The wild species reported as hosts of *P. infestans* in Peru include 24 tuber-bearing species and four non-tuber-bearing species (Table S1). Wild species are adapted to a wide variety of climates including coastal deserts, high elevation cold grasslands and lowland tropical rainforests. They commonly grow in secondary vegetation and in disturbed areas, including areas near agricultural fields. Due to their proximity to cultivated crops, these alternative hosts may act as refuges where *P. infestans* may survive outside the cultivated crop's growing season.

Global populations of *P. infestans* have been characterized in detail by molecular characterization and sequencing of mitochondrial DNA haplotypes (Griffith

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& Shaw, 1998; Avila-Adame *et al.*, 2006) and identification of clonal lineages through RFLP banding pattern with the RG57 probe (Goodwin *et al.*, 1992; Forbes *et al.*, 1998), multilocus genotyping (Gomez-Alpizar *et al.*, 2007), AFLP (Flier *et al.*, 2003) or simple sequence repeats (SSR; Lees *et al.*, 2006; Li *et al.*, 2013; Saville *et al.*, 2016). More recently, new techniques such as next-generation sequencing of whole genomes (Martin *et al.*, 2016), mitochondrial genome sequencing (e.g. Yoshida *et al.*, 2013; Martin *et al.*, 2014), transcriptome sequencing (Pais *et al.*, 2018), and genotyping by sequencing (GBS) (Grünwald *et al.*, 2017) have also been applied, but the 12-plex SSR microsatellite markers have become the global standard for quickly genotyping large numbers of samples and classifying lineages (Li *et al.*, 2013).

A comprehensive analysis of the diversity of the late blight pathogen in Peru showed that Peruvian *P. infestans* populations consist of clonally reproducing lineages belonging to a single mating type A1 (Pérez *et al.*, 2001). Clonal lineages EC-1 and US-1 were reported on modern varieties, and PE-3 and PE-7 on native varieties (Pérez *et al.*, 2001) or wild Solanaceae species (Garry *et al.*, 2005a). EC-1, which is also present in Ecuador and Colombia (Cárdenas *et al.*, 2011; Delgado *et al.*, 2013), has been the dominating lineage for several decades (Pérez *et al.*, 2001). According to mitochondrial haplotypes, the Peruvian *P. infestans* lineages were either Ia (PE-3, PE-7), Ib (US-1) or IIa (EC-1) (Pérez *et al.*, 2001; Garry *et al.*, 2005a). The US-1 clonal lineage was dominant in Peru until the late 1990s when EC-1 and PE-3 were found (Pérez *et al.*, 2001). Since then, EC-1 spread rapidly and US-1 was only dominant in Puno (Pérez *et al.*, 2001).

The evolution of these lineages was recently studied by gene sequencing and SSR genotyping and comparison was made with the lineages of *P. infestans* in Mexico (Goss *et al.*, 2014; Saville *et al.*, 2016). The results suggest that US-1 diversified before EC-1, PE-3 and PE-7 and the earliest Andean lineage was the FAM-1 lineage found in Colombia and central America in the early 20th century. The US-1 lineage dominated worldwide populations from the mid-twentieth century until the late 1990s (Goodwin *et al.*, 1994). US-1 is an older lineage in the Andean region and may have its origins in a secondary metapopulation outside Mexico (Yoshida *et al.*, 2013); it was also found in herbarium samples collected from Bolivia (1967) and Ecuador (1944; May & Ristaino, 2004). The metapopulation that was the source of US-1 most probably originated in the Andean region (Saville *et al.*, 2016; Saville & Ristaino, 2019).

Haplotype Ia (PE-3 and PE-7) is the same haplotype as the lineage that caused the Irish potato famine (Gomez-Alpizar *et al.*, 2007; Martin *et al.*, 2014), while US-1 is more recently derived (Martin *et al.*, 2016). Both PE-3 and PE-7 most probably originated via admixture or hybridization; PE-3 is possibly an admixture of US-1 and an unknown population, while PE-7 has a more complex ancestry (Goss *et al.*, 2014). However, more sequencing is needed to determine their precise evolutionary history.

Considering the large geographical area where potato is cultivated in the Peruvian Andes and the many alternative solanaceous hosts that grow in the proximity of potato fields, this study was undertaken to test the hypothesis that the *P. infestans* population is substructured by geography and by host species. In addition, the hypothesis that the population is clonal and potentially new clonal lineages have emerged was tested. To this end, *P. infestans* isolates were sampled from cultivated potato and alternative hosts from the Solanaceae in the main potato cultivation areas in the Peruvian Andes. The *P. infestans* isolates sampled, as well as isolates available in the CIP *Phytophthora* collection, were genotyped using 12-plex SSR markers and a subset of the new isolates was phenotyped for mefenoxam sensitivity, host range, virulence and mating type.

Materials and methods

Sampling and sample processing

Leaf samples with late blight-like symptoms were collected during 2016–2017 from the potato production areas in the Peruvian Andes between the latitudes 13° S and 5° S at 1844–4125 m elevation. Potato fields and surrounding areas were surveyed for all wild and cultivated Solanaceae species that could serve as alternative hosts of *P. infestans*. The species were identified by Solanaceae taxonomic specialists (authors P. Gonzáles, T. Särkinen and M. Cueva). Voucher samples of flowers and/or fruits were collected from all wild Solanaceae species encountered; these were pressed, dried and stored in herbaria. Species were identified using taxonomic keys, updated literature (Edmonds, 1972; Brako & Zarucchi, 1993; Ochoa, 1999; Knapp, 2002; Anderson *et al.*, 2006; Peralta *et al.*, 2008; Särkinen *et al.*, 2015), type specimens available online (Global Plants JSTOR https://plants.jstor.org/), and comparison with herbarium samples named by taxonomic experts available at the Natural History Museum of the National University of San Marcos (USM). The classification system followed APG III (Angiosperm Phylogeny Group, 2009) and Olmstead *et al.* (2008) for groups within the family. All vouchers were deposited at USM herbarium, Lima.

Plants of potato and all Solanaceae species were inspected for late blight-like symptoms and leaves with single lesions were collected. Lesions were pressed on FTA cards following the instructions specified at http:/euroblight/protocols/.

All samples from the alternative hosts and at least one sample from each potato field were placed abaxial side up in a sealed Petri dishes containing a layer of 1.5% water agar and brought to the laboratory for isolation of pure cultures. In the laboratory, the sporangia were suspended in distilled water, filtered through cheesecloth, collected on a 10 μm filter and rinsed with sterile water. The filter system allowed for efficient recovery of sporangia, even when the infected tissue was several days old and contaminated with bacteria and saprophytes. The sporangial suspension was refrigerated at 5–8 °C to promote the liberation of zoospores. Potato tuber slices (*Solanum tuberosum* Chaucha group cv. Huayro) were inoculated with 20 μL of the zoospore suspension per slice and incubated at 18 °C for 5–7 days in a moist chamber with photoperiod of 12 h. Mycelial fragments

were transferred aseptically to rye B agar and V8 agar. After 1–2 weeks, the growing colonies were transferred to rye A agar and maintained at 15 °C.

