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Mitochondrial DNA variability in *Fusarium proliferatum* (*Gibberella intermedia*)

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Abstract

Restriction fragment length polymorphisms (RFLP) were used to assess genetic diversity of mitochondrial DNA (mtDNA) among 184 isolates of *Fusarium proliferatum* recovered from maize, asparagus, palms and reed. All strains were cross-fertile with standard mating type tester strains of *Gibberella intermedia*. Sixteen mitochondrial haplotypes were identified following digestion of DNAs with *Hae*III, with seven, seven, five and six different haplotypes from maize, asparagus, palms and reed, respectively. Four haplotypes (I, III, IV and VII) were found on more than one host. Of these four, haplotype I was dominant on maize, representing 71% of the isolates. The banding patterns for haplotypes III and IV were >90% similar to the banding pattern of haplotype I. Haplotypes I, III and IV accounted for 87% of the isolates from maize, but were less common on the other hosts, accounting for 70%, 52% and 33% of the isolates from asparagus, palms and reed, respectively. Thirteen of the 16 haplotypes were recovered from only a single host plant species. When comparing the banding patterns and frequencies of these haplotypes, at least five were recovered at a higher frequency from one host relative to the others. Our results suggest that mtDNA RFLP analysis is a useful indicator of genetic divergence in *Fusarium proliferatum*.

Introduction

Fusarium proliferatum (*Gibberella intermedia*) is a widespread pathogen of agriculturally-important plants, including maize (Logrieco et al., 1995), rice (Desjardins et al., 1997), asparagus (Elmer, 1990), sorghum (Leslie, 1995), and date palm (Abdalla et al., 2000; Armengol et al., 2002). The species is toxigenic, with some strains producing several mycotoxins, including fumonisin B-1 (Nelson et al., 1992), moniliformin (Marasas et al., 1984), beauvericin (Moretti et al., 1996), fusaric acid (Bacon et al., 1996), and fusaroproliferin (Ritieni et al., 1995). Despite its substantial importance both as a plant pathogen and as a producer of mycotoxins, there is limited information regarding population structure and genetic diversity in this fungus at a molecular level. It is not known whether all isolates of *F. proliferatum* belong to a single population, or if isolates from different hosts or locations represent genetically discrete populations. Both β-tubulin and mitochondrial small subunit ribosomal DNA (mtSSU rDNA) sequences have been analyzed from several *F. proliferatum* isolates (O'Donnell et al., 1998), but no clearly identifiable host-related variation has been reported.

Many molecular methods can be used to generate data on genetic variation in *Fusarium* populations, e.g. isozymes (Bosland and Williams, 1987; Elias and Schneider, 1992; Huss et al., 1996; Láday and Szécsi, 2001), restriction fragment length polymorphism (RFLP) of both nuclear and mitochondrial (mt) DNA (Kistler et al., 1987; Jacobson and Gordon, 1990; Kim et al., 1993; Fernandez et al., 1994), and random amplified polymorphic DNA (RAPD) patterns (Amoah et al., 1995; Bentley et al., 1998; Vakalounakis and Fragkiadakis, 1999). Sexual recombination is relatively common in *F. proliferatum* (Leslie and Klein,

1996), which means that haplotypes based on nuclear genes are numerous and that individual haplotypes generally are of limited importance. MtDNA evolves more quickly than nuclear genes, but compared to the variation observed in haplotypes of a polymorphic sexually reproducing population, it is relatively stable. Due to the rapid evolution and clonal inheritance of mtDNA, RFLP of mtDNA provides a molecular method suitable for analyses of divergence at the subspecies level (Avise, 1989).

The objectives of this study were (i) to identify restriction enzymes that could be used to produce mtDNA RFLP profiles in *F. proliferatum*, and (ii) to compare genetic diversity and divergence among isolates of *F. proliferatum* from various host plants in different geographic locations by comparing their mitochondrial RFLP profiles. Of particular interest were the distribution of mitochondrial haplotypes of *F. proliferatum* and their relationship with the host plants.

