European Journal of Plant Pathology **110:** 551–562, 2004.

Genetic diversity of *Fusarium graminearum* in Europe and Asia

T. Yu. Gagkaeva[1] and T. Yli-Mattila[2]
[1]*Laboratory of Mycology and Phytopathology, All-Russian Institute of Plant Protection (VIZR), 196608 St-Petersburg-Pushkin, Russia (Fax: +7-812-4705110; E-mail: gagkaeva02@yahoo.com);* [2]*Department of Biology, Laboratory of Plant Physiology and Molecular Biology, FIN-20014 University of Turku, Finland*

Key words: IGS, isozyme, population, β-tubulin

Abstract

The biodiversity and phylogeny of *Fusarium graminearum* isolates originating from different geographical areas were analyzed using isozyme variation, β-tubulin and IGS sequences. Geographically distinct groups of isolates originating from different areas of the Russian Federation, China, Germany and Finland were studied. The complex enzymes (α- and β-esterases, aspartate aminotransferase and superoxide dismutase) and the IGS sequences demonstrated a high level of genetic diversity in *F. graminearum*. Diversity in the Asian population was higher than in the European one. There was a correlation between genetic clusters of the IGS sequences and geographical origin in *F. graminearum*. Knowledge of biodiversity and identification of some phylogenetic lineages in *F. graminearum* will be useful in defining the risk of pathogen evolution as well as benefiting disease management strategies.

Introduction

Fusarium head blight (FHB) caused by *Fusarium graminearum* (sexual state *Gibberella zeae*) is an important disease of cereals that causes serious losses in both yield and quality of grain. The trichothecene and estrogenic mycotoxins produced by this fungus are hazardous to people and animals, while the trichothecenes are also virulence factors during pathogenesis in plants (Proctor et al., 1995). *Fusarium graminearum* is an important pathogen within Europe (Bottalico and Perrone, 2002); in Bulgaria (Mladenov and Karadzhova, 1978), Austria (Lew et al., 2001), the Czech Republic (Ostry and Ruprich, 2001), Croatia (Cosic and Jurkovic, 2000), France (Bakan et al., 2001), central and northern Germany (Ellner, 2001), Romania (Ittu, 2001) and southwestern Ukraine (Klechkovskaya, 1999). In Asia, this fungus has great importance in China (Zheng et al., 1983; Chen et.al., 2000; Gale et al., 2002) and Japan (Koixumi et al., 1991). In Russia, *F. graminearum* is common in the southern region between the Black and Caspian Seas and in the Far East region close to the Pacific Ocean, but has not been found in the northwestern and central parts, or in Siberia (Levitin et al., 1994, 2000). In Finland, *F. graminearum* is not detected every year, but it can be common during warm and rainy years, especially on oats (Ylimäki et al., 1979; Yli-Mattila, 2002b).

Variations in cultural, pathogenic and other characteristics are a common phenomenon in *F. graminearum* (Oswald, 1949; Cullen et al., 1982; Sugiura et al., 1990; Bai and Shaner, 1996). Isozyme analysis has been a valuable tool for inferring relationships at the inter- and intra-specific level in several fungi, including *Fusarium* (Reddy and Stahmann, 1972; Yli-Mattila et al., 1996; Paavanen-Huhtala et al., 1999; Laday et al., 2000). In addition, molecular techniques, in particular sequences of variable regions (β-tubulin, ITS, IGS) have used to assess of diversity within *Fusarium* (Schilling et al., 1997; Aoki and O'Donnell, 1999; Logrieco et al., 1999; Roux et al., 2001; Yli-Mattila et al., 2002a).

The purpose of this study was to analyze the biodiversity and phylogeny of isolates originating from different geographical areas by using isozymes variation, β-tubulin and IGS sequences.

Material and methods

Fungal material

Forty-eight isolates of *F. graminearum* obtained from wheat grain were studied and their sources are listed in Table 1: 11 isolates were from far east Russia, 13 from southern Russia, 9 from north-eastern China and 10 from Germany. Four isolates were collected from wheat, barley and oats in Finland. Cultures were identified and single-spore isolates were produced. All the *F. graminearum* isolates are in the collection of *Fusarium* fungi at the All-Russian Institute of Plant Protection (VIZR).

Extraction, gel electrophoresis and staining of proteins

The isolates were grown for one week in 250 ml of liquid Czapek medium containing yeast extract (0.1%). The mycelium was harvested, washed several times in distilled water, dried on paper and frozen at −20 °C. After this, 50 mg of mycelium was ground in 1.5 ml Eppendorf tubes containing 300 µl cold (4 °C) extraction buffer (0.1 M Tris–HCl buffer (pH 8.0), 0.5 sucrose, 0.1% ascorbic acid and 0.1% hydrochloride cysteine). The tubes were centrifuged at 10,000 rpm for 15 min at 4 °C and the supernatant stored at −80 °C. The electrophoretic profiles of enzymes were detected by vertical polyacrylamide gel electrophoresis (Honold et al., 1966; Mayrer, 1971; Yli-Mattila et al., 1996). Electrophoresis was performed on a thin (0.5 mm) lower running gel (7.5%) and an upper concentrated gel (1%) onto which 50 µl of the sample were loaded. Electrophoresis was carried out on vertical gels with 0.01 M Tris–glycine buffer (pH 8.3). The upper chamber contained 0.001% bromophenol blue as a marker dye. The samples were first run through the upper gel at 1.5 mA and then through the lower gel at 2 mA until the marker dye front reached a level of 1 cm from the bottom of the gel. After electrophoretic separation, the gels were stained (Honold et al., 1966; Korochkin et al., 1977). The polymorphic enzymes used in the analysis were α- and β-esterases (E.C.3.1.1.1), aspartate aminotransferase (E.C.2.6.1.1) and superoxide dismutase (E.C.1.15.1.1). The position of each isozyme band was calculated as the ratio between the distance moved by the isozyme band and the distance moved by the bromphenol blue front; this ratio was designated as the R_f value. Each gel was repeated at least twice for each sample and only reproducible results were accepted.