Genotypic characterization

Cultures of *P. infestans* were grown on pea broth (filtrate from 120 g autoclaved frozen peas per litre). For each isolate, four or five Petri dishes of pea broth were inoculated with mycelium from a colony actively growing on rye B agar and incubated at 18 °C in darkness without shaking. After 10 days, the hyphal tissue was harvested by vacuum filtration, enveloped in filter paper, and dried at room temperature using silica gel for 7–10 days. Dry tissue was ground in liquid nitrogen and DNA was extracted using the DNeasy Plant Mini kit (QIAGEN).

SSR genotypes were identified using the 12-plex microsatellite assay (Table S2; Li *et al.*, 2013). The Type-It Microsatellite PCR kit (QIAGEN) was used for PCRs following a modified protocol described by Saville *et al.* (2016). PCR conditions were as follows: 95 °C for 5 min; 28 cycles (33 cycles for FTA cards) of 95 °C for 30 s, 58 °C for 90 s, and 72 °C for 20 s; and a final extension at 60 °C for 30 min. PCR amplicons were sent to Jean Ristaino's laboratory at NC State or to Arizona State University for post-PCR processing in 96-well plates. For fragment analysis, 1–2 μL of PCR product was added to a 10.3 μL reaction mix consisting of 10 μL highly deionized formamide and 0.3 μL LIZ500 size standard (Applied Biosystems). Fragments were then analysed on an Applied Biosystems 3730xl DNA analyzer at the Genomic Sciences Laboratory at North Carolina State University or Arizona University. SSR alleles were sized by GENEMARKER v. 1.9 software (SoftGenetics) using the allele sizing parameters defined by Li *et al.* (2013) and a reference dataset consisting of Peruvian *Phytophthora* isolates previously genotyped by David Cooke's laboratory at The James Hutton Institute, UK. A set of 206 isolates from earlier collections (1996–2013) was included in the diversity analysis by SSR. Reference isolates from Peru with known SSR genotypes were included on each PCR plate and were used in the analysis (EC-1: POX067; US-1: PPU103; PE-7: PCA023; and PE-3: PCZ007).

Data analysis

All data analyses were conducted using the statistical software R v. 2.14.0 (R Core Team, 2011). Locus and population statistics were calculated using POPPR (Kamvar *et al.*, 2014). The hypothesis of clonality was tested using the index of association and the statistical significance was tested based on 999 permutations of each of the subsets of data using a *t*-test.

The population structure was determined using discriminant analysis of principal components (DAPC) in the ADEGENET 4 package (Pritchard *et al.*, 2000; Jombart *et al.*, 2010; Grünwald & Goss, 2011) following the detailed commands and instructions at https://grunwaldlab.github.io/Population_Genetics_in_R/DAPC.html. *K*-means clustering based on residual variance (within-groups sum of squares) was used to determine the structure in the dataset and thereby to group the isolates within clonal lineages. An iterative process was applied considering the genotypic classification of the control isolates and the assumption that the isolates similar to those need to form clear groups. *K*-means clustering was done by running successive *K*-means with an increasing number of clusters (*k*), after transforming data using a principal component analysis (PCA). PCA was based on the genetic distance metric of Bruvo *et al.* (2004) implemented in the POLYSAT package (Clark & Jasieniuk, 2011). The optimal number of clusters was determined by WSS and diffNgroup options. The cluster assignments of the isolates were then used as populations in the DAPC to partition the variance in the sample into between-group and within-group components. The appropriate number of principal components (PC) retained for the analysis was determined by DAPC cross-validation.

The population differentiation by geography was tested for the clone-corrected data using analysis of molecular variance (AMOVA) in the ADEGENET 4 package and considering the allele frequencies in calculating the distance matrix. Samples were grouped hierarchically into the main Andean biogeographical regions (North, Centre and South) and political departments (Amazonas, Ancash, Arequipa, Cajamarca, Cusco, Huancavelica, Huánuco, Junín, La Libertad, Lima, Pasco, Piura, Puno) based on geographical locations of the sampling sites as detailed in Table S3.

The analytical scripts and the data are available at https://github.com/hlindq/Pinf_Peru.

Phenotypic characterization

Mating type was determined by pairing each isolate in the collection with two isolates of known A1 and A2 mating types (Peruvian isolates POX067 from *S. tuberosum* and POX119 from *Solanum betaceum*, respectively) on 10% clarified V8 agar. Plates were placed in an incubator at 15 °C in the dark and examined for the presence of oospores after 4 weeks.

Metalaxyl resistance was measured with a procedure used previously for isolates collected from potato in Peru (Pérez *et al.*, 2001; Garry *et al.*, 2005a). Each isolate was plated on 10% V8 agar containing metalaxyl at concentrations of 0, 5, 50 and 100 ppm. A plug of mycelium was placed in the centre of the plate, and plates were incubated in darkness at 18 °C for 15 days before radial growth was measured. Isolates were classified as resistant (R), moderately resistance (MR) or sensitive (S) to the systemic fungicide as described by Therrien *et al.* (1993).

The specific virulence of 161 isolates was determined by inoculation of detached leaflets of a differential set of potato cultivars carrying the 11 known *Solanum demissum R* genes for resistance to *P. infestans*. Each virulence assay was repeated at least twice. Differentials were obtained from the Research Institute for Plant Protection, Wageningen, Netherlands: *R1* (CEBECO-43154-5; CIP800986), *R2* (CEBECO-44158-4; CIP800987), *R3* (CEBECO-4642-1; CIP800988), *R4* (CEBECO-4431-5; CIP800989), *R5* (Black 3053-18; CIP800990), *R6* (Black XD2-21; CIP800991), *R7* (Black 2182ef(7); CIP800992), *R8* (Black 2424a(5); CIP800993), *R9* (Black 2573; CIP800994), *R10* (Black 3618ad(1); CIP800995) and *R11* (Black 5008ab(6); CIP800996). In each experiment, at least one susceptible local variety was included as a control.

Inoculum for virulence tests was obtained from infected tuber slices incubated for 6–7 days at 18 °C in a moist chamber, with photoperiod of 12 h. Three leaflets were collected from the middle part of 45–60-day-old plants of each differential cultivar. Leaflets were placed in the lids of inverted Petri dishes lined with 1.5% water agar. A 20 μL drop of sporangial suspension in distilled water (approximately 5×10^3 sporangia mL^{-1}) was placed on the abaxial surface of each leaflet. Petri dishes were kept in darkness for 24 h at 15–18 °C, followed by 6 days with a photoperiod of 12 h (Pérez *et al.*, 2001). Isolates were considered virulent to a given *R* gene when the interaction resulted in a sporulating lesion. Isolates were considered avirulent when

there were no lesions or there were clear necrotic spots without sporulation.

