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Phylogenetic relationships of *Fusarium poae* based on *EF-1α* and *mtSSU* sequences

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ABSTRACT

A molecular phylogenetic analysis of *Fusarium poae* isolates from South America (Argentina) and Europe (mainly England, Germany, Italy) was performed using 98 *F. poae*, four *Fusarium culmorum*, two *Fusarium sporotrichioides* and one *Fusarium langsethiae* isolates. Phylogenetic analyses were performed using nuclear (translation elongation factor 1-alpha, *EF-1α*) and mitochondrial (mitochondrial small subunit rDNA, *mtSSU*) sequences. Partitioned (each dataset separately) and combined (*EF-1α* + *mtSSU*) analyses did not reveal any clear correlations from the inferred branching topology, between the distribution of observed haplotypes and the geographic origin and/or host species. Results from the present study confirmed that isolates from *F. poae* form a monophyletic group, and the low variability within isolates from a broad geographic range suggests a common lineage history. Among *F. poae* isolates from Argentina, however, some were found to possess an insert within *mtSSU* with structural similarities to group IC2 introns. *F. poae* isolates differing by the presence/absence of a *mtSSU* insertion were characterized further by analysis of a portion of the *Tri5* gene, but this sequence was unable to reveal variability. The presence of this insert only within isolates from Argentina suggests that evolutionary events (insertions/deletions) are probably taking place within the Argentinian *F. poae* isolates, and that the acquisition of this insert occurred after geographic isolation of the Argentinian and European populations.

Introduction

Fusarium head blight (FHB) is a disease caused by a complex of fungal species and has a severe impact through reductions in yield and grain quality and mycotoxin contamination of cereal grains. The predominant FHB mycotoxin producer worldwide is *Fusarium graminearum*, while *Fusarium culmorum* and *Fusarium avenaceum* are common in regions with cooler/wetter

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environments (Parry *et al.* 1995). In some drier warmer environments another species *Fusarium poae*, can predominate (Xu *et al.* 2005, 2008; Bourdages *et al.* 2006; Buttner 2006). Among the mycotoxins produced by these *Fusarium* species, trichothecenes are especially important, as they are potent inhibitors of eukaryotic protein synthesis (Bennett & Klich 2003). Trichothecenes can cause a wide range of acute and chronic effects in humans and animals through ingestion of food and feed prepared from contaminated cereal crops (D'Mello *et al.* 1999). Type A trichothecenes such as T-2 toxin, HT-2 toxin, neosolaniol (NEO) and diacetoxyscirpenol (DAS) are more acutely toxic than type B trichothecenes such as DON (deoxynivalenol) and nivalenol (NIV) (Rotter *et al.* 1996; Desjardins 2006). *F. poae* has been reported to produce type A trichothecenes DAS, NEO, T-2 toxin, HT-2 toxin, scirpentriol (SCR), monoacetoxyscirpenol (MAS), as well as the type B trichothecenes DON, NIV and fusarenone-X (FX) (Salas *et al.* 1999; Thrane *et al.* 2004). In contrast to *F. graminearum* and *F. culmorum*, *F. poae* is a relatively weak pathogen of cereal plants and the lack of extensive visible symptoms on plants after infection constitutes a potential risk to both humans and animals (Wong *et al.* 1992; Xu *et al.* 2007).

A number of studies have been reported for *F. graminearum* (O'Donnell *et al.* 2000, 2004), *F. culmorum* (Miedaner *et al.* 2001; Toth *et al.* 2004) and *F. avenaceum* (Yli-Mattila *et al.* 1996), but information relating to the population biology and genetic diversity of *F. poae* is currently lacking. Although both the *MAT-1* and *MAT-2* mating types present in heterothallic *Fusarium* species occur and are transcribed in *F. poae* (Kerènyi *et al.* 2004), the teleomorph is not known. Increased knowledge about the phylogeny, genetic diversity and population biology of *F. poae* could help in understanding the epidemiology and control of FHB disease (Stenglein 2009).

In many organisms, mitochondrial DNA has a higher rate of evolution than nuclear DNA (Brown *et al.* 1979). It has been reported that the substitution rate is roughly 16-fold greater in the mitochondrial small subunit (*mtSSU*) rDNA gene than in the nuclear small subunit rDNA gene in 10 species of mushroom (Bruns & Szaro 1992). Recently, a phylogenetic analysis of *Fusarium* species using partial sequences of the translation elongation factor 1-alpha (*EF-1α*) gene revealed that *EF-1α* sequence motifs can be used for *Fusarium* lineage and species identification (Geiser *et al.* 2004; Kristensen *et al.* 2005). Comparison of phylogenetic markers in *Fusarium* showed that the *EF-1α* gene possesses considerably more phylogenetic information than the *mtSSU* (O'Donnell *et al.* 1998). The same study showed that the combined *EF-1α* and *mtSSU* provided much better resolution of relationships among and within lineages than other loci, which provided little (β-tubulin and calmodulin genes) or no resolution (ITS and 5′-end of the nuclear 28S rDNA).

The *Tri5* gene encodes trichodiene synthase, an enzyme which catalyses the transformation of farnesyl pyrophosphate to trichodiene as the first specific step in the biosynthesis of trichothecene mycotoxins (Hohn & Beremand 1989). Beside highly conserved regions present in the *Tri5* gene, Niessen *et al.* (2004) analyzed this gene in *Fusarium kyushuense*, *Fusarium langsethiae*, *F. poae* and *Fusarium sporotrichioides* isolates, and reported several short portions of a 60 bp intron that vary and make the intron particularly useful for the study of intra and interspecific phylogenetic relationships.