DNA extraction, PCR and PCR product purification and sequencing

The mycelium for DNA extraction was grown in the same way as for protein extraction. Total DNA was isolated using two methods (Lee and Taylor, 1990; Möller et al., 1992), but in both cases fresh-frozen mycelium (0.2 g) was used. The DNA pellet was dissolved in 100 µl TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). DNA concentrations were estimated by electrophoresis using 0.8% agarose gels stained with ethidium bromide by comparison with DNA standards. The gels were viewed under UV light and PCR products were detected visually from the photographs of the gels. Amplification for PCR reactions was performed in 25 µl of 1× Dynazyme reaction buffer (10 mM Tris–HCL pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100), 150 µM of each deoxyribonucleotidetriphosphate (dNTP), ca. 60 ng forward and reverse primers, 0.8 U Dynazyme polymerase (Finnzymes, Espoo, Finland) and 1–10 ng of template DNA (Paavanen-Huhtala et al., 2000; Yli-Mattila et al., 2002a). Primer sequences and PCR amplification conditions for β-tubulin (500 bp) and the IGS region were as described in Yli-Mattila et al. (2002a). PCR reactions were performed in a PTC 200 DNA Engine thermal cycler (MJ Research, Watertown, MA, USA). The amplification products (5µl) were separated in 1% agarose gel by electrophoresis in 1× TBE buffer. PCR products were purified and sequenced (Yli-Mattila et al., 2002a; Yli-Mattila et al., in press). All new sequences were submitted to GenBank.

Data analysis

Sequences were aligned using the Clustal W program (Thompson et al., 1994) on the server of the DNA Data Bank of Japan and edited visually. The programs DNADIST (Kimura) and NEIGHBOR (UPGMA) of PHYLIP 3.5 (Felsenstein, 1993) were used for phenetic analyses. In addition, the POY 2.7 program (Wheeler, 1996; Gladstein and Wheeler, 2001) in the computers of CSC (Scientific

Table 1. Isolates of *Fusarium graminearum*

No.	Isolate	Geographic origin		Host/substrate	Year	Accession number
		Country	Territory			
1	Pr12	Russia	Far East, Primorsk	Wheat, seed	1998	
2	Pr13	Russia	Far East, Primorsk	Wheat, seed	1998	AY360075
3	Pr14	Russia	Far East, Primorsk	Wheat, seed	1998	
4	Pr81	Russia	Far East, Primorsk	Wheat, seed	1998	
5	Pr82	Russia	Far East, Primorsk	Wheat, seed	1998	AY360078
6	Chab11	Russia	Far East, Khabarovsk	Wheat, seed	1998	
7	Chab14	Russia	Far East, Khabarovsk	Wheat, seed	1998	
8	Chab6-2	Russia	Far East, Khabarovsk	Wheat, seed	1998	
9	Chab2-13	Russia	Far East, Khabarovsk	Wheat, seed	1998	AY360071
10	Chab10-2	Russia	Far East, Khabarovsk	Wheat, seed	1998	AY360079
11	Chab507	Russia	Far East, Khabarovsk	Wheat, spikelet	1998	AY360072
12	Ch1	China	North-East part, Harbin	Wheat, seed	1998	
13	Ch13	China	North-East part, Harbin	Wheat, spikelet	1999	
14	Ch14	China	North-East part, Harbin	Wheat, spikelet	1999	AY360072
15	Ch25	China	North-East part, Harbin	Wheat, seed	1999	
16	Ch31	China	North-East part, Harbin	Wheat, seed	1999	
17	Ch39	China	North-East part, Harbin	Wheat, seed	1999	AY360064
18	Ch311	China	North-East part, Harbin	Wheat, seed	1999	
19	Ch317	China	North-East part, Harbin	Wheat, seed	1999	
20	Ch324	China	North-East part, Harbin	Wheat, seed	1999	AY360076
21	CO2-2	Russia	North Ossetia	Wheat, seed	1998	
22	CO3-1	Russia	North Ossetia	Wheat, seed	1998	
23	CO3-7	Russia	North Ossetia	Wheat, seed	1998	AY360077/ AY360063
24	CO4-2	Russia	North Ossetia	Wheat, seed	1998	AY360066
25	CO4-5	Russia	North Ossetia	Wheat, seed	1998	AY360064
26	CO5-1	Russia	North Ossetia	Wheat, seed	1998	
27	CO5-4	Russia	North Ossetia	Wheat, seed	1998	
28	CO5-5	Russia	North Ossetia	Wheat, seed	1998	AY360073/ AY360063
29	Kr1	Russia	Krasnodar	Wheat, seed	1997	
30	Kr10	Russia	Krasnodar	Wheat, seed	1997	
31	Kr14	Russia	Krasnodar	Wheat, seed	1997	AY360070
32	Kr15	Russia	Krasnodar	Wheat, seed	1998	
33	Kr18	Russia	Krasnodar	Wheat, seed	1998	AY360065
34	Kr19	Russia	Krasnodar	Wheat, seed	1998	AY360065
35	F27, original code 92027	Finland	Pori	Wheat, root	1986	
36	F28, original code 92028	Finland	Jalasjärvi	Barley, stem base	1986	
37	F29, original code 92029	Finland	Espoo	Barley, root	1986	AY360068
38	F1	Finland	Ylistaro	Oat, stem base	1993	

Table 1. (Continued)

No.	Isolate	Geographic origin		Host/substrate	Year	Accession number
		Country	Territory			
	G1-15	Germany	Falkenhagen	Wheat, seed	1998	
39						
40	G3-4	Germany	Falkenhagen	Wheat, seed	1998	
41	G3-5	Germany	Falkenhagen	Wheat, seed	1998	AY360074
42	G3-6	Germany	Falkenhagen	Wheat, seed	1998	AY360073
43	G8-6	Germany	Falkenhagen	Wheat, seed	1998	
44	G8-8	Germany	Falkenhagen	Wheat, seed	1998	AY360069
45	G23-1	Germany	Reinshof	Wheat, seed	1998	AY360067
46	G23-3	Germany	Reinshof	Wheat, seed	1998	
47	G23-4	Germany	Reinshof	Wheat, seed	1998	
48	G 50	Germany	Rocking	Wheat, seed	1998	

Computing, Espoo) was used for analyzing IGS sequences without previous alignment. The X^2 test was used to determine the significance of differences in diversity between Asian and European populations (Szecsi et al., 1976).

