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Genetic structure of the population of *Phytophthora infestans* attacking *Solanum ochranthum* in the highlands of Ecuador

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Abstract

Thirty-nine isolates of *Phytophthora infestans* were collected from the wild host *Solanum ochranthum* in the highland tropics of Ecuador and characterized with a set of phenotypic and molecular markers (mating type, metalaxyl sensitivity, the allozyme loci *Gpi*, and *Pep*, mitochondrial DNA haplotype, RFLP, and SSR), as well as for pathogenicity on various hosts. Three groups of isolates (A, B, and C) were identified based on their multilocus genotypes and variable abilities to cause disease on different hosts. Group A had a combination of alleles for the *Gpi* (86/100), *Pep* (96/100) and mtDNA (Ia) loci, as well as an RFLP fingerprint, that have not been reported for *P. infestans* in Ecuador, or elsewhere. Group B shares many marker characteristics with the US-1 lineage described in Ecuador on tomato, pear melon (*S. muricatum*), and *S. caripense*, but has SSR alleles not present in typical US-1 isolates. Group C for all markers tested is identical to the EC-1 lineage described on cultivated and wild potatoes in Ecuador. All isolates from *S. ochranthum* were able to re-infect their host of origin in the detached leaf assay; however, we did not draw clear conclusions as to the relative aggressiveness of the three groups on this host. Isolates of group A were the most specialized and were generally non-pathogenic or weakly pathogenic on all hosts other than *S. ochranthum*. Groups B and C infected tuber-bearing hosts, including the cultivated potato but were generally non-pathogenic on other non-tuber bearing hosts. *Solanum ochranthum* was infected by isolates coming from tuber-bearing *Solanum* hosts (i.e., the EC-1 lineage of *P. infestans*) and some US-1 isolates from non-tuber bearing hosts. Thus, in nature this species might be a potential reservoir of inoculum of different pathogen populations able to infect the cultivated hosts potato, tomato and pear melon (*S. muricatum*). Unlike potato and tomato in Ecuador, each of which is primarily attacked by a highly specialized pathogen population, *S. ochranthum* appears to harbour at least three pathogen groups of different genetic make-up. The unresolved issue of potential host specificity in isolates found on *S. ochranthum* could complicate efforts to use this species in tomato improvement.

Introduction

The oomycete pathogen *Phytophthora infestans* causes late blight, the most important disease of potato (*Solanum tuberosum*) worldwide and a very severe disease of tomato (*S. lycopersicum*). Most research on this pathogen has been conducted in the temperate zone on potato and

tomato, but the pathogen is reported to infect more than 100 hosts, most of them in the family Solanaceae, occurring in many parts of the world (de Abad and Henfling, 1982; Erwin and Ribeiro, 1996; Patiño et al., 1999). In recent studies in the tropical highlands of Ecuador we identified at least 18 host species in the Solanaceae with blight-like symptoms in nature (Adler et al., 2004). Some of the pathogen genotypes attacking these hosts are atypical and may eventually be described as one or more new species of *Phytophthora* (Adler et al. 2004). For the purpose of this paper, however, all will be considered *P. infestans*, the species that most adequately accommodates these pathogen genotypes.

In Ecuador, five groups of *P. infestans* have been detected (Erselius et al., 1999; Chacón et al., 2002; Adler et al., 2004). Three of these appear to be clonal lineages, each characterized by a particular mating type, mitochondrial DNA (mtDNA) haplotype, nuclear RFLP fingerprint and isoenzyme electrophoretic pattern. Each of the three lineages has been described in detail: EC-1 (Forbes et al., 1997), US-1 (Oyarzun et a1., 1998), and EC-3 (Adler et al., 2004). Another pathogen group was described as the EC-2 clonal lineage (Ordoñez et al., 2000) but we have since identified new genotypes of *P. infestans* similar to EC-2 but differing in mating type and mtDNA haplotype. Clearly, the EC-2 group with its two mating type subgroups does not fit the definition of a clonal lineage and this population has subsequently been referred to as the *Anarrhichomenum* group, in reference to the series in the genus *Solanum* comprising the varied and poorly defined plant species that these pathogens attack (Adler et al., 2004).

The hosts of *P. infestans* in Ecuador can be attacked by different pathogen lineages or groups, but generally only one of these pathogen groups is highly aggressive on each host species (Forbes et al., 1997; Oyarzun et al., 1998; Erselius et al., 1999). This observation holds for most pathosystems we have studied, but not all. The cultivated pear melon (*S. muricatum*) was thought to be attacked exclusively by isolates belonging to the US-1 lineage (Erselius et al., 1999), but recently pathogen genotypes belonging to the *Anarrhichomenum* group were found on this host in two separate epidemics, which occurred in the same geographic area but in subsequent years. Re-inoculation of these isolates on pear melon demonstrated that they were highly aggressive (Adler et al., 2002). US-1 and the *Anarrhichomenum* group are genetically distant and have different mating types (Adler et al., 2004).

