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ORIGINAL RESEARCH

Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany

Firas Talas · Heiko K. Parzies · Thomas Miedaner

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Abstract Fusarium head blight (FHB) is one of the most destructive diseases of wheat. Twelve small commercial wheat fields (size 1–3 hectares) were sampled in Germany for *Fusarium* populations at three spots per field with 10 heads each. PCR assays using generic primers confirmed 338 isolates as *F. graminearum* sensu stricto (s.s.) (64.9%) out of 521 *Fusarium spp*. that were further analyzed. Populations of *F. graminearum* s.s. in Germany contain three types of trichothecenes with a dominancy of 15-acetyldeoxynivalenol chemotype (92%) followed by 3-acetyldeoxynivalenol chemotype (6.8%) and a few isolates of nivalenol chemotype (1.2%). All these isolates were genotyped using 19 microsatellite loci. The 12 populations showed a high genetic diversity within the small scale sampling areas resulting in 300 different haplotypes. Genetic diversity within populations (71.2%) was considerably higher than among populations (28.8%) as shown by analysis of molecular variance. Gene flow (Nm) between populations ranged from 0.76–3.16. Composition of haplotypes of one population followed over 2 years changed considerably. No correlation between genetic and geographical distance was found. In conclusion, populations of *F. graminearum* s.s. in Germany display a tremendous genetic variation on a local scale with a restricted diversity among populations.

Keywords DON · Fusarium head blight · Mycotoxin · Gene flow · Population genetics

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F. Talas · T. Miedaner (✉)
State Plant Breeding Institute (720),
Universität Hohenheim,
Fruwirthstr. 21,
70593 Stuttgart, Germany
e-mail: miedaner@uni-hohenheim.de

F. Talas
National Commission of Biotechnology (NCBT),
P.O.Box. 31902, Damascus, Syria

H. K. Parzies
Institute of Plant Breeding, Seed Science and Population Genetics, Universität Hohenheim (350),
Fruwirthstr. 21,
70593 Stuttgart, Germany

Introduction

Fusarium head blight (FHB) is one of the most destructive pathogens reported on wheat and other small grains (Shaner 2003). Worldwide, the most important causal agent of this disease is *Gibberella zeae* (anamorph *Fusarium graminearum*), while in Europe additionally other species such as *F. culmorum*, *F. avenaceum* and *F. poae* may play a role (Nicholson et al. 2003). Infected spikelets initially appear as water soaked and then lose their chlorophyll, become straw coloured and turn to pinkish-red

colour during the flowering period combined with warm, humid weather. Mycelium and conidia develop abundantly in infected spikelets and the infection will spread to adjacent spikelets or even to the entire head of susceptible varieties. FHB can cause significant yield losses up to 50% (Xu et al. 2008). In some areas where corn is extensively grown alternating with wheat, infection of FHB becomes more severe. It has been reported that 70% of overwintered cornstalk residues were colonized by *G. zeae* (Stack 2003). *G. zeae* is a homothallic fungus with the ability of outcrossing (Zeller et al. 2004). In Germany, FHB epidemics occur mostly on a regional basis when rain prevails at flowering. In 2008, for example, most wheat fields in southern Germany with susceptible varieties were visibly infected by FHB whereas no natural infection occurred in northern Germany.

Infected kernels of wheat contain mycotoxins such as deoxynivalenol (DON) or nivalenol (NIV) that are toxic to humans and animals (Agrios 2004). Isolates of *F. graminearum* produce three profiles of trichothecene chemotypes (Miller et al. 1991; Ward et al. 2002): (i) NIV chemotype producing nivalenol and acetylated nivalenol derivatives, (ii) 3-ADON chemotype producing deoxynivalenol and predominantly 3-acetyldeoxynivalenol, (iii) and 15-ADON chemotype producing deoxynivalenol and predominantly 15-acetyldeoxynivalenol. A distinct shift from the original 15-ADON to 3-ADON chemotype occurred in Canada in the last decade (Ward et al. 2008). In the UK, it has been reported that among *F. graminearum* isolates of the DON chemotype, 95% were 15-ADON chemotype and just 5% were 3-ADON chemotype (Jennings et al. 2004). The NIV chemotype was detected in the same study in 25% of *F. graminearum* isolates, thus being lower than the proportion of NIV chemotype in *F. culmorum* populations (43%) sampled in the same locations and years. In Germany, Miedaner et al. (2000) found both DON and NIV chemotypes of *F. graminearum* in their collection without differentiating the two types of DON. Overall, detailed analysis of natural populations of *F. graminearum* for chemotype in Central Europe is missing.