Sequence of the *EF-1α* and *mtSSU* has been used in *Fusarium* phylogenetic analyses (O'Donnell *et al.* 1998; Li *et al.* 2000; Knutsen *et al.* 2004; Kristensen *et al.* 2005; Mbofung *et al.* 2007). However, extensive studies that combined *EF-1α* and *mtSSU* sequences of *F. poae* have not been reported. The aims of our study were (i) to analyze the molecular differences of *F. poae* isolates originating from two different regions, South America (Argentina) and Europe (mainly England, Germany, Italy), coming from wheat and barley (we included one isolate obtained from tomato, Stenglein *et al.* 2008) , on the basis of *EF-1α* and *mtSSU* sequences and (ii) to examine whether intraspecific variation could be correlated with geographic and/or host origin. In addition we compared the *Tri5* sequences of *F. poae* isolates that possessed or lacked an insertion within the *mtSSU* region.

Materials and methods

Fungal isolates, DNA extraction, and DNA amplification and sequencing

A total of 98 *Fusarium poae*, four *Fusarium culmorum*, two *Fusarium sporotrichioides* and one *Fusarium langsethiae* monosporic isolates were used in this study (Table 1; Fig 1). Genomic DNA was extracted using the CTAB method described by Nicholson *et al.* (1997) and Stenglein & Balatti (2006). PCRs were performed with specific primers to amplify four targets: a *F. poae*-specific 220 bp fragment, portions of *EF-1α* and *mtSSU*, and a part of *Tri5* (Table 2). PCR amplification of the *mtSSU* region was attempted initially using the MS1–MS2 and NMS1–NMS2 primers, which were designed to amplify a portion of the *mtSSU* gene in ascomycetes (White *et al.* 1990; Li *et al.* 1994). However, these primers did not work consistently for some *F. poae* isolates used in this study and a new set of primers were designed (Table 2).

Each PCR reaction was performed in a 50 μl mixture that contained 20–30 ng of genomic DNA, 100 μM each of dATP, dCTP, dGTP, and dTTP, 10X PCR buffer (Boehringer Mannheim), 100 nM each of forward and reverse primers, 0.8 U *Taq* DNA polymerase, 0.05 % (w/v) Tween 20 and 0.05 % (w/v) Nonidet P-40. PCR reactions were carried out in a MJ Research PTC-225 thermal cycler (Waltham, MA) using the following cycling protocol: an initial denaturation step of 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 62 °C (*F. poae*-specific primers) for 30 s, 72 °C for 45 s; final extension of 72 °C for 2 min. Annealing temperatures were 54 °C for *EF-1α* and 60 °C for *mtSSU* and *Tri5*. Successful amplifications were confirmed by gel electrophoresis. PCR products were purified with the aid of a QIAquick PCR purification kit (Qiagen Inc., Stanford, CA). DNA sequencing, from both the sense and antisense ends, of the 105 *mtSSU*, 105 *EF-1α* and 24 *Tri5* fragments from *Fusarium* isolates were carried out using Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA) (Table 1).

Sequence data analyses

DNA sequences were edited using Vector NTI software (Invitrogen) and aligned using the multiple sequence alignment

Table 1 – *Fusarium* isolates used in this study and associated haplotypes.

Isolate[a]	Geographic origin[b]	Host	Haplotype[c]		
			mtSSU	*EF-1α*	*Tri5*
Fusarium poae					
HSu1a	C. Suarez/AR	Barley	1	1	1
HSu1b	C. Suarez/AR	Barley	1	1	ND
HPu1a	Puán/AR	Barley	1	1	1
HPu2a	Puán/AR	Barley	1	1	1
HPu3a	Puán/AR	Barley	1	1	ND
HPu4a	Puán/AR	Barley	1	6	ND
HPu5a	Puán/AR	Barley	1	1	ND
HPu5b	Puán/AR	Barley	1	1	ND
HPu5c	Puán/AR	Barley	1	1	ND
HPu5d	Puán/AR	Barley	1	1	ND
HPu5e	Puán/AR	Barley	1	1	ND
HPu5f	Puán/AR	Barley	1	1	ND
HTA1a	Tres Arroyos/AR	Barley	1	1	ND
HBe1a	Bellocq/AR	Barley	1	1	ND
HBe1b	Bellocq/AR	Barley	2	1	1
HBe1c	Bellocq/AR	Barley	1	1	ND
HBe1d	Bellocq/AR	Barley	3	1	1
HBig1a	Bellocq/AR	Barley	2	1	ND
TCa1a	Castelar/AR	Wheat	2	1	1
TSS1a	Sancti Spiritu/AR	Wheat	1	1	ND
TSS1b	Sancti Spiritu/AR	Wheat	1	1	ND
TSS2a	Sancti Spiritu/AR	Wheat	2	1	ND
TSS2b	Sancti Spiritu/AR	Wheat	4	1	1
TSS2c	Sancti Spiritu/AR	Wheat	1	4	ND
THo1a	Los Hornos/AR	Wheat	1	1	ND
THo1b	Los Hornos/AR	Wheat	1	10	ND
THo1c	Los Hornos/AR	Wheat	2	7	1
THo1di	Los Hornos/AR	Wheat	1	1	1
THo2a	Los Hornos/AR	Wheat	1	1	ND
TPu1a	Puán/AR	Wheat	1	1	ND
TPu1b	Puán/AR	Wheat	1	1	ND
TPu1c	Puán/AR	Wheat	1	1	ND
TPu2a	Puán/AR	Wheat	1	1	ND
TPu3a	Puán/AR	Wheat	1	1	ND
TPu4ai	Puán/AR	Wheat	1	1	1
TSa1a	Saladillo/AR	Wheat	1	9	ND
TSa1b	Saladillo/AR	Wheat	1	1	ND
TSm1ai	San Manuel/AR	Wheat	1	1	1
TSm2a	San Manuel/AR	Wheat	1	1	ND
TBig1a	Bigand/AR	Wheat	1	1	ND
TBig1b	Bigand/AR	Wheat	1	1	ND
TBig2ai	Bigand/AR	Wheat	1	1	1
TMa1a	25 de Mayo/AR	Wheat	1	1	1
TMa1b	25 de Mayo/AR	Wheat	1	1	ND
TJu1a	Junín/AR	Wheat	1	1	1
TJu1b	Junín/AR	Wheat	1	1	ND
TPe1a	Pergamino/AR	Wheat	1	1	ND
LSP1a	San Pedro/AR	Tomato	1	5	1
3/4766	Sussex/UK	Wheat	1	3	ND
4/3084/1	Surrey/UK	Wheat	1	1	ND
4/3084/2	Surrey/UK	Wheat	1	3	ND
4/3084/3	Surrey/UK	Wheat	1	3	ND
4/3084/4	Surrey/UK	Wheat	1	3	ND
4/4343/12	Suffolk/UK	Wheat	1	1	ND
4/4343/10	Suffolk/UK	Wheat	1	3	ND
F600	UK	Wheat	1	1	ND
F18	Cambridge/UK	Wheat	1	1	2
Fu53	Norfolk/UK	Wheat	1	8	1
Fu53/2	Norfolk/UK	Wheat	1	1	ND
Fusarium poae					
4/3	UK	Wheat	1	1	ND
CSL6	Herefordshire/UK	Wheat	1	2	ND

