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Large subclonal variation in *Phytophthora infestans* populations associated with Ecuadorian potato landraces

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The population of *Phytophthora infestans* on potato landraces in three provinces (Carchi, Chimborazo and Loja) of Ecuador was analysed. All isolates ($n = 66$) were of the A1 mating type. Simple sequence repeats (SSR) were used to assess the genetic diversity of the isolates. The *P. infestans* isolates from the potato landraces grouped in a single clade together with reference isolates belonging to the clonal lineage EC-1. In the 66 SSR profiles obtained, 31 multilocus genotypes were identified. The 66 isolates constituted 49 different races according to the *Solanum demissum* differential set (*R1* to *R11*). The *P. infestans* population was complex and virulent on 4 to 11 *R* genes. Analysis showed that the subclonal variation in the Ecuadorian EC-1 clone is increasing over time and is much larger than clonal variation in lineages in the Netherlands and Nicaragua, suggesting high mutation rates and little or no selection in Ecuador.

Keywords: diversity, late blight, *Phytophthora andina*, SSRs, virulence

Introduction

The oomycete *Phytophthora infestans* is the causal agent of late blight and attacks a range of species in the Solanaceae family. It is the most important disease on potato in Ecuador and worldwide (Oyarzún *et al*., 2002). Diversity within and among *P. infestans* populations has been studied using phenotypic and genotypic markers. Among the phenotypic markers used are mating type and virulence. The mating type is based on the fact that *P. infestans* is heterothallic, so two types, A1 and A2, are necessary for sexual reproduction (Forbes, 1997). For virulence studies, a set of potato genotypes with different *R* genes is used (Black *et al*., 1953; Malcolmson & Black, 1966). This evaluation is based on the reaction of *P. infestans* isolates with *Solanum* plants carrying specific resistance genes, and grouping those with the same virulence phenotype as races. Among the genotypic markers used are allozymes, restriction fragment length polymorphisms (RFLPs), mitochondrial haplotypes, amplified fragment polymorphisms (AFLPs) and simple sequence repeats (SSRs or microsatellites) (Fry *et al*., 2009). Studies on *Phytophthora* associated with Solanaceae in Ecuador showed the presence of four clonal lineages based on the RFLP fingerprinting pattern (Forbes *et al*., 1997; Adler *et al*., 2004). Two clonal lineages, EC-1 and US-1, both of the A1 mating type, have been identified already in the previous century (Forbes *et al*., 1997). The EC-1 lineage is predominant on potatoes and the US-1 on tomatoes (Forbes *et al*., 1997; Oyarzún *et al*., 1998). More recently, two additional lineages were found in Ecuador, EC-2 and EC-3, which cause late blight on non-tuber bearing *Solanum* species such as *S. betaceaum*, *S. quitoense*, *S. hispidum* and *S. muricatum* (Ordoñez *et al*., 2000; Adler *et al*., 2004). Oliva *et al*. (2010) formally described EC-2 and EC-3 as a distinct species, *Phytophthora andina*. However, the species status of *P. andina* is questioned by others (Cárdenas *et al*., 2012); it may actually be a hybrid between *P. infestans* and an as yet unknown lineage. Support for this also comes from a study by Blair *et al*. (2012).

Studies on the race structure of *P. infestans* populations isolated from cultivated potatoes in Ecuador showed an increase in the complexity from a predominance of avirulent isolates (INIAP, 1974, 1975, 1976) to complex races, which was suggested to have resulted from the replacement of the US-1 lineage on potatoes by the EC-1 lineage (Forbes *et al*., 1997). Additionally, EC-1 was found to be more aggressive on potato than isolates of the US-1 clonal lineage (Oyarzún *et al*., 1998). Forbes *et al*. (1997) found 24 races infecting potatoes in Ecuador, 14 in Carchi, 14 in Chimborazo and eight in Loja. More recently, in commercial varieties and selected clones from INIAP's national potato breeding programme, 27, 17 and 37 races were found in Carchi, Cotopaxi and Pichincha provinces, respectively (Tello, 2008). In Peru and Colombia three clonal lineages were identified that infected potato landraces (Garry *et al*., 2005; Vargas *et al*., 2009) and, as in Ecuador, the domi-

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nant lineage was EC-1. Some studies on *P. infestans* populations indicated a low genetic diversity, represented by a single dominant clonal lineage but with high virulence diversity (Forbes *et al.*, 1997; Guo *et al.*, 2009). In regions where sexual reproduction occurs, such as the Nordic countries, populations had a high genetic diversity (Brurberg *et al.*, 2011).

Despite all previous studies on *Phytophtora* spp., there are no specific studies on populations of *P. infestans* associated with Ecuadorian potato landraces and it is unknown if there are any other lineages or species present on them.

There are more than 400 potato landraces in Ecuador (Cuesta *et al.*, 2005). These landraces are the result of selection and conservation carried out by small-scale farmers in the highlands (Cuesta *et al.*, 2005). In contrast to conventional potato cultivation, these potatoes are cultivated on small acreages, with low input of pesticides and often several landraces grown together (Cuesta *et al.*, 2005; Monteros & Reinoso, 2010). The landraces belong to three *Solanum* species: the diploid *S. phureja*, the triploid *S. chaucha* and the tetraploid *S. andigena* (Monteros-Altamirano, 2011). They constitute a potential source of genetic variation for breeding purposes, in characters such as quality, earliness and resistance to biotic and abiotic stresses. Evaluations showed that most landraces in Ecuador were highly susceptible to late blight, but some landraces with field resistance to late blight were also identified (Cañizares & Forbes, 1995; Revelo *et al.*, 1997; Monteros-Altamirano, 2011).