Pathogenicity test and host range

To demonstrate the pathogenicity of *P. infestans* isolates obtained from alternative hosts, Koch's postulates were tested. Seeds and cuttings of alternative hosts were obtained from the same collection sites as the isolates and were propagated and kept in the greenhouse. To determine the host range, isolates were inoculated onto plants of *Solanum lycopersicum* 'Rio Grande', *S. tuberosum* 'Yungay', *Datura stramonium* and *Physalis peruviana*. Whole plants were inoculated before flowering with a suspension of sporangia (3×10^3 sporangia mL^{-1}), which was sprayed over the entire leaf area of the plants until they were completely wet (run-off). This was performed twice per isolate. The plants were evaluated from the third day post-inoculation, noting the presence of necrotic spots or sporulation on leaflets and stems.

Results

Samples recovered

Although the potato fields inspected had only a few symptoms due to efficient management of late blight with treatments applied by farmers, samples were recovered from nearly all potato fields inspected, 686 samples in total from cultivated potato. Solanaceae species were collected in the proximity of 85 potato fields located in eight departments mainly in the north and south of the country: Amazonas, Apurímac, Arequipa, Ayacucho, Cajamarca, Cusco, La Libertad and Piura. A total of 53 species were identified, which, according to the most current classification, corresponded to three subfamilies and 14 genera: Solanoideae (11 genera, 48 species), Nicotianoideae (one genus, two species) and Cestroideae (two genera, three species), the genus *Solanum* being the most diverse with 24 species (Table S4). Most Solanaceae species appeared free of late blight symptoms and *P. infestans* was successfully isolated only from nine wild species, resulting in a total of 34 samples from the alternative hosts (Table 1, Table S3). Three wild species were discovered for the first time to be hosts to *P. infestans* (*Solanum zahlbruckneri*, *Solanum grandidentatum* and *Iochroma grandiflorum*).

Host specificity analysis

Koch's postulates and host range tests were completed for the isolates originating from the alternative hosts. Plants of *I. grandiflorum* could not be grown in the greenhouse and therefore no pathogenicity tests were carried out with this species. The remaining species determined to be alternative hosts showed disease symptoms 5–6 days after inoculation (Fig. 1). The first symptoms consisted of necrotic spots on leaflets, young stems and petioles and sporulation mostly on the underside of leaves. With time, the symptoms became more severe. The pathogen was reisolated from the infected plants, and kept in a pure culture medium, under the same conditions as the original isolate.

When isolates obtained from alternative hosts were inoculated to determine their host range, all 34 isolates were found to be pathogenic on cultivated potato, but none could infect *P. peruviana*. Cultivated tomato was susceptible to isolates from *S. huancabambense* and *S. muricatum* and resistant to some isolates from *I. grandiflorum*, *S. candolleanum*, *S. caripense*, *S. ochranthum* and *S. zahlbruckneri*, displaying a hypersensitive reaction (HR). *Datura stramonium* was infected by all isolates obtained from *S. muricatum* but in contrast, isolates from *I. grandiflorum*, *S. acaule*, *S. candolleanum*, *S. grandidentatum*, *S. ochranthum* and *S. zahlbruckneri* failed to infect (Table 2).

Genotypic characterization

Identification of clonal lineages

A total of 720 isolates from the current population and 206 isolates collected between 1996 and 2013 were characterized for diversity based on SSR markers (Table S3). Previous studies using RFLP, SSR and mitochondrial haplotyping have identified four different clonal lineages of *P. infestans* in Peru: EC-1 (IIa), US-1 (Ib), PE-3 (Ia) and PE-7 (Ia) (Pérez *et al.*, 2001). An iterative process with three rounds of *K*-means clustering identified four groups that correspond to the previously described clonal lineages (Table 3) and form four clearly separate clusters in DAPC (Fig. 2).

A total of 260 multilocus genotypes (MLG) were identified in the whole dataset. The hypothesis of clonality was tested by standardized index of association ($\bar{r}_d$), which indicated that, as expected, all populations (lineages) were reproducing clonally (Table 3). Genotypic richness (eMLG) and genotypic evenness (E_5) were highest in the PE-3 population, indicating that this population is the most diverse of the four (Table 3).

Population substructuring

EC-1-IIa clonal lineage showed the widest distribution across all three Andean regions, whereas PE-3 and US-1 lineages were generally found in lower frequency in all sampled areas (Fig. 3). A few notable exceptions included Tacna and Arequipa, which were dominated by the PE-3 lineage, and Puno where PE-7 was found to dominate (Fig. 3). AMOVA showed that geographical variation is partitioned hierarchically by lineage (EC-1, US-1, PE-3 and PE-7), region (North, Centre, South) and department (see the list of departments and their relation to the regions in Table S3). As expected, most of the variation was found between clonal lineages (65%). Geographical differentiation was significant within lineage ($P = 0.001$) as well as for samples within regions ($P = 0.023$; Table 4).

The effect of host species on population differentiation could not be reliably tested by AMOVA because there were too few samples collected from most of the

Region	Department	Cultivated potato	Other species	Species names
Centre	Ancash	85	1	*Solanum candolleanum*
	Huancavelica	130	4	*S. acaule*, *S. brevicaule*, *S. grasilifrons*
	Huánuco	81	0	—
	Junín	152	12	*S. candolleanum*, *S. chrysotrichum*, *S. paucissectum*
	Lima	3	17	*S. cantense*, *S. caripense*, *S. habrochaites*, *S. hypacrarthrum*, *S. medians*, *S. pennellii*, *S. peruvianum*, *S. wittmackii*
	Pasco	26	14	*S. chiquidenum*, *S. lycopersicum*, *S. muricatum*
North	Amazonas	64	10	*S. ochranthum*, *S. zahlbruckneri*
	Cajamarca	67	13	*S. caripense*, *S. habrochaites*, *S. peruvianum*, *S. lycopersicum*
	La Libertad	43	7	*S. caripense*, *S. chomatophilum*, *S. habrochaites*, *S. mochiquense*, *S. sogarandinum*
	Piura	68	21	*Iochroma grandiflorum*, *S. caripense*, *S. chiquidenum*, *S. grandidentatum*, *S. habrochaites*, *S. huancabambense*, *S. lycopersicum*
South	Apurímac	2	0	—
	Arequipa	3	0	
	Cusco	64	0	
	Puno	38	0	
	Tacna	1	0	
Grand total		827	99	

Table 1 Number of *Phytophthora infestans* isolates obtained from cultivated potato and other Solanaceae host species from the different geographical locations in Peru.

This table includes isolates from old (1997–2013) and recent (2016–2017) collections.