Materials and methods

Fungal strains and mycelium preparation

One hundred and eighty-four strains of *F. proliferatum* were obtained from the culture collection at the Institute of Sciences of Food Production, Bari, Italy (Table 1). Most of the isolates were collected from different fields though some were collected from the same region. Isolates were cultured from single conidia. For molecular analysis, isolates were grown initially in 10 ml carboxymethyl cellulose (CMC) broth (15 g l^{-1} CMC, 0.1% NH_4NO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.1% yeast extract) in a 50 ml Erlenmeyer flask inoculated with three mycelial discs (5 mm diameter) from young colonies growing on potato dextrose agar (Nelson et al., 1983). Cultures were incubated for three days on an orbital shaker (120 rpm) at 25°C, then 10 ml of culture were transferred to 100 ml YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2.0% D-glucose) in a 250 ml Erlenmeyer flask and incubated for two additional days on an orbital shaker (120 rpm) at 25 °C. Mycelia were vacuum-filtered on Whatman No. 1 filter paper, washed three times with 50 ml of distilled water and used immediately for mtDNA isolation. For other analyses the mycelial mat was lyophilized, ground with a mortar and pestle, and stored at −20 °C until needed for DNA extraction.

Fertility tests

Strains were crossed as male parents on carrot agar with standard tester strains of mating populations A–G (Klittich and Leslie, 1988). Mated cultures were considered fertile if perithecia and ascospores formed within six weeks. All strains were crossed twice with both testers from each mating population.

DNA isolation

Mitochondrial DNA was isolated using the method of Láday et al. (2001) and total DNA was isolated using the method of Leach et al. (1986). For total DNA isolation, 30 mg lyophilized mycelium powder was used and extracted DNA was diluted to a final volume of 100 µl with TE (150–200 ng DNA μl^{-1}).

Restriction enzyme digestion, electrophoresis and gradient ultracentrifugation

Restriction enzymes (New England Biolabs Inc., Beverly, MA, USA) were used to digest the purified mtDNA for 2 h in buffers recommended by the manufacturer using 3 µl of the isolated mtDNA preparation. For total DNA preparations, 15 µl samples were digested in a final volume of 100 µl overnight. Analyses were performed in 0.8% horizontal agarose gels overnight (≅16 h). Gels were stained with ethidium bromide and visualized by UV transillumination. CsCl gradient ultracentrifugation was performed as described by Williamson and Fennell (1974).

Data analysis

The molecular weights of the DNA bands were estimated with a ChemiImager 4000 Low Light Imaging System (Alpha Innotech Corporation, CA, USA). The data were organized into a binary matrix, and a pairwise similarity matrix was generated with the Nei and Li coefficient (1979) using the PhylTools programme (version 1.32; Laboratory of Plant Breeding, Wageningen University

Table 1. Isolates of *F. proliferatum* used in this study and their corresponding mitochondrial DNA haplotypes