Table 1 – (*continued*)

Isolate[a]	Geographic origin[b]	Host	Haplotype[c]		
			mtSSU	*EF-1α*	*Tri5*
CSL8	Scotland/UK	Wheat	1	1	ND
CSL10	Yorkshire/UK	Wheat	1	1	ND
CSL28	Hertfordshire/UK	Wheat	1	1	ND
1021	Lancashire/UK	Wheat	1	4	ND
1020	Cheshire/UK	Wheat	1	1	ND
1019	W. Yorkshire/UK	Wheat	1	1	ND
887	N. Yorkshire/UK	Wheat	1	1	ND
751	Dorset/UK	Wheat	1	1	ND
750	Dorset/UK	Wheat	1	1	ND
743	Hertfordshire/UK	Wheat	1	3	ND
736	Norfolk/UK	Wheat	1	3	ND
735	Bedfordshire/UK	Wheat	1	1	ND
731	Hereford & Worcester/UK	Wheat	1	1	ND
721	Humberside/UK	Wheat	1	1	ND
720	Merseyside/UK	Wheat	1	1	1
718	Northumberland/UK	Wheat	1	1	ND
563	Oxfordshire/UK	Wheat	1	3	ND
552	Surrey/UK	Wheat	1	1	ND
522	Cambridgeshire/UK	Wheat	1	1	ND
506	Somerset/UK	Wheat	1	1	ND
444	Hereford/UK	Wheat	1	1	1
303	Kent/UK	Wheat	1	1	ND
747	Buckinghamshire/UK	Wheat	1	2	ND
507	Lincolnshire/UK	Wheat	1	1	ND
504	Suffolk/UK	Wheat	1	1	ND
301	Lincolnshire/UK	Wheat	1	1	1
300	Nottinghamshire/UK	Wheat	1	1	ND
295	Norfolk/UK	Wheat	1	1	ND
173	Hampshire/UK	Wheat	1	1	ND
141	Wiltshire/UK	Wheat	1	1	ND
4/4343/1	Suffolk/UK	Wheat	1	1	ND
33c/1	UK	Wheat	1	2	ND
I14-99	Italy	Wheat	1	1	ND
F62	France	Wheat	1	2	1
F730	Germany	Wheat	1	2	1
F732	Germany	Wheat	1	2	1
F733	Germany	Wheat	1	1	2
Fusarium langsethiae					
CC321	UK	Wheat	5	11	ND
Fusarium sporotrichioides					
F627	France	Wheat	6	12	ND
F95	Poland	Wheat	6	12	ND
Fusarium culmorum					
4/3155	UK	Wheat	7	13	ND
CC42	UK	Wheat	7	13	ND
CC52	UK	Wheat	7	13	ND
4/3153/1	UK	Wheat	7	13	ND

a In Argentinean isolates the numbers identify different samples and the small letters identify isolates of a sample. In THo1di, TPu4ai, TSm1ai and TBig2ai, the 'i' indicates that the isolate possess a second fragment of the *mtSSU* with an insert.
b AR, Argentina; UK, United Kingdom.
c *mtSSU* sequences have GenBank codes EU744624–EU744738. *EF-1α*, EU744739–EU744849. *Tri5*, EU744850–EU744872. ND, not determined.

program CLUSTAL W (Thompson *et al.* 1994). Molecular diversity for *Fusarium poae* isolates were estimated through Haplotype (H) and Nucleotide Diversity (π) indices in DnaSP v. 4.50.3 (Rozas *et al.* 2003). Nucleotide proportion was estimated using MEGA v. 3.1 (Kumar *et al.* 2004).). Ambiguously aligned regions were excluded from the datasets for phylogenetic analyses. Both datasets were analyzed as partitions (each dataset separately) and simultaneously (*EF-1α* + *mtSSU*) in a total evidence analysis (*sensu* Nixon & Carpenter 1996). Each phylogenetic analysis was performed by maximum parsimony and Bayesian inference. All *F. poae* isolates were used as ingroup, and the remaining as outgroups. We followed the approach of Nixon & Carpenter (1993), *i.e.* we used several outgroup species as a tool to test the monophyly of the ingroup species.