Another host apparently attacked with regularity in nature by two or more pathogen groups is *S. ochranthum* Dunal, a woody vine growing in moist habitats of the northern Andes (Stommel, 2001). The initial isolates of *P. infestans from S. ochranthum* were identified as either EC-1 or US-1. Re-inoculation studies indicated that the US-1 isolates were more aggressive on *S. ochranthum* and were therefore considered the primary pathogen group (Erselius et al., 1999). However, subsequent isolations and pathogenicity studies were inconsistent with original observations, and new EC-1 isolates were found that were aggressive after re-inoculation. We had hypothesized that EC-1 was introduced into the Andes from Europe during the last two decades and replaced US-1 as the major pathogen lineage on potato (Forbes et al., 1997) but not on tomato (Erselius et al., 2000). Based on recent isolations of EC-1 from *S. ochranthum*, lineage displacement on that host also appeared to be a plausible hypothesis.

Solanum ochranthum and three other species, *S. juglandifolium*, *S. lycopersicoides* and *S. sitiens* form the series *Juglandifolia* in the section *Petota*, subgenus *Potatoe* (Hawkes, 1990). *Potatoe* is the group most closely related to tomato (Rick, 1979), with *S. juglandifolium* and *S. ochranthum* being the closest species (Peralta and Spooner, 2001). *Solanum ochranthum* can potentially be used in tomato improvement as a source of resistance to insects, bacteria, fungi, viruses (Rick, 1986; Rick et al., 1990), and *P. infestans* (Kobayashi et al., 1994). Resistance in *S. ochranthum* could also be transferred to potato via genetic engineering.

It is important to elucidate the complex and potentially dynamic relationship between *S. ochranthum* and *P. infestans in* Ecuador for several reasons. First, the utility of *S. ochranthum* as a source of resistance to *P. infestans* will be enhanced by understanding the host–pathogen relationship of this plant species. Observation of disease caused by an alternative and weak pathogen population may lead to spurious conclusions regarding the degree of resistance. Knowledge of the host/parasite relationship for *S. ochranthum* is also important because this plant species grows in

the same environment as potato and tomato and may play a role as an inoculum reservoir. Finally, the *S. ochranthum* story is one more piece in the complex puzzle of interactions between solanaceous plants and *P. infestans* in the Ecuadorian Andes, which has intrigued us for the last decade (Adler et al., 2004).

The objective of this study was to provide further information about the *S. ochranthum*/*P. infestans* pathosystem. Specifically, with this study we attempted to test the hypothesis that *S. ochranthum* is attacked by several groups of *P. infestans*, but one of these is more highly adapted to the host than the others. This was done by enlarging the sample size of isolates through new explorations and comparing all isolates with a suite of molecular and phenotypic markers. Isolates were also compared for pathogenicity on *S. ochranthum* and several other hosts of *P. infestans*.

Materials and methods

Pathogen collection

Thirty-nine isolates of *P. infestans* (Table 1) collected from *S. ochranthum* in the Ecuadorian provinces of Carchi, Imbabura, and Pichincha between 1997 and 2004 (Figure 1) were evaluated in this study. From 2001 on, collection sites were geo-referenced with GPS. In most cases, isolates were collected on plants with sporulating foliar lesions that resemble those produced by *P. infestans* on potato. In all cases, an effort was made to isolate from tissue containing only one lesion. Isolation was done by trapping with potato tuber slices (Forbes et al., 1997) or with a selective medium (Oyarzun et al., 1998). Isolates were maintained for short periods on Rye A and Rye B medium (Caten and Jinks, 1968) at 18 °C in the dark, and stored for longer periods on Rye A agar slants at 15 °C with 12-h photoperiod. The isolates collected before 2001 were described previously, although in less detail (Erselius et a1., 1999; Ordoñez et al., 2000; Chacón et al., 2002; Adler et al., 2004).

Table 1. Isolates of *Phytophthora infestans* collected from *Solanum ochranthum* between 1997 and 2004 in the tropical highlands of Ecuador

Year	Province	Collection site	Number	Group[a]
1997	Pichincha	Cutuglahua	1	A
1998	Pichincha	Cutuglahua	1	A/C
2001	Pichincha	Cutuglahua	3	A
2003	Pichincha	Cutuglahua	1	C
2004	Pichincha	Cutuglahua	4	A
1997	Carchi	El Angel	21	C
1997	Imbabura	Otavalo	1	B
1999	Imbabura	Otavalo	1	B
2002	Imbabura	Otavalo	1	C[b]
2003	Imbabura	Angochagua	5	C

[a]Group designation based on *Gpi*, *Pep*, mtDNA, RFLP fingerprint, and SSR alleles.
[b]This is a different collection location from the others in Otavalo.