Host[1]	Location	Haplotype	Item nos.	Source[2]
Zm	Canada, West of Clinton	I	1682	KS
Zm	Canada, South of Dublino	I	1687	KS
Zm	Canada, South of Tavistock	I	1688	KS
Zm	Canada, Flomborough	I	1689	KS
Zm	Canada, North Bowmanville	I	1690	KS
Zm	Canada, South of Suly's Bay	I	1691	KS
Zm	Canada, North of Deshwood	III	1684	KS
Zm	Canada, Manatick	IV	1692	KS
Zm	Canada, South east of Whitechurch	VI	1686	KS
Zm	USA, Iowa	I	2215, 2216, 2218, 2219–2221, 2287, 2291, 2292, 3821, 3822, 3825, 3830–3833	GM
Zm	USA, Iowa	III	3824, 3826–3829	GM
Zm	USA, Iowa	VI	2293, 2294	GM
Zm	USA	I	2400–2403, 2407, 2408	JFL
Zm	Argentina, Cordoba	I	2433, 2434, 2436, 2437, 2447–2449, 2451–2455	FSC
Zm	Argentina, Cordoba	IV	2446, 2450, 2456	FSC
Zm	Argentina, Rio Cuarto	I	1916, 1918, 1920	FSC
Zm	Argentina, Rio Cuarto	IV	1919	FSC
Zm	Italy, Lombardy	I	1493	AL
Zm	Italy, Tuscany, Arezzo	I	1494	AL
Zm	Italy, Milan	I	1504, 1505, 2026, 2027, 2030, 2032	AL
Zm	Italy, Asti	I	2185, 2186, 2187, 2188, 2189, 2190, 2192	AL
Zm	Italy, Asti	III	2191	AL
Zm	Italy, Pordenone	I	2246, 2249, 2251, 2262	AL
Zm	Italy, Pordenone	III	2263	AL
Zm	Italy, Bergamo	I	2297, 2299	AL
Zm	Italy, Bergamo	II	2298, 2300	AL
Zm	Italy, Cremona	V	1506	AL
Zm	Italy, Campania	V	1528	AL
Zm	Italy, Sassari	I	1719, 1722, 1723, 1725, 1727, 1749	AL
Zm	Italy, Sassari	VI	1726	AL
Zm	Italy, Sassari	VII	1748, 1750, 1751, 1752	AL
Zm	Italy, Cagliari	I	1762, 1763, 1764	AL
Zm	Italy, Cagliari	III	1761	AL
Zm	Italy, San Giuliano	IV	2105	AL
Zm	Slovakia	I	2631, 2635	AS
Zm	Slovakia	IV	2620	AS
Zm	Slovakia	VI	2649	AS
Zm	Slovakia	VII	2644	AS
Ao	Italy, Matera	I	1448	AL
Ao	Italy, Potenza	I	1449, 1482, 1491	AL
Ao	Italy, Potenza	III	1475, 1477–1479, 1483, 1484	AL
Ao	Italy, Potenza	VII	1480	AL
Ao	Italy, Potenza	VIII	1456, 1457	AL
Ao	Italy, Montescaglioso	I	1450	AL
Ao	Italy, Montescaglioso	X	1451	AL
Ao	Italy, Laterza	I	1452, 1453	AL
Ao	Italy, Stigliano	IV	1454	AL
Ao	Italy, Stigliano	VIII	1455	AL
Ao	Italy, Treia	IX	1485	AL
Ao	Italy, Asti	I	1488	AL
Ao	Italy, Asti	IX	1486	AL
Ao	Italy, Piacenza	III	1489, 1492	AL
Ao	Italy, Foggia	I	2366	AL
Ao	Italy, Foggia	IV	2368	AL
Ao	Italy, Foggia	X	2365	AL

Table 1. (Continued)

Host[1]	Location	Haplotype	Item nos.	Source[2]
Pd	Saudi Arabia, Buriedah	I	2339	MYA
Pd	Saudi Arabia, Buriedah	VII	2342	MYA
Pd	Saudi Arabia, Buriedah	XI	2383, 2386, 2387	MYA
Pd	Saudi Arabia, Buriedah	XI/a	2343	MYA
Pd	Saudi Arabia, Buriedah	XII	2341	MYA
Pd	Saudi Arabia, Bakireia	XII	2336	MYA
Pd	Saudi Arabia, Al Monawara	XII	2337	MYA
Pc	Spain, Alicante	I	4284, 4286, 4304, 4305	*JA*
W sp.	Spain, Alicante	I	4292	JA
Ch	Spain, Alicante	I	4293	JA
Pd	Spain, Alicante	I	4296–4298	JA
Pd	Spain, Alicante	I/a	4303	JA
Ch	Spain, Alicante	I/a	4294, 4295	JA
Pc	Spain, Alicante	XI	4285	JA
Pr	Spain, Alicante	XIII	4291	JA
Pc	Spain, Valencia	I	4299–4301	JA
Pc	Spain, Valencia	XI	4287–4290	JA
Wf	Spain, Tarragona	VII	4306	JA
Ad	Italy, Brindisi	XIV	1590	AL
Ad	Italy, Pescara	VII	1606	AL
Ad	Italy, Pescara	XIV	1609–1611	AL
Ad	Italy, Lecce	XVI	1796	AL
Ad	Italy, Porto Torres	XIV	1799	AL
Ad	Italy, Sassari	VII	1800	AL
Ad	Italy, Sassari	XIV	1801, 1808	AL
Ad	Italy, Bari	IV	1870	AL
Ad	Italy, Bari	XIV	1871	AL
Ad	Italy, Bari	XV	1872, 1874	AL
Ad	Italy, Foggia	I	2113, 2114, 2116	AL
Ad	Italy, Foggia	IV	2111, 2112, 2115	AL