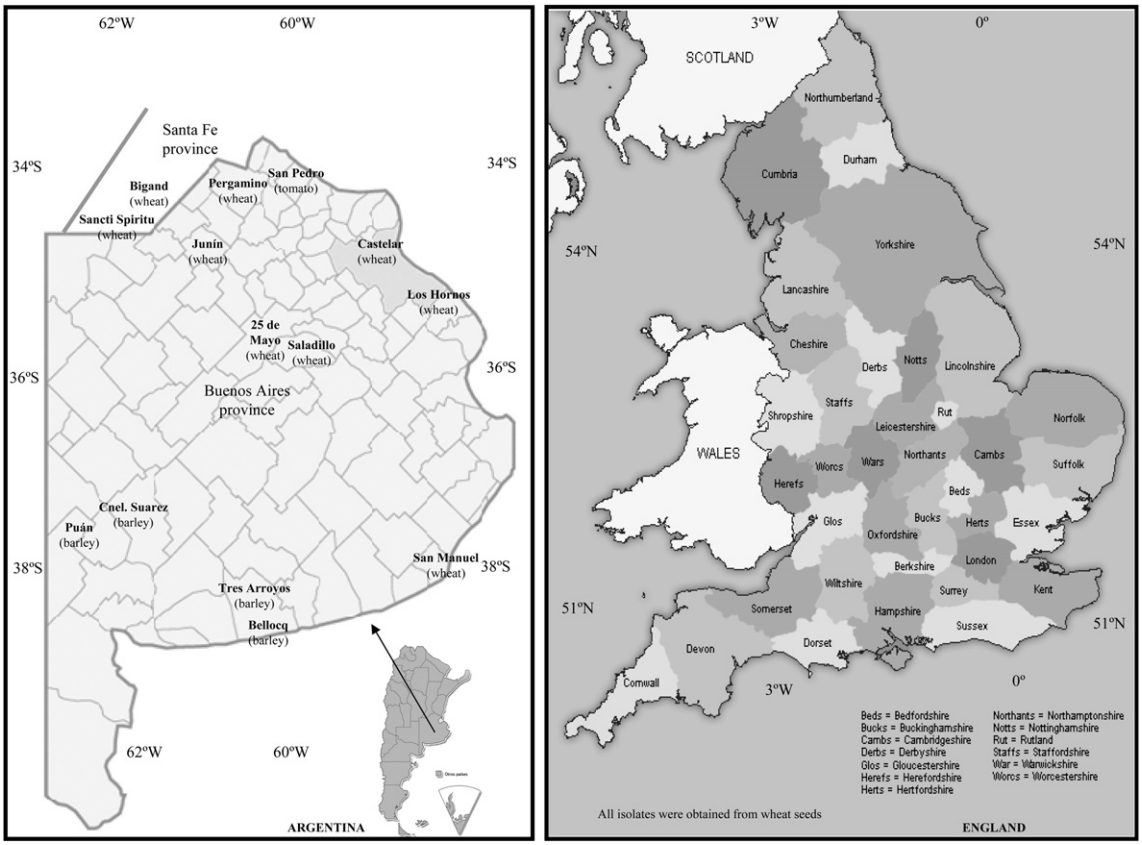


Fig 1 – Geographic location and host of *Fusarium poae* isolates from Argentina and England.

Molecular datasets were first analyzed using the TNT program ver. 1.1 (Goloboff *et al.* 2003), with different weighting strategies: equal weights and differential transversion/transition ratio (TV/TS) transformation costs. Four values of TV/TS were explored for every dataset and the total evidence analysis, to avoid using just one '*a priori*' arbitrary ratio (sensitivity analysis *sensu* Wheeler 1995). The different costs tested were: TV/TS: 2/1, 4/1, 6/1 and 8/1. Gaps were treated as a fifth state. Clade stability was assessed by 10 000 parsimony bootstrap replications (Felsenstein 1985). Analyses were conducted using implicit enumeration (number of taxa <25). Preceding the combined analysis, the congruence among datasets was measured by the 'Incongruence Length Difference' test ILD (Farris *et al.* 1995) using the WinClada program ver. 1.00.08 (Nixon 2002). The evolutionary model GTR + I (Tavaré 1986) for *EF-1α* and F81 + I (Felsenstein 1981) for *mtSSU* sequences were selected based on Akaike Information Criterion (AIC) (Akaike 1973), an optimal strategy for model selection (Posada & Buckley 2004), using MrModelTest v. 2.2 (Nylander 2004) through the interface MrMTgui v. 1.0 (Nuin 2005).