Pathogen characterization

Isolates were characterized for mating type, metalaxyl resistance, glucose-6-phosphate isomerase (*Gpi*), peptidase (*Pep*), mitochondrial haplotype (mtDNA), DNA fingerprint (RFLP), and microsatellites (SSR).

Mating type was determined by pairing agar plugs of each isolate with known A1 and A2 isolates on 10% clarified V8 agar (Goodwin et al., 1992b). After 14 days at 18 °C, paired isolates were assessed for the presence of oospores in the zone of interaction by visual examination under a dissecting microscope. Isolates that produced oospores with the known A1 tester were designated A2 mating type and those that produced oospores with the A2 tester were designated A1 mating type.

Isolates were tested for resistance to 5 and 100 mg ml^{-1} of metalaxyl in 10% unclarified V8 medium and classified as sensitive, intermediate, or resistant. Conditions of the test and classification criteria were described previously (Forbes et al., 1997). Isolates were considered sensitive to metalaxyl if radial growth on 5 ppm reached 40% of the 0 ppm control; intermediate if radial growth was greater than 40% of the control on 5 ppm but not on 100 ppm; and resistant if radial growth on 100 ppm was greater than 40% of the control.

Isozyme electrophoresis for *Gpi* and *Pep* was done on starch (Spielman, 1991) and polyacrylamide gels. Polyacrylamide gel electrophoresis (PAGE) was carried out as described by Adler et al. (2004). Mitochondrial haplotypes were also determined as described previously (Adler et al., 2004) and classified as Ia, Ib, IIa, IIb (Carter et al.,

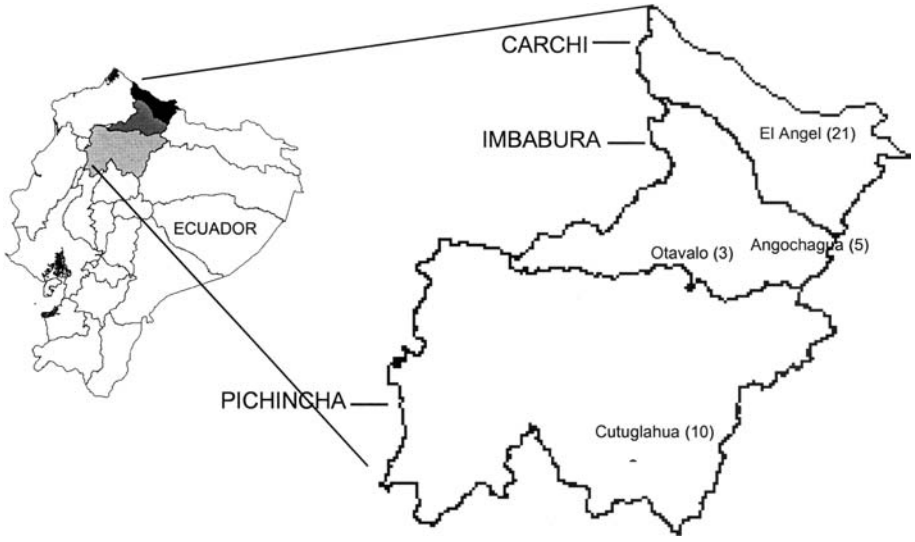

Figure 1. Collection sites of isolates of *Phytophthora infestans* from *Solanum ochranthum* in Ecuador between 1997 and 2004. Number of isolates at each locality in parentheses.

1990; Griffith and Shaw, 1998) or Ic (Oliva et al., 2002).

Restriction fragment length polymorphisms (RFLP) were obtained using the moderately repetitive probe RG-57 (Goodwin et al., 1992a) and the non-radioactive kit ECL (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The DNA marker λ, *Hin*dIII (10 μg ml^{-1} Amersham Pharmacia Biotech) was used as reference for relative band sizes.