Population structure is the result of evolutionary forces acting on the population in space and time. Mutation, recombination, and migration enhance the genetic variance of a population and random drift and selection reduce it (McDonald and Linde 2002). Determining the genetic variance within and among populations and detecting migration between populations or subpopulations is an essential parameter of population ecology (Hartl and Clark 2007). Microsatellite (also called SSR, single-sequence repeat) markers provide an ideal tool to study population structure due to their random distribution throughout the genome and absence of selecting forces (Lowe et al. 2005).

F. graminearum complex has been subdivided into 14 cryptic species according to phylogenetic analysis of DNA sequences (O'Donnell et al. 2008; Yli-Mattila et al. 2009). All species together subsequently have been named *F. graminearum* clade (*Fg* clade). *F. graminearum* sensu stricto (*F. graminearum* s.s.) is the main member of the *Fg* clade in Europe (O'Donnell et al. 2004), the United States and Canada (Zeller et al. 2003; Gale et al. 2007). High genetic diversity within 15 populations of *F. graminearum* s.s. has been found with a significant differentiation between some of these populations among Canadian locations (Guo et al. 2008). Recent US studies demonstrated the presence of genetically different subpopulations within *F. graminearum* s.s. (Gale et al. 2011). Furthermore, a high genetic diversity has been found at extremely small spatial scales, *e.g.* individual fields or even 0.25 m^2 plots within a field (Zeller et al. 2003). As a consequence, a small number of isolates should be sufficient to estimate the genetic diversity within a field. To our knowledge, no comprehensive study on the population structure of *F. graminearium* s.s. in Germany or even Central Europe across several populations has been published to date.

The objectives of this study were to: (i) identify the dominant *Fusarium* species in Germany, (ii) predict their chemotype by chemotype-specific primers, (iii) assess population structure of *F. graminearum* s.s. in Germany among 12 natural populations collected from neighboring and distant geographical areas, and (iv) study the genetic diversity within and among these *F. graminearum* s.s. populations.

Materials and methods

Sampling and isolation

Twelve naturally infected, commercial fields of winter wheat (*Triticum aestivum* L.) were sampled in Germany to establish populations of *F. graminearum* (Table 1).

Table 1 Information on sampling sites of *F. graminearum* sensu stricto collected from soft wheat in 12 locations in Germany

Location	Group	No.	Population	Location	Year of collection	State	Latitude	Longitude	No. of heads	No. of *Fg* s.s. isolates	No. of different haplotypes[c]
South Germany	I	1	HOH	Hohenheim	2008	B.W[a]	N 48 42′ 50″	E 9 12′ 58″	30	30	28
	I	2	PLN	Plieningen	2008	B.W	N 48 42′ 2″	E 9 12′ 54″	30	27	27
	I	3	BIR	Birkach	2008	B.W	N 48 43′ 19″	E 9 12′ 30″	30	29	29
	II	4	TUB	Tübingen	2008	B.W	N 48 31′ 22″	E 9 3′ 7″	30	30	28
	II	5	NUF	Nufringen	2008	B.W	N 48 37′ 0″	E 8 52′ 59″	30	16	16
	II	6	ENT	Entringen	2008	B.W	N 48 33′ 14″	E 8 58′ 22″	30	16	14
	II	7	HER	Herrenberg	2008	B.W	N 48 35′ 46″	E 8 52′ 12″	30	17	12
	II	8	BOL	Bohlingen	2008	B.W	N 48 16′ 59″	E 8 50′ 59″	30	26	25
	III	9	KEL	Kehl	2008	B.W	N 48 34′ 59″	E 7 49′ 0″	30	26	24
North Germany	IV	10	SCHICK	Schickelsheim	2007	N.S[b]	N 52 15′ 16″	E 10 51′ 54″	15	31	23
	IV	11	WET1	Wetze	2006	N.S	N 51 44′ 27″	E 9 54′ 34″	30	30	27
	IV	12	WET2	Wetze	2009	N.S	N 51 44′ 27″	E 9 54′ 34″	60	60	47
SUM									375	338	300

[a] Baden-Württemberg

[b] Niedersachsen (Lower Saxony)