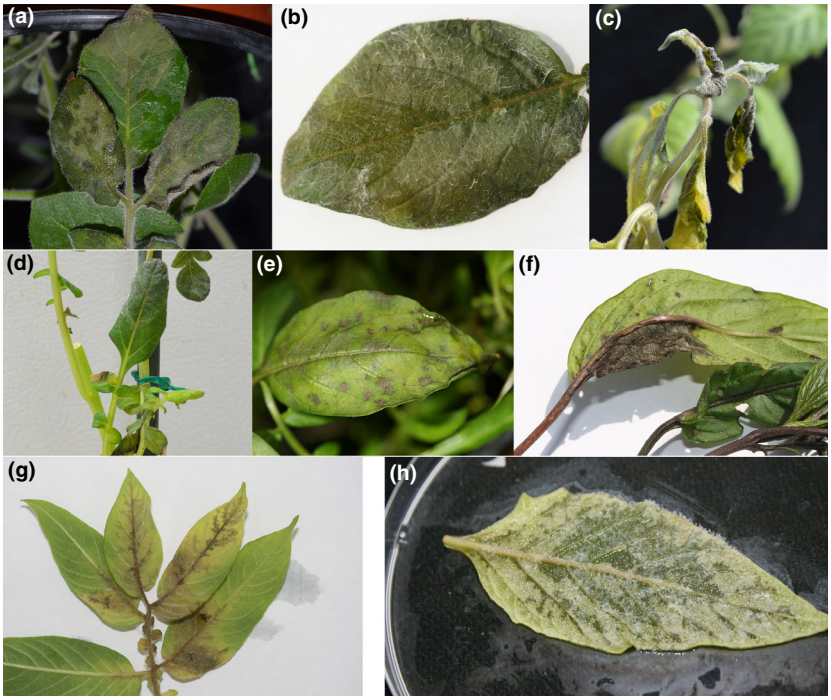

Figure 1 Symptoms of late blight (caused by *Phytophthora infestans*) on alternative hosts after reisolation and inoculation: (a) *Solanum caripense*, (b) *S. candolleanum*, (c) *S. grandidentatum*, (d) *S. acaule*, (e) *S. huancabambense*, (f) *S. muricatum*, (g) *S. ochranthum*, (h) *S. zahlbruckneri*. High resolution versions of the photographs have been deposited at https://doi.org/10.6084/m9.figshare.9636941.v2. [Colour figure can be viewed at wileyonlinelibrary.com].

alternative host species (Table S3). Most of the alternative host species that were sampled more than once were represented by more than one MLG. *Solanum caripense* had most MLGs, while *S. chrysotrichum* had most MLGs relative to the sample size. *Solanum muricatum* was the least diverse as the 11 isolates collected belonged to only two MLGs. According to the rarefaction analysis, larger sample sizes from the alternative hosts would

Table 2 Host specificity of the *Phytophthora infestans* isolates collected from alternative hosts in Peru in 2016–2017.

Host	Isolate	Potato cv. Yungay	Tomato cv. Rio Grande	*Datura stramonium*	*Physalis peruviana*
Solanum zahlbruckneri	PAMA1	+	+	−	−
	PAMA2	+	HR	−	−
	PAMA3	+	+	−	−
	PAMA4	+	+	−	−
	PAMA5	+	+	−	−
Solanum acaule	PHCA116	+	+	−	−
Iochroma grandiflorum	PPI71	+	+	−	−
	PPI72	+	HR	−	−
Solanum grandidentatum	PPI73	+	+	−	−
	PPI74	+	+	−	−
Solanum ochranthum	PAMA35	+	HR	−	−
	PAMA36	+	HR	−	−
	PAMA37	+	+	−	−
	PAMA38	+	HR	−	−
	PAMA39	+	+	−	−
Solanum candolleanum	PCO133	+	HR	−	−
Solanum caripense	PPI103	+	HR	+	−
	PPI104	+	+	+	−
	PPI105	+	−	+	−
	PPI106	+	−	+	−
	PPI107	+	−	+	−
	PPI108	+	HR	+	−
	PPI109	+	+	−	−
Solanum huancabambense	PPI110	+	+	+	−
	PPI111	+	+	−	−
	PPI112	+	+	−	−
	PPI113	+	+	−	−
Solanum muricatum	PPA87	+	+	+	−
	PPA88	+	+	+	−
	PPA89	+	+	+	−
	PPA90	+	+	+	−
	PPA91	+	+	+	−
	PPA92	+	+	+	−
	PPA93	+	+	+	−

+, compatible interaction; −, incompatible interaction; HR, hypersensitive response.

Table 3 Diversity statistics for microsatellite data from 12 microsatellite loci in populations of *Phytophthora infestans* from Peru by clonal lineage.

Population (clonal lineage)	*N*	MLG	eMLG	SE	*H*	*G*	λ	E_5	H_{exp}	$\bar{r}_d$	$P(\bar{r}_d)$
EC-1	763	178	23.006	2.778	3.806	14.584	0.931	0.309	0.409	0.068	0.001
PE-3	80	55	31.265	1.993	3.794	32.990	0.970	0.737	0.380	0.055	0.001
US-1	43	21	19.950	0.805	2.703	11.072	0.910	0.724	0.586	0.457	0.001
PE-7	40	6	6.000	0.000	1.197	2.703	0.630	0.737	0.572	0.963	0.001
Total	926	260	26.295	2.795	4.290	20.981	0.952	0.278	0.480	0.494	0.001

Clonal lineages were identified by *K*-means clustering. *N*, number of individuals; MLG, number of multilocus genotypes; eMLG, expected number of MLGs at smallest size of at least 10; SE, standard error; *H*, Shannon–Weiner index of MLG diversity; H_{exp}, Nei's 1978 expected heterozygosity; $\bar{r}_d$, standardized index of association.

probably lead to the discovery of further MLGs. Only *S. muricatum* was an exception, having a fully converged rarefaction curve (Fig. 4).

Variation at the field level

The current *P. infestans* populations were used to estimate variability at the field level. In total, 116 fields were sampled with an average of 6.2 samples and 3.9 MLGs per field. Although the genotypic richness was often directly proportional to the sample size, in some fields this was not the case and only few MLGs were found regardless of thorough sampling; this indicates that there may be differences in genotypic diversity among the fields (Fig. 5). The genotypic variation was partitioned by field

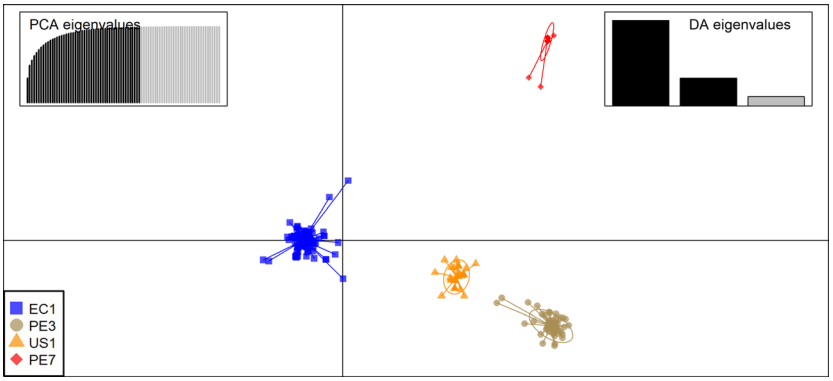

Figure 2 Discriminant analysis of principal components (DAPC) plot based on the microsatellite marker analysis separating the *Phytophthora infestans* isolates into four clonal lineages EC-1, PE-3, US-1 and PE-3. [Colour figure can be viewed at wileyonlinelibrary.com].