[1] Zm: *Zea mays*; Ao: *Asparagus officinalis*; Pd: *Phoenix dactylifera*; Pc: *Phoenix canariensis*; Pr: *Phoenix reclinata*; Ch: *Chamaerops humilis*; W: *Washingtonia*; Wf: *Washingtonia filifera*; Ad: *Arundo donax*.

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(http://www.dpw.wau.nl/pv)). Dendrograms were constructed using the software package PHYLIP 3.5 (J. Felseinstein, Washington University, Seattle). A data set containing only the value '0' was used as an outgroup. Cluster analysis (UPGMA) was carried out from the distance matrix using the NEIGHBOR programme with random input order.

Statistical analyses on frequencies of different haplotypes were developed by the computer software of JMP (version 4, SAS Institute, Cary, NC) using the distribution menu.

Results

Mating population and mating type determination

One hundred and eighty-four isolates (105 from maize (*Zea mays*), 27 from asparagus (*Asparagus officinalis*), 31 from palms (*Chamaerops humilis*, *Phoenix dactilyfera*, *Phoenix canariensis*, *Phoenix reclinata*, *Washingtonia filifera*, and *Washingtonia* sp.), 21 from reed (*Arundo donax*) were assayed for sexual compatibility. All isolates were crossed with standard testers of *G. intermedia*.

*Hae*III RFLP patterns of total DNA and purified mtDNA for isolates ITEM-1456, -1485, -1483, -1800, -1801, -2105, -2216, -2337, -2343, -2383 and -4294 were compared. The two methods resulted in identical banding patterns (Figure 1). When total DNA was used, however, the smaller RFLP fragments were less visible due to the nuclear DNA smear; therefore only bands >1000 bp were scored.

Sixteen mtDNA haplotypes were detected among the 184 *F. proliferatum* isolates (Table 1, Figure 2) and were assigned them roman numerals (I–XVI). Forty-nine polymorphic RFLP bands were identified among a total of 53 bands generated following digestion with *Hae*III. A great deal of heterogeneity was observed among isolates from each host with seven (I, II, III, IV, V, VI, VII), seven (I, III, IV, VII, VIII, IX, X), five (I, VII, XI, XII, XIII), and six (I, IV, VII, XIV, XV, XVI) haplotypes for maize, asparagus, palms and reed, respectively.

Of the 16 haplotypes detected, only four (I, III, IV and VII) were recovered from more than one host. Isolates of haplotype I were the most common containing 103 isolates (56%) from the total of 184 isolates. Haplotypes III and IV were very similar (>90%) to the banding pattern of haplotype I and contained an additional 30 isolates (16%) from maize, asparagus and reed. Isolates from haplotype VII also were recovered, at a low

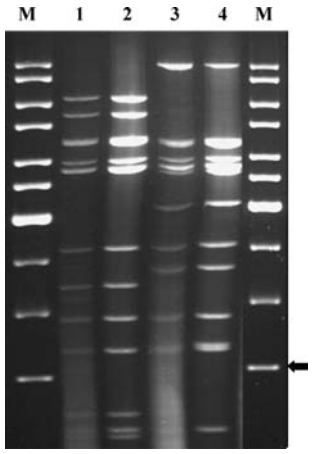

Figure 1. Agarose gel electrophoresis of *Hae*III digested DNA from isolates Item 2336 (1–2) and Item 2383 (3–4). Lanes 1 and 3 are from total DNA digestions, and lanes 2 and 4 are from purified mtDNA digestions. M – 1-kb DNA ladder (Fermentas, Vilnius, Lithuania). The arrow indicates a 1000 bp band in the marker lane.

frequency, from all four hosts (11/184 isolates). The RFLP pattern of haplotype VII was quite different (<70% similarity) to that of haplotypes I, III and IV (Figures 2 and 3). All other haplotypes were recovered from only a single host species.