Bayesian phylogenetic analyses were performed using the 'metropolis-coupled Markov chain Monte Carlo' (MCMCMC or MC3) algorithm implemented in MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003). Program defaults were used for estimation of priors. Two independent analyses were run using a random starting tree over 1 000 000 generations sampling every 100 generations for all datasets. For the combined analysis, a partitioned algorithm was used to account for heterogeneity

Table 2 – Primers used in this study for DNA amplification and sequencing.

Locus	Primer name	Primer sequence (5′–3′)	Reference
F. poae–220 bp	Fp8F	ACGACGAAGGTGGTTATG	Parry & Nicholson (1996)
	Fp8R	GAAGAGCCTGTTTGCTTG	Parry & Nicholson (1996)
EF-1α	EF1	ATGGGTAAGGA(A/G)GACAAGAC	O'Donnell *et al.* (1998)
	EF2	GGA(A/G)GTACCAGT(G/C)ATCATGTT	O'Donnell *et al.* (1998)
mtSSU	MS3F	TAACGGCTGAACTGGCAAC	This study
	MS3R	CCTGCGTTGCAACATTACTC	This study
Tri5	Tri5F	AGCGACTACAGGCTTCCCTC	Nicholson *et al.* (2004)
	Tri5R	AAACCATCCAGTTCTCAATCTG	Nicholson *et al.* (2004)

between the two datasets. Tree space was explored using one cold chain and three incrementally heated ones. We evaluated 'burn-in' plots by plotting log-likelihood scores, tree lengths, and all model parameter values against generation to assess stationarity of the cold Markov chain for all MrBayes analyses. Stationarity was inferred when values reached an asymptote. Also, we evaluated convergence by performing the cumulative analysis of posterior probabilities of all the nodes to verify that these values were stable across all post-burn-in generations within each analysis (the *cumulative* command) using the online convergence program Are We There Yet? (AWTY) (Wilgenbusch *et al.* 2004). Node posterior probabilities were compared between the two independent runs using the *compare* command in AWTY. A run was assumed to reach stationarity when all of these criteria yielded patterns congruent with stationarity. All posterior samples of a run prior to this point were discarded as burn-in. Remaining trees were used to construct a 50 % majority-rule consensus tree with mean branch length estimates. The frequency of all observed bipartitions was used to assess the level of support for each node (Huelsenbeck & Ronquist 2001). DNA sequences of both datasets were combined in a total evidence matrix and concordance was measured by the ILD tests using the program Winclada.

To reduce the complexity of the matrix for phylogenetic analyses, a single representative of each *EF-1α* (*i.e.* ten terminal taxa for the ingroup and three for the outgroup) and *mtSSU* haplotypes (*i.e.* four terminal taxa for the ingroup and three for the outgroup) were retained (Table 1). In the combined analyses, all the possible combinations of nuclear and mitochondrial haplotypes were included in the phylogenetic analyses (*i.e.* 13 terminal taxa for the ingroup and three for the outgroup) (Table 1).

A large insertion within the *mtSSU* sequence was excluded from the datasets for phylogenetic analyses. Similarities of the *mtSSU* insert with previously published sequence data were examined with BLASTn and BLASTx (Altschul *et al.* 1990) and secondary structure comparison with group I and II introns using this insert sequence as a query. Among the bioinformatics approaches to intron identification, we used Rfam (http://www.sanger.ac.uk/Software/Rfam) (Griffiths-Jones *et al.* 2003) and RNAweasel (http://www.megasun.bch.umontreal.ca/RNAweasel) (Lang *et al.* 2007).

Results

PCR amplification

All isolates determined to be *Fusarium poae* on the basis of morphological characteristics produced a fragment of 220 bp identical to that observed by Parry & Nicholson (1996).

Sequence analyses

The *EF-1α* sequence alignment included a total of 98 *Fusarium poae* isolates (consisting of 10 haplotypes), four *Fusarium culmorum* (1 haplotype), two *Fusarium sporotrichioides* (1 haplotype), and one *Fusarium langsethiae* (1 haplotype). Sequences from the four *Fusarium* species ranged between 637 and 693 bp in length. The total proportions of nucleotides were 22.8 % A, 29.2 % C, 21.6 % G, 26.4 % T. With the exception of a single isolate, all *F. poae* isolates produced a fragment of 660 bp. The remaining isolate (TSa1a), had a deletion of 23 bp, producing a fragment of 637 bp. No insertions or deletions (own of the gene) were observed in the four exon regions.

All 98 *F. poae* isolates and the other *Fusarium* species amplified a product from the *mtSSU* rDNA region using primers MS3. The *mtSSU* sequences were 578–581 bp in length and included 4 *F. poae* haplotypes, and 1 haplotype for *F. culmorum*, *F. langsethiae*, and *F. sporotrichioides* species. The majority of *F. poae* isolates produced a fragment of 578 bp. The total proportions of nucleotides were 32.9 % A, 15.2 % C, 24.1 % G, 27.8 % T. Five of the Argentinian isolates studied (HBe1b, HBe1d, TCa1a, TSS2b and THo1c) yielded a 1945 bp product and four (THo1d, TPu4a, TSm1a and TBig2a) yielded both the 578 bp product and the 1945 bp product. A BLASTx search using public databases and the 1367 bp insert sequence (located between positions 268–269) as the query sequence, showed an *E* value $= 2\,e^{-74}$ and $6\,e^{-72}$ with LAGLIDADG endonuclease from *Podospora anserina* and *Gibberella zeae*, respectively. Further bioinformatic analyses using the Rfam and RNAweasel programs revealed that the insert sequence conformed to that of a group I catalytic IC2 intron.

