Genetic Diversity of *Phytophthora infestans* sensu lato in Ecuador Provides New Insight Into the Origin of This Important Plant Pathogen

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ABSTRACT

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The metapopulation structure of *Phytophthora infestans* sensu lato is genetically diverse in the highlands of Ecuador. Previous reports documented the diversity associated with four putative clonal lineages of the pathogen collected from various hosts in the genus *Solanum.* This paper simultaneously analyzes diversity of the complete collection of isolates, including a large number that had not yet been reported. This analysis confirmed the existence of three pathogen populations, which all appear to be clonal lineages, and that correspond to those previously reported as US-1, EC-1, and EC-3. No evidence was found from the analyses of recently collected isolates that would contradict earlier reports about these three lineages. In contrast, new data from a group of isolates from several similar hosts caused us to modify the previous description of clonal lineage EC-2 and its previously proposed hosts, *S. brevifolium* and *S. tetrapetalum.* Given the uncertainty associated with the identification of these hosts, which all belong to the section *Anarrhichomenum*, we refer to them as the *Anarrhichomenum* complex, pending further taxonomic clarification. New pathogen genotypes associated with the *Anarrhichomenum* complex were isolated recently that are A1 mating type and Ia mitochondrial DNA (mtDNA) haplotype, and therefore differ from the previously described EC-2 lineage, which is A2 and Ic, respectively. Because of uncertainty on host identification, we do not know if the new genotypes are limited to one host species and therefore represent yet another host-adapted clonal lineage. For now, we refer to the new genotypes and previously described EC-2 genotypes, together, as the pathogen group attacking the *Anarrhichomenum* complex. Two A2 isolates identical to the previously described EC-2 archetype were collected from severely infected plants of pear melon (*S. muricatum*). Pear melon is generally attacked by US-1, and this is the first clear case we have documented in which two distinct pathogen genotypes have caused severe epidemics on the same host. Based on presence of unique marker alleles (restriction fragment length polymorphism [RFLP] and mtDNA) and genetic similarity analysis using RFLP and amplified fragment length polymorphism data, EC-3 and isolates from the *Anarrhichomenum* complex are genetically distinct from all genotypes of *P. infestans* that have been reported previously. No current theory of historical migrations for this pathogen can adequately support a Mexican origin for EC-3 and genotypes of the *Anarrhichomenum* complex and they may, therefore, be palaeoendemic to the Andean highlands. To date, we have identified 15 hosts in the genus *Solanum*, in addition to the *Anarrhichomenum* complex, and some unidentified species of *P. infestans* sensu lato in Ecuador. Five of the *Solanum* hosts are cultivated. One isolate was collected from *Brugmansia sanguinea*, which represents the first report from Ecuador of a host of this pathogen that is not in the genus *Solanum.* However, *P. infestans* sensu lato was only found on flower petals of *B. sanguinea.* This study provides new insights into the population structure of highly specialized genotypes of *P. infestans* sensu lato in the Andean highlands. The results are discussed in light of previous hypotheses regarding the geographic origin of the pathogen.

The late blight pathogen, *Phytophthora infestans* (Mont.) de Bary, which has a global distribution on potatoes and tomatoes, is believed to have originated in the highlands of central Mexico (27,29), causing mild epidemics on native wild tuber-bearing *Solanum* sp. (37). Mexico is considered the center of origin of *P. infestans* because both mating types (A1 and A2) are present and there is a high genetic diversity for this pathogen in this region (25,29,32,42). In contrast, only the A1 mating type and low genetic diversity had been found outside Mexico until the mid-1980s (27,29,31). An alternative hypothesis proposed the Andes, the center of origin of the cultivated potato, as the center of origin for *P. infestans* (1). This hypothesis is based primarily on historical accounts of potato disease in the Andes, and appears to have little support in the scientific literature.

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Late blight of potato first appeared in the United States and Europe in the middle 1800s. Devastating late blight epidemics destroyed potato crops in Ireland during 1845 and 1846, contributing to poverty, starvation, and emigration. Until recently, most isolates of *P. infestans* found outside North America belonged to the US-1 clonal lineage. This led to a hypothesis that US-1 had caused the original epidemics in Europe in the 1800s (24) and then spread globally, presumably with seed trade (21). Recent analyses, however, of mitochondrial DNA (mtDNA) of *P. infestans* in herbarium material presented evidence that a genotype different from US-1 was involved in the original epidemics in Europe (38).

During the 1980s, the A2 mating type of *P. infestans* was detected in Europe (31) along with several new alleles for known markers (12). A second global migration from Mexico had taken place. The pathogen population in Europe is now highly diverse and there is evidence for sexual reproduction in several European countries (3,11,44). As a variant to the hypothesis for the first and second global migrations of *P. infestans*, some authors proposed that earlier migrations also took place from Mexico to South America (4,43). One hypothesis also advances the idea that *P. in-*

festans was originally introduced into Europe from South America and not directly from Mexico (4,36).