The aims of this study were to: (i) characterize *P. infestans* populations associated with Ecuadorian potato landraces in three areas of Ecuador, (ii) compare these to populations previously reported on commercial potatoes, and (iii) assess the impact of landraces on the *P. infestans* population. The genotypic diversity found in the EC-1 lineage present in Ecuador was also compared to the variation found elsewhere.

Materials and methods

Isolate collection

Phytophthora infestans isolates were collected from potato landraces present in three regions of Ecuador: the provinces of Carchi in the north, Chimborazo in the centre and Loja in the south (Monteros *et al.*, 2008). For each potato landrace present on a farm, five to 10 leaves with a single lesion were sampled. Leaves were kept at 4°C until isolation (Forbes *et al.*, 1997). The name and ploidy level of the landrace sampled, farm owner, location, GPS coordinates and altitude were recorded (Table S1). The pathogen was isolated either from infected leaves or from small pieces of necrotic leaves, which were placed between potato slices of the susceptible cv. Superchola and incubated in a humid chamber at 16°C with a 12 h photoperiod. Once mycelium was visible on leaves or slices, it was transferred to Rye B Agar plates (Caten & Jinks, 1968) with antibiotics (Oyarzún *et al.*, 1998). Purified isolates of *P. infestans* were maintained on Rye A medium (Caten & Jinks, 1968).

Isolate characterization

The mating type was determined for each isolate by pairing it with known A1 (EC3090 or EC3690) and A2 (EC3260) isolates (provided by the International Potato Center (CIP), Lima, Peru) on 10% clarified V8 agar (Forbes, 1997) at 18°C. After 2–3 weeks each plate containing the paired isolates was assessed for the presence of oospores. Isolates that produced oospores in the presence of a known A2 tester were designated as the A1 mating type, and vice versa (Forbes, 1997).

Virulence was determined using a differential set of potato clones containing 11 major *P. infestans* resistance genes from *S. demissum* (Malcolmson & Black, 1966). *R1* (CIP 801038), *R3* (CIP 801041), *R4* (CIP 801042), *R5* (CIP 801043), *R7* (CIP 801045), *R8* (CIP 801046) and *R9* (CIP 800994) differential cultivars were provided by CIP. *R2* (CIP 800987), *R6* (CIP 800991), *R10* (CIP 800995) and *R11* (CIP 800996) differentials, and the susceptible cv. Bintje, which has no known *R* genes and was used as a control, (Montarry *et al.*, 2010) were obtained from Wageningen University. This set of differentials is commonly used to characterize *P. infestans* isolates. Each isolate was inoculated on the differentials. From each plant, three leaflets were taken and placed in inverted Petri dishes containing water agar. On the abaxial surface of each leaflet, two 20 μL drops containing 25×10^3 sporangia mL^{-1} were placed at each side of the mid-vein. The inoculated leaves were placed in a climate chamber at 16°C for 6 days with a 12 h photoperiod, after which the reaction was scored. The reaction was considered compatible if a necrotic lesion and/or sporulation was observed on a leaflet, and as incompatible when a hypersensitive reaction was seen or no lesion was visible.

DNA extraction

All the *P. infestans* isolates obtained from potato landraces were grown for 10–14 days on Pea broth (Forbes, 1997). The mycelium was harvested and lyophilized. DNA was extracted from each sample with AGOWA sbeadex Maxi Plant kit using a KingFisher96 robot (Thermo Fisher Scientific).

SSR amplification and genetic data analysis

Twelve SSRs were used in this study: Pi04, Pi63, Pi70, G11, D13, Pi4B (Lees *et al.*, 2006); PinfSSR2, PinfSSR3, PinfSSR4, PinfSSR6, PinfSSR8 and PinfSSR11 (Li *et al.*, 2010). These were selected from previously published sets according to their map position, ease of scoring and allelic diversity (van der Lee *et al.*, 2004; Knapova & Gisi, 2002; Lees *et al.*, 2006; Li *et al.*, 2010). Amplifications were run in a PTC200 thermocycler (MJ Research), with an initial denaturation at 95°C for 15 min, followed by 30 cycles of 95°C for 20 s, 58°C for 90 s, and 72°C for 60 s, and a final extension at 72°C for 20 min (Li *et al.*, 2010). A number of isolates from previous surveys carried out in Ecuador were included in the analysis for reference (Table S2). The resulting amplification products were sized by capillary electrophoresis on an ABI 3730 sequencer using the molecular size standards GeneScan-500 ROX, and scored using GeneMapper v. 3.7 (Applied Biosystems). Within the GeneMapper software kits, panels and bin sets were generated, defining the markers and their known allele bins (provided by Plant Research International, PRI, Wageningen, the Netherlands). The known panels and bin sets for the 12 SSRs have been used to size SSR alleles in thousands of isolates from different countries by PRI and the James Hutton Institute, UK. Most *P. infestans* isolates

possessed a maximum of two alleles per locus, as expected for a diploid organism. However, in a number of cases more than two alleles were found, which may result from aneuploidy or polyploidy (see Fig. S1 for examples). This complicates the analysis because the analysis tools assume haploid or diploid data. Therefore, the detected SSR fragments (alleles) were converted to binary presence (1) or absence (0) data. From this a neighbour-joining tree was produced using PAUP* v. 4.0 Beta software. Robustness of the phylogram branches was inferred from jackknife values after 10 000 replicates (Swofford, 2002).

Diversity analysis

Race and genotypic diversity were estimated using Shannon's index (H_S) as $H_S = -\sum (p_i \times \ln p_i)$, where p_i is the frequency of the race or genotype. Evenness (E) was estimated by the formula: $E = H_S/\ln(n)$, where n is the total number of isolates of the sample and H_S is the Shannon index. E varies from 0 to 1, with 1 representing a situation in which all races or genotypes are equally abundant (Magurran, 1988).