[c] Haplotypes that differ at least in one band from each other

Collections were performed by picking arbitrarily 30 visually infected heads per field with 10 heads each from the front, middle, and end of the field. Sampling was done at milk-ripening stage where the typical bleaching of FHB could be clearly seen. Due to the law of succession in southern Germany, fields are small sized, ranging from one to three hectares. Population sampling was done in geographical groups, Group I–III were sampled in the state of Baden-Württemberg in 2008 with the following structure: Group I covered fields in a diameter of about 5 km around the University of Hohenheim (HOH, PLN, and BIR). Group II covered fields in a diameter of about 45 km south of Stuttgart (TUB, NUF, ENT, and HER) in addition to a field collected from Lake Constance in Bohlingen (BOL). Group III consisted of a single field from the western Baden-Württemberg at Kehl/Rhine (KEL). The final group IV contained field samples from northern Germany in Wetze in 2006 (WET1), 2009 (WET2) and Schickelsheim 2007 (SCHICK) in the state of Niedersachsen (Lower Saxony). Both samples from Wetze were from susceptible wheat varieties sown into naturally infected maize stubble. Geographical road distances from Stuttgart to Bohlingen and Kehl/Rhine are 200 and 147 km, respectively, from Stuttgart to Wetze 430 km, from Stuttgart to Schickelsheim 532 km and from Wetze to Schickelsheim 113 km.

All heads were frozen at −20°C until isolation. For isolation, one visibly infected spikelet per head was disinfected with sodium hypochlorite (2%) for 10 min, followed by rinsing with sterile distilled water, and by sulfate-streptomycin (0.8%) for another 10 min and final rinsing with sterile distilled water (modified from MacDonald and Chapman 1997). Each spikelet was placed on an individual Petri dish (Ø 2.5 cm) with SNA (synthetic nutrient agar) supplemented by 50 mg/l streptomycin sulfate (modified from Knoll et al. 2002). Plates were incubated in the dark at 24°C for 2 days followed by exposure to continuous black light (Philips TLO, 40 W/80, Royal Philips Electronics, Amsterdam, Netherlands) at 20°C for 4–5 days. Resulting macroconidia were transferred onto water agar (WA) plates (Ø 9 cm) to spread the spores on the surface. One single spore was picked out under the microscope, marked, and transferred onto a SNA (Ø 2.5 cm) plate. After 2–3 days of mycelium growth on agar plates, several agar plugs were transferred to 2 ml Eppendorf tubes filled with sterile water and kept at 6°C as a stock collection for long-term storage (Leslie and Summerell 2006). Subsequently, the same SNA plate was placed under continuous black light to induce massive sporulation. From each plate some conidia samples were transferred to water dropped on a glass slide and the spores were identified by use of a light microscope with 200 x magnification (Zeiss, Axioskop, Oberkochen, Germany) using the taxonomical key of Nelson et al. (1983).

DNA extraction and molecular identification of *F. graminearum*

To obtain mycelium for DNA extraction, isolates morphologically classified as *F. graminearum* sensu lato were incubated on potato dextrose agar (PDA/Ø 9 cm) plates for 7–8 days at 24°C starting with an agar plug from the stock collection. Mycelium of each isolate (about 50 µg) was scraped off, ground into fine powder using liquid nitrogen in a small porcelain mortar (Ø12 cm) and agitated with 200 µl of extraction solution containing 2% Triton X-100, 100 mM NaCl, 10 mM Tris-Cl pH 8, 1 mM EDTA pH 8, and equal volume of 25:24:1 v/v of phenol-chloroform-isoamyl alcohol. The mixture was centrifuged at 14,000 rpm at 4°C and an aliquot of 200 µl was transferred into a new tube and mixed with 24:1 v/v chloroform-isoamyl alcohol and cold centrifuged as above. The resulting aliquot was mixed with a double volume of cold isopropanol and 20 µl of sodium acetate. Precipitation was enhanced by placing the tubes for 1 h or more on ice. The DNA pellet was washed two times with 70% cold ethanol, air dried and then dissolved in 150 µl double distilled water. The quantity and quality of DNA was estimated by comparing1µl of DNA with Lambda DNA of known quantity on a 0.8% agarose gel (Hoffman and Winston 1987: modified protocol).