Isolates were also evaluated with four SSR primers (4B, 4G, 1F, and 2D) previously published by Knapov et al. (2001). PCR amplifications were performed in a 10 μl volume containing 5 ng of genomic DNA of *P. infestans*, 1 μl of 10× PCR buffer (Amersham Pharmacia Biotechnology Inc.), 0.1 mM of each dNTP, 0.2 μM each of forward and reverse primers, and 0.7 U of *Taq* DNA polymerase (Amersham Pharmacia Biotechnology Inc.). One quarter of the forward primer was end-labelled with (γ-^{33}P) ATP (1000–3000 Ci/mmol; Amersham Pharmacia Biotech Ltd.). PCR was performed in an OmniGene cycler (MWG-Biotech) under the following conditions: 33 cycles of 40 s at 94 °C, 40 s at 60 °C and 20 s at 72 °C, with a final extension of 10 min at 72 °C. Before loading, the radio-labelled PCR products were denatured by adding one volume of denaturing 6× gel loading buffer (15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol FF) and heating at 94 °C for 5 min. Microsatellite alleles were separated by running the reactions on a 6% denaturing acrylamide gel (SequaGel XR, National Diagnostic) in 1× TBE buffer using an IBI DNA sequencing unit (BioMax STS45i; Kodak/International Biotechnology Inc.) at 1700 V. After this run, gels were transferred onto Whatman 3 mm paper (Whatman International Ltd.), dried at 80 °C in a gel dryer (Bio-Rad Laboratories) for 1–2 h, and exposed for 24–48 h to an X-ray film (Kodak Biomax MR; Eastman Kodak Company). Films were developed in a CURIX 60 (AGFA) developing machine.

Pathogenicity tests

Phytophthora infestans isolates collected from *S. ochranthum* were re-inoculated on *S. ochranthum* and also on several other hosts from the highlands of Ecuador, some of which are tuber-bearing (hereafter referred to as *Petota* hosts) and others that are non-tuber bearing (hereafter referred to as non-*Petota* hosts). Isolates collected on these hosts (*Petota* and non-*Petota* isolates, respectively) were also inoculated on *S. ochranthum*.

Leaflets used in the detached leaf assays came from different sources: host plants collected directly in their natural environments, plants derived from true potato seed (from berries collected in the field) and seed tubers, plants growing near

the CIP station in Quito, Ecuador, and plants bought in markets. *Solanum ochranthum* leaflets were collected from plants growing naturally in a gorge near the CIP station. For most species, including *S. ochranthum*, the first pair of fully expanded lateral leaflets was taken from a healthy leaf, and in the case of potted plants this was taken from the upper third of the plant. For other species, such as *S. betaceum*, *S. quitoense*, *S. caripense*, and *S. paucijugum*, whole leaves were used, although hereafter we refer to all tissues as leaflets. Leaflets were washed with tap water, towel dried and stored abaxial side up in the lids of inverted Petri dishes which contained water agar (4%) in the base. Petri dishes were subsequently used as high humidity chambers for inoculation and incubation.

Petota isolates were first inoculated on leaflets of potato cultivars or of their original hosts to restore aggressiveness after cultivation on rye agar. Non-*Petota* isolates were multiplied on green tomato fruits or, when these were not available (for *S. ochranthum* and *S. quitoense*), on Rye A medium. Some attempts were made to multiply isolates from *S. ochranthum* and *S. quitoense* on host leaf tissue, but because of long incubation periods, contamination and scarce sporulation (*S. quitoense*) this approach failed to consistently produce sufficient inoculum. Whether from living tissue or Rye A medium, sporangia were rinsed several times with distilled water over a 12 μm filter. Attempts to standardize inoculum concentrations were unsuccessful so variable concentrations were used, which ranged from 6,000 to 30,000 sporangia ml^{-1}. One 10 μl drop was placed on the abaxial side of each leaflet close to the midrib. Petri dishes containing inoculated leaflets were incubated at 15 ± 2 °C with 14 h of fluorescent light per day. Evaluations were made 5, 6, 7 and 10 days after inoculation. Pathogenicity, or ability to cause infection, was based on presence of signs (sporangiophores and/or sporangia) within a 10-day period. All inocula were checked for inherent pathogenicity by inoculation on their host of origin.

Results

Pathogen characterization

All *S. ochranthum* isolates tested were of the Al mating type. The majority of isolates were sensitive to metalaxyl, as only two had an intermediate resistance. These two were collected in Carchi and belonged to group C (grouping described below).

All isolates of *S. ochranthum* except one fell into one of three distinct groups, designated A, B, or C, based on mtDNA haplotype, isoenzyme pattern, RFLP fingerprint, and SSR alleles (Table 2). The

Table 2. Multilocus genotypes of *Phytophthora infestans* isolates collected from *Solanum ochranthum* in Ecuador between 1997 and 2004

Group[a]	Isolate #	Province	*Gpi* *Pep*	mtDNA RFLP fingerprint[b]	SSR[c]		
					4B	4G	1F
A	8	Pichincha	86/100 96/100 Ia	New 101 010 100 100 110 101 111 001 1; 00100	*212* 216	161	*128 166*
B	2	Imbabura	86/100 92/100 Ib	US-1 101 010 101 100 110 100 011 001 1; 00100	216 220	161	*112* 118 *164*
C	28	Carchi (21) Imbabura (6) Pichincha (2)	90/100 96/100 IIa	EC-1 111 010 100 100 110 100 011 001 1; 00101	*206* 216 220 *157* 161[d]		*120*[e]
A/C[f]	1	Pichincha	86/100 96/100 IIa	New 101 010 100 100 110 101 111 001 1; 00100	206 216 220 157 161		120