The significance of Shannon indices was assessed with Hutcheson's t-test (t_H) (Hutcheson, 1970). It was calculated as follows: $t_H = (H_1 - H_2)/[S^2_{H1} + S^2_{H2}]^{1/2}$, where $H_1 - H_2$ is the difference of the Shannon indices between two samples. The variance of each sample (S^2) was estimated with the formula: $S^2 = [\sum p_i(\ln p_i)^2 - (\sum p_i \ln p_i)^2/n]/n^2$, where p_i and n are as described above.

The degrees of freedom (df) for each pairwise comparison were calculated using the formula: $df = [S^2_{H1} + S^2_{H2}]^2/[((S^2_{H1})^2/n_1) + ((S^2_{H2})^2/n_2)]$.

To examine the distribution of genetic variation within and among populations, analysis of molecular variance (AMOVA) using ARLEQUIN v. 3.5 (Excoffier *et al.*, 2005) was carried out with the binary data set as input. Population differentiation (F_{ST}) was calculated using ARLEQUIN.

To compare the subclonal variation in the Ecuadorian to other populations, the genotyping data of 219 Dutch isolates was used. These isolates were collected during the period 2004–2009 in the Netherlands and genotyped with the same 12 SSR markers. The genotyping data and isolate information can be found in Li *et al.* (2012a). In addition the Shannon index and the evenness were calculated using the frequency data in Nicaragua reported by Blandón-Díaz (2011). These were based on four multilocus genotypes obtained with six SSRs (D13, G11, Pi04, Pi4B, Pi63 and Pi70) on 165 *P. infestans* isolates collected from potatoes in Nicaragua.

Results

Isolate collection

A total of 66 *P. infestans* isolates were collected during the years 2009–2010 from 16 farms (Table 1; details of the isolates are provided in Table S1). The reason for the low number collected in Loja was a severe drought that occurred in that province at the time of collection, making it difficult to obtain samples.

Isolate characterization using potato *R* gene differentials

All isolates were of the A1 mating type. There were 49 different races in total (Table 1). The number of races collected and associated virulence factors are detailed in Table S3. Twenty-seven races were identified in Carchi, 18 in Chimborazo and seven in Loja. The *P. infestans* population observed was complex, and virulent on between four and 11 *R*-genes. One race (1,3,4,5,6,7,8, 9,10,11) was present in all three provinces; the other races were restricted to one province (Table S3).

Table 1 Diversity of the *Phytophthora infestans* population associated with potato landraces in three provinces of Ecuador based on number of races and SSR profiles

	Carchi	Chimborazo	Loja	All sites
No. of farms	8	3	5	16
No. of isolates	37	20	9	66
Virulence phenotype				
No. of races	27	18	7	49
H_S	3·15a	2·86b	1·89c	3·78
E	0·88	0·95	0·86	0·91
SSR genotype				
No. of genotypes	17	9	8	31
H_S	2·44a	1·94b	2·04b	3·03
E	0·68	0·66	0·93	0·72

H_S: Shannon index.
E: evenness.
Values followed by same letter within one line are not significantly different ($\alpha = 0.05$) according to Hutcheson's t-test for pairwise comparisons.

Isolate characterization using SSR markers

Eight of the 12 SSR markers were polymorphic among the *P. infestans* isolates collected. Markers Pi04, Pi63, Pi70 and PinfSSR8 were monomorphic (Table S4).

For some markers three or even four alleles were observed in a particular isolate. In the *P. infestans* isolates from potato landraces, three alleles were observed with Pi4B and PinfSSR4 markers (Table S4). In the reference *P. infestans* isolates, three alleles were detected with markers Pi4B, Pi63, PinfSSR4 and PinfSSR8 (Table S5). In the case of *P. andina*, three to four alleles were detected with the Pi4B marker (Table S5). Several private and monomorphic alleles were detected. Allele 279 from locus Pi63 was detected in all *P. infestans* isolates. Allele 188 from locus Pi70 was present in all US-1 reference isolates and absent from the rest of the *P. infestans* isolates. In total, 31 different genotypes were observed among the 66 isolates (Table 1) using the SSRs.

Relationship among the materials collected

The phylogenetic tree obtained from the SSR profiles showed that the *P. infestans* isolates grouped together in a large clade (Fig. 1; STRUCTURE analysis in Fig. S2). This clade also included the reference isolates known to belong to the clonal lineage EC-1. They were clearly distinct from the reference isolates identified as US-1 and *P. andina*. There was no relationship between the multi-

locus genotypes and the race phenotype. Isolates that belonged to the same race could possess different multilocus genotypes and vice versa. Even isolates obtained from the same landrace and sharing the same virulence phenotype could possess different SSR multilocus genotypes (Table S1).

Diversity among regions and development of diversity over time

In total, 31 different multilocus genotypes were identified. Only one genotype (EC-1_001) occurred in all three provinces, but it predominated in Carchi; another one (EC-1_003) was found in two provinces (Carchi and Chimborazo). The others were restricted to one province (Table S4). To examine the distribution of genetic variation among and within subpopulations, as defined by the three geographical regions, AMOVA was performed. This showed that 82% of the variance was present within subpopulations whereas the remaining 18% was among the three populations. There was a clear relationship between genotype and geographic origin (F_{ST} = 0·18; P < 0·0001).

Carchi had the highest Shannon diversity index for race phenotype (3·15) and it was significantly more diverse than Chimborazo (t_H = 23·28, df = 46·29, P < 0·0001) and Loja (t_H = 7·31, df = 15·32, P < 0·001; Table 1). Chimborazo, with a Shannon index of 2·86, was more diverse than Loja (t_H = 17, df = 17·13, P < 0·0001), which showed the lowest diversity value (1·89). The evenness among provinces showed slight, non-significant, differences.