Polymerase chain reaction (PCR) using species-specific primers Fg16F/Fg16R and FC01/FC01R for *F. graminearum* and *F. culmorum,* respectively, were performed (Table S1). Expected products were a polymorphic band of 400–500 bp and a monomorphic 570 bp fragment, respectively (Nicholson et al. 1998; Demeke et al. 2004). PCR for single-species identification was performed in 0.2 ml tubes with 25 µl of the reaction mixture containing of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.2 mM of each dNTPs, 0.4 µM of each oligonucleotide primer, and 0.75 units of *Taq* DNA polymerase (Genaxxon BioScience

GmbH, Ulm, Germany). DNA amplification was performed using an initial denaturation temperature of 95°C for 3 min followed by 35 cycles as 95°C for 30 s, annealing temperature 58°C (*Fg*) or 62°C (*Fc*) for 50 s and elongation on 72°C for 60 s, followed by a final extension at 72°C for 7 min using a thermocycler (BIORAD DNA engine cycler®, PTC-200, USA). PCR products were analyzed by electrophoresis in 1.5% w/v agarose gels buffered in TAE (40 mM Tris-acetate, 2 mM Na-EDTA), stained with 0.2 μg/ml ethidium bromide and were visualized on a transilluminator.

Identification of *F. graminearum* s.s.

Generic primers based on the translation elongation factor 1-alpha (TEF-1α) gene (Yang et al. 2008) were used to identify *F. graminearum* s.s.(Table S1). The primers consist of two pairs: the first (FgTEFf124 and FgTEFFr590) gives an amplicon of 484 bp for the *Fg* clade and the other (Fg7TEFf364, and Fg5TEFr411) gives an amplicon of 260 bp for *F. graminearum* s.s. Multiplex PCR conditions were: 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 57°C for 50 s, 72°C for 1 min and a final extension at 72°C for 10 min.

Prediction of chemotype in *F. graminearum* s.s.

Multiplex PCR was performed using the previously designed primers N11, 15D11, 3D11, and 11R (Table S1), which are based on the *Tri11* gene sequence (Zhang et al. 2010b). Expected PCR products were a fragment of 643 bp for NIV-type isolates, of 424 bp for 15-ADON-type isolates, and of 342 bp for 3-ADON-type isolates. A PCR mixture of 0.4 μM from each primer was used in 25 μl final volume. PCR conditions were as described above, using 60°C as annealing temperature.

Generation of SSR allele data

Nineteen SSR markers (Giraud et al. 2002; Suga et al. 2004; Karugia et al. 2009) were used for the molecular analysis of the *F. graminearum* s.s. isolates (Table S1). PCR conditions were like those of species-specific primers using a suitable annealing temperature for each pair of primer. Products were visualized in 3% ultra-pure agarose gels (Genaxxon BioScience GmbH, Ulm, Germany) to determine accurate allele sizes.

Statistical analyses

Coordinates of each sampling location were determined using the online published program ACME Mapper 2.0 which is based mainly on Google maps (Internet source: http://mapper.acme.com, 2006). Genetic data of 338 *F. graminearum* s.s. isolates generated by 19 microsatellite markers were analyzed by Arlequin 3.5 program (Excoffier and Lischer 2010). The number of alleles at each locus was calculated by GenALEx.6.3 (Peakall and Smouse 2006). Private alleles in each population and Normalized Shannon index were computed for each population based on allele frequencies and calculated for haploid data ($sHa = -1 \times sum[Pi \times \log 2(Pi)]$ "Log base = 2.718"), where Pi is the frequency of the *i*-th allele, averaged over populations, using GenALEx 6.3. The fixation index (F_{st}) between populations was computed using Arlequin 3.5 based on the number of different alleles and significance level was estimated by setting 1,000 permutations at $P<0.05$. Gene flow (Nm) was derived by the formula $Nm = \frac{[(\frac{1}{F_{st}})-1]}{2}$ for haploid species (Lowe et al. 2005). Pairwise values of F_{st} and Nm were converted into a black-white contrast graph using R 2.11 package (http://www.R-project.org). Nei's gene diversity within populations and, genetic diversity among and between studied populations by Analysis of Molecular Variance (AMOVA) were computed using Arlequin 3.5. Mantel correlation coefficient also calculated with GenALEx 6.3. using the formula $r_{xy} = \frac{SP_{xy}}{\sqrt{SS_x SS_y}}$, where SP_{xy} is the sum of cross products of corresponding elements of X and Y matrices, SS_x is the sum of products of X matrix elements and SS_y that of Y matrix elements (Mantel 1967). As a prerequisite for Mantel correlation coefficient, pairwise unbiased Nei's distance between populations was calculated with GenALEx 6.3. according to $Nei_D = -In\left(\frac{J_{xy}}{\sqrt{J_x J_y}}\right)$, where J_{xy}, J_x, and J_y are the sum of the frequency of the *i*-th allele in population *x* and *y* over all loci and alleles divided by the number of loci (Hedrick 2000).