Results

Electrophoretic separation

Polymorphic patterns of α- and β-esterase were revealed in 15 and 13 distinct bands, respectively. The isozyme profiles of aspartate aminotransferase and superoxide dismutase consisted of 5 and 8 bands, respectively (Figure 1). Based on the frequency of each isozyme band, coefficients of similarity were calculated and a dendrogram was constructed (Figure 2). The enzymes used revealed a high level of phenotypic variation: 31 different electrophoretic phenotypes (single clonal lineages based on 100% similarity) were detected among 48 isolates. These phenotypic lineages consist of 1–6 members. Isolates of *F. graminearum* were divided into four clusters at a similarity level of 83%. The first cluster consists of 15 Asian and 22 European isolates and included 77% of the analyzed isolates. Cluster I was divided, at a similarity level of 87%, into two subclusters. Within subcluster IA, all European isolates (10), except one isolate from North Ossetia (CO4-2), were grouped together with the Asian isolates (12). In subcluster IB, the European isolates (12) prevailed over Asian (3) isolates. Clusters II and III consist of a small number of isolates, 4 (2 Asian and 2 European) and 2 (Asian) isolates, respectively. Cluster IV, which contained all four isolates from Finland and one from China (Ch13), was least similar to the other isolates. The diversity in Asian population was significantly higher than in European population ($X^2_{\text{calculated}} = 16.13$, $X^2_{\text{tabulated}} = 12.6$ at 95% and 16.8 at the 0.99% level of significance, df = 6).

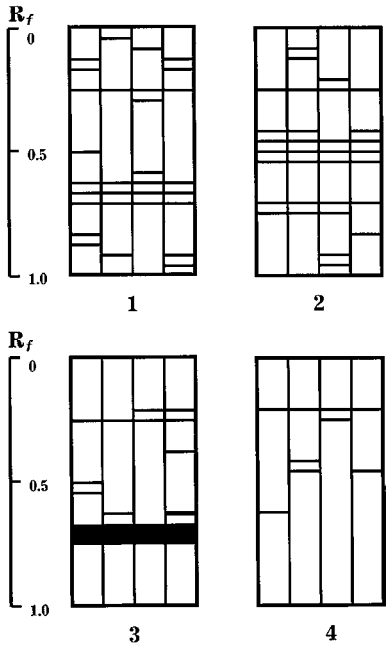

Figure 1. Electrophoresis patterns of enzymes detected in *Fusarium graminearum*. 1 = α-esterase, 2 = β-esterase (E.C.3.1.1.1), 3 = superoxide dismutase (E.C.1.15.1.1), 4 = aspartate aminotransferase (E.C.2.6.1.1).

β-tubulin sequences

The β-tubulin sequences (500 bp) of 15 *F. graminearum* isolates (Kr14, Kr18, Kr19, Pr81, Pr82, G3-5, G3-6, G23-4, F27, F28, Chab507, Chab10-2, Chab2-13, Ch13, Ch39) representative of the different location were identical to each other and to known *F. graminearum* sequences (e.g. accession number AF107861). Only isolates from the North Ossetia CO3-7 (AY360077) and CO5-5 (AY360073) had one common point mutation differing from other isolates. They were found to have identical β-tubulin sequences with an Iranian *F. flocciferum* (accession number AF006363; O'Donnell et al., 1998) isolate, which was later reidentified as *F. graminearum* of lineage 7 (O'Donnell et al., 2000).

Ribosomal DNA internal spacers

IGS haplotypes partially differentiated the geographical groups of isolates. Five main clusters were found in the UPGMA dendrogram at a similarity level of 99.2% (Figure 3). Clusters III and IV were Asian, while cluster V was European and clusters I and II contained isolates from both Europe and Asia. Cluster II could be divided into three subclusters at a similarity level of 99.48%; one of the subclusters was European, one Asian and the third mainly European. Isolates CO4-5, Ch39 (cluster I), Kr19, Kr18, CO4-2, G23-1, Kr14 (cluster IIA), F29, G8-8, F27 (cluster V) and Chab2-13 (cluster IV) had a long deletion (80 bp),

which was partly common with the shorter deletion (69 bp) of isolates Chab507 (cluster IIB) and Pr82 (cluster IIC). Isolate groups CO4-5, Ch39, Kr19, Kr18, CO4-2, G23-1 and Kr14 (clusters I and IIA) and Ch324, Ch14 and Chab10-2 (cluster III) had their own deletions, which were 18 and 28 bp, respectively. POY analysis gave 53 shortest trees with a length of 409 steps for IGS sequences.