[a]Group designation based on *Gpi*, *Pep*, mtDNA, RFLP fingerprint, and SSR alleles. All isolates were A1 mating type.
[b]RFLP banding pattern based on probe RG-57 (Goodwin et al., 1992a). Presence or absence of bands is indicated by 1 and 0, respectively. From left to right, the bands refer to band positions 1 to 25, followed by bands 1a, 8a, 14a, 20a, and 24a.
[c]Microsatellite primers described by Knapova et al. (2001). Unique alleles for each primer are shown in italics.
[d]One isolate from Carchi was 157.
[e]One isolate from Carchi was 119 120. One isolate from Imbabura was 118 120.
[f]Intermediate type between A and C groups.

SSR primer 2D was not polymorphic and therefore was not included in the analysis.

Group A had a combination of alleles not reported previously for any pathogen genotype in Ecuador (Adler et al., 2004). The eight isolates in this group were characterized by *Gpi* 86/100, *Pep* 96/100, Ia mtDNA haplotype and an RFLP fingerprint different from any described previously (Table 2, Figure 2). The fingerprint lacked bands 2 and 24a, present in the EC-1 lineage, and had bands 18 and 19, previously described for the RFLP fingerprint EC-2.1 (*Anarrhichomenum* group) (Ordoñez et al., 2000). Isolates in group A were collected in the province of Pichincha, near the CIP station, between 1997 and 2004 (Table 1).

The second group, B, comprised two isolates similar to the US-1 lineage, that were collected in the province of Imbabura (close to Otavalo) in 1997 and 1999 (Table 1). These isolates had marker data identical to US-1, including *Gpi* 86/100, *Pep* 92/100, Ib haplotype, and an RFLP fingerprint identical to that of US-1 (Table 2). However, two of the SSR alleles associated with these isolates have not been found in other US-1 isolates we have tested (unpublished data).

The third group, C, included isolates collected in Carchi (El Angel), Imbabura (Otavalo), and Pichincha (CIP station) between 1997 and 2003 (Table 1). Based on the markers we used, these isolates were identical to previous descriptions of EC-1 (Table 2, Figure 2). Our unpublished data indicate that these isolates are also identical to EC-1 for SSR alleles.

The SSR characterization revealed 12 alleles for the loci 4B, 4G, and IF (Table 2) with loci 4B and IF being more polymorphic than 4G. The three isolate groups could be distinguished based on the combination of alleles and, in most cases, each group had a specific genotype at each locus (Table 2). Some alleles were associated only with a particular isolate group, with locus IF having the highest number of these. At the 4B locus, alleles 212 and 206 were exclusive of groups A and C, respectively (Figure 3). At locus 4G, allele 157 was exclusive of C. At locus IF, all three groups had at least one unique allele: alleles 128 and 166 for A; 112 and 164 for B; and 120 for C.

Isolate 3222, collected in 1998, had common characteristics between A and C groups (Table 2). It had the isoenzyme pattern and the RFLP