Isolates belonging to haplotype I were the most common on maize (75/105 isolates; 71%). Together with the closely related haplotypes III and IV, haplotypes I, III and IV dominated on maize (87%), and represented at least 80% of isolates at the 95% confidence level. Isolates from haplotype

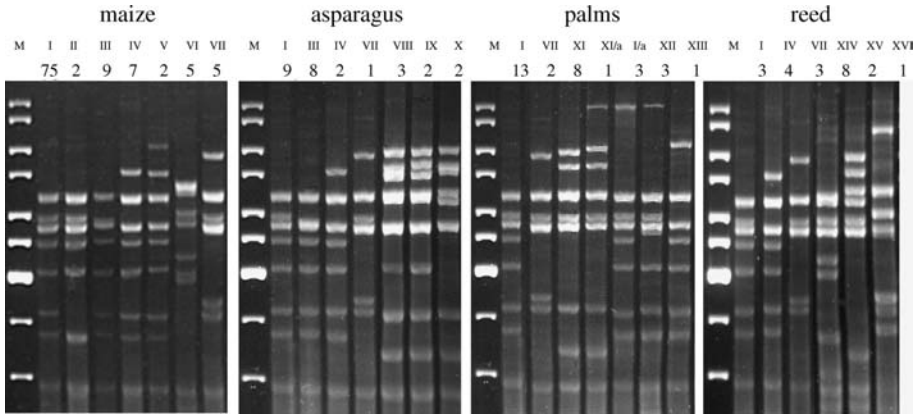

Figure 2. MtDNA haplotypes (I–XVI) detected after *Hae*III restriction enzyme digestions of *F. proliferatum* isolates from maize, asparagus, palms, and reed. Numbers immediately above each lane indicate the number of isolates in which a haplotype was detected. Mating standard testers of the D-04853 (D2) and D-04854 (D1) had a pattern of haplotype I. M – 1-kb DNA ladder (Fermentas).

VII also were recovered at low frequencies (5/105 isolates) from maize. Haplotypes II, V, and VI were exclusively found on maize and accounted for only nine isolates (9%) of the 105 total isolates.

Haplotype I isolates were also recovered from asparagus (9/27 isolates), palms (16/31 isolates), and reed (3/21 isolates); haplotype III was recovered from asparagus (8/27 isolates), and haplotype IV was recovered from asparagus (2/27 isolates) and reed (4/21 isolates). Although haplotypes I, III and IV dominated on maize (87%), these haplotypes were collectively less frequent on other hosts, and made up only 70% (19/27 isolates), 52% (16/31), and 33% (7/21 isolates) of the isolates from asparagus, palms, and reed, respectively. Based on 95% confidence intervals, haplotypes I, III and IV represent at most 81%, 68% and 55% of the total isolates from asparagus, palms, and reed, respectively. Haplotype VII was also recovered, at low frequencies (3–14%), from all three of these hosts. All other haplotypes were recovered from only a single host species. Haplotypes VIII, IX and X were recovered only from asparagus. These three haplotypes had similar banding patterns (>89%) and constituted 26% (7/27 isolates) of the isolates from asparagus. Haplotypes XI, XII and XIII were found only on palms. Haplotype XI was the second most common banding pattern on palms, and the nine isolates (29%) with this haplotype were found in both Saudi Arabia and Spain (Table 1). Three isolates from Saudi Arabia had the haplotype XII banding pattern while haplotype XIII was represented by only a single isolate. Haplotype XIV was detected exclusively on reed and dominated on this host. Of the 21 isolates, eight (38%) had the banding pattern of haplotype XIV. Two isolates from reed had a banding pattern of haplotype XV, and an additional haplotype unique to isolates from reed was identified as haplotype XVI. Based on the 95% confidence intervals, haplotypes VIII, IX and X represented at least 14% of the isolates from asparagus, while haplotype XI represented at least 16% of the isolates from palms. Similarly, isolates with haplotype XIV constituted at least 21% of the isolates from reed.