Results

Five hundred and twenty one single-spore isolates were obtained; most of the isolates could be identified to species level. *F. graminearum* s.s. was the predominant species with 338 isolates (64.9%) followed by *F. culmorum* (26.1%). *F. poae*, *F. tricinictum*, *Microdochum*

nivale and others were also identified morphologically in small amounts. According to our research objectives, only *F. graminearum* s.s. isolates were analyzed further.

Prediction of chemotype

Isolates of *F. graminearum* s.s. were chemotaxonomically classified into NIV, 15-ADON, and 3-ADON types according to amplified DNA products of 643, 424, and 342 bp, respectively. Out of 338 *F. graminearum* s.s. isolates (100%), only four isolates were classified as NIV types (1.2%) and 23 isolates as 3-ADON types (6.8%) while the majority of 311 (92%) isolates were typed as 15-ADON. Isolates typed as NIV were detected in the populations HOH, WET1, and WET2. *F. graminearum* s.s. isolates typed as 3-ADON were detected in almost all populations at a rate of one to two isolates per population, except for WET2, where the 3-ADON type was not recovered.

Population analysis

Average number of alleles varied among *F. graminearum* s.s. populations from 2.8 to 3.9 with a total average of 3.2 (Table 2). Accordingly, the normalized Shannon index resulted in high values in all populations ranging from 0.53 (SCHICK) to 0.96 (NUF). Nei's gene diversity ranged from 0.30 in SCHICK to 0.58 in NUF. A high number of private alleles was detected in KEL (A=10).

Pairwise comparisons of population genetic differentiation (F_{st}) and gene flow (Nm) between populations ranged from 0.14–0.40 and 0.76–3.16, respectively (Fig. 1). All F_{st} values were statistically significant ($p<0.001$). Estimates of gene flow corresponded to F_{st} values. A considerably higher amount of genetic variance was found within populations than among populations (Table 3).

Discussion

F. graminearum s.s. as tested by the TEF primer set of Yang et al. (2008) was the most prevalent *Fusarium* species isolated from infected wheat ears in Germany. Prediction of the chemotype by multiplex PCR revealed the predominance of the 15-ADON type in Germany as has been previously reported from the UK (Jennings et al. 2004). This is consistent with the finding that the older Canadian *F. graminearum* isolates consisted only of the 15-ADON type, but in sharp contrast to the newly occurring isolates of the western Canadian provinces and Japan where 3-ADON type of *F. graminearum* s.s. now prevails (Guo et al.

Table 2 Estimates of average number of alleles per locus, number of private alleles, normalized Shannon's index and Nei's genetic diversity and their standard errors (± SE) in 12 *F. graminearum* s.s. populations sampled from South and North Germany revealed by alleles at 19 microsatellite loci

Population[a]	Average no. of alleles ± SE	No. of private alleles[b]	Normalized Shannon index ± SE	Nei's gene diversity ± SE
HOH	3.9±2.4	3	0.85±0.14	0.44±0.07
PLN	3.8±2.8	3	0.80±0.14	0.43±0.07
BIR	2.8±1.9	1	0.61±0.12	0.34±0.16
TUB	2.8±2.0	4	0.59±0.10	0.36±0.06
NUF	3.5±1.3	4	0.96±0.09	0.58±0.04
ENT	3.0±1.4	3	0.87±0.10	0.54±0.35
HER	2.8±1.4	7	0.78±0.11	0.49±0.06
BOL	2.8±2.0	5	0.62±0.13	0.35±0.07
KEL	3.3±2.1	10	0.73±0.12	0.40±0.07
SCHICK	2.8±1.2	1	0.53±0.09	0.30±0.05
WET1	3.4±1.9	1	0.74±0.11	0.41±0.06
WET2	3.4±2.5	3	0.70±0.14	0.37±0.07
Total	3.2±1.9	45	0.73±0.03	0.42±0.01

[a] For abbreviation of the populations please refer to Table 1

[b] Alleles occurring only in the respective population