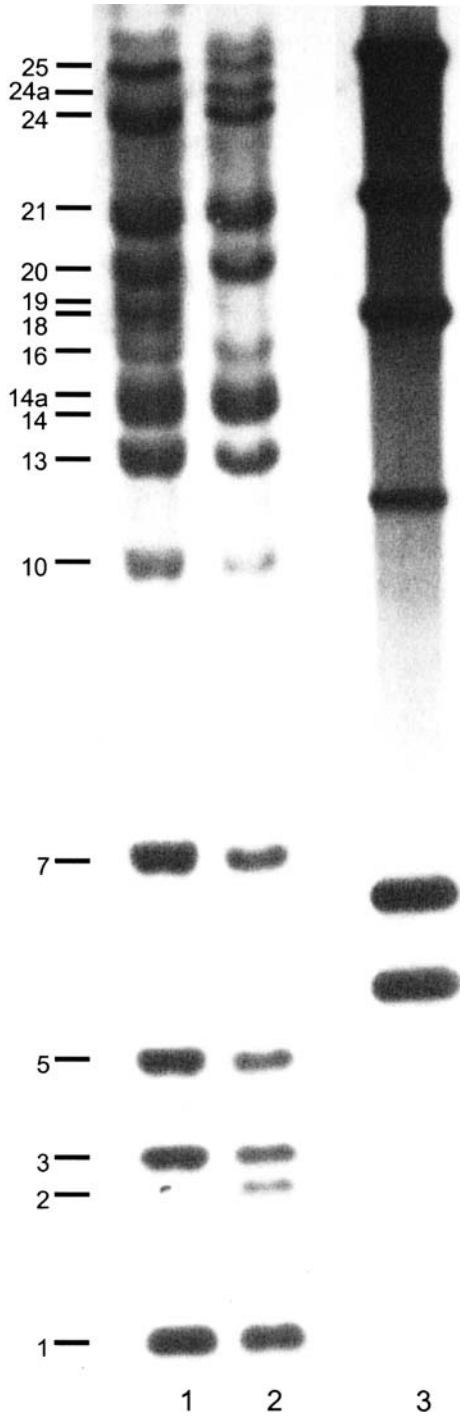

Figure 2. RELP fingerprints (with RG-57) *of Phytophthora infestans* isolates collected on *Solanum ochranthum* in Ecuador. Lane 1 = group A (new), lane 2 = group C (EC-1), lane 3 = λ DNA marker. Band numbers are listed on the left side.

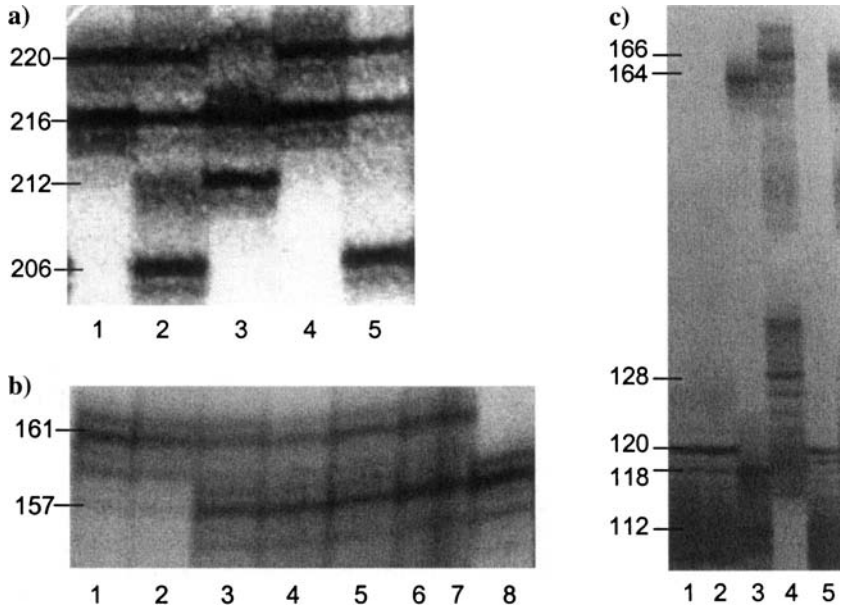

Figure 3. Microsatellite (SSR) banding patterns of *Phytophthora infestans* isolates collected from *Solanum ochranthum* in Ecuador. (a) Primer 4B: lane 1 = US-1 collected from *Solanum lycopersicum*, lane 2 = EC-1 collected from *S. paucijugum* (*Petota*), lane 3 = group A, lane 4 = group B, and lane 5 = group C; (b) Primer 4G: lane 1 = group A, lane 2 = group B, lanes 3 to 6 = group C, lane 7 = EC-1 collected from *S. tuquerrense*, and lane 8 = group C; (c) Primer 1F: lane 1 = EC-1 collected from *S. paucijugum*, lane 2 = group C, lane 3 = group B, lane 4 = group A, and lane 5 = group C. The size of bands (bp) is noted on the left side of each image.

fingerprint of the A type, but the mtDNA and SSR alleles were the same as group C.

Pathogenicity tests

Most isolates tested, including those from *S. ochranthum*, were able to re-infect their host of origin in the detached leaf assay. The only exception was the intermediate isolate described above (no. 3222), which did not re-infect *S. ochranthum*. Group A isolates were the most specific among those from *S. ochranthum* and generally did not infect hosts other than *S. ochranthum*, although one isolate did cause a sporulating lesion on the potato (*S. tuberosum*) cultivar INIAP-Catalina. All other inoculation attempts of group A on *Petota* and non-*Petota* materials generally caused no symptoms or signs of infection, except that in a very few occasions necrotic spots were observed.

The other two groups (B and C) were generally pathogenic on *Petota* species, but for the most part did not cause sporulation on non-*Petota* hosts within 10 days of inoculation except for two cases in which small lesions with some sporulation were visible. Most reactions between B and C groups and non-*Petota* hosts caused no visual symptoms.