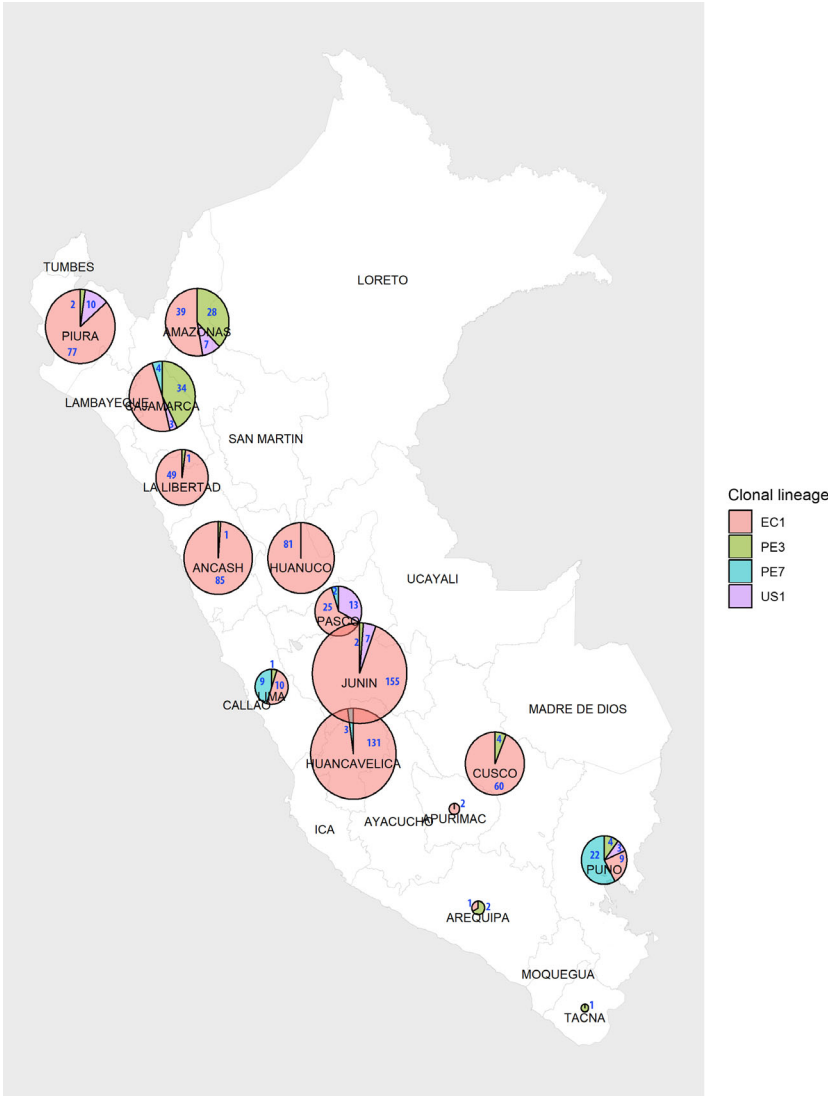

Figure 3 Geographical distribution of the clonal lineages EC-1, PE-3, PE-7 and US-1 among the *Phytophthora infestans* isolates (n = 926) collected in Peru between 1996 and 2017. The sizes of the pie charts are relative to the sample size. [Colour figure can be viewed at wileyonlinelibrary.com].

and host plants within field using AMOVA, and while no significant differences were found between hosts within the field (P = 0.459), the differences between fields were significant (P = 0.064; Table 5). The genotypic richness within fields also included different clonal lineages. Diversity of MLGs per field was sometimes highest in cultivated potatoes (e.g. field 182 in Amazonas in northern Peru) and other times highest in wild Solanaceae species (e.g. field 94 in Piura in northern Peru). Some MLGs were found only in wild Solanaceae species in a few fields in

Table 4 Hierarchical analysis of molecular variance (AMOVA) among isolates of *Phytophthora infestans* from Peru by clonal lineage (EC-1, PE-3, PE-7, US-1), region (North, Centre, South) and political department (Amazonas, Ancash, Apurimac, Arequipa, Cajamarca, Cusco, Huancavelica, Huanuco, Junin, La Libertad, Lima, Pasco, Piura, Puno, Tacna).

				Components of covariance		Monte-Carlo test for significance nrepet = 999			
	df	SS	MS	σ	%	Obs	Std. Obs.	Alter.	*P*-value
Between lineage	3	221.720	73.907	1.466	65.579	1.466	3.287	Greater	0.001
Between region within lineage	8	29.952	3.744	0.121	5.422	0.121	4.191	Greater	0.001
Between samples within region	24	24.978	1.041	0.048	2.145	0.048	2.292	Greater	0.023
Within samples	298	178.869	0.600	0.600	26.854	0.600	−39.661	Less	0.001
Total	333	455.519	1.368	2.235	100				

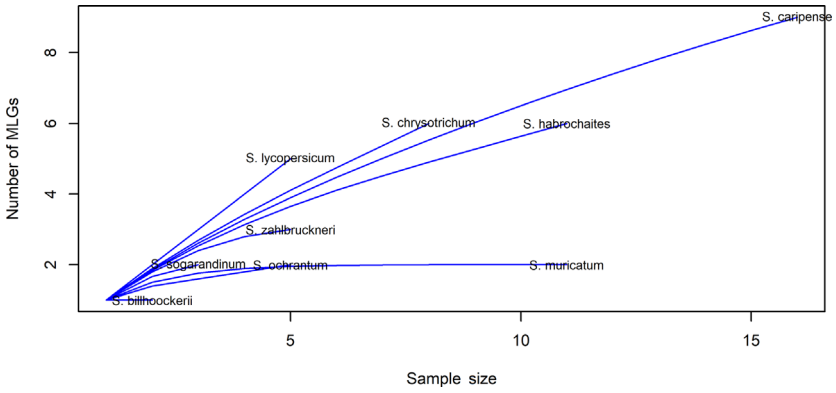

Figure 4 Rarefaction analysis of the genotypic richness encountered among the *Phytophthora infestans* isolates sampled from alternative host *Solanum* species. Genotypic richness is expressed as number of multilocus genotypes (MLG) identified in relation to the number of *P. infestans* isolates sampled. The genotypic richness of the isolates sampled from host species *Solanum huancabambense* and *S. peruvianum* fall on the same curve as those sampled from *S. lycopersicum*. The labels of the first two species are excluded from the plot for clarity. [Colour figure can be viewed at wileyonlinelibrary.com].

northern Peru, but were common in cultivated potato in several fields in central Peru, for example MLG221 – EC-1 was found in 21 potato fields in central Peru in cultivated potatoes, and in only one field in wild species, while in northern Peru the same MLG was found only in two fields in wild species (Fig. 6b, c; Table S3). Rarefaction analysis suggested that further MLGs could be discovered by increasing the number of isolates collected from most of the fields (Fig. 5).