A single PCR product (516 bp) was amplified from the 98 *F. poae*, four *F. culmorum*, two *F. sporotrichioides* and one *F. langsethiae* isolates using the *Tri5* primer set. The *Tri5* product was sequenced for a sub-set of 24 isolates of *F. poae* representing those with and without the *mtSSU* insertion. The *Tri5* sequence was identical for most isolates and only two isolates (F18 and F733) were found to be different. Three polymorphic sites were identified for this region (all of them synonymous substitutions).

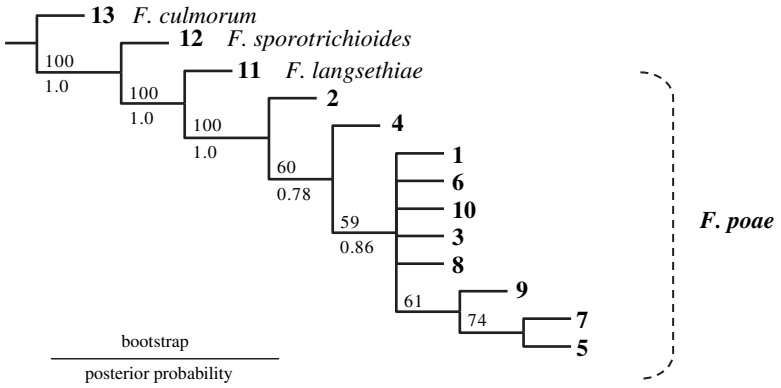
Phylogenetic analyses

Two independent datasets were examined representing the nuclear (*EF-1α*) and mitochondrial (*mtSSU*) genomes. The *EF-1α* dataset consisted of 692 nucleotide characters, of which 92 were parsimony informative. Sequences comprising the 667 *mtSSU* dataset contained 32 parsimony informative characters.

Molecular diversity values indicated a lower level of genetic variation for the *mtSSU* than the *EF-1α* dataset. The value of H (haplotype diversity) for *EF-1α* was 0.499 while that for *mtSSU* was 0.249 and the value of π (nucleotide diversity) for *EF-1α* was 0.032 while that for *mtSSU* was 0.006. According to the results of the ILD test, the nuclear and mitochondrial characters were not significantly incongruent ($P = 1.0$).

Maximum parsimony searches yielded a single most parsimonious tree (MPT) for the *EF-1α* dataset ($L = 205$ for equal transformation costs between tranversions and transitions). The same topology was obtained for every transformation cost scheme. Hence, the only one reported here is TV = TS (Fig 2).

Convergence of the Bayesian analysis for the *EF-1α* dataset appeared complete by 1 000 000 generations, based on an inspection of a burn-in plot of log-likelihood scores, tree lengths, all model parameters and the cumulative analysis of posterior probabilities. Therefore, we discarded the first 2500 samples from each analysis, resulting in two posterior distributions



Haplotype 1: Argentinian isolates obtained from barley (C. Suarez, Puán, Tres Arroyos and Bellocq) and wheat (Castelar, Sancti Spiritu, Los Hornos, Puán, Saladillo, San Manuel, Bigand, 25 de Mayo, Junín, Pergamino). English isolates obtained from Surrey, Suffolk, Norfolk, Scotland, Yorkshire, Hertfordshire, Cheshire, Dorset, Bedfordshire, Hereford & Worcester, Humberside, Merseyside, Northumberland, Cambridgeshire, Somerset, Hereford, Kent, Lincolnshire, Nottinghamshire, Wiltshire). Italy and Germany. **Haplotype 2:** English isolates from Herefordshire and Buckinghamshire, and isolates from France and Germany. **Haplotype 3:** English isolates from Sussex, Surrey, Suffolk, Hertfordshire, Norfolk, Oxfordshire. **Haplotype 4:** English isolate obtained from Lancashire and Argentinian isolate obtained from wheat (Sancti Spiritu). **Haplotype 5:** Argentinian isolate obtained from tomato (San Pedro). **Haplotype 6:** Argentinian isolate obtained from barley (Puán). **Haplotype 7:** Argentinian isolate obtained from wheat (Los Hornos). **Haplotype 8:** English isolate obtained from Norfolk. **Haplotype 9:** Argentinian isolate obtained from wheat (Saladillo). **Haplotype 10:** Argentinian isolate obtained from wheat (Los Hornos).

Fig 2 – Most parsimonious tree for the *EF-1α* sequences. *Fusarium culmorum* (1 haplotype), *Fusarium sporotrichioides* (1 haplotype), *Fusarium langsethiae* (1 haplotype), and *Fusarium poae* (10 haplotypes). Bootstrap values are indicated above branches and posterior probabilities below them.

containing 7500 samples each. A plot of the posterior probabilities of all splits from the two separate MC3 runs for this dataset demonstrated a linear relationship, suggesting that our analyses were not restricted to local optima.

Bayesian analysis yielded trees topologically concordant with *EF-1α* maximum parsimony analysis (data not shown, but see posterior probabilities values in Fig 2). No outgroup species broke off the monophyly of *Fusarium poae*. In fact, monophyly of the ingroup in the *EF-1α* dataset was strongly supported by bootstrapping (100 %) and posterior probability (1.0) (Fig 2). Within the ingroup, some relationships are resolved on the basis of both phylogenetic analyses (Fig 2). Bayesian analysis suggested that divergence among *F. poae* isolates is low (short branches practically equally sized, data not shown).