The brief description of the origins and migrations of *P. infestans* provided can be found in much greater detail in recent reviews (4,23). Nonetheless, the conclusions presented in these reviews are based primarily on studies involving isolates of *P. infestans* originating from potato, and to a lesser extent tomato. This is probably because late blight is an economically important disease on potatoes worldwide and possibly also because there appear to be few alternative hosts, apart from tomato, in the temperate zone where most of the research on the pathogen was done. In other parts of the world however, *P. infestans*, or species similar to *P. infestans*, are known to attack several other wild and cultivated hosts in the family Solanaceae. In Mexico, *P. infestans* attacks several wild tuber-bearing species of *Solanum.* Evidence from recent studies in Mexico indicates that these wild *Solanum* spp. are all attacked by *P. infestans* (29), although only limited gene flow exists between the subpopulations of the pathogen on wild *Solanum* spp. and cultivated potatoes (17). A new homothallic *Phytophthora* sp. closely related to *P. infestans* was recently described as *P. ipomoeae* (18). In the Toluca Valley of central Mexico, epidemics on solanaceous hosts are caused exclusively by *P. infestans* because the host range of *P. mirabilis* (22) and *P. ipomoeae* is restricted to nonsolanaceous hosts.

The situation appears to be more complex in South America where a *Phytophthora* sp. very similar to *P. infestans* was found on hosts then described as *S. brevifolium* and *S. tetrapetalum* (34). This pathogen group was designated the EC-2 clonal lineage of *P. infestans* sensu lato and it does not attack cultivated potatoes or tomatoes. Four host-adapted groups of *P. infestans* sensu lato have recently been described in Ecuador. Little variability was found within these groups, and there appeared to be little or no gene flow among them, so all were considered to be clonal lineages. Their designations were EC-1, found on potato and tuber-bearing wild *Solanum* spp. (19); EC-2, described previously; US-1, found on tomato, cultivated pear melon (*S. muricatum*), and *S. caripense* (19,35); and EC-3, found on cultivated tree tomato (*S. betaceum*) (13). Novel restriction fragment length polymorphism (RFLP) bands were found in fingerprints of EC-2 and EC-3 genotypes (13,34), and the EC-2 lineage was characterized by a new mtDNA haplotype (34), subsequently designated as Ic (33). These results demonstrate that the Ecuadorian highlands harbor wide genetic diversity within *P. infestans* sensu lato that has not been detected in studies done elsewhere on pathogen populations attacking potato and tomato. Furthermore, preliminary assessment of recent isolates collected in Ecuador indicated that the full extent of pathogen diversity has not yet been described. In the current study, we describe genotypes of *P. infestans* sensu lato collected recently from several cultivated and wild hosts in the highlands of Ecuador. The new data have caused us to modify some previous conceptions about the *P. infestans* sensu lato in Ecuador. The origin and historical migrations of this pathogen are discussed in light of our current understanding of the pathogen population structure in the Ecuadorian highlands.

MATERIALS AND METHODS

Definitions. An operational definition for the taxonomic status of host-adapted populations of *P. infestans* sensu lato was applied throughout this paper. There is evidence suggesting that certain host-adapted clonal lineages present in Ecuador may belong to one or more *Phytophthora* spp. that have not yet been formally described (34). The *P. infestans* sensu lato metapopulation is defined as a group of local pathogen populations with varying levels of genetic isolation based on geographic distance, temporal effects, and host specialization. Nonetheless, for the purpose of this publication, we consider these populations to be part of *P. infestans* sensu lato because no other described species accommodates them better.

Collection and isolation of *P. infestans* sensu lato. We collected and assessed with at least three markers over 450 single lesion isolates throughout the highlands of Ecuador from 1995 to 2002 (Table 1). Except for specific studies on pathogen populations attacking potato (19) and tomato (14,35), no specific sampling plan was followed for the collection. Collections from wild tuber-bearing species were oriented on previous trips by plant taxonomists (41), but we also visited previously unexplored areas. Researchers from the National Agricultural Research Institute

TABLE 1. Number of isolates of *Phytophthora infestans* sensu lato from different hosts in Ecuador evaluated for different markers

Host species	RFLP classification	Base[a]	MtDNA[b]	RFLP[c]	AFLP[d]	Metalaxyl[e]		
						R	I	S
Tuber-bearing								
Solanum andreanum	EC-1, US-1	17	15	7	…	…	2	10
S. colombianum	EC-1	91	71	60	2	2	5	12
S. minutifoliolum	EC-1	11	9	11	2	…	…	4
S. paucijugum	EC-1	19	17	15	3	1	…	8
S. regularifolium	EC-1	1	1	1	1	…	1	…
S. solisii	EC-1	1	1	1	…	…	…	1
Solanum spp.		14	13	8	2	…	1	4
S. tuberosum, *S. phureja* (potato)	EC-1	49	43	22	5	11	8	19
S. tuquerrense	EC-1	9	8	5	…	…	…	9
Non-tuber-bearing								
Brugmansia sanguinea	EC-2	1	1	1	…	…	…	1
S. betaceum (tree tomato)	EC-3	34	31	17	5	3	3	19
Anarrhichomenum complex	EC-2, EC-2.1[f]	67	66	29	13	…	1	36
S. caripense	US-1	25	22	8	1	…	10	7
S. lycopersicum (tomato)	US-1	77	49	13	4	2	21	28
S. muricatum (pear melon)	US-1, EC-2.1[f]	19	18	11	…	…	3	4
S. ochranthum	US-1	16	15	4	…	…	2	13
S. radicans	?	1	1	…	…	…	…	…
Total		452	381	213	38	…	…	…

[a] The base set of markers consists of mating type and banding patterns for *glucose-6-phosphate isomerase* and *peptidase*.
[b] Mitochondrial DNA haplotype.
[c] Restriction fragment length polymorphism (RFLP) fingerprint.
[d] Amplified fragment length polymorphism fingerprint.
[e] R = resistant to metalaxyl, I = intermediate, and S = sensitive.
[f] The EC-2.1 sublineage was described previously (34). The RFLP fingerprint of EC-2.1 differs from EC-2 by three bands.