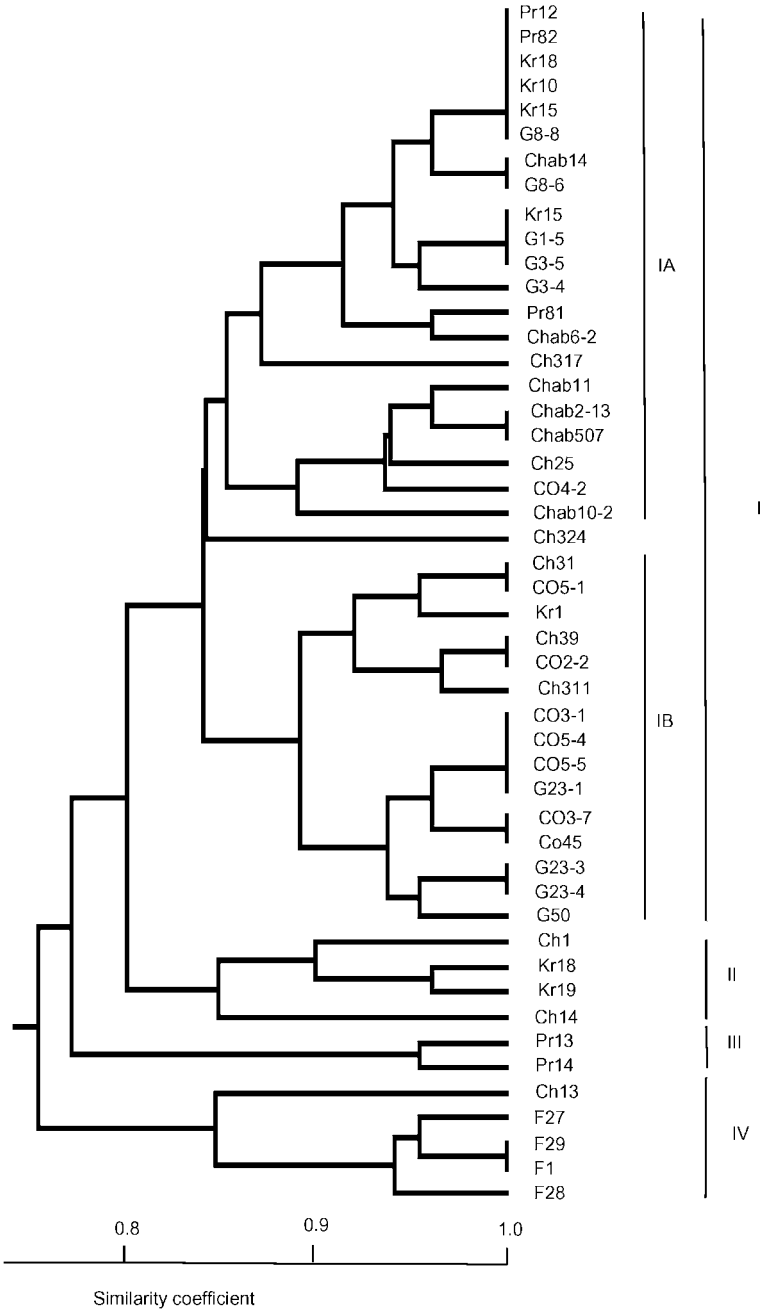

Figure 2. UPGMA dendrogram for combined isozyme data based on coefficient of similarity (DICE). Clusters I, II, III and IV are marked.

In the POY consensus tree (Figure 4) clusters I, III, IV and V were well supported, while the large cluster II was divided into subclusters, of which IIA and IIB were similar to those in the UPGMA dendrogram. The three European isolates of cluster IIA had a basal position to other *F. graminearum* isolates. The diversity in the Asian population was significantly higher than in the European one ($X^2_{\text{calculated}} = 16.09$, $X^2_{\text{tabulated}} = 12.6$ at 95% and 16.8 at the 0.99% level of significance, df = 6).

Discussion

The population structure of *F. graminearum* is poorly understood. Miedaner et al. (2001) found a high molecular genetic diversity and variation for aggressiveness in *Fusarium graminearum* population originating from Europe. Walker et al. (2001) demonstrated a high level of genotypic and phenotypic diversity in populations of *F. graminearum* in North Carolina. Quellet and Seifert (1993), on the other hand, characterized *F. graminearum*