Isolates from *Petota* hosts were of variable pathogenicity on *S. ochranthum*. Twelve of 18 EC-1 isolates we tested, coming from the nine *Petota* species, caused sporulating lesions within 10 days. Isolates from non-*Petota* hosts were generally non-pathogenic on *S. ochranthum*. The *Anarrhichomenum* group isolates, involving both Al and A2 mating types, and one isolate from the flower of *Brugmansia sanguinea*, caused no lesions on *S. ochranthum*. Only one isolate from EC-3, which attacks *S. betaceum*, was inoculated on *S. ochranthum*, and it did not cause a lesion. Likewise, two isolates from *S. quitoense* caused no lesions on *S. ochranthum*. US-1 isolates coming from several hosts (*S. caripense*, *S. lycopersicum*, and *S. muricatum*) were variable with about half causing lesions, which remained small and had reduced sporulation. There was no apparent association between the origin of the US-1 isolate and its pathogenicity on *S. ochranthum*. Therefore, overall, US-1 isolates generally appeared to be pathogenic but of limited aggressiveness.

Discussion

The hypothesis we proposed to test, that *S. ochranthum* is attacked by more than one pathogen group of *P. infestans* in Ecuador and that one of these is most highly adapted, was only partially tested. It is clear that different pathogen groups were associated with this host but it is not clear that a particular one was highly adapted to the host. Therefore, we cannot exclude the possibility that *S. ochranthum* is not a primary host of *P. infestans* in Ecuador and infections may represent selection of occasional pathogenic clones from the population of airborne spores. Further research on a larger sample is needed to determine whether there is a population of *P. infestans* that has adapted over time to *S. ochranthum*.

Our efforts to enlarge the collection of isolates of *P. infestans* from *S. ochranthum* were only partially successful. This is primarily due to the scarcity of the plant in nature and the even greater patchiness of the disease. Ironically, this uncommon species grows in a humid ravine within 400 m of the CIP experiment station. In that location it almost always has lesions, although two different pathogen groups were collected there in different years (see collection site Cutuglahua, Table 1). *Solanum ochranthum* also was found in a number of locations but without disease (not reported here). Even after extensive searching, the 39 isolates we characterized represent only five locations, some of which were visited in subsequent years. In spite of this reduced number of collection sites, we found a variable pathogen population associated with this host species.

The three pathogen groups we found on *S. ochranthum* are distinguishable by their multilocus genotypes. With the markers most commonly used for this pathogen species (e.g., isozymes, mtDNA, RFLP), two of the pathogen groups described on *S. ochranthum* could be placed into two previously described lineages of the pathogen. Group B shares characteristics with the US-1 lineage described in Ecuador on *S. lycopersicum*, *S. muricatum*, and *S. caripense* (Oyarzun et al., 1998) and elsewhere (Goodwin et al., 1994); however, the two US-1 isolates we collected from *S. ochranthum* have two SSR alleles (112 and 118 from locus 1F) not found in other US-1 isolates from Ecuador (unpublished data). Therefore, it would appear that Group B is a sub-group of US-1. This hypothesis is consistent with the low pathogenic aggressiveness of typical US-1 isolates on *S. ochranthum*.

Group C is identical for all markers tested, including SSR alleles (G. Chacón, unpublished) to the EC-1 lineage described on cultivated and wild potatoes in Ecuador (Forbes et al., 1997; Adler et al., 2004). Group C is the only one comprised of isolates with intermediate sensitivity to metalaxyl (all isolates of the other two groups were sensitive). The intermediate group C isolates were collected in Carchi, the main potato-producing province in Ecuador, where resistance to this fungicide has been described in EC-1 isolates from potato (Forbes et al., 1997). Our inoculation studies would support (although not conclusively prove) the hypothesis that EC-1 from cultivated and wild potatoes can multiply on *S. ochranthum*. However, it is unclear why the EC-1 isolates we isolated from *S. ochranthum* did not infect tomato in the pathogenicity test. Though not the primary pathogen lineage attacking tomato in Ecuador, EC-1 does infect tomato and detached leaf inoculations in the past identified only small differences in pathogenic aggressiveness between EC-1 and the tomato-adapted US-1 (Oyarzun et al., 1998). Nonetheless, in that study, a few of the potato isolates did not infect any of the tomato differentials that were used.

Unlike the two groups described above, group A is not associated with a known pathogen lineage. In this group, the combination of alleles at the *Gpi* (86/100), *Pep* (96/100) and mtDNA (Ia) loci, as well as the RFLP fingerprint, have not been reported for *P. infestans* in Ecuador, nor elsewhere. This group also appeared to be the most highly specialized of the three, attacking only *S. ochranthum* among the potential hosts we tested (Table 2). In an earlier study, Group A was probably misidentified as typical US-1 based on its 86/100 *Gpi* pattern (Erselius et al., 1999).