For the *mtSSU* dataset, something similar occurred regarding transformation costs for maximum parsimony analysis, although for TV/TS = 4/1 and TV/TS = 8/1 we preferred not to interpretate those results because we had violated the triangle inequality for some characters. Therefore, we analyzed the phylogenetic relationships among ingroup isolates on the basis of the single MPT obtained under equal transformation costs for TV and TS (L = 127) (Fig 3). In this case, the monophyletic ingroup is strongly supported (Fig 3), and outgroup species did not break this monophyly. Within the ingroup, no better relationships than *EF-1α* dataset could be resolved from the *mtSSU* dataset, maybe owing its lower variability regarding *EF-1α* sequences.

Convergence of Bayesian analysis was reached by 1 000 000 generations. Hence, we discarded the first 2500 samples from each analysis, resulting in two posterior distributions containing 7500 samples each. For this analysis, as with maximum parsimony, the monophyletic ingroup is strongly supported (data not shown). However, for this dataset, the phylogenetic relationships presented some discrepancies related to the former analysis, because the isolate of *Fusarium culmorum* have broke off the ingroup monophyly (data not shown). Nevertheless, such outgroup isolate (*F. culmorum*), is highly divergent from the *F. poae* isolates included in the ingroup (data not shown).

Regarding the total evidence analyses, two MPTs were obtained (*L* = 335). The strict consensus can be seen in Fig 4. Both maximum parsimony and Bayesian analyses of the *EF-1α* + *mtSSU* dataset yielded the same topology, which is similar with that from the Bayesian *EF-1α* analysis (see Fig 2), although the posterior probabilities of the combined analysis was higher, maybe because of the high number of characters.

For the *EF-1α* and the total evidence analyses, some relationships could be resolved within the ingroup (Figs 2 and 4). The more exclusive group contained eight of the different haplotypes for the *EF-1α* gene, including isolates coming from different hosts and geographic regions (Table 1; Fig 1). No relationships regarding geographic origin and/or host species could be discerned from our phylogenetic analyses. In fact, autapomorphies differentiated the haplotypes from each other.

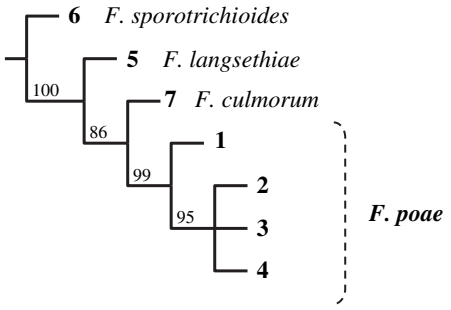


Fig 3 – Most parsimonious tree for the *mtSSU* sequences. *Fusarium culmorum* (1 haplotype), *Fusarium sporotrichioides* (1 haplotype), *Fusarium langsethiae* (1 haplotype), and *Fusarium poae* (4 haplotypes). Bootstrap values are indicated above branches.

Discussion

In the present study, isolates of *Fusarium poae* from two different regions, one from South America (Argentina), and the other one from Europe (mainly England) were compared on the basis of *EF-1α* and *mtSSU* sequences. Individual and total evidence analyses (*EF-1α + mtSSU*) did not reveal any clear correlations, from the inferred branching topology, between the distribution of observed haplotypes and the geographic origin and/or host species. A low genetic variation was remarkable for both datasets, whereas a small number of haplotypes was observed for *F. poae* isolates from different geographic regions. The *EF-1α* region had a better resolution than the *mtSSU* and provided a better phylogenetic resolution among the species examined. Although largely congruent with both data partitions, combined analyses only provide a slightly better resolution of relationships among and within isolates than the individual analyses. It is probable that the *mtSSU* region did not produce a strong phylogenetically informative signal. Hence, the resulting topology for the total evidence analysis (*EF-1α + mtSSU*) resembled that of the *EF-1α* dataset. In concordance with this, O'Donnell *et al.* (1998) demonstrated that the *EF-1α* gene possesses more phylogenetic signal than the *mtSSU* region in *Fusarium oxysporum*. However, in their study they obtained much better resolution with analysis of the total evidence analysis (*EF-1α + mtSSU*) for *F. oxysporum*.

Results from the present study confirmed that isolates from *Fusarium poae* conform to a monophyletic group, and the limited variability within isolates from a broad geographic range suggests a common lineage history. The *EF-1α* and *mtSSU* sequences have proved to be useful in phylogenetic studies for resolving *Fusarium* species and also for resolving relationships within the *F. oxysporum* complex (O'Donnell *et al.* 1998; Kristensen *et al.* 2005; Mbofung *et al.* 2007). In the present study, however these gene regions were relatively conserved among the *F. poae* isolates examined herein.

Although *Fusarium culmorum* (section *Fusarium*) is highly divergent from *F. poae* (section *Sporotrichiella*) isolates, and maximum parsimony analyses clearly indicate that *F. poae* is a monophyletic group, isolates of *F. culmorum*, at least for *mtSSU* Bayesian analysis, were not clearly separated from the *F. poae* isolates (data not shown). Curiously, the bootstrap value for the ingroup in the maximum parsimony analysis of the *mtSSU* dataset is 99 %. Then, in the 1 % of the replicates of the bootstrap analysis any of the outgroup species could break the ingroup monophyly. A possible explanation would be that the *mtSSU* gene of *F. culmorum* and *F. poae* isolates has a lower rate of evolution than *EF-1α* and that, as a result of this, these species were not clearly separated. Nevertheless, the *F. culmorum* isolate was largely divergent from the *F. poae* strain on the basis of the mitochondrial dataset (data not shown).