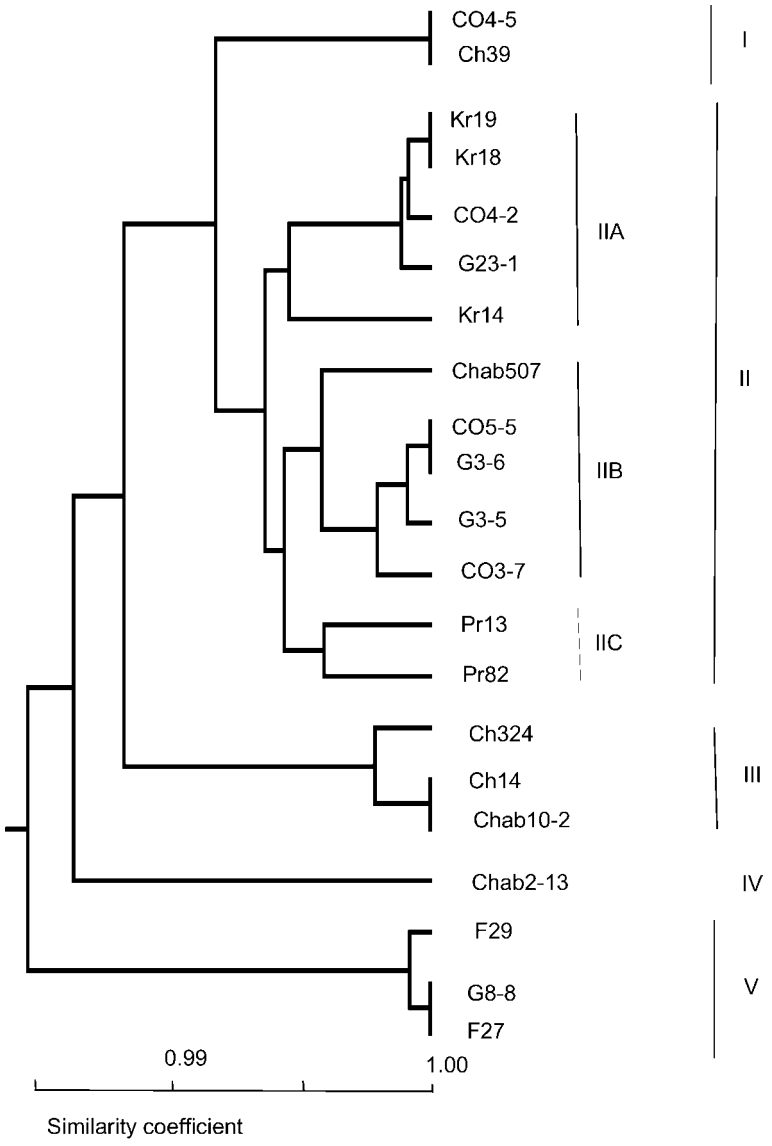

Figure 3. UPGMA dendrogram for IGS sequences based on similarity (DICE) coefficient. Clusters I, II, III, IV and V and subclusters IIA, IIB and IIC are marked.

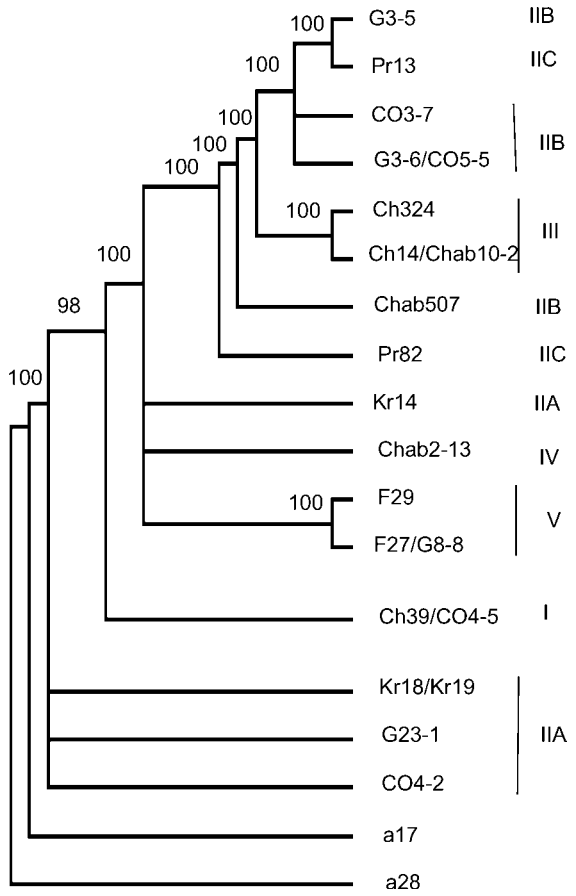

Figure 4. POY consensus tree of 53 shortest trees with a length of 409 steps for IGS sequences. Clusters and subclusters marked as in Figure 2. Only branches present in more than 50% of the trees are shown. IGS sequences of *F. avenaceum* isolates a28 and a17 (Yli-Mattila et al., 2002a) were used as outgroups.

isolates from Canada using RAPD and restriction analysis of amplified fragments from the PCR and demonstrated a relatively low amount of genetic diversity, which could not be grouped according to host or geographic origin. The analysis of 72 isolates from Canada by RAPD showed that all isolates were genetically distinct; 90.56% of the genetic variability was explained by within-region variation (Dusabenyagasani et al., 1999). Bowden et al. (2000) using AFLP analysis to determine genetic diversity in *F. graminearum* from Kansas and North Dakota, found a high degree of homogeneity between subpopulations. Fernando et al. (2003) confirmed that diversity between Canadian isolates was more closely related to their aggressiveness and toxin production than to geographic location or to the host from which the pathogen was isolated.

O'Donnell et al. (2000) have identified at least seven biogeographically structured lineages of *F. graminearum* from different regions of the world. They suggested that such lineages may be due to longstanding geographic isolation or other reproductive barriers. According to O'Donnell et al. (2000), the most basal (oldest) phylogenetic lineages of *F. graminearum* can be found in the southern hemisphere. Nicholson et al. (2002) showed that three distinct genetic groups (one in northwestern Europe and USA and two in Nepal) exist within *F. graminearum*, and demonstrated that these groups have different biological properties, especially with respect to their pathogenicity on wheat and maize.

A considerable genetic resemblance was found by RAPD between 34 isolates from northeastern and northwestern China. Grouping of isolates was not related to pathogenicity or to host cultivar (Liu et al., 2002). Investigation of *F. graminearum* isolates from the southeastern area of China using RFLP established that all isolates belonged to lineage 6, and revealed a homogeneous population (Gale et al., 2002).

The intensive mycogeographic surveys for more than 10 years involved sampling of grain throughout the Russian Federation. The results revealed two suitable areas where *F. graminearum*. was distributed: the South European (North Caucasus) and the Far East regions. The isolation and identification of a large number of *Fusarium* isolates has given considerable confidence to conclusions drawn about the absence of *F. graminearum* between the 50° and 120° meridians of Russia. The distribution of the pathogen is obviously associated with climate, since the area where the cereal crop is grown is larger than the distribution of *F. graminearum*. The area, where *F. graminearum* is absent in Russia, is about 7000 km long and it separates the European and Asian populations of the fungus. If the spores (conidia or ascospores) were dispersed by air currents from one of these areas to the other one, it should be possible to isolate *F. graminearum* from cereal plants in central Russia, but this is not the case. There is also not much exchange of cereal seeds between the European part and the far east of Russia. The apparent geographical isolation over a long period could cause dissimilarity between the genetic

structures of the species between the different to geographical areas.