Variation at lineage level

EC-1 was the dominant clonal lineage of *P. infestans* in Peru (Fig. 3). A large amount of genetic variation was found within the population, with 178 MLGs (Table 3), of which many were sampled more than once. The four most frequent MLGs were MLG179 (isolated 153 times), MLG257 (80 times), MLG221 (74 times) and MLG177 (35 times); all of these MLGs were present in the current as well as the old collections (Fig. 6a), were also found on hosts other than potato (Fig. 6b) and were sampled in at least two regions (Fig. 6c). MLG257 and MLG177 were found from all of the host groups except for cultivated tomato. MLG221 and MLG177 were sampled from wild potato, cultivated potato and wild species. In total, 24 MLGs were found from hosts other than cultivated potato and nine MLGs were found from multiple hosts (Table S3).

The clonal lineage PE-3 had 55 MLGs in total (Table 3) and was sampled from cultivated potato (51 MLGs), cultivated tomato (one MLG), a wild potato relative (one MLG) and wild Solanaceae species (two MLGs, all from old collections). The two most frequently sampled MLGs (MLG46 and MLG74) were sampled in several potato fields in northern Peru. No MLGs were detected across regions, host groups, or old and current collections. PE-3 was found mostly in northern Peru (Fig. 3), where 42 MLGs were found on cultivated potato (six in old and 37 in current collections) and one on cultivated tomato in the old collections (Table S3). MLGs from wild species were only present in the old collections and only found in central Peru. Nine MLGs were found in southern Peru and only in the old collections.

US-1 had 21 MLGs in total and was sampled from cultivated potato (five MLGs from old collections), cultivated tomato (two from old collections), wild potato (one from old collections), wild tomato (three; one from current, two from old collections), pear melon (five; two from current, three from old collections) and wild species (seven; two from current, five from old collections; Table S3). No MLGs were detected across regions, or old and current collections but two MLGs were shared among host groups. MLG69 was found on both pear melon and wild potato from northern Peru in the current collection and MLG80 was found on pear melon and cultivated potato from central Peru in the old collections. Lineage US-1 was found in northern (10 MLGs) and in central (eight MLGs) Peru from both old and current collections and in southern Peru (three MLGs) only

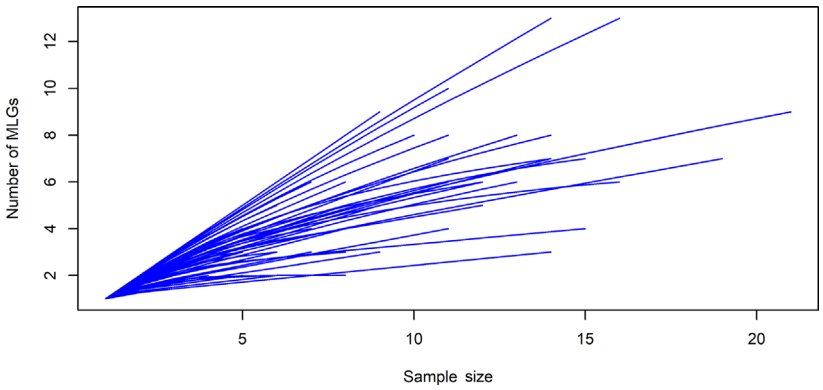

Figure 5 Rarefaction analysis of the genotypic richness of the *Phytophthora infestans* isolates at the field level. Genotypic richness is expressed as number of multilocus genotypes (MLGs) identified among the number of isolates sampled from each geographical location. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 5 Hierarchical analysis of molecular variance (AMOVA) among isolates of *Phytophthora infestans* from Peru (collected in 2016–2017) by geographical location (potato field) and host species.

				Components of covariance		Monte Carlo test of significance nrepet = 999			
	df	SS	MS	σ	%	Obs	Std. Obs.	Alter.	*P*-value
Between field	115	180.655	1.571	0.299	27.098	0.299	1.330	Greater	0.064
Between sample within field	5	3.079	0.616	−0.143	−12.914	−0.143	0.187	Greater	0.459
Within sample	332	314.677	0.948	0.948	85.816	0.948	−6.771	Less	0.001
Total	452	498.411	1.103	1.104	100				

among the old collections. In the current collections US-1 was found in six locations, only on wild Solanaceae and not on cultivated plants.

Clonal lineage PE-7 had six MLGs in total (Table 3) and was sampled from wild tomato (two MLGs), cultivated potato (four MLGs), wild potato (one MLG), cultivated tomato (two MLGs) and pear melon (one MLG). Only MLG5 was discovered on various hosts but was only present in the old collections. In the current collections a variant of PE-7 (PE-7.1) was discovered only in southern Peru, in Puno, where one MLG (MLG222) was sampled 18 times from cultivated potato.

Phenotypic characterization

None of the isolates produced oospores when paired with an isolate of A1 mating type (POX067) but oospores were observed when they were paired with A2 isolate of *P. andina*, POX119, indicating that the isolates were all A1 mating type.

Of the 232 isolates analysed, 197 (85%) were resistant to the fungicide metalaxyl while nine isolates were moderately resistant (MR), and 26 were sensitive (S). Clonal lineage EC-1 had the highest proportion of metalaxyl-resistant isolates, but resistant isolates were also found among those belonging to the PE-3 and US-1 lineages (Fig. 7). No clear phenotypic differentiation in metalaxyl resistance was evident between the most common MLGs of the EC-1 clonal lineage.

In total, 161 isolates were analysed for virulence. As in previous studies (Pérez *et al.*, 2001), isolates belonging to the EC-1 and PE-3 lineages showed broad-spectrum virulence, overcoming the resistance of, on average, 6.3 and 5.5 of the 11 differentials, respectively, and the US-1 isolates were virulent on an average of 2.5 differentials. One third of all isolates from cultivated potato and alternative hosts were compatible with differentials carrying *R1*, *R3*, *R4*, *R7*, *R10* and *R11*. The differentials carrying *R5*, *R6* and *R9* were infected by 16%, 39% and 2%, respectively, of all isolates tested. However, the isolates collected in 1997–1999 were most frequently compatible with *R1*, *R4*, *R8*, *R10* and *R11*, only a few isolates could infect the *R5* and *R6* differentials and none could cause sporulate on the *R9* differential.