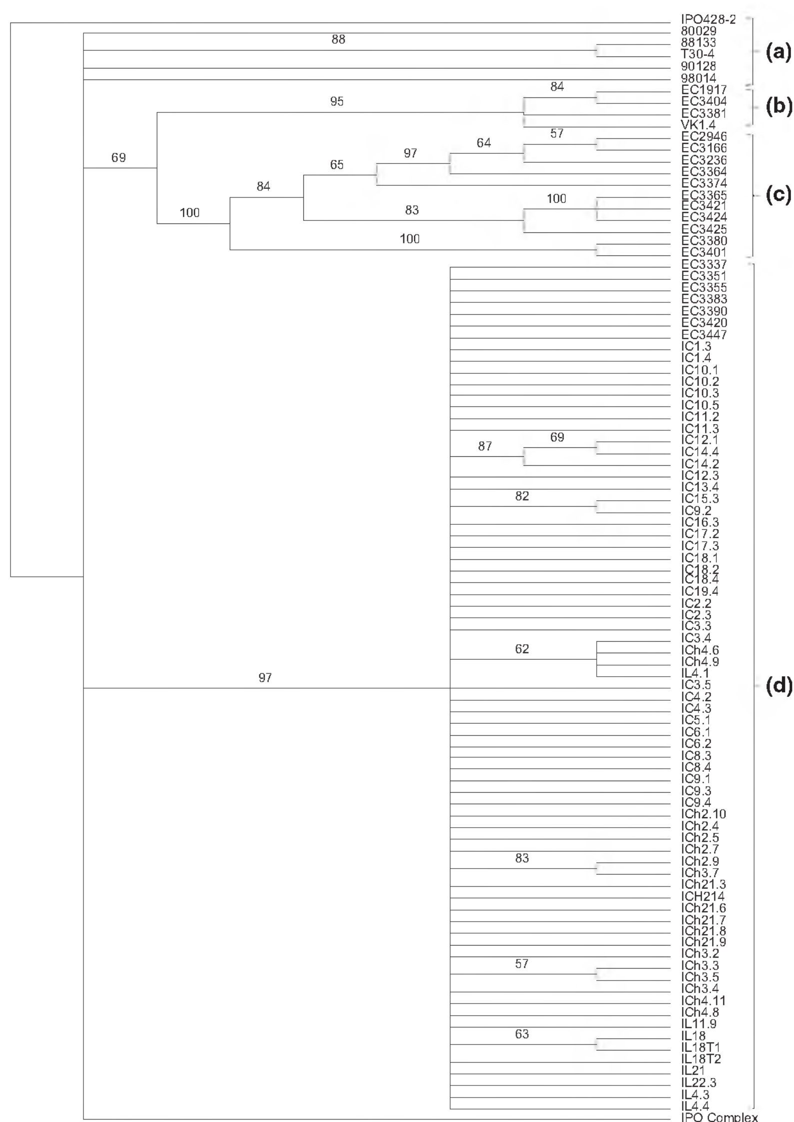

Figure 1 Neighbour-joining tree of the Ecuadorian *Phytophthora infestans* populations associated with potato landraces, based on 12 SSRs. Numbers at the nodes are jackknife values supporting the branches based on 10 000 replicates. (a) European *P. infestans* isolates; (b) *P. infestans* isolates, clonal lineage US-1; (c) *P. andina* isolates; (d) *P. infestans* isolates, clonal lineage EC-1.

Based on the SSR markers, the *P. infestans* population in Carchi ($H_S = 2{\cdot}44$) was genetically more diverse than the population in Chimborazo ($t_H = 18{\cdot}56$, df = 30·39, $P < 0{\cdot}001$) and Loja ($t_H = 5{\cdot}48$, df = 9·43, $P < 0{\cdot}001$). The populations in Chimborazo and Loja were not significantly different from each other ($t_H = -0{\cdot}80$, df = 10·73, $P = 0{\cdot}372$; Table 1). The total race diversity ($H_S = 3{\cdot}78$; Table 1) was significantly higher than the genotypic diversity ($H_S = 3{\cdot}03$; $t_H = 12{\cdot}66$, df = 66·28, $P = 0{\cdot}007$; Table 1). Analysis of the genotype changes over time in the Ecuadorian population of *P. infestans* indicated there was an increase in diversity, evenness and number of genotypes (Table 2).

Comparison of the variation within EC-1 in Ecuador to other clonal lineages

Genetic variation was also compared within the Ecuadorian population (the three regions taken together) with the variation in a clonal lineage that is dominant in Europe. In total, 219 Dutch isolates from the clonal lineage Blue_13 were studied with the same set of SSR markers (Li *et al.*, 2012a). The markers split the Blue_13 clonal lineage into 32 different subclones. The genotypic diversity ($Hs = 1{\cdot}98$, $E = 0{\cdot}37$) in the Dutch clonal lineage was significantly ($t_H = -5{\cdot}00$, df = 177·88, $P = 1{\cdot}36 \times 10^{-6}$) lower than the diversity ($Hs = 3{\cdot}03$, $E = 0{\cdot}72$) in the Ecuadorian population. The diversity present in the Nicaraguan population, based on the data provided by Blandón-Díaz (2011) (165 isolates, 4 SSR multilocus genotypes, $Hs = 0{\cdot}19$, $E = 0{\cdot}04$), was also much lower than in the Ecuadorian population measured with the same set of SSRs ($Hs = 1{\cdot}89$, $E = 0{\cdot}45$, $t_H = 46{\cdot}38$, df = 94·86, $P < 0{\cdot}0001$).