The present work established genetic variation between and within geographical groups of *F. graminearum*. The complex enzymes (α- and β-esterases, aspartate aminotransferase and superoxide dismutase) and the IGS sequences demonstrated a high level of genetic diversity in *F. graminearum*. The partial sequences of the β-tubulin gene demonstrated high similarity of the *F. graminearum* isolates, except for the two isolates originating from the North Ossetia, Russia. These were later found to have β-tubulin sequences identical to an Iranian isolate of *Fusarium graminearum* (NRLL 13383), known to be of lineage 7. This isolate produces zearalenone, but not deoxynivalenol or nivalenol (O'Donnell et al., 2000). In the IGS dendrogram and POY consensus tree CO3-7 and CO5-5 were, however, clustered in the same group as isolates G3-6 and G3-5, which having a typical *F. graminearum* β-tubulin sequence, but not with isolate NRLL 13383.

Some grouping among geographically similar isolates of *F. graminearum* was observed, but it was not sharply defined. For example, the Asian cluster III and European clusters IIA and IIB were well-supported in the IGS dendrogram and in the phylogenetic IGS POY consensus tree. When the partial IGS sequences of the present study were compared to unpublished *F. graminearum* IGS sequences obtained from O'Donnell, more strains of clusters I (NRRL 29169), IIB (NRRL 5883, NRRL 28063 and NRRL 31084), IV (NRRL 6394) and V (NRRL 28336 and NRRL 13383) were found. The isolates NRRL 29169, NRRL 5883, NRRL 28063, NRRL 31084 and NRRL 28063 are from USA, NRRL 6394 from Hungary and NRRL 13383 from Iran and they all belong to lineage 7 (O'Donnell, personal communication). Thus, it seems that most of our isolates belong to the northern lineage 7 of *F. graminearum* (O'Donnell et al., 2000).

On the basis of the enzyme and IGS sequence data, Asian populations are more heterogeneous than European ones. Under laboratory conditions, all the isolates studied formed perithecia and were homothallic (Gagkaeva et al., 2001). However, the same fungus can display different modes of reproduction in different geographic locations and at different times. In the southern and far eastern region of Russia the sexual stage (*G. zeae*) is frequently observed as perithecia on the heads and the debris of cereals.

Perithecia formation was not observed in Finland. The absence of a sexual stage in Finland could be one of the causes of the similarity of the isolates. The appearance of *F. graminearum* in Finland is relatively recent, caused by the spreading of highly adapted clones of the pathogen from the warmer areas. The Finnish isolates, for instance, formed a cluster with the German isolate G8-8 in the IGS dendrogram and in the IGS POY consensus tree. The Finnish isolates were also identical in the isozyme analysis and were in the same dendrogram cluster as one isolate from China (Ch13, cluster IV). This cluster had a low level of similarity with other *F. graminearum* isolates. They are also very similar based on the RAPD analysis of Yli-Mattila et al. (1996). Thus, Finland may be a marginal region for *F. graminearum* and this may explain the low level of genetic variability.

It appears that *F. graminearum* occurred in the Asian region earlier than in the other regions included in this work. In the eastern part of Russia, for example, the scientific investigation of *Fusarium* head blight began at the end of the 19th century (Voronin, 1890, 1891; Paljchevskiy, 1891). Long before that, local people had already observed the pink scab with small black spots on the head of cereals. This suggestion is supported by the high frequency of the FHB resistant plant genotypes originating from the Asian region (Gocho, 1985; Takeda and Heta 1989; Snijders, 1990; Gagkaeva et al., 2002), as the result of host-pathogen coexistence over a long period.

The results of the present study are in agreement with previous studies of these isolates (Gagkaeva et al., 2001). Significant quantitative variation in aggressiveness was observed within populations, but this did not differ between Asian and European populations. Variation in the *in vitro* sensitivity of isolates to fungicides (Sportak, Folicur, Benzimidazole) exists and the Asian subgroup is less sensitive to fungicides than European isolates. Variation in the sensitivity of the Asian isolates to fungicides was considerably higher than European group. Vegetative compatibility studies revealed a high degree of genetic diversity among these isolates, 29 VCGs were found among 32 isolates. Only one complex VCG was found and it included isolates originating from distant locali-

ties, two isolates from Asia (China and the Far East of Russia) and one from Europe (Germany). Another similarity between these isolates was their low aggressiveness. The ERICs fingerprint revealed that all the isolates of *F. graminearum* belong to two major molecular types, in which the isolates were grouped according to their aggressiveness. Isolates from the complex VCG positioned in one subcluster (Gagkaeva et al., 2001).

The genetic structure within *F. graminearum*, which is determined by the evolution history of pathogen, is defined as the amount and distribution of genetic variability. Since *F. graminearum* is found in different regions throughout the world it makes sense to ignore administrative barriers and to bring together to join the efforts of a large number of qualified research groups in studying its genetic status. Knowledge of its biodiversity and identification of the phylogenetic lineages in *F. graminearum* will be useful in both defining the risk of pathogen evolution and facilitating efficient disease management.

Acknowledgements

This study was supported financially by the Raisio Group and Turku University Foundations, by travel grants from the Academy of Finland to T. Gagkaeva and T. Yli-Mattila and by the Russian Foundation for Basic Research (No. 00-04-49406, 03-04-49082). We also want to thank Dr. Kerry O'Donnell for unpublished IGS sequence data. The English was checked by Ellen Valle of the Department of English, University of Turku.

References

Aoki T and O'Donnell K (1999) Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the group 1 population of *F. graminearum*. Mycologia 91: 597–609.

Bai GH and Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Disease 80: 975–979.

Bakan B, Cahagnier B and Melcion D (2001) Natural occurrence of *Fusarium* toxins in domestic wheat and corn harvested in 1996 and 1997 – production of mycotoxin by *Fusarium* isolates from these samples. In: Logrieco A (ed.) Cost Action 835 Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe (pp 51–53) European Communities, Belgium.

Bottalico A and Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small grain cereals in Europe. European Journal of Plant Pathology 198: 611–624.