Discussion

The current diversity of the late blight pathogen *P. infestans* infecting cultivated potato and alternative hosts growing near fields in the main potato-growing areas of the Peruvian Andes was investigated. The diversity discovered was compared to the diversity of *P. infestans* isolates collected in Peru between 1997 and 2013, which had been maintained in cryostorage at the International Potato Center (referred to as the old isolates). Many of the old isolates included in this study have already been analysed using different techniques (Pérez *et al.*, 2001; Garry *et al.*, 2005a; Goss *et al.*, 2014; Martin *et al.*, 2014), but not with the complete 12-plex SSR set. Therefore, to be able to compare the genetic diversity among the old isolates and the newly collected isolates, both were genotyped using this set of markers. The results showed that the same four clonal lineages EC-1, PE-3, PE-7 and US-1 were present among the current and old

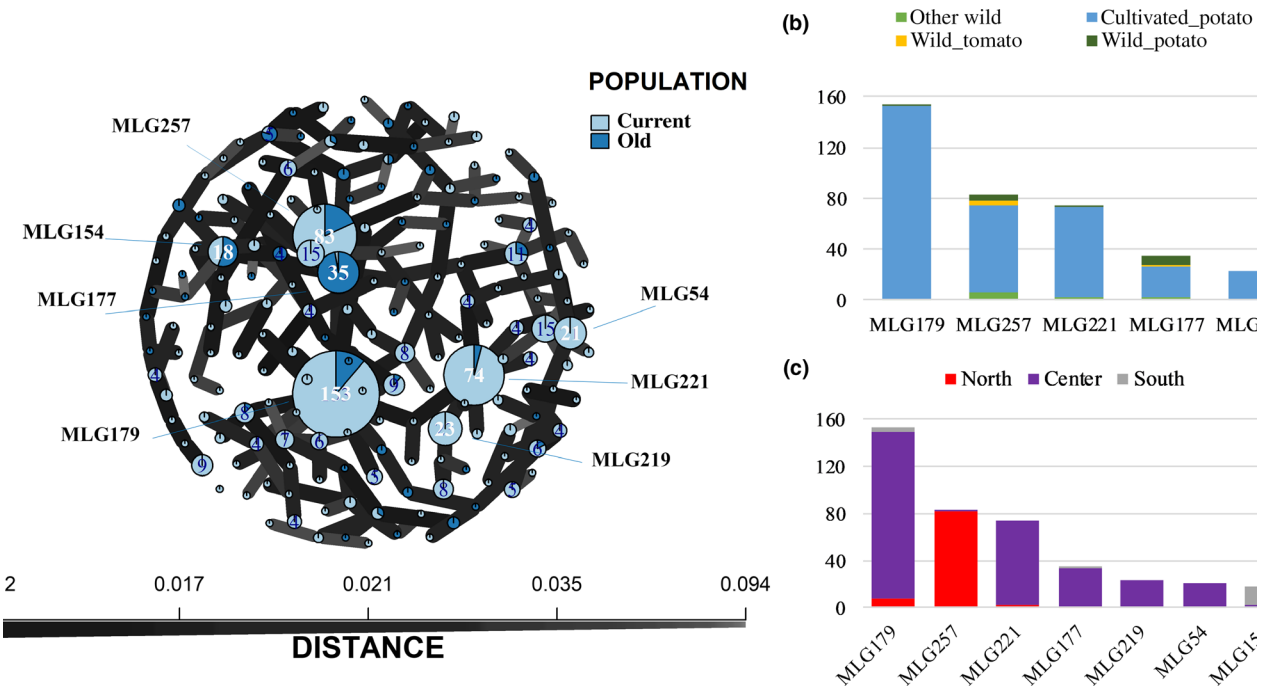

Figure 6 Minimum spanning network (MSN) of multilocus genotypes (MLGs) of *Phytophthora infestans* clonal lineage EC-1 from Peru, sampled from current (2016–2017) and old (1997–2013) collections (a); host range of the five most frequently sampled MLGs (b); and geographical distribution of the most seven frequently sampled MLGs (c). The host range included cultivated potato; wild potato (*Solanum acaule*, *S. brevicaule*, *S. candolleanum*, *S. cantense*, *S. chiquidenum*, *S. chomatophilum*, *S. huancabambense*, *S. hypacrarthrum*, *S. medians*, *S. mochiquense*, *S. paucissectum*, *S. sogarandinum*, *S. wittmackii*); wild tomato (*S. habrochaites*, *S. ochranthum*, *S. peruvianum*); and other wild species (*Iochroma grandiflorum*, *S. caripense*, *S. chrysotrichum*, *S. grandidentatum*, *S. gracilifrons*, *S. zahlbruckneri*). [Colour figure can be viewed at wileyonlinelibrary.com].

collections and that EC-1 was the predominant lineage found on cultivated and alternative hosts throughout the areas sampled.

Recent studies worldwide have reported rapid changes in the structure of *P. infestans* populations, in which lineages with better fitness are replacing the old predominant lineages (Chowdappa *et al.*, 2013; Fry *et al.*, 2015; Saville *et al.*, 2016). Much of the change can be attributed to the global exchange of tuber seed, fruits or plant parts (Chowdappa *et al.*, 2013). This study found no evidence for the existence of additional clonal lineages than those previously reported. The seed potato sector in Peru is mostly informal and import of seed potato from other continents is currently nonexistent; therefore, the risk of new lineages from overseas is low. The bordering countries in the north (Ecuador and Colombia) have reported the same dominating lineage, EC-1, mostly infecting potato (Vargas *et al.*, 2009; Delgado *et al.*, 2013). Venezuela has also reported isolates of A1 mating type with Ia haplotype (Briceño *et al.*, 2009). In Argentina since 2011, the oldest lineage (AG-1_A2) has been displaced by EU-2_A1, which is also present in Chile, suggesting introduction from Europe (Lucca *et al.*, 2018). Brazil (Forbes *et al.*, 1998) and Uruguay (Deahl *et al.*, 2003) have reported having a different lineage (BR-1) belonging to the A2 mating type, but the authors are not aware of potato seed trade or exchange between Peru and these countries. One potential risk area for migration of new lineages is at the Peru–Bolivia border as it has been reported that the lineage in Bolivia is mating type A2 (Goodwin *et al.*, 1994; Fernández-Northcote & Plata, 1998). So far, no *P. infestans* isolate of the A2 mating type has been discovered in Peru. However, another species, *P. andina*, which is A2 mating type and a hybrid between *P. infestans* and an unknown species (Goss *et al.*, 2014), has been found infecting tree tomato in Peru (Forbes *et al.*, 2016).

In the present study, MLGs belonging to the EC-1 clonal lineage, as well as MLGs that grouped together with the PE-7 clonal lineage in the DAPC, were found in potato-growing areas of Puno, *c.* 40 km from the Bolivian border. Further studies are under way to sample more of the diversity in this area and conduct phenotypic studies of the mating type and virulence of the isolates. Generally speaking, the current *P. infestans* population of Puno is different from the old population of only US-1 and PE-3 isolates, suggesting that a displacement of the old dominating lineages has taken place.