Discussion

Sixty-six isolates of *P. infestans* were obtained from 16 farms in three provinces of Ecuador. As a first step in the characterization, their virulence was analysed on a set of differential genotypes containing the *P. infestans* resistance genes *R1* to *R11* (Black *et al.*, 1953; Malcolmson & Black, 1966), as these genes and functional homologues are widely distributed in *Solanum* spp. (Vleeshouwers *et al.*, 2011). Using this differential set of genotypes, 49 (75%) of the isolates were found to be unique races. Simple races capable of overcoming one to three *R* genes were not observed. A similar high frequency of different races among isolates has been reported from Costa Rica (37 out of 40; Barquero *et al.*, 2005). However, lower frequencies were observed in other studies, e.g. Nepal 30 out of 251, Estonia 86/432, Finland 66/269, Norway 38/105, Nordic countries (Denmark, Finland, Norway and Sweden) 31/177 and China 61/125 races (Hermansen *et al.*, 2000; Ghimire *et al.*, 2001; Lehtinen *et al.*, 2008; Li *et al.*, 2009; Runno-Paurson *et al.*, 2010). It should be noted that in Denmark, Estonia, Finland, Norway and Sweden sexual populations are dominant, and the populations seems to be clonal in China and Nepal (although both mating types can be found), whereas the population in Ecuador is clonal.

The variation in the Ecuadorian population may be maintained due to a lack of selection for a particular race. Most of the Ecuadorian potato landraces are highly susceptible to late blight (Cañizares & Forbes, 1995; Revelo *et al.*, 1997; Monteros-Altamirano, 2011). In addition, fungicides are rarely used in the small-scale farming system used for these potato landraces. Grünwald *et al.* (2006) reported that populations of *P. infestans* not exposed to the fungicide metalaxyl showed more genetic diversity than exposed ones.

The current study has confirmed that *P. infestans* attacking potato in the Ecuadorian highlands is of the EC-1 clonal lineage. Notwithstanding the fact that all isolates from potato landraces belonged to the same clonal lineage, genetic variation was detected using the SSRs and by phenotypic characterization. Thirty-one multilocus SSR genotypes were detected among the 66 isolates. This variability is large for a clonal lineage. Clonal lineages have been identified in Europe (Cooke *et al.*, 2006, 2012) and Nicaragua (Blandón-Díaz *et al.*, 2012) using microsatellites. Guo *et al.* (2009) identified a single clonal lineage in northern China using two SSRs; Li *et al.* (2012b) identified three clonal lineages in western China using ten SSRs. In the Nordic countries (Denmark, Finland, Norway and Sweden), 169 SSR multilocus genotypes were identified using seven microsatellite markers from a sample of 191 *P. infestans* isolates. This high genetic diversity observed in Nordic countries is attributed to the sexual reproduction of the pathogen (Brurberg *et al.*, 2011). Comparing the results of the current study to published data is not easy as different sampling strategies and different numbers of SSR markers have been used: the best comparison is with the European Blue_13 lineage as this study was carried out with the same set of SSR markers. The variation in the Ecuadorian EC-1 lineage was significantly greater than the diversity in Blue_13 as measured by the Shannon index and

Table 2 Changes in number of races, Shannon index (H_S) and evenness (E) of *Phytophthora infestans* populations over the years in Ecuador

		Provinces		
	Year	Carchi	Chimborazo	Loja
H_S	1990–1993[a]	2·33	2·12	1·55
	2007[b]	2·81	n.e.[d]	n.e.
	2009–2010[c]	3·15	2·86	1·89
E	1990–1993[a]	0·64	0·58	0·45
	2007[b]	0·67	n.e.	n.e.
	2009–2010[c]	0·88	0·95	0·86
Races	1990–1993[a]	14 (n = 39)	14 (n = 38)	8 (n = 31)
	2007[b]	27 (n = 68)	n.e.	n.e.
	2009–2010[c]	27 (n = 36)	18 (n = 20)	7 (n = 9)

[a]Calculated from data of Forbes *et al.* (1997).
[b]Calculated from data of Tello (2008).
[c]Data from this research.
[d]Not evaluated.

evenness. When the results of the current study are compared to the population present in Nicaragua (only considering markers that were present in both studies), the variation in the Ecuadorian population was greater than that in the Nicaraguan population. The high diversity in Ecuador might be related to a high mutation frequency, e.g. due to increased UV radiation as potatoes are grown in Ecuador at high altitudes (>2400 m). It is also possible that the greater diversity in the EC-1 population than in the clonal lineage Blue_13 is due to the age of the population: Blue_13 was detected for the first time in samples from 2004 in the Netherlands (Li *et al.*, 2012a), whereas EC-1 was described in the 1990s (Forbes *et al.*, 1997).

Mechanistically, the subclonal variation may partly be explained by loss of chromosome regions or mitotic recombination. Nevertheless, new alleles were detected that can only be explained by changes in the number of repeat units during mitosis. The presence of more than two peaks in some of the SSR profiles supports the hypothesis that polyploidization or gene duplication followed by mutations caused the genetic diversity observed in the *P. infestans* population of Ecuador (Tooley & Therrien, 1991; Cooke *et al.*, 2012). The occurrence of more than two alleles at a specific locus has been reported before (Knapova & Gisi, 2002; Lees *et al.*, 2006; Chacón Acosta, 2007; Akino *et al.*, 2009; Oliva Pérez, 2009; Cooke *et al.*, 2012; Li *et al.*, 2012a,b). The same mechanism seems to be active in *P. andina* also, where four alleles were observed (Fig. S1). Changes in virulence spectrum have been attributed to (partial) chromosomal deletions (van der Lee *et al.*, 2001). Also, mutations in avirulence genes have been found to cause changes in the virulence phenotype (Armstrong *et al.*, 2005). Copy number variations, amino acid replacements, and gene gains and losses have been suggested as sources of the variability within the clonal lineage Blue_13 in the UK (Cooke *et al.*, 2012). The high race and SSR diversity observed in the Ecuadorian population might be related to the cultivation of potatoes all year round, so several generations of the pathogen can occur, increasing the chance of the appearance of new genotypes.