Our sample was too small for conclusions about the population dynamics of *P. infestans* on this host, but there was, nonetheless, apparent geographic sub-structuring in the population. Group A was only found in the Cutuglahua site and group B was only found in Otavalo. Group C was more generalized but was principally found in the north in Carchi and Angochagua. One clear C isolate was also found in Cutuglahua.

In addition to the three groups we identified, all apparently clonal, we identified one isolate that

appeared to be intermediate between groups A and C. This isolate was found in Cutuglahua, a location where both groups A and C were also collected. Therefore it appears possible that the intermediate isolate may be a product of asexual recombination (Shaw, 1991). With this isolate, we were unable to cause infection on leaves of *S. ochranthum* (also taken from Cutuglahua), which would indicate the isolate is not, or is no longer, pathogenic. We propose that the A and C isolate is neither a heterokaryon nor a mixture of isolates because either would have produced a multilocus marker genotype that included all alleles in both A and C.

Due to problems of inconsistent inoculum production, variable host tissue age, necrosis associated with some lesions and long incubation periods in the leaf assay, we could not draw clear conclusions as to the relative aggressiveness of the three pathogen groups on *S. ochranthum*. Pathogenicity tests were used as a general indicator of pathogenicity and not for quantifying differences in aggressiveness among isolates or groups. Since only five locations were found where isolates could be collected, predominance in the field is also not a good indicator of adaptation.

In Ecuador, *S. ochranthum* is a third example of a host species that has been associated with repeated isolations of more than one pathogen group or clonal lineage of *P. infestans* in nature. The first case was *S. andreanum*, where both the EC-1 and US-1 lineages were isolated (Erselius et al., 1999) and the second was *S. muricatum*, where both the US-1 and EC-2 isolates were identified (Adler et al., 2002, 2004). Interestingly, all isolations from *S. andreanum* subsequent to the study mentioned above produced only EC-1 isolates, so the isolation of US-1 appears to be a rare event. Similarly, although EC-2 isolates were found twice on *S. muricatum*, they were not found in subsequent studies, even in the same geographic area. These cases may represent occasional opportunistic infection from other hosts; however, the case of *S. muricatum* is nonetheless significant because the two pathogen populations were of different mating type. A common host for different mating types could increase the probability of sexual reproduction.

The relatively clear situation for potato and tomato and the less clear situation for the other hosts mentioned here may reflect peculiarities, already highlighted, of disease systems that occur sporadically in nature, and in some cases on hosts that are found in a limited number of locations (e.g., *S. ochranthum* and *S. andreanum*). In addition to host adaptation, the presence of a given pathogen genotype on a host may be due to availability of inoculum after periodic dry spells. Even in a relatively ubiquitous cultivated host like potato, founder effects and other fitness characteristics may help preserve less aggressive isolates in a pathogen population (Lebreton et al., 1999).

Potential variation in the host may also influence the pathogen population structure in Ecuador. Variability has been described among genotypes of *S. ochranthum* (Correll, 1962; Hawkes, 1990) and although we were not able to distinguish different genotypes in our collection, this possibility cannot be ruled out. However, as noted, two pathogen groups (A and C) were sequentially isolated in different years from the same plants growing in Cutuglahua near the CIP station (Table 1). Thus, in this case the host genotype did not appear to be a determining factor in the presence of any particular group, at least for groups A and C.

This study showed that *S. ochranthum* can be infected by EC-1 isolates from potato and to a lesser extent by US-1 isolates from tomato and *S. muricatum*. Thus, in nature this species might be a potential reservoir of inoculum of different lineages able to infect these cultivated crops. *Solanum ochranthum* as a perennial is available as an alternative host of *P. infestans* at any time of the year. The tendency of this species to grow close to sources of water makes it an ideal refuge for the pathogen during dry spells. Even during a drought period when disease pressure is low, conditions inside the lush vines may be suitable for the pathogen to survive for extended periods.

The potential use of *S. ochranthum* as a source of resistance to *P. infestans* should be considered with caution until more isolates are screened and the host–pathogen interactions between *P. infestans* and this host species are clarified. Typical US-1 isolates, representing the primary pathogen lineage of tomato in Ecuador, were weakly pathogenic on *S. ochranthum* in our test. The two isolates like US-1 that we found on this host may represent a specialized sub-population of the lineage that had partially adapted to the host. If *S. ochranthum* were used as a source of resistance,

segregating progeny should be screened against an aggressive pathogen population. The suggestion of Flier and Turkensteen (1999), that resistance screening should be done with several diverse pathogen genotypes, may be the best option for this pathosystem until more is known about pathogen diversity on *S. ochranthum*.

Isolates representing the different groups are available from G. Forbes.

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