Bowden RL, Zeller KA and Leslie JF (2000) Population structure of *Gibberella zeae* in the Great Plains of North America. In: Proceedings of the International Symposium on Wheat Improvement for Scab Resistance (pp 211–213) 5–11 May 2000, China.

Chen LF, Bai GH and Desjardins AE (2000) Recent advances in wheat head scab researches in China. In: Proceedings of the International Symposium on Wheat Improvement for scab Resistance (pp 258–273) 5–11 May 2000, China.

Cosic J and Jurkovic D (2000) Biological characterization of *Fusarium graminearum* Schw. Isolated from different host plants. In: 6th European *Fusarium* Seminar and Third COST 835 Workshop of Agriculturally Important Toxigenic Fungi, Book of Abstracts: 79–80.

Cullen D, Caldwell RW and Smalley EB (1982) Cultural characteristics, pathogenicity and zearalenone production by strains of *Gibberella zeae* isolated from corn. Phytopathology 72: 1415–1418.

Dusabenyagasani M, Dostaler D and Hamelin RC (1999) Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. Canadian Journal of Plant Pathology 21: 308–314.

Ellner FM (2001) Levels of mycotoxins in cereals of various regions of Germany in the 1990 harvest. In: Logrieco A (ed.) Cost Action 835 Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe (pp 59–60). European Communities, Belgium.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package), version 3.5. Department of Genetics: University of Washington, Seattle, USA.

Fernando WGD, Ramarathnam J, Gilbert J and Clear R (2003) Genetic diversity of *Fusarium graminearum* isolates from wheat, barley, and corn. In: Proceedings of International Congress of Plant Pathology (pp 107) 2–8 February 2003, New Zealand.

Gagkaeva TY, Koopmann B and Wolf GA (2001) Biodiversity of *F. graminearum* isolates from different geographical locations. In: Proceedings of the 8th Aschersleben Symposium "New Aspects of Resistance Research on Cultivated Plants" (pp 11–15) Germany.

Gagkaeva TY, Levitin MM, Zuev E and Terentjeva I (2002) Evaluation of genetic resources of wheat and barley from far east of Russia for resistance to *Fusarium* head blight. Journal Applied Genetics 43A: 229–236.

Gale LR, Chen L-F, Hernick CA, Takamura K. Kistler HC (2002) Population analysis of *Fusarium graminearum* from wheat fields in eastern China. Phytopathology 92: 1315–1322.

Gladstein D and Wheeler W (2001) POY (Phylogeny Reconstruction via Direct Optimization of DNA data). Version 2.7. Department of Invertebrates, American Museum of Natural History, Central Park West, 79th St. New York, NY 10024–5192, USA.

Gocho H (1985) Wheat breeding for scab resistance. Wheat Information Service 60: 41.

Honold GR, Farkas GL and Stahmann MA (1966) The oxidation-reduction enzymes of wheat. Cereal Chemistry 43: 517–529.

Ittu M (2001) Occurrence of FHB in Romania and control strategy. In: Logrieco A (ed.) Cost Action 835 Occurrence of

Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe (pp 147–150) European Communities, Belgium.
Klechkovskaya H (1999) Ecological and biochemical characteristics of *Fusarium spp*. on winter wheat in steppe area close to Black Sea in Ukraine. Mycology and Phytopathology 33: 280–289 (in Russian).
Koixumi S, Kato H, Yoshino R, Hayoshi N and Ichinoe M (1991) Distribution of causal fusaria of wheat and barley scab in Japan. Annals of the Phytopathological Society of Japan 57: 165–173.
Korochkin LI, Serov OL and Pudovkin AI (1977) Genetic of enzymes. Moscow, Russia: 1–278 (in Russian).
Laday M, Bagi F, Mesterhazy A and Szecsi A (2000) Isozyme evidence for two groups of *Fusarium graminearum*. Mycological Research 104: 788–793.
Lee SB, Taylor JW (1990) Isolation of DNA from fungal mycelia and single spores. In: Innis MM, Gelfand DH, Sninsky JJ and White JW (eds) PCR Protocols, a Guide to Methods and Applications (pp 282–287) Academic Press, San Diego, USA.
Levitin MM, Ivashchenko VG, Shipilova NP, Gagkaeva TY (1994) *Fusarium* head blight of cereals and problems of breeding for resistance. Plant Science (Sofia) 31: 158–161.
Levitin M, Ivashenko V, Shipilova N and Gagkaeva T (2000) *Fusarium* head blight of the cereal crops in Russia. Plant Protection 51: 111–122.
Lew H, Adler A, Thimm N, Kriska G and Schuh M (2001) Occurrence of toxigenic fungi and related mycotoxins in plants, food and feed in Austria. In: Logrieco A (ed.) Cost Action 835 Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe (pp 3–12) European Communities, Belgium.
Liu W-Ch, Xi J-H, Li H-Y, Pan H-Yu, Hu H-Q, Guo Y-L and Bai R-L (2002) RAPD analysis of isolates from *Fusarium* spp. causing wheat head blight in northeast China. Mycosystema 21: 63–70.
Logrieco A, Altomare C, Mule G and Moretti A (1999) Molecular and biochemical characterization of toxigenic *Fusarium* species. Recent Research Developments in Microbiology 3: 135–145.
Mayrer G (1971) Disk-Electrophoresis. Moscow, Russia: 1–247 (in Russian).
Miedaner T, Schilling AG and Geiger HH (2001) Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *F. culmorum* sampled from wheat fields in different countries. Journal of Phytopathology 149: 641–648.
Mladenov M and Karadzhova I (1978) Investigation of *Fusarium* species on wheat in Bulgaria. Rastenievdni Nauk 15: 170–175.
Möller EM, Bahnweg G, Sandermann H and Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissue. Nucleic Acids Research 20: 6115–6116.
Nicholson P, Carter JP, Chandler E and Simpson S (2002) Pathogenicity and genetic diversity in *Fusarium graminearum* and relationship to nivalenol and deoxynivalenol. In: Abstracts of 7th European seminar on *Fusarium*-mycotoxins, taxonomy and pathogenicity and WG-4 COST 835 Action Workshop (pp 18) Poznan, Poland.
O'Donnell K, Cigelnik E and Casper HH (1998) Molecular, phylogenetic, morphological, and mycotoxin data support reidentification of the Quorn mycoprotein fungus as *Fusarium venenatum*. Fungal Genetics and Biology 23: 57–67.
O'Donnell K, Kistler, HC, Tacke BK and Casper HH (2000) Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceedings of the National Academy of Sciences, USA 97: 7905–7910.
Ostry V and Ruprich J (2001) Fumonisins in corn-based products and *Fusarium* occurrence in wheat grains in the Czech Republic. In: Logrieco A (ed.) Cost Action 835 Occurrence of Toxigenic Fungi and Mycotoxins in Plants, Food and Feed in Europe (pp 25–36) European Communities, Belgium.
Oswald J W (1949) Cultural variation, taxonomy and pathogenicity of *Fusarium* species associated with cereal root rots. Phytopathology 39: 359–376.
Paavanen-Huhtala S, Hyvönen J, Bulat SA and Yli-Mattila T (1999) RAPD-PCR, isozyme, rDNA RFLP and rDNA sequence analyses in identification of Finnish *Fusarium oxysporum* isolates. Mycological Research 103: 625–634.
Paavanen-Huhtala S, Avikainen H and Yli-Mattila T (2000) Development of strain-specific primers for a strain of *Gliocladium catenulatum* used in biological control. European Journal of Plant Pathology 106: 187–198.
Paljchevskiy NA (1891) Disease of cereal grain in the South-Ussuriiskiy region. SPb, Russia: 1–43 (in Russian).
Proctor RH, Hohn TM and McCormick SP (1995) Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Molecular Plant-Microbe Interactions 8: 593–601.
Quellet T and Seifert KA (1993) Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathology 83: 1003–1007.
Reddy MN and Stahmann MA (1972) Isozyme patterns of *Fusarium* species and their significance in taxonomy. Phytopathologische Zeitschrift 74: 115–125.
Roux J, Steenkamp ET, Marasas WFO, Wingfield MJ and Wingfield BD (2001) Characterization of *Fusarium graminearum* from *Acacia* and *Eucalyptus* using beta-tubulin and histone gene sequences. Mycologia 93: 704–711.
Schilling AG, Moller EM and Geiger HH (1997) Molecular differentiation and diagnosis of the cereal pathogens *Fusarium culmorum* and *F. graminearum*. Sydowia 30: 71–82.
Snijders CA (1990) Genetic variation for resistance to *Fusarium* head blight in bread wheat. Euphytica 50: 171–179.
Sugiura Y, Watanabe Y, Tanaka T, Yamamoto S and Ueno Y (1990) Occurrence of *Gibberella zeae* strains that produce both nivalenol and deoxynivalenol. Applied and Environmental Microbiology 56: 3047–3051.
Szersi A, Szentkiralyi F and Koves-Pechy Ch (1976) Comparison of esterase patterns of *Fusarium culmorum* and *Fusarium graminearum*. Acta Phytopathologica 11: 183–203.
Takeda K and Heta H (1989) Establishing the testing method and a search for resistant varieties to *Fusarium* head blight in barley. Japanese Journal Breeding 39: 203–216.
Thompson JD, Higgins DG and Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap

penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

Voronin M (1890) About intoxicating corn in the South-Ussuriiskij region. Botanical notes. SPb, Russia 3: 13–21 (in Russian).

Voronin M (1891) Ueber das ''Taumelgetreide'' in Sud-Ussurien. Botanische Zeitung, Leipzig 49: 81–93.

Walker SL, Leath S, Hagler WMJ and Murphy JP (2001) Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina. Plant Disease 85: 404–410.

Wheeler WC (1996) Optimization alignment. The end of multiple sequence alignment in phylogenetics? Cladistics 12: 1–9.

Ylimäki, A, Koponen, H, Hintikka, E-L, Nummi, M, Niku-Paavola, M-L, Ilus, T, Enari, TM (1979) Mycoflora and occurrence of *Fusarium* toxins in Finnish grain. In: Technical Research Centre of Finland, Materials and Processing Technology Publication, Vol. 21 pp. 1–28, Valtion Painatuskeskus, Helsinki, Finland.

Yli-Mattila T, Paavanen-Huhtala S, Hannukkala A, Papikka P, Tahvonen R and Karjalainen R (1996) Isozyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathology 45: 126–134.

Yli-Mattila T, Paavanen-Huhtala S, Bulat SA, Alekhina IA and Nirenberg HI (2002a) Molecular, morphological and phylogenetic analysis of *Fusarium avenaceum*/*F. arthrosporioides*/*F. tricinctum* species complex – a polyphasic approach. Mycological Research 106: 655–669.

Yli-Mattila T, Paavanen-Huhtala S, Parikka P, Konstantinova P, Gagkaeva T, Eskola M, Jestoi M and Rizzo A (2002b) Occurrence of *Fusarium* fungi and their toxins in Finnish cereals in 1998 and 2000. Journal of Applied Genetics 43A: 207–214.

Yli-Mattila T, Mach R, Alekhina IA, Bulat SA, Koskinen S, Kullnig-Gradinger CM, Kubicek C. and Klemsdal SS (in press) Phylogenetic relationsip of *Fusarium langsethiae* to *Fusarium poae* and *F. sporotrichioides* as inferred by IGS, ITS, β-tubulin sequence and UP-PCR hybridization analysis. International Journal of Food Microbiology.

Zheng YM, Lin ZF and Zhu ZD (1983) Study on pathogenic species and form of wheat scab fungi in Fijian Province. Acta Phytopathologica Sinica 13: 53–59.