Primers MS1–MS2 and NMS1–NMS2 (White *et al.* 1990; Li *et al.* 1994), designed to amplify a portion of the *mtSSU* gene in ascomycetes, failed to amplify the intended region for several *F. poae* isolates. We designed a pair of primers, MS3F and MS3R that consistently amplified the *mtSSU* region of *F. poae* and the other *Fusarium* species used in this study. Although

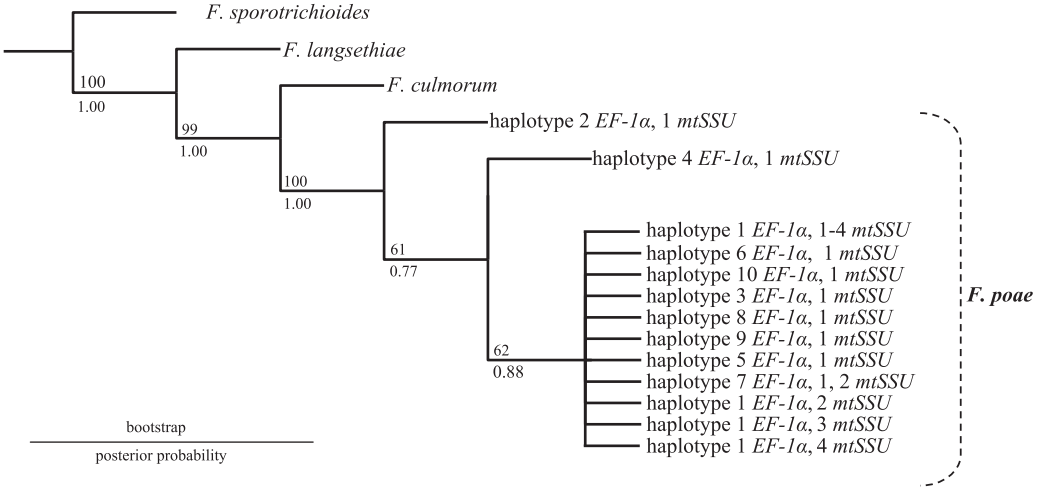


Fig 4 – Most parsimonious tree for the combined data analysis (*EF-1α + mtSSU*). *Fusarium culmorum* (1 haplotype), *Fusarium sporotrichioides* (1 haplotype), *Fusarium langsethiae* (1 haplotype), and *Fusarium poae* (13 haplotypes). Bootstrap values are indicated above branches and posterior probabilities below them.

the priming sites of this mitochondrial locus are conserved among ascomycetes, our results indicated that the intervening region is sufficiently polymorphic to permit differentiation of the species tested herein. One surprising result of the current study is the presence of an insert in the *mtSSU* region in some Argentinian *F. poae* isolates. Isolates with the expected 578 bp product and isolates with the insert where isolated from different Argentinian localities from both wheat and barley, and did not reveal any clear correlations between the distribution of isolates with or without the insert and the geographic origin and/or host species. Furthermore, isolates producing either a 578 bp product, a 1945 bp or both products were present in a single grain sample, for example, isolates THo1a, THo1c and THo1d, respectively obtained from Los Hornos, Argentina.

Isolates of *F. poae* differing by the presence/absence of the *mtSSU* insertion were characterized further by analysis of a portion of the *Tri5* gene. Although the *Tri5* gene has been shown to display variability between *Fusarium kyushuense*, *Fusarium langsethiae*, *F. poae* and *Fusarium sporotrichioides* (Niessen *et al.* 2004), this sequence was highly conserved across all *F. poae* isolates irrespective of their *mtSSU* haplotype. Group I introns have been described in protein and rRNA encoding genes in nuclear and mitochondrial genomes from various fungal species (Carbone *et al.* 1995; Gonzalez *et al.* 1997; Dusabenyagasani *et al.* 2002). The presence of this insert with secondary structure similarities to group IC2 introns in the *mtSSU* sequence suggests that evolutionary events (insertions/deletions) are probably taking place within Argentinian *F. poae* isolates, and that the acquisition of this insert occurred after geographic isolation of the Argentinian and European populations. Group I introns residing in mitochondrial genes have approximately 140 > 3000 bases and may contain long insertions that include either an open reading frame (ORF) or consist of noncoding sequences similar to intergenic spacers (Lang *et al.* 2007). The first report of an insert of 1380 bp characterized as a group I intron in the *mtSSU* rDNA was described in *Sclerotinia sclerotiorum*, showed close similarity in secondary structure to IC2 introns, and encodes a putative protein which contains two copies of the LAGLIDADG motif (Carbone *et al.* 1995). The insert that we found in some Argentinian *F. poae* isolates had a significant sequence (1367 bp), similarity with LAGLIDADG endonuclease, and secondary similarities to group I catalytic IC2 introns. A possibility is that these introns descended from a common ancestral filamentous fungus that had an IC2 intron within the mitochondrial genome. Chen *et al.* (1998) proposed that the group I intron in the nuclear *SSU* rDNA of *Phialophora gregata* f. sp. *adzukicola* could be an useful genetic marker for the separation between *P. gregata* f. sp. *adzukicola* and f. sp. *sojae*. On the basis of the results from the present study, however, it is not possible to establish whether this insert is of significance for distinguishing *F. poae* isolates with particular characteristics.

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