There was no correlation between the phenotypic and genotypic diversity. Isolates of a particular race showed different multilocus genotypes. These occurred even in isolates obtained from the same landrace or farm (Table S1). Thus, the observed variation appears to be produced randomly with no selective pressure. The high race and SSR diversity observed in this study contrasts with populations from USA, France and China, where clonal lineages were shown to possess a wide diversity in races but a low genetic diversity (Abu-El Samen *et al.*, 2003; Montarry *et al.*, 2006; Guo *et al.*, 2009).

The genetic diversity was highest in the Carchi province and significantly different from Chimborazo and Loja (Table 1). The F_{ST} analysis showed clear differentiation of the populations in the three regions and most of the variation was present within populations. This difference in diversity observed in Carchi could be due to the higher number of isolates and farms sampled. The highest evenness value was measured in Loja (0·93), although the number of samples was small (Table 1) and the value probably reflects under-sampling. The one genotype that occurred in all three provinces might have migrated through the Ecuadorian highlands, perhaps as a result of the exchange of landrace seeds among farmers (Monteros-Altamirano, 2011). Alternatively, this genotype may have arisen multiple times independently. The microsatellite analysis also showed some *P. infestans* lineage- and species-specific alleles. These may be used to distinguish clonal lineages (Akino *et al.*, 2009). The SSR markers used in this study reflected the genetic diversity within the current *P. infestans* population in Ecuador: in future they may be used to monitor changes in the population and displacement of pathogen genotypes across the country.

Comparisons with previous studies have to be treated with caution because of the different sampling strategies used. However, an increase in the Shannon index and evenness over time was apparent, as was an increase in the number of races. The differences in Carchi in 2007 and 2009–2010 may be due to the fact that the present study survey included eight farms, whereas Tello (2008) collected all the samples from just one farm in the province and some races were much more frequent than others. The evenness values are close to 1 for all provinces, which was not the situation in 1990–1993, where in Carchi two races represented 40% of the isolates. For Chimborazo and Loja, the situation was similar, two races at each location representing 55 and 70% of the isolates sampled (Forbes *et al.*, 1997).

All *P. infestans* isolates from potato landraces grouped in a clade together with known EC-1 isolates, separate from US-1 and *P. andina* isolates (Fig. 1). There is currently some discussion as to whether *P. andina* should be considered a separate species or a hybrid. Although SSR markers are not particularly suitable for species identification, some of the alleles identified in *P. andina* are rare and have never been reported in *P. infestans* isolates. In addition, in *P. andina* there were four alleles at locus Pi4B and null alleles for locus Pi70 which have not been found for other *P. infestans* isolates (Y. Li & T. van der Lee, Plant Research International, Wageningen, The Netherlands, personal communication). This is not a typical pattern for a simple hybridization, but clearly distinguished *P. andina* isolates from *P. infestans* isolates.

This study has identified genetic variation within the *P. infestans* clonal population in Ecuador. The high number of races and their complexity constitute a challenge for late blight management in the country. It is necessary to incorporate new *R* genes not belonging to the *S. demissum* group into the National Breeding Programme. Other sources of resistance (Wang *et al.*, 2008; Jacobs *et al.*, 2010; Lokossou *et al.*, 2010) may be useful for potato breeding against *P. infestans* in Ecuador. However, one promising broad-spectrum resistance gene, *Vnt1* (Pel, 2010) cannot be used because the avirulence gene triggering the defence response is not expressed in EC-1 genotypes.

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References

Abu-El Samen FM, Secor GA, Gudmestad NC, 2003. Variability in virulence among asexual progenies of *Phytophthora infestans*. *Phytopathology* **93**, 293–304.

Adler NE, Erselius LJ, Chacón MG *et al.*, 2004. Genetic diversity of *Phytophthora infestans* sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. *Phytopathology* **94**, 154–62.

Akino S, Baba Y, Kondo N, 2009. Use of microsatellite marker Pi26 for rapid discrimination of five Japanese genotypes of *Phytophthora infestans*. *Journal of General Plant Pathology* **75**, 349–52.

Armstrong MR, Whisson SC, Pritchard L *et al.*, 2005. An ancestral oomycete locus contains late blight avirulence *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *Proceedings of the National Academy of Sciences, USA* **102**, 7766–71.

Barquero M, Brenes A, Gómez L, 2005. Complejidad fisiológica de *Phytophthora infestans* en Costa Rica. *Agronomía Costarricense* **29**, 21–9.

Black W, Mastenbroek C, Mills WR, Peterson LC, 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* **2**, 173–9.

Blair JE, Coffey MD, Martin FN, 2012. Species tree estimation for the late blight pathogen, *Phytophthora infestans*, and close relatives. *PLoS ONE* **7**, e37003.

Blandón-Díaz JU, 2011. *Insights into Population Structure and Epidemiology of* Phytophthora infestans *from Nicaragua*. Uppsala, Sweden: Swedish University of Agricultural Sciences, PhD thesis.

Blandón-Díaz JU, Widmark AK, Hannukkala A, Andersson B, Högberg N, Yuen JE, 2012. Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. *Phytopathology* **102**, 323–30.

Brurberg MB, Elameen A, Le VH *et al.*, 2011. Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology* **115**, 335–42.

Cañizares G, Forbes G, 1995. Foliage resistance to *Phytophthora infestans* (Mont.) de Bary in the Ecuadorian national collection of *Solanum phureja* ssp. *phureja* Juz. & Buk. *Potato Research* **38**, 3–10.