The US-1 lineage was widespread in potato in old collections but in current collections it was found only in wild Solanaceae species. This change can perhaps be attributed to the low level of virulence of this lineage in cultivated potato and, in addition, >50% of the US-1 isolates were sensitive to the fungicide metalaxyl. Nevertheless, US-1 is being maintained in the wild species as well as in cultivated pear melon and has accumulated several

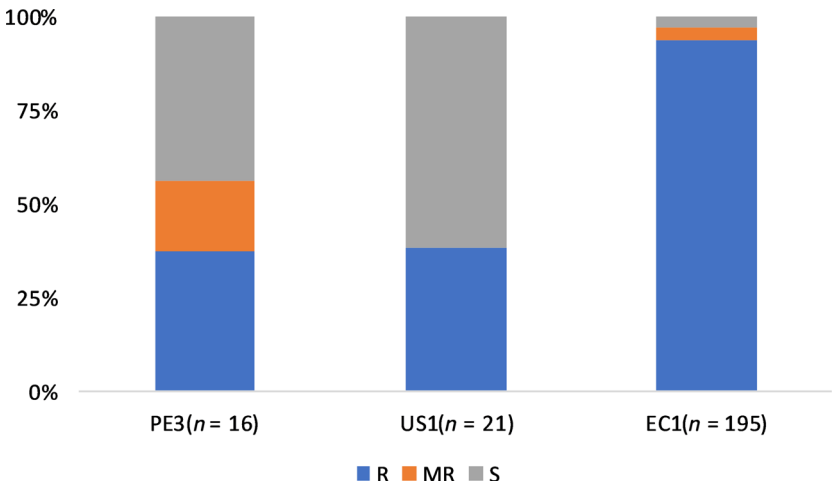

Figure 7 Sensitivity of isolates of *Phytophthora infestans* from Peru to the fungicide metalaxyl, determined by laboratory assay. The isolates were grouped according to clonal lineage: PE-3, US-1 and EC-1. The number of isolates in each lineage group is indicated in parentheses. R, resistant; MR, moderately resistant; S, sensitive. [Colour figure can be viewed at wileyonlinelibrary.com].

mutations resulting in new MLGs that were not found in the old collections. In Ecuador, the US-1 lineage has been found on pear melon, *S. caripense* and cultivated tomato (Adler *et al.*, 2002). In the old collection, isolates of the US-1 lineage were collected from tomato; however, as no samples were collected from cultivated tomato in the current survey, it is not possible to determine whether US-1 is still predominant in tomato in Peru. In other parts of the world, the US-1 lineage has been largely displaced by new lineages with better fitness (Yuen & Andersson, 2013).

Geographical substructuring is attributed to the clonal lineages other than EC-1, as these are found in more isolated pockets. Surprisingly, the PE-3 lineage has appeared in the north of the country in Amazonas and Cajamarca, where it was nearly as frequently sampled as the EC-1 lineage. Because metalaxyl resistance is not as prominent among the isolates of the PE-3 lineage, this has implications for disease management in this area and the continued monitoring of the *P. infestans* population is recommended.

The very large subclonal variation identified in the SSR fingerprints of the EC-1 clonal lineage is not surprising considering the time the lineage has dominated the area. EC-1 was first discovered in Ecuador in the 1990s and subsequently found in Peru and Colombia (Forbes *et al.*, 1998; Garry *et al.*, 2005a; Vargas *et al.*, 2009). Interestingly, some of the EC-1 MLGs have persisted for a long time and are present in high frequency in the current population. These would be expected to have fitness advantages over the other less frequent MLGs. Representative isolates from different frequently sampled MLGs were all resistant to metalaxyl, which could be linked to their overall fitness. Metalaxyl-resistant isolates have been shown to form larger lesions faster or produce more spores than metalaxyl-sensitive isolates (Kadish & Cohen, 1988). Comparison of parasitic fitness of *P. infestans* isolates from the US-1, EC-1 and PE-3 populations has shown that there were no differences in the incubation period, latent period, sporulation capacity and lesion growth rate among the lineages that could be linked with the displacement of the US-1 lineage in Peru (Andrade Piedra *et al.*, 1997). Instead, the authors of that report discovered that the sporulation capacity of the old lineage was significantly greater than that of the new population, leading the authors to suggest that the greater aggressiveness of the new population could be due to improved adaptation to environmental factors such as temperature or humidity.

The EC-1 lineage has a broad host range and in most of the cases where alternative hosts were infected by the EC-1 lineage, the same MLG was also found in the neighbouring potato field. This is expected because the short distance dispersal of asexual sporangia can reach hundreds of metres to kilometres (Fry *et al.*, 2013). Andean potato farmers cultivate dozens of different landraces as well as modern potato varieties, of which most are highly susceptible to late blight. This combined with the constant presence of alternative hosts can probably explain the persistence of MLGs over years.

Phytophthora infestans was found infecting a broad range of hosts, including species not previously considered as hosts. The new host species include *S. zahlbruckneri*, *S. grandidentatum* and *I. grandiflorum*, which all occupy mid-elevation habitats. *Solanum zahlbruckneri* and *S. grandidentatum* belong to the Morelloid clade of *Solanum* and are relatively closely related to tomato and potato, while *Iochroma* represents a more distantly related species of Solanaceae from the Iochrominae subtribe (Särkinen *et al.*, 2013). *Solanum zahlbruckneri* is a relatively weedy shrub from northern Peru, and *S. grandidentatum* a weedy herbaceous species. Both species occur in mid- to high elevations (1300–4600 m a.s.l.). *Iochroma grandiflorum* is a tree from northern Peru, also found in Ecuador and Colombia, and occurs at 2000–3200 m a.s.l. In most hosts the foliar symptoms caused by *P. infestans* are like those on potato and tomato and include dark expanding lesions surrounded by water-soaked areas as well as, if conditions are favourable, a halo of sporulation. In perennial hosts the lesions forming in the woody tissues may remain latent for long periods and provide a survival strategy (Forbes *et al.*, 2013).

To the best of the authors' knowledge this study is the first systematic survey of late blight symptoms across Solanaceae species in the wild. Within the flowering plants, the Solanaceae family is a moderately diverse group with *c*. 100 genera and 3000–4000 species, but economically, it is one of the most important in the world. The greatest diversity of the Solanaceae family is found in Central and South America and most of its species, both cultivated and wild, are concentrated in the Andean region. In Peru, cultivated Solanaceae represent 2% of the total diversity of the family, while 98% correspond to wild species with more than 540 species currently known from the country.

Discovering late blight infections in new hosts is not an easy task and most of the Solanaceae plants examined were healthy at the time of inspection. Therefore, it is possible that even further species may be alternative hosts for *P. infestans* under favourable epidemiological conditions.

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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. Solanaceous species reported as hosts of *Phytophthora infestans* in Peru (Junchaya, 1983; Garry *et al*., 2005b).

Table S2. Details of the SSR markers of the 12-plex microsatellite assay used for the characterization of *Phytophthora infestans* isolates.

Table S3. All data of the *Phytophthora infestans* isolates characterized in this study.

Table S4. The registered Solanaceae species identified in this study (current collection).