(INIAP, Quito, Ecuador) provided useful information on the locations of plantations of the cultivated hosts pear melon (*S. muricatum*) and tree tomato (*S. betaceum*). In general, Ecuador is a center of diversity for the genus *Solanum* (30) and most of the wild non-tuber-bearing hosts we describe are common. Therefore, numerous trips to unexplored areas were planned based on knowledge of climatic conditions favorable for late blight development. Isolations were made by trapping with potato tuber slices (19) or with a selective medium (34,35). Some isolates originating from plants of the *Anarrhichomenum* complex (34) and from tree tomato (13) were described previously. Some of the isolates from potato and tomato were also described using several genetic and phenotypic markers (19,35). A brief description of two isolates with A2 mating type isolated from pear melon (*S. muricatum*) was published recently (2). All isolates were maintained for short periods on rye A or rye B medium (8) at 18°C in the dark. Isolates are being stored for longer periods on rye A agar slants at 15°C with a 12 h photoperiod in Quito, Ecuador (contact G. A. Forbes) and in liquid nitrogen at Plant Research International in Wageningen, the Netherlands (contact W. G. Flier).

Characterization of isolates. All isolates reported here (452) were tested for mating type, *glucose-6-phosphate isomerase* (*Gpi*), and *peptidase* (*Pep*) (Table 1). A majority of isolates from each host was also tested for mtDNA haplotype. Smaller samples from each host were fingerprinted using RFLP and amplified fragment length polymorphism (AFLP) technology. Metalaxyl resistance was tested for a subset of isolates from most hosts. At least two isolates from each of the major pathogen groups were compared for sporangial dimensions and papilla shape using light microscopy and a video image system.

Metalaxyl resistance. Isolates were tested for resistance to 5 and 100 µg of metalaxyl per ml of 10% unclarified V8 medium and classified as resistant, intermediate, or sensitive. Conditions of the test and criteria for classification were described previously (19).

Isozyme electrophoresis. Isozyme electrophoresis for the enzymes *Gpi* and *Pep* was done on starch gels (40) and on polyacrylamide gels. Polyacrylamide gel electrophoresis (PAGE) was done using 1 mm thick 7.5% gels with 25 mM Tris and 0.19 M glycine, pH 8.8, as separating gel and electrode buffer. Bands were clearer when a 1-cm stacking gel (2.5% acrylamide 0.06 M Tris-HCl, pH 6.7) was used (10). PAGE was run with a constant current of 5 mA for 1 h and then increased to 10 mA. Voltage rose continuously throughout, from about 50 to 280 V. Electrophoresis was terminated when the bromophenol blue dye reached the bottom of the gel, at about 16 cm running length. Allozyme genotypes (inferred from banding pattern phenotypes) were scored as described by Spielman et al. (40). Scorings represent mobility of the enzyme alleles relative to an allele designated arbitrarily as 100. Isolates with known alleles from the collection of W. E. Fry, Cornell University, were used for comparison.

DNA extraction. Isolates were grown for 10 to 14 days at 20°C in pea broth prepared by autoclaving 120 g of frozen peas in 1 liter of water. The peas were removed by filtering through cheesecloth and the broth was autoclaved again. The mycelium was grown on pea broth, harvested, lyophilized, and stored at –80°C. Lyophilized mycelium (10 to 20 mg) was ground in microcentrifuge tubes with a pestle and sterile sand. Total DNA was extracted with the Puregene Kit (Gentra/Biozym, Landgraaf, the Netherlands) according to manufacturer's instructions or as previously described (34). DNA was dissolved in 100 µl of TE (10 mM Tris-HCl [pH 8.0] and 1 mM EDTA [pH 8.0]) and stored at –20°C.

mtDNA haplotype. mtDNA haplotypes were determined by amplification of DNA of each isolate using primers designed for specific regions of the mitochondrial genome of *P. infestans* (28). Polymerase chain reaction (PCR) was performed in a thermocycler (PTC100; MJ Research, Waltham, MA). Digestion of the amplified regions with *Cfo*I, *Msp*I, and *Eco*RI restriction enzymes yielded restriction patterns by which the isolates could be classified into five haplotypes: Ia, Ib, IIa, IIb (7,28), and Ic (33).

RFLP fingerprinting. A total of 213 isolates were characterized using the moderately repetitive clone RG57 (26) and the nonradioactive enhanced chemiluminescence (ECL) kit (Amersham, Eindhoven, the Netherlands) according to the manufacturer's instructions.

AFLP fingerprinting. DNA (250 ng) was digested in a 50-µl reaction volume with *Eco*RI (10 units) and *Mse*I (10 units) for 6 h at 37°C in restriction ligation buffer (10 mM Tris/Ac [pH 7.5], 10 mM MgAc, 50 mM KAc, 5 mM dithiothreitol, and 50 ng μl^{-1} bovine serum albumin). Digestion was confirmed on agarose gels. Restriction fragments were ligated to *Mse*I adapters (5′-GACGATGAGTCCTGAT/CTACTCAGGACTAGC-5′) and *Eco*RI adaptors (5′-CTCGTAGACTGCGTACC/CATCTGACGCATGGTTAA-5′) using 0.1 µM *Eco*RI adapter, 1.0 µM *Mse*I adapter, 0.2 mM ATP, and 2.4 units of T4 DNA ligase (Amersham Pharmacia Biotech, Uppsala, Sweden) (5). Ligation was performed overnight at 10 to 12°C, and the ligation products were diluted 10 times with filtered ultra pure water. Nonselective PCR amplification was performed using primers E00 (5′-GACTGCGTACCAATTC-3′) and Mse00 (5′-GATGAGTCCTGAGTAA-3′) for all restriction fragments. Nonselective PCR amplifications were performed in a PTC200 Thermocycler as described previously (5). The amplified restriction fragment products were checked on 1.0% agarose gels. Selective PCR was performed in a 50-µl reaction volume with 5 µl of 20× diluted amplification products with 200 µM dNTP and 5 ng of Cy5-labeled fluorescent Eco21 primer (5′-CTCGTAGACTGCGTACC) and 30 ng of Mse16 primer (5′-GATGAGTCCTGAGTAACC). Products were loaded on Sequagel (Gentra/Biozym) polyacrylamide gels and run on an ALFexpress Automatic Sequencer (Amersham Pharmacia Biotech). Conditions were 1,500 V, 60 mA, 35 W, and 55°C. On each gel, 36 samples were loaded together with flanking Cy5-labeled fluorescent 50-bp ladders (Amersham, Eindhoven, the Netherlands) and two reference isolates (Pic99016 and VK6C).

Data analysis. A subset of 37 recently collected isolates from a wide range of host plant species was selected for comparison of the resolution of multilocus and AFLP genotypes. Isozyme and mating type data were combined with RG57 RFLP fingerprints as described previously (20) to create multilocus genotypes. AFLP patterns were analyzed using Imagemaster ID software (Amersham, Eindhoven, the Netherlands). A total of 67 distinct and reproducible AFLP bands were identified using the primers Eco21 and Mse16. Bands were treated as putative single AFLP loci, and a binary matrix containing the presence or absence of these reproducible bands was constructed and used for further analysis. The AFLP marker matrix is available upon request (W. G. Flier). Multilocus and AFLP genotypes were in separate cluster analyses using GENSTAT 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). Similarity matrixes of multilocus and AFLP genotypes were constructed using the Jaccard method (46); and similarity between and within *P. infestans* populations was estimated using the HDISPLAY procedure in GENSTAT (Genstat 5, release 3) according to manufacturer's directions. Trees were constructed from the distance matrixes with the unweighted pair-group method of averages algorithm and visualized with TREECON version 1.3b (Konstanz, Germany) software (45).

RESULTS

Collections of *P. infestans* sensu lato were made in all parts of the Ecuadorian highlands but were concentrated in the central provinces of Tungurahua and Pichincha (Fig. 1). Locations for each collection event were logged, and after 1999, each event was geo-referenced with a global positioning system; all data are stored in a database. Disease is ubiquitous and generally serious

on potato and tomato during each of the two rain periods in the highlands, but especially in the most intense rainy season occurring between February and May. The situation was quite different for other hosts. Disease may be severe on the other cultivated hosts but it is generally sporadic. Disease is sporadic to rare on wild hosts, although at times severe epidemics were encountered. Our sample size was too small to make observations on the epidemiological dynamics of the diverse pathosystems we studied.

Hosts of *P. infestans* sensu lato. All but one of the hosts we found for *P. infestans* sensu lato are in the genus *Solanum*, including five cultivated *Solanum* spp.: tomato (*S. lycopersicum*), tree tomato (*S. betaceum*), potato (*S. tuberosum* and *S. phureja*), pear melon (*S. muricatum*); nine wild species: *S. regularifolium*, *S. paucijugum*, *S. caripense*, *S. colombianum*, *S. minutifoliolum*, *S. tuquerrense*, *S. ochranthum*, *S. radicans*, and *S. andreanum*; and one complex of wild species similar to *S. brevifolium* that we refer to as the *Anarrhichomenum* complex, pending further taxonomic clarification. One isolate was collected from *Brugmansia sanguinea* in 2001. All the wild species that we studied grow in the highlands of Ecuador (Fig. 1), but sometimes at different altitudes. Many tuber-bearing species and some wild species, such as *S. caripense* and the *Anarrhichomenum* complex, grow at higher altitudes between 2,500 and 4,000 m above sea level. Tree tomato is generally grown somewhat lower, between 1,500 and 2,500 m above sea level. The identification of host species was based on published descriptions (9) and, when possible, verified by taxonomists.

Phenotypic description of *P. infestans* sensu lato in Ecuador. Most foliar lesions from which *P. infestans* sensu lato isolates were taken closely resembled those produced on potato after infection with *P. infestans*, although some wild host species showed slightly differing symptoms in the field. Leaves of plants in the *Anarrhichomenum* complex, for instance, which are small and thin (most are less than 3 cm long), blackened rapidly and sporulation was usually visible only at the edges of lesions. Leaflets of *S. ochranthum*, which are up to 20 cm long and fleshy, had extensive chlorosis associated with infection. The morphology of

Fig. 1. Collection sites in Ecuador and hosts of the collected isolates of *Phytophthora infestans* sensu lato obtained between 1995 and 2002. Each number symbolizes a specific host: 1 = potato (*Solanum tuberosum* or *S. phureja*), 2 = tomato (*S. lycopersicum*), 3 = *S. andreanum*, 4 = *S. colombianum*, 5 = *S. minutifoliolum*, 6 = *S. paucijugum*, 7 = *S. regularifolium*, 8 = *S. solisii*, 9 = *Solanum* spp., 10 = *S. tuquerrense*, 11 = *Brugmansia sanguinea*, 12 = tree tomato (*S. betaceum)*, 13 = *Anarrhichomenum* complex, 14 = *S. caripense*, 15 = pear melon (*S. muricatum*), 16 = *S. ochrantum*, and 17 = *S. radicans.*

sporangia from all of the isolates that we studied was typical of *P. infestans*: limoniform with a short pedicel (15). Sporangial dimensions were consistent with those published for *P. infestans* (15) for all isolates, except some from tomato, tree tomato, and pear melon (US-1 clonal lineage), which were all larger than those reported previously.

Metalaxyl resistance. Except for the population attacking potato, *P. infestans* sensu lato in Ecuador appears to be generally sensitive or of intermediate resistance to metalaxyl (Table 1). A high proportion of isolates from the population attacking potato was also found to be resistant to metalaxyl in an earlier study in Ecuador (19). In contrast to the potato-attacking population, a high proportion of isolates in the US-1 lineage from tomato and *S. caripense* were of intermediate sensitivity, but a few were resistant (Table 1). The number of isolates assayed for most hosts was small, making it difficult to do further comparisons. Nonetheless, it is interesting that only 2 of the 19 isolates assayed from *S. colombianum* were resistant, a much lower proportion than that found in isolates from cultivated potato. *S. colombianum* generally grows close to potato production zones and isolates coming from this wild species could not otherwise be differentiated from those on potato with the markers we used.

Genetic markers. Based on mating type, mtDNA haplotype, and RFLP fingerprint, isolates from all hosts, except the *Anarrhichomenum* complex, fell into one of three distinct groups, US-1, EC-1, or EC-3 (Table 2). We consider these groups to be clonal lineages because all are A1 mating type, each has at least one unique marker allele, and very little polymorphism was found within each group. All three lineages were described previously for all markers except AFLP (13,33,34), and the recent study did not produce any marker information that was inconsistent with the previous descriptions.

In contrast, isolates collected recently from plants in the *Anarrhichomenum* complex provided data that were not consistent with our previous descriptions. We have reported previously on isolates from *S. brevifolium* and *S. tetrapetalum* (34), for which host identifications were based on published descriptions (9). It now appears, however, that one or more new host species may be involved and the identification of these hosts is uncertain, although all are vines with roots growing from nodes and belong to the section *Anarrhichomenum* of the genus *Solanum* (L. Bohs, *personal communication*). Pending further taxonomic clarification, we refer to this group of hosts as the *Anarrhichomenum* complex,

It is now evident that the pathogen population associated with the *Anarrhichomenum* complex is more genetically diverse than we had thought. Some isolates, a total of 57, collected recently are similar to those reported previously as EC-2 (34) in that they are A2 mating type, 100/100 and 76/100 for *Gpi* and *Pep*, respectively, Ic mtDNA haplotype (33), and have one of two similar RFLP fingerprints (34). However, other isolates collected recently from the *Anarrhichomenum* complex are A1 mating type and Ia mtDNA haplotype and are therefore quite distinct from earlier EC-2 isolates. Nonetheless, these Ia haplotype isolates have the typical RFLP fingerprint of the previously described EC-2 clonal lineage (34). Furthermore, although the sample of isolates from the *Anarrhichomenum* complex is small, there appears to be more polymorphism for *Gpi* (Table 2) than found in the other groups of *P. infestans* sensu lato in Ecuador. Two isolates from the *Anarrhichomenum* complex had the 86/100 genotype for *Gpi* and one had the 90/100 genotype. The 86/100 genotype is generally associated with the US-1 lineage, which has been found worldwide (24), and the 90/100 genotype is found in the EC-1 lineage and genotypes in Europe (19).

AFLP analysis. The AFLP analysis demonstrated that all isolates from the Ecuadorian highlands can be classified in one of two major clusters (Fig. 2). Isolates from potato and its tuber-bearing wild relatives (EC-1 lineage) and tomato (US-1) formed one cluster, which is genetically distant to isolates from tree tomato (EC-3) and *Anarrhichomenum* complex, the latter two forming the second major cluster. Each of these major clusters was divided into minor clusters. The first major cluster was clearly divided into EC-1 and US-1 lineages; the second clearly distinguished EC-3 from isolates attacking the *Anarrhichomenum* complex. Furthermore, the AFLP analysis separated isolates from the *Anarrhichomenum* complex into two groups, each one associated with a particular mtDNA haplotype and mating type combination.

Both EC-1 and US-1 minor clusters had isolates from several different hosts and there was no evidence of host-related grouping, because *S. tuberosum* was scattered across the EC-1 cluster. The taxonomy of the *Anarrhichomenum* complex is too obscure at this stage to tell whether any particular host species is associated with either of the pathogen groups isolated from the host complex. Results of the cluster analysis of the multilocus genotypes involving isozyme banding patterns, mating type, and RFLP fingerprints are not presented here because they were generally similar to the results from the AFLP analysis.

The AFLP analysis also indicated that isolates from the *Anarrhichomenum* complex and EC-3 have a low genetic similarity with potato-tomato related groups of *P. infestans* sensu lato, ranging from 0.488 to 0.536 and 0.380 to 0.435 for US-1 and EC-1, respectively (Table 3). High genetic similarities were calculated for isolates within EC-3 and within the two groups attacking the *Anarrhichomenum* complex.

A2 isolates, identical for marker data with the original EC-2 archetypal description (34), were isolated from severely infected plants of pear melon. Prior to this, only US-1 isolates had been

TABLE 2. Some characteristics of subpopulations of *Phytophthora infestans* sensu lato found in Ecuador since 1995 and host species from which they were isolated

RFLP[a]	Clonal lineage[b]	Mating type	*Gpi*[c]	*Pep*[c]	mtDNA[c]	Hosts
US-1	US-1	A1	86/100	92/100	Ib	Tomato (*Solanum lycopersicum*), pear melon (*S. muricatum*), *S. caripense*, *S. ochranthum*, and *S. andreanum*[d]
EC-1	EC-1	A1	90/100	96/100	IIa	Potatoes (*S. tuberosum* and *S. phueja*) and wild potatoes (*Solanum* spp. in section Petota)
EC-2, EC-2.1[e]	?	A2	100/100[f]	76/100	Ic	*Anarrhichomenum* complex[g], pear melon (*S. muricatum*)
EC-2	?	A1	100/100[h]	76/100	Ia	*Anarrhichomenum* complex, *Brugmansia sanguinea*
EC-3	EC-3	A1	86/100	76/100	Ia	Tree tomato (*S. betaceum*)

[a] Restriction fragment length polymorphism (RFLP) fingerprints are named according to the clonal lineage with which they were associated in previous publications.
[b] Designations for populations with very little variability and for which there is no evidence of sexual reproduction.
[c] *Gpi* = *glucose-6-phosphate isomerase*; *Pep* = *peptidase*; and mtDNA = mitochondrial DNA haplotype.
[d] *S. andreanum* is a tuber-bearing species in the section Petota. It is also attacked by isolates of EC-1.
[e] The EC-2.1 sublineage was described previously (34). The RFLP fingerprint of EC-2.1 differs from EC-2 by three bands.
[f] Two isolates of the *Anarrhichomenum* complex with the Ic mtDNA haplotype were 86/100 for *Gpi*.
[g] Specific species in this complex have not yet been determined.
[h] One isolate of the *Anarrhichomenum* complex with the Ia mtDNA haplotype was 90/100 for *Gpi*.

collected from pear melon. One isolate with Ia haplotype was isolated from a lesion on flower petals of *B. sanguinea*. This is the first isolate of *P. infestans* sensu lato that we have collected from a host outside the genus *Solanum*.

DISCUSSION

This study confirmed earlier studies in several ways but also provided new insights into the population structure of *P. infestans* sensu lato in Ecuador. The present study confirmed the presence and previous description of clonal lineages EC-1, US-1, and EC-3 (13,33,34). Data derived from some isolates collected recently, however, are not consistent with the previous description of the putative clonal lineage EC-2 (34). EC-2 isolates collected previously were all A2 mating type and had 100/100 and 76/100 for *Gpi* and *Pep*, respectively. Furthermore, all had a new mtDNA haplotype that was subsequently described as Ic (33) and one of two similar RFLP fingerprinting patterns (34). Strains with the variant fingerprint were collected in the valley of Nono and were designated EC-2.1 as a subgroup within the clonal lineage EC-2 (34). Genotypes in the EC-2 lineage were believed to indiscriminately attack two host species, which were then identified as *S. brevifolium* and *S. tetrapetalum*. In recent collection trips, we found plants that do not appear to be either *S. brevifolium* or *S. tetrapetalum*; however, all are woody vines with roots growing from nodes and belong to the section *Anarrhichomenum* in the

TABLE 3. Genetic similarity between and within groups of Ecuadorian isolates of *Phytophthora infestans* sensu lato based on amplified fragment length polymorphism fingerprinting

Group	US-1	EC-1	Ic	Ia	EC-3
Tomato (*S. lycopersicum*) (US-1)	0.826	…	…	…	…
Solanum spp. section Petota (EC-1)	0.651	0.834	…	…	…
Anarrhichomenum complex Ic (Ic)[a]	0.488	0.380	0.944	…	…
Anarrhichomenum complex Ia (Ia)[a]	0.536	0.435	0.834	0.955	...
Tree tomato (*S. betaceum*) (EC-3)	0.504	0.433	0.787	0.759	0.924

[a] Isolates from a complex of hosts similar to *Solanum brevifolium.* One group of isolates has the Ic mitochondrial haplotype and another group has the Ia haplotype.

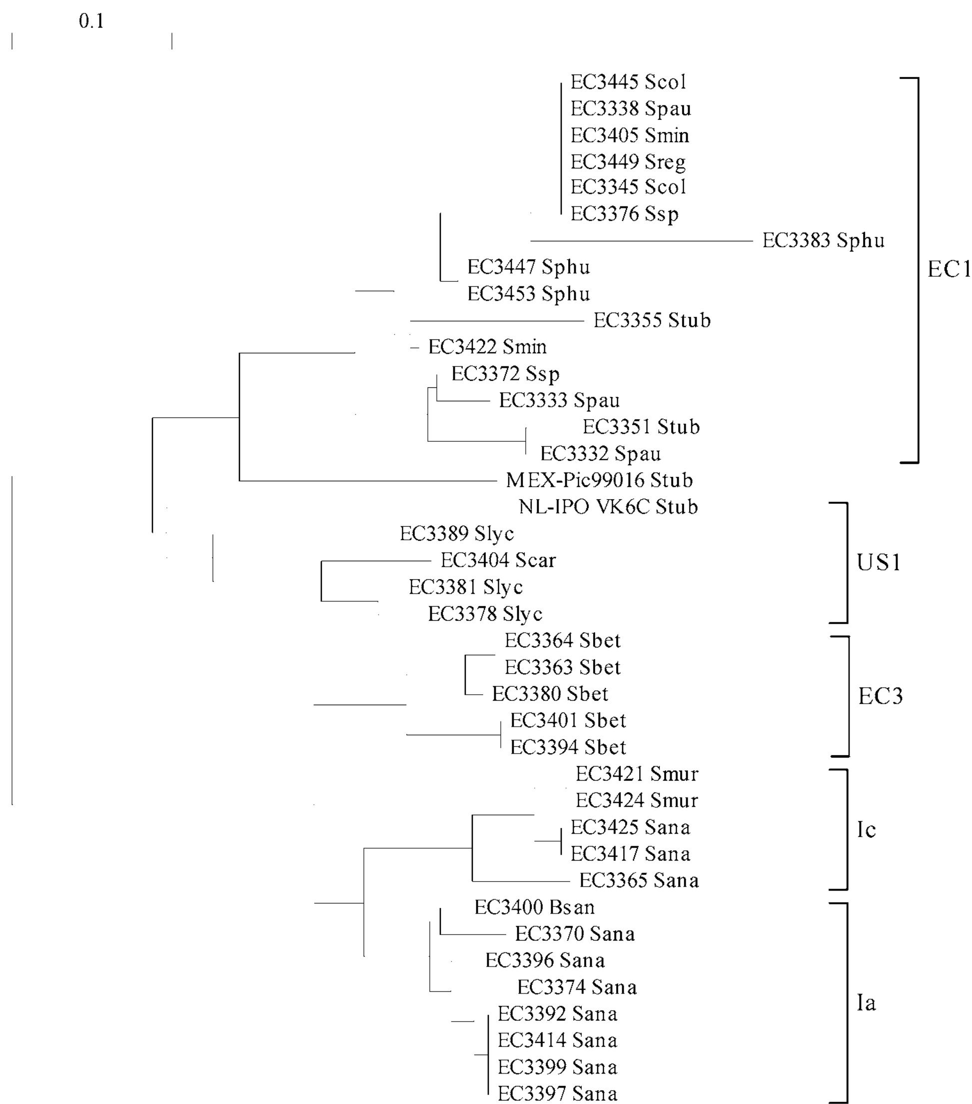

Fig. 2. Phenogram of cluster analysis of amplified fragment length polymorphism banding patterns for 37 isolates of *Phytophthora infestans* sensu lato collected in Ecuador between 1995 and 2002. EC-1, US-1, and EC-3 refer to putative clonal lineages of the pathogen; anar Ic and anar Ia are two pathogen groups that together attack hosts in *Anarrhichomenum* complex. Ic and Ia refer to the mitochondrial haplotype of isolates in each group. Mex-Pic99016 and NL-IPO-VK6C are from Mexico and the Netherlands, respectively. The latter was previously determined to be in the US-1 lineage. Bsan = *Brugmansia sanguinea*, Sana = *Anarrhichomenum* complex, Sbet = *Solanum betaceum* (tree tomato), Scar = *S. caripense*, Scol = *S. colombianum*, Slyc = *S. lycopersicum* (tomato), Smin = *S. minutifoliolum*, Smur = *S. muricatum* (pear melon), Spau = *S. paucijugum*, Sphu = *S. phureja* (potato), Ssp = *Solanum* spp., Stub = *S. tuberosum* (potato), and Sreg = *S. regularifolium.*

genus *Solanum*. Further collection and taxonomic evaluation is needed to clarify which host species in section *Anarrhichomenum* occur in Ecuador and are hosts of *P. infestans* sensu lato.

Analysis of isolates collected recently from these hosts revealed greater diversity than previously described for EC-2 (34). Some new isolates are A1 mating type and Ia mtDNA haplotype. Although these represent important differences with the EC-2 lineage as described previously (A2 mating type and Ic mtDNA haplotype), the new A1 isolates have RFLP fingerprints identical to archetype RFLP fingerprint of EC-2 (34). Furthermore, recent isolates from the *Anarrhichomenum* complex that have an A2 mating type and Ic haplotype have the EC-2.1 fingerprint, which we previously thought was only associated with the pathogen populations in the relatively isolated valley of Nono (34). To add to the confusion, two isolates with A2 mating type and Ic haplotype have the 86/100 genotype for *Gpi* and one isolate (A1 mating type and Ia haplotype) is 90/100 for *Gpi*. Each of these *Gpi* genotypes occurs in one or more of the three clonal lineages found in Ecuador: US-1, EC-1, and EC-3. With our limited sample, we cannot speculate on how these *Gpi* genotypes occurred in the *Anarrhichomenum* complex, but gene flow between this group and the more common potato and tomato types of *P. infestans* cannot be excluded until further studies are carried out.

Previous studies established a one-host/one-pathogen hypothesis for the host pathogen relationship of *P. infestans* sensu lato in Ecuador. A particular pathogen lineage could be associated with several hosts, but each host species was associated with one primary pathogen lineage. This was particularly true for potato (EC-1 lineage) and tomato (US-1), but also for tree tomato (EC-3), *S. caripense* (US-1), *S. colombianum*, and other tuber-bearing Solanaceae (attacked by EC-1). Other pathogen genotypes may infect a host as alternative pathogens, but these are weak and not epidemiologically significant (13,19,34,35). For example, both EC-1 and US-1 had been isolated from *S. ochranthum*, but EC-1 appeared to be a weak pathogen in the field, and this was subsequently confirmed by inoculations on detached leaves (13). EC-1 was probably only evident on *S. ochranthum* in the absence of inoculum from the primary pathogen lineage.

Recent observations indicate that cultivated pear melon may be an exception to the one-host/one-pathogen hypothesis. Pear melon is generally attacked by genotypes of the US-1 lineage in all areas where we have collected in Ecuador, including near the town of Baños. However, a few isolates taken from severely infected plants near Baños were identical to those previously described as EC-2 lineage (A2 mating type, Ic haplotype). The RFLP fingerprint was also identical to EC-2.1 (34). These isolates were subsequently found to be highly aggressive on pear melon in detached-leaf inoculations. This observation is important for two reasons. First, it is the only case we have documented in Ecuador of major epidemics being caused on one host species by two pathogen genotypes, which apparently come from genetically isolated pathogen groups. Second, the two pathogen genotypes attacking pear melon are A1 and A2 mating type, respectively, greatly increasing the potential for sexual reproduction (2). Reduced viability and low pathogenicity of offspring from *P. infestans* sensu lato crosses made in Ecuador (33) are consistent with earlier work on oospore viability in *P. infestans* (16). The hypothesis that the risk of recombination may be greater between co-existing genotypes that are genetically distant or between local and introduced genotypes (6) is pertinent to the Ecuadorian situation because genotypes infecting pear melon are genetically distant.

Other hosts may also be attacked by more than one pathogen group, but we have not found them because of limited sample size. On one occasion, we isolated both US-1 and EC-1 lineages from plants of *S. andreanum* (Table 2) all growing within a 50-m radius. Since that time, however, all isolates taken from *S. andreanum* have been EC-1, and we have no clear explanation for the earlier presence of US-1.

The genetic diversity of *P. infestans* sensu lato in wild *Solanum* sp. found in Ecuador is extremely wide, although more different genotypes are found in sexual reproducing populations (e.g., Mexico and the Netherlands). Three clonal lineages and one heterogeneous group as well as a high diversity in the mtDNA were found in association with different host species in the genus *Solanum* and beyond. The pathogen groups attacking the *Anarrhichomenum* complex and the EC-3 lineage are quite different from any genotypes of *P. infestans* described to date. It appears highly unlikely that either was introduced on potato seed, which is considered the primary means of long-distance transport of this pathogen (21). An earlier study on isolates from the *Anarrhichomenum* complex, which were then designated as EC-2 clonal lineage, compared them with genotypes from a global database using multilocus marker data (34). EC-2 was different from any genotype of *P. infestans* reported previously. The AFLP analysis done here confirmed the earlier study in that it showed that isolates from the *Anarrhichomenum* complex are distinct from genotypes of *P. infestans* found on potato and tomato. Therefore, it is extremely difficult to speculate on the time when *Anarrhichomenum* complex pathogens and EC-3 were possibly introduced into South America or on the mechanism of introduction. A more plausible alternative hypothesis to explain the presence of these distinctive groups is that they are indigenous to the Andean highlands of South America, surviving in humid refugia during dry seasons. The hypothesis that these pathogen groups represent a palaeoendemic population is supported by the fact that several of their alleles, including the mitochondrial haplotype of some isolates, have not yet been reported for other populations of the *P. infestans*, not even those studied in Mexico (20). The taxonomic status of the isolates assigned to the *Anarrhichomenum* complex and EC-3 clonal lineage is still unresolved. A detailed taxonomic study including quantitative assessment of gene flow and construction of a molecular phylogenetic tree based on conserved DNA sequences may elucidate their relatedness to populations of the pathogen that attack potato and tomato.

Based on our limited study of resistance to the systemic fungicide metalaxyl, it would appear logical that high resistance was found in isolates coming from cultivated crops that are regularly sprayed with fungicides (potato, tomato, and tree tomato). The apparent difference in frequency of resistance between wild tuber-bearing hosts and potato is interesting and merits further consideration. However, the difference could be due to sampling in some potato fields where metalaxyl had been used, which would increase the frequency of resistant isolates.

The economic importance and social significance of *P. infestans* have been historically aligned with the disease it causes on potato, and to a lesser extent tomato (39). The potato and tomato populations, however, appear to represent only two of several specialized forms of this pathogen. In terms of significance for humans, other specialized forms that attack tree tomato and pear melon are extremely important in the areas where these crops are produced and are in fact among the most eminent threats to the survival of these native crops in the Andean highlands of Ecuador.

It is important to note that our study was not exhaustive and even greater genetic diversity in *P. infestans* sensu lato probably exists in Ecuador. Furthermore, because of the relatively small sample size that we have been able to analyze, we do not know to what extent the new diversity we report here is due to geographic substructuring or is temporal in nature. For that reason, it is difficult to conclude whether the population of *P. infestans* sensu lato in Ecuador is stable or changing. The subpopulations on potato and tomato appear to be stable (14,19,35), but we do not know if this is the case for new diversity found in subpopulations on pear melon or the *Anarrhichomenum* complex.

At this time, the diversity of *P. infestans* sensu lato reported in Ecuador is much greater than that reported in other Andean countries. We assume that, to some extent, the diversity exists but

has not been described in other countries. We know, for example, that tree tomato is severely affected by late blight in Colombia although we are not aware of reports demonstrating that the pathogen is EC-3. Furthermore, plant species in the *Anarrhichomenum* complex are found throughout the Andes (9) and these may be attacked by *P. infestans* sensu lato. For these reasons, we are unable to comment at this time on the geographic limits of the diversity we have found.

Our ability to manage this important plant pathogen may be greatly enhanced when we better appreciate its genetic potential and further investigation in the Andean highlands is clearly warranted.

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