Cárdenas M, Tabima J, Fry WE, Grünwald NJ, Bernal A, Restrepo S, 2012. Defining species boundaries in the genus *Phytophthora*: the case of *Phytophthora andina*. A response to '*Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands' (Oliva *et al.*, 2010). *Plant Pathology* **61**, 215–20.

Caten CE, Jinks JL, 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Canadian Journal of Botany* **46**, 329–48.

Chacón Acosta MG, 2007. *Variability of* Phytophthora infestans *sensu lato in the Highland Tropics of Ecuador*. Zurich, Switzerland: Swiss Federal Institute of Technology Zurich, PhD thesis.

Cooke LR, Carlisle DJ, Donaghy C, Quinn M, Perez FM, Deahl KL, 2006. The Northern Ireland *Phytophthora infestans* population 1998–2002 characterized by genotypic and phenotypic markers. *Plant Pathology* **55**, 320–30.

Cooke DEL, Cano LM, Raffaele S *et al.*, 2012. Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. *PLoS Pathogens* **8**, e1002940.

Cuesta X, Castillo C, Monteros C, 2005. Biodiversidad de las papas nativas ecuatorianas. In: Monteros C, Cuesta X, Jiménez J, López G, eds. *Las Papas Nativas en el Ecuador*. Quito, Ecuador: INIAP, 6–10.

Excoffier L, Laval G, Schneider S, 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Forbes GA, 1997. *Manual for Laboratory Work on* Phytophthora infestans. Quito, Ecuador: Centro Internacional de la Papa.

Forbes GA, Escobar XC, Ayala CC *et al.*, 1997. Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* **87**, 375–80.

Fry WE, Grünwald NJ, Cooke DEL, McLeod A, Forbes GA, Cao K, 2009. Population genetics and population diversity of *Phytophthora infestans*. In: Lamour K, Kamoun S, eds. *Oomycete Genetics and Genomics: Diversity, Interactions and Research Tools*. Hoboken, NJ, USA: John Wiley & Sons, 139–64.

Garry G, Salas A, Forbes GA, Perez W, Santa CM, Nelson RJ, 2005. Host specialization not detected among isolates of the EC-1 lineage of *Phytophthora infestans* attacking wild and cultivated potatoes in Peru. *European Journal of Plant Pathology* **113**, 71–81.

Ghimire SR, Hyde KD, Hodgkiss IJ, Liew ECY, 2001. Race diversity and virulence complexity of *Phytophthora infestans* in Nepal. *Potato Research* **44**, 253–63.

Grünwald NJ, Sturbaum AK, Romero Montes G, Garay Serrano E, Lozoya-Saldaña H, Fry WE, 2006. Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. *Phytopathology* **96**, 1397–403.

Guo J, van der Lee T, Qu DY *et al.*, 2009. *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biology* **11**, 57–67.

Hermansen A, Hannukkala A, Hafskjold NR, Brurberg MB, 2000. Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* **49**, 11–22.

Hutcheson K, 1970. A test for comparing diversities based on the Shannon formula. *Journal of Theoretical Biology* **29**, 151–4.

INIAP (Instituto Nacional de Investigaciones Agropecuárius), 1974. *Informe Anual de Actividades*. Departamento de Fitopatología. Quito, Ecuador: INIAP.

INIAP (Instituto Nacional de Investigaciones Agropecuárius), 1975. *Informe Anual de Actividades*. Departamento de Fitopatología. Quito, Ecuador: INIAP.

INIAP (Instituto Nacional de Investigaciones Agropecuárias), 1976. *Informe Anual de Actividades*. Departamento de Fitopatología. Quito, Ecuador: INIAP.

Jacobs MMJ, Vosman B, Vleeshouwers VGAA, Visser RGF, Henken B, van der Berg R, 2010. A novel approach to locate *Phytophthora infestans* resistance genes on the potato genetic map. *Theoretical and Applied Genetics* **120**, 785–96.

Knapova G, Gisi U, 2002. Phenotypic and genotypic structure of *Phytophthora infestans* populations on potato and tomato in France and Switzerland. *Plant Pathology* **51**, 641–53.

van der Lee T, Testa A, van 't Klooster J, van der Berg-Velthuis G, Govers F, 2001. Chromosomal deletion in isolates of *Phytophthora infestans* correlates with virulence on R3, R10 and R11 potato lines. *Molecular Plant–Microbe Interactions* **14**, 1444–52.

van der Lee T, Testa A, Robold A, van 't Klooster J, Govers F, 2004. High-density genetic linkage maps of *Phytophthora infestans* reveal trisomic progeny and chromosomal rearrangements. *Genetics* **167**, 1643–61.

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams NA, Cooke DEL, 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology* 55, 311–19.

Lehtinen A, Hannukkala A, Andersson B *et al*., 2008. Phenotypic variation in Nordic populations of *Phytophthora infestans* in 2003. *Plant Pathology* 57, 227–34.

Li B, Chen Q, Lv X *et al*., 2009. Phenotypic and genotypic characterization of *Phytophthora infestans* isolates from China. *Journal of Phytopathology* 157, 558–67.

Li Y, Govers F, Mendes O *et al*., 2010. A new set of highly informative SSR markers for *Phytophthora infestans* population analysis assembled into an efficient multiplex. *Molecular Ecology Resources* 10, 1098–105.

Li Y, van der Lee T, Evenhuis A *et al*., 2012a. Population dynamics of *Phytophthora infestans* in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. *Genes, Genomes, Genetics* 2, 1529–40.

Li Y, van derLee T, Zhu JH *et al*., 2012b. Population structure of *Phytophthora infestans* in China – geographic clusters and presence of the EU genotype Blue_13. *Plant Pathology*. Doi: 10.1111/j.1365-3059.2012.02687.x.