CsCl gradient ultracentrifugation

Banding patterns of seven isolates from palms (all three patterns of haplotype XIII, three patterns of haplotype I (I/a) and one pattern of haplotype XII (XII/a)) shared a band with an estimated size of 10 kb. Strains containing this 10 kb band were isolated in both Saudi Arabia and Spain (Table 1, Figure 2). MtDNA from strains ITEM-2343 (haplotype XI/a), ITEM-4294 (haplotype I/a), and ITEM-2337 (haplotype XII) was banded by CsCl gradient ultracentrifugation resulting in two bands for all three mtDNA samples. Digestion of the DNA from the upper band of the CsCl gradient with *Hae*III resulted in only one, approximately 10 kb long fragment, while similar digestion of the DNA from the lower bands yielded the same banding patterns as those from digestion of the total DNA except that the 10 kb band was missing (data not shown). Thus, the 10 kb fragments probably represent a mitochondrial plasmid-like DNA sequence with no *Hae*III cleavage site.

Cluster analysis

The relatedness among RFLP haplotypes was analyzed using UPGMA and Nei and Li's similarity matrix based on the presence and/or absence of mtDNA bands. The bands of the putative plasmid were not included in the analysis. The most common haplotype, haplotype I was in the largest subcluster with haplotypes II, III, IV and XIII with an average similarity of 89%. Haplotypes V and XIV were in single-haplotype subgroups with similarity coefficients of 85%, and 76%, respectively to the haplotype I subcluster (Figure 3). Haplotypes XI and VII grouped together with 64% similarity, and were 62% similar to the subcluster of haplotype I. Haplotypes VIII, IX and X from asparagus clustered together with a similarity of 88% and were more distant (58%) from the haplotype I subcluster. The remaining haplotypes, XIV, XV, VI and XVI had an average similarity of 54%, 49%, 42% and 31% respectively to the haplotype I subcluster.

Discussion

Molecular markers have often been used to investigate diversity among different populations of *Fusarium* spp. MtDNA RFLP analysis has been used to estimate genetic variability within *Fusarium* populations. A high variability was observed in the *F. oxysporum* complex based on VCG and mtDNA RFLP (Kistler et al., 1987; Jacobson and

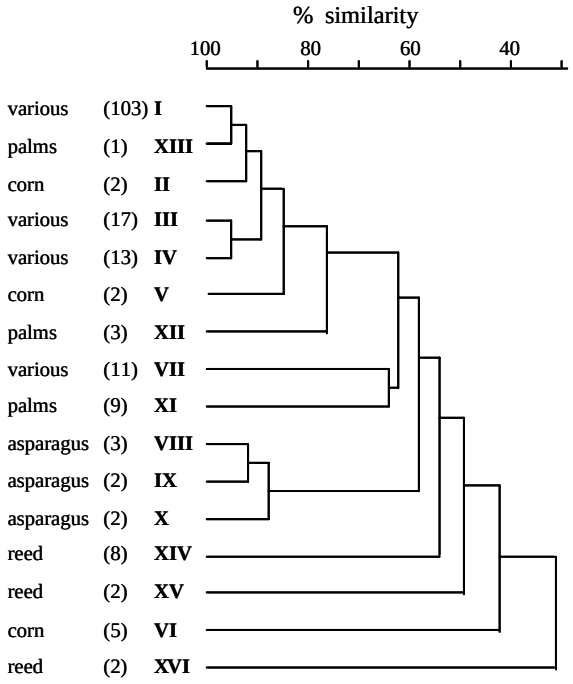

Figure 3. Dendrogram showing the genetic relationship of 16 mtDNA RFLP haplotypes. Numbers in parentheses are the number of isolates in which a haplotype was detected.

Gordon, 1990; Gordon and Okamato, 1992; Tantaoui et al., 1996). Analysis of mtDNA RFLP data provided information regarding the genetic similarity of individuals in different VCGs (Jacobson and Gordon, 1990; Kim et al., 1993). Only limited data on both mtDNA RFLPs and VCG are available for the *G. fujikuroi* species complex. Correll et al. (1992) found that all the isolates of *F. subglutinans* f.sp. *pini* (syn. *F. circinatum*) had the same, unique mtDNA RFLP profile, while 45 VCGs were identified among a collection of 116 isolates from Florida, and a large number of unique VCGs were found in each population examined.

