Phytophtora infestans sensu lato in South America Population substructuring through host-specificity

N. Adler[1], G. Chacón[1], G. Forbes[1,2], W. Flier[3]

The causal organism of late blight, *Phytophthora infestans,* has a pan global distribution. It is thought that *P. infestans* originates from the central highlands of Mexico where high levels of genetic variation are being maintained in sexually reproducing populations on both cultivated potato and several native *Solanum* species.

Although our understanding of recent migrations and global population architecture has been improved considerably in recent years, little is known on the population biology of *P. infestans* in the Andean highlands of South America. The Andes are known as the center of diversity of solanaceous plant species (Spooner et al., 1992).

Late blight research in South America does not differ much from the rest of the world in that the majority of research is done on isolates obtained from potato and tomato, the cultivated host plants of greatest economic interest. Therefore, it is not surprising that little is known about the population biology of *Phytophthora* on other hosts. This may be one reason that the genetic diversity of clonally reproducing *P. infestans* populations in South America reported thus far is low and apparently stable (Table 1).

Diversity of *P. infestans* on solanaceous plants

In Ecuador, known for its highly variable ecosystems, *P. infestans* can be found on many host plant species belonging to the genus *Solanum*. Solanaceous plants can be subdivided in tuber-bearing (section "Petota") and non tuber-bearing ("Etuberosum") species. Ecuador offers a wide range of cultivated solanaceous hosts, such as potato (*S. tuberousm*), tomato (*S. lycopersicon*), tree tomato (*S. betaceum*), pear melon (*S. muricatum*) and naranjilla (*S. quitoense*) and wild species like *S. andreanum*, *S. caripense*, *S. brevifolium*-complex, *S. tuquerrense* and many more. There are four described clonal lineages for *P. infestans*, an exception to the general situation in South America (Table 1), which gives an indication of the diversity of *P. infestans* that might be revealed when population studies include samples from host plant species other than potato and tomato (Table 2).

Table 1. Genetic diversity of *P. infestans* population in South America.

Country	Plant species studied	Mating-types	Haplotypes[a]	Clonal lineages[b]
Argentina	Potatoes, tomatoes	A1, A2	Ia, IIa	AR-1, AR-2, AR-3, AR-4, AR-5, BR-1
Bolivia	Potatoes	A2	Iia	BR-1
Brazil	Potatoes, tomatoes	A1, A2	Ia, IIa	BR-1
Chile	Potatoes	A1	Ib	US-1
Colombia	Potatoes, tomatoes	A1	Iia, Ib	US-1, EC-1
Ecuador	solanaceous	A1, A2	Ia, IIa, Ib, Ic	US-1, EC-1, EC-2, EC-3,
Peru	Petota, tomatoes, S. *caripense*	A1	Ia, IIa, Ib	US-1, EC-1, PE-3, PE-5, PE-6
Uruguay	Potatoes	A2	Iia	BR-1
Venezuela	Potatoes	A1	Iia, Ib	US-1, EC-1

[a]Mitochondrial DNA haplotype
[b]Clonal lineage as defined by marker data.
Sources: Deahl et al. in press, Erselius et al. 2000, Forbes et al. 1998, Gonzales Castano and Garcia 1998, Ñústez 1999, Pérez et al. 2001.

[1] International Potato Center (CIP), Quito, Ecuador
[2] Now at International Potato Center (CIP) HQ, Peru
[3] Plant Research International, Wageningen, The Netherlands

Table 2. Diversity of *P. infestans* on solanaceous plants in Ecuador.

Lineage [a]	Haplo-type [b]	Mating type	Gpi [c]	Pep [d]	Hosts
US-1	Ib	A1	86/100	92/100	*S. ochrantum*, *S. lycopersicon*, *S. caripense, S. muricatum*[e]
EC-1	IIa	A1	90/100	96/100	*S. tuberosum*, all Solanum of the section "Petota" (tuber-bearing-potatoes)
?[a]	Ia	A1	100/100	76/100	*S. brevifolium*-complex[f], *Datura bicolour*
EC-2	Ic	A2	100/100	76/100	*S. brevifolium*-complex[f], *S. muricatum*[g]
EC-3	Ia	A1	86/100	76/100	*S. betaceum*

[a]Clonal lineage as defined by marker data. The Ia mitochondrial DNA haplotype isolates attacking *S. brevifolium* complex collected to date have the archetype EC-2 RFLP fingerprint but differ for other markers.
[b]Mitochondrial DNA haplotype
[c]Glucose phosphate isomerase banding pattern
[d]Peptidase banding pattern
[e]Before 2001 only US-1 was found on *S. muricatum.*
[f]Host species of this complex have not yet been identified.
[g]Two isolates with this genotype were taken from a heavily infected field of *S. muricatum* near Baños.

All isolates described so far in Ecuador can be placed in either of these clonal lineages; US-1, EC-1, EC-2 or EC-3. However, there is some confusion as to the degree of diversity in the group of isolates described previously as EC-2. This will be discussed below.

Ongoing research on the population biology of *P. infestans* attacking species in the genus *Solanum* demonstrates the level of host-specificity that can be found within the meta-population of *P. infestans* in the Ecuadorian highlands. In spite of a high level of diversity in the Ecuadorian meta-population of *P. infestans,* almost all the isolates coming from tuber-bearing species of *Solanum* (section Petota) are genetically similar, With only minimal polymorphism, this group, designated the EC-1 clonal lineage, is characterized by the A1 mating type, *Gpi* 90/100 and *Pep* 96/100 allozyme genotype and the IIa mitochondrial DNA (mtDNA) haplotype. The diversity in *P. infestans* coming from the non-tuber-bearing hosts is higher (Table 2), occurring principally among and not within host-specific groups. One exception is the group of isolates associated with the *S. brevifolium*-complex. Plants belonging to this host-group including *S. brevifolium*, *S. tetrapetalum*, *S. oblongifolium* and few other plants that do not fit the description of these species could not always be clearly identified at the species level**.**

To date there are at least two distinct pathogen groups associated with the *S. brevifolium*-complex. One has generally been associated with a plant phenotype tentatively identified as *S. oblongifolium*. This pathogen group is characterized by the A1 mating-type, *Gpi* (100/100) and *Pep* (76/100) allozyme genotype and the Ia mtDNA haplotype. A second group of isolates characterized by the same allozyme genotype, but with the A2 mating type and Ic mtDNA haplotype (Oliva et al, 2002), is associated with other phenotypes in the host complex, probably *S. brevifolium* and *S. tetrapetalum*. However, host specificity within the pathogen group attacking *S. brevifolium* complex can only be considered a hypothesis at this time because of confusion about the identities of the different host species.

The relationship between the two pathogen groups attacking the *S. brevifolium* complex is confounded even further by RFLP fingerprint data. The Ic mtDNA haplotype group appears to belong to the EC-2 clonal lineage as described previously (Ordoñez et al., 2000), but it is now generally found with an RFLP fingerprint assigned previously to the sub-lineage EC-2.1 (Ordoñez et al., 2000). Surprisingly, the Ia mtDNA haplotype group, which by mating type and mtDNA haplotype does not resemble EC-2, has the archetype RFLP fingerprint of EC-2. More sampling and marker evaluation is needed to determine how these two groups are related. Future research on isolates coming from the *S. brevifolium*-complex will focus on whether they are indeed isolates of *P. infestans* or if they belong to another taxon related to *P. infestans*.

Isolates with the genetic profile of the Ic mtDNA haplotype group were also found in two ocassions on cultivated pear melon plants (*S. muricatum*), implying that some apparently host-specific sub-populations of *P. infestans* are still capable of successfully colonizing new hosts. Viable oospores were found in the fruits of pear melon after infection with isolates of opposite mating types originally obtained from this host plant. Sexual reproduction between apparently host specific populations of *P. infestans* on "bridging hosts" would lead to increased variability in the pathogen population, and host switching would lead to a more complex population structure and could be of great importance in population biology of *P. infestans* in Ecuador.

In general, host specificity is still a driving force for maintaining genetic isolation between sub-populations (clonal lineages) of *P. infestans* in Ecuador (Table 3). For example, it is impossible to infect plants in the *S. brevifolium* complex (hosts of Ic mtDNA group of isolates) with *P. infestans* isolates obtained from a host of the Petota section, and vice versa. The results of cross inoculation experiments demonstrate that, in general,

each clonal lineage, except EC-3 that is only found on *S. betaceum* (tree tomato) plants, is associated with more than one host, but rarely are hosts associated with more than one lineage. The only exception appears to be pear melon, where two lineages have been found causing epidemics.

AFLP analysis divided all Ecuadorian isolates into two major clusters. The US-1 and EC-1 lineages formed one major cluster, while the *S. brevifolium* complex isolates and EC-3 isolates belonged to the second major cluster. Within these major clusters, AFLP analysis also discriminated among clonal lineages. Furthermore, within the *S. brevifolium* complex group, the Ic mtDNA isolates clustered separately from the Ia mtDNA isolates. This is consistent with the hypothesis that two distinct groups attack the *S. brevifolium* complex. However, as noted above, these groups have similar RFLP fingerprints.

The separation of the *S. brevifolium* complex group and the EC-3 lineage from the other lineages raises the question of origin. It is possible that these two lineages are relatives of an "ancient" *Phytophthora* population that is indigenous to Ecuador or immigrated into Ecuador long before the first reported migration from Mexico took place.

The sexual frontier

In pear melon plants a coexistence of the "old" population US-1 (A1) and the EC-2 (A2) was reported, while in other hosts of the section Petota the US-1 lineage was replaced by the EC-1 lineage (Forbes et al., 1997). Therefore gene flow between host-specific populations of *P. infestans* might take place in the Andes when bridging hosts exist.

Apart from genetic isolation through host specificity, geographical isolation between late blight populations has been observed in South America. This was found at the frontier of Peru and Bolivia next to Lake Titicaca. The *P. infestans* population found in potato cultivars of Peru is of the A1 mating type and principally the EC-1 lineage, while Bolivian isolates on potato are predominantly A2 mating type and belong to the BR-1 clonal lineage. No interactions were ever observed between the two clonal lineages in potato crops along the border between the two countries. Nonetheless, meiotic recombination of the two lineages may take place on other solanaceous hosts growing along the border of the lake. Although hosts generally only have one principal pathogen population, at least two cases of host switching have been detected so far in Ecuador. The EC-1 lineage is believed to have displaced US-1 on potato (Forbes, et al, 1997) and both A1 and A2 genotypes were found to cause epidemics on pear melon. More fieldwork and population studies should be carried out to determine whether sexual recombination is occurring in the region of Late Titicaca.

Knowledge of the genetic structure of *P. infestans* on all Andean solanaceous hosts, especially the cultivated hosts, gives new insight into the historical and present changes in the pathogen population. This information is also important in breeding for durable resistance because the mechanisms of host specificity are not known and may mask or confound resistance.

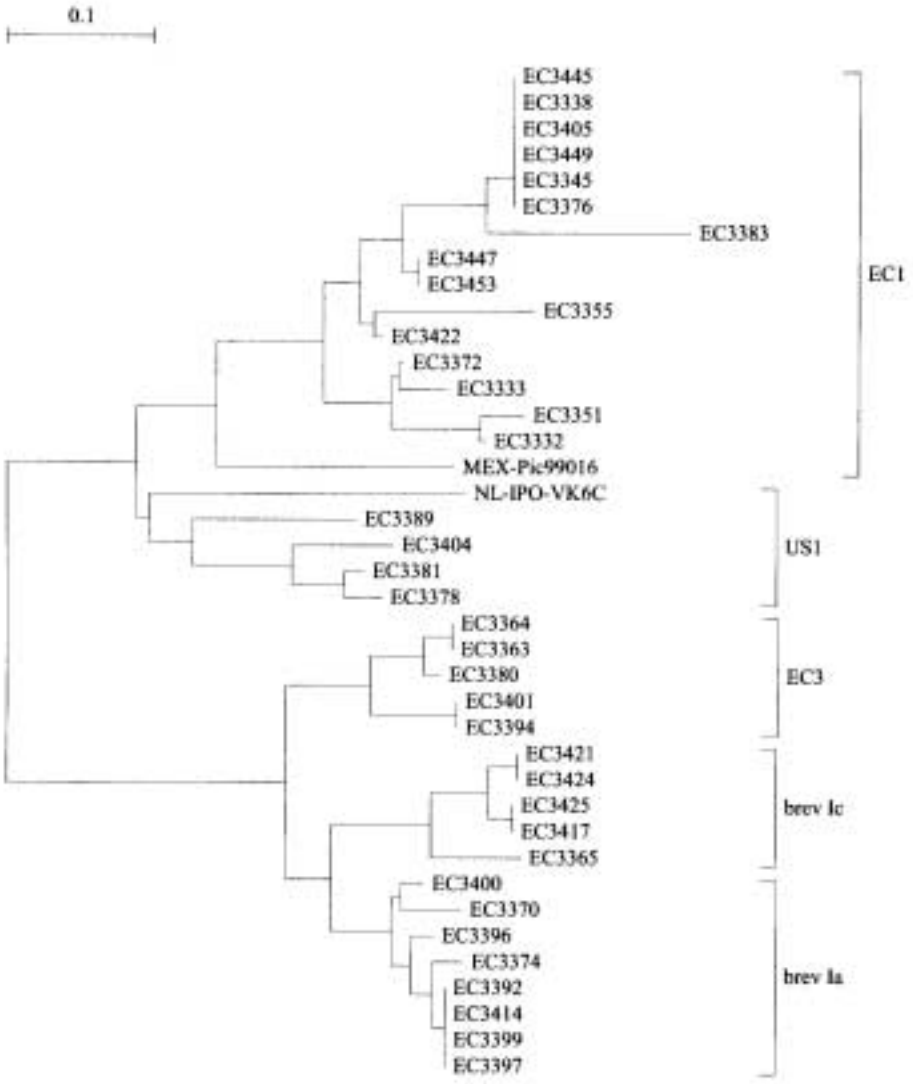
Table 3. Cross inoculations of sub-populations of *P. infestans* on different hosts ("**+" =** infection, "-" = no infections or hypersensitive reactions detached-leaf tests).

Isolates from Hosts	"Petota"- (EC-1)	S. brevifolium-complex	*S. betaceum* (EC 3)	*S. lycopersicon, S. muricatum, S. caripense* (US-1)
"Petota" group	+	-	-	+[a]
S. brevifolium - complex	-	+	-	-
S. betaceum	-	-	+	
US-1 group (*S. lycopersicon, S. muricatum, S. caripense*)	+[a]	*S. muricatum*[b]	-	+

[a]US-1 and EC-1 isolates can be cross inoculated on their alternative hosts,but are more aggressive on their primary hosts.

[b]Two isolates typical of those from *S. brevifolium* complex were collected from a heavily infected field of *S. muricatum*.

Figure 1: AFLP analysis of isolates of *P. infestans* isolated from different solanaceous hosts in Ecuador.



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