Lokossou AA, Rietman H, Wang M *et al*., 2010. Diversity, distribution, and evolution of *Solanum bulbocastanum* late blight resistance genes. *Molecular Plant–Microbe Interactions* 23, 1206–16.

Magurran AE, 1988. *Ecological Diversity and its Measurement*. Princeton, USA: Princeton University Press.

Malcolmson JF, Black W, 1966. New *R* genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15, 199–203.

Montarry J, Corbière R, Lesueur S, Glais I, Andrivon D, 2006. Does selection by resistant hosts trigger local adaptation in plant–pathogen systems? *Journal of Evolutionary Biology* 19, 522–31.

Montarry J, Hamelin FM, Glais I, Corbière R, Andrivon D, 2010. Fitness costs associated with unnecessary virulence factors and life history traits: evolutionary insights from the potato late blight pathogen *Phytophthora infestans*. *BMC Evolutionary Biology* 10, 283.

Monteros-Altamirano AR, 2011. *Potato Landraces: Description and Dynamics in Three Areas of Ecuador*. Wageningen, The Netherlands: Wageningen University, PhD thesis.

Monteros C, Reinoso I, 2010. Biodiversidad y oportunidades de mercados para las papas nativas ecuatorianas. In: *Memorias XXIV Congreso Latinoamericano de la Asociación Latinoamericana de la Papa*. Cusco, Perú: INIA, 447–8.

Monteros A, Vosman B, van der Berg R, 2008. Estudios preliminares para la descripción de papas nativas en tres microcentros de diversidad. In: *Memorias XXIII Congreso Latinoamericano de la Asociación Latinoamericana de la Papa/VI Seminario Latinoamericano de Uso y Comercialización de la Papa, 2008*. Mar del Plata, Argentina: Universidad Nacional de Mar del Plata, 205–6.

Oliva Pérez R, 2009. *Occurrence of Sympatric* Phytophthora *Species in the Highland of Ecuador*. Zurich, Switzerland: Swiss Federal Institute of Technology Zurich, PhD. thesis.

Oliva RF, Kroon LPNM, Chacón G, Flier WG, Ristaino JB, Forbes GA, 2010. *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. *Plant Pathology* 59, 613–25.

Ordoñez ME, Hohl ME, Velasco JA *et al*., 2000. A novel population of *Phytophtora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. *Phytopathology* 90, 197–202.

Oyarzún PJ, Pozo A, Ordoñez ME, Doucett K, Forbes GA, 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* 88, 265–71.

Oyarzún P, Gallegos P, Asaquibay C *et al*., 2002. Manejo integrado de plagas y enfermedades. In: Pumisacho M, Sherwood S, eds. *El Cultivo de la Papa en Ecuador*. Quito, Ecuador: INIAP/CIP, 85–169.

Pel MA, 2010. *Mapping, Isolation and Characterization of Genes Responsible for Late Blight Resistance in Potato*. Wageningen, The Netherlands: Wageningen University, PhD thesis.

Revelo J, Garcés S, Andrade J, 1997. Identification of sources of horizontal resistance to *Phytophtora infestans* free of major genes. *CIP Circular* (April) 22, 14–17.

Runno-Paurson E, Fry WE, Remmel T, Mänd M, Myers KL, 2010. Phenotypic and genotypic characterisation of Estonian isolates of *Phytophthora infestans* in 2004–2007. *Journal of Plant Pathology* 92, 375–84.

Swofford DL, 2002. *PAUP*4.0 Beta, Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, USA: Sinauer Associates.

Tello C, 2008. Identificación de aspectos epidemiológicos relacionados con la expresión de resistencia de la papa (*Solanum tuberosum*) para las poblaciones de *Phytophthora infestans* predominantes en tres localidades de la Sierra Ecuatoriana. Quito, Ecuador: Universidad Central del Ecuador. [http://books.google.com.ec/books/about/Identificacion_de_aspectos_epidemiologic.html?id=eoszAQAAMAAJ&redir_esc=y.]. Accessed 09 January 2013.

Tooley PW, Therrien CD, 1991. Variation in ploidy in *Phytophthora infestans*. In: Lucas JA, Shattock RC, Shaw DS, Cooke LR, eds. *Phytophthora*. Cambridge, UK: Cambridge University Press, 204–17.

Vargas AM, Quesada OL, Céspedes MC *et al*., 2009. Characterization of *Phytophthora infestans* populations in Colombia: first report of the A2 mating type. *Phytopathology* 99, 82–8.

Vleeshouwers VGAA, Raffaele S, Vossen JH *et al*., 2011. Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology* 49, 507–31.

Wang M, Allefs S, van der Berg R, Vleeshouwers VGAA, van der Vossen E, Vosman B, 2008. Allele mining in *Solanum*: conserved homologues of *Rpi-blb1* are identified in *Solanum stoloniferum*. *Theoretical and Applied Genetics* 116, 933–43.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. *Phytophthora infestans* isolates associated with potato landraces in Ecuador.

Table S2. Reference *Phytophthora* spp. isolates used in the genetic study.

Table S3. Races of *Phytophthora infestans* associated with potato landraces in Ecuador.

Table S4. SSR multilocus genotypes of *Phytophthora infestans* isolates associated with potato landraces in Ecuador.

Table S5. SSR genotypes observed in reference isolates.

Figure S1. Electropherograms of the fluorescent amplification products for locus Pi4B in isolates of *Phytophthora andina*; (a) EC3365; (b) EC3380.

Figure S2. STRUCTURE analysis of the Ecuadorian *Phytophthora infestans* populations associated with potato landraces, based on 12 SSRs; (a) the whole Ecuadorian population, (b) structure of the clonal lineage.