In the present study a considerable number of mtDNA polymorphisms were detected: with 16 haplotypes among 184 isolates of *F. proliferatum*. Closely related haplotypes I, III and IV were found on different hosts and were dominant on maize, and less common on the other hosts. The relatively distant haplotype VII was also recovered, at low frequencies, from all four hosts studied, but the other 12 haplotypes were recovered from only a single host species. Of the 27 isolates from Italian asparagus, seven were in the closely related haplotypes VIII, IX and X, but these haplotypes were not found in any of the other 67 Italian isolates from maize and reed. Similarly, haplotype XIV was common (38%) amongst Italian isolates from reed, but was not recovered from the 74 Italian fungal samples from maize and asparagus. It is particularly interesting that of the four isolates from Sardinian reed three were haplotype XV, while none of the 15 isolates from Sardinian maize belonged to this haplotype. If haplotype XIV is present at the same level on Sardinian maize as it is on reed, at least three isolates of haplotype XIV should have been detected in the Sardinian maize isolates ($\alpha = 0.05$). Moreover, none of the 163 other isolates had the banding patterns of haplotype XIV. Haplotype XI was only found on palms (9/31 isolates) but was found in both Spain and Saudi Arabia. Haplotypes VIII, IX and X from asparagus, XI from palms and XIV from reed occur more frequently on their respective hosts than on the other hosts studied. Maize also appears to be the preferred host for the relatively widespread haplotype I. Therefore, mtDNA RFLP data indicate a significant level of heterogeneity in *F. proliferatum* haplotypes both among host species and within any given host species (Figure 2). Heterogeneity among host species was due mainly to the presence of haplotypes that were found only on a single host species. These population patterns could have arisen partially as the result of host-fungus coevolution.

The distribution of haplotype XI may also reflect a geographic difference between the populations of *F. proliferatum*, because no other host was examined from both Saudi Arabia and Spain. The most common haplotype I, however, was also detected in both countries, and the relatively widespread haplotype VII was also found. These facts indicate that neither the Saudi Arabian nor the Spanish populations of *F. proliferatum* expressed a geographically separated population structure based on mtDNA RFLP patterns of isolates.

Some haplotypes were isolated from different hosts. Strains that have successfully adapted to one host may also survive beyond the point of introduction to other host environments and be maintained stably. This possible change in the host environment for an isolate may contribute to the increase in the diversity of the fungal population.

For example, haplotype I was dominant on maize, but this haplotype was also found on the other host species at lower, but significant, frequencies. This pattern is consistent with such a change in the host environment. It is also possible that infrequent or unique haplotypes, such as haplotype XIII, XV, and XVI, represent introductions from other environments.

A common, 10 kb long mitochondrial plasmid was detected as a part of three different haplotypes (Figure 2; I/a, XI/a, XII). The transfer of mitochondrial plasmids between fungal strains has been demonstrated for some ascomycetes *in vitro* through sexual reproduction, vegetative fusion or simple hyphal contact (May and Taylor, 1989; Collins and Saville, 1990; Kempken, 1995). Our data on the distribution of the mitochondrial plasmid are consistent with the horizontal transfer of this mitochondrial plasmid between co-existing strains. Such transfer also increases the diversity of the *F. proliferatum* populations, because the presence of this plasmid in some haplotypes provides additional mitochondrial variants (Figure 2).

The mtDNA RFLP data indicated a significant level of heterogeneity in *F. proliferatum* isolates obtained from the same host species as well as among isolates from different host species. These results may have important practical implications for the control of *F. proliferatum* diseases and mycotoxin contamination in infected plants. It is important to evaluate the pathogenicity/toxigenicity and endophytic properties of different haplotypes for each host in order to gather information for plant breeders. Finally, additional data on the cross-fertility and toxigenicity of the haplotypes are needed to further characterize the different populations of this important pathogen and evaluate the effect of plant host exposure to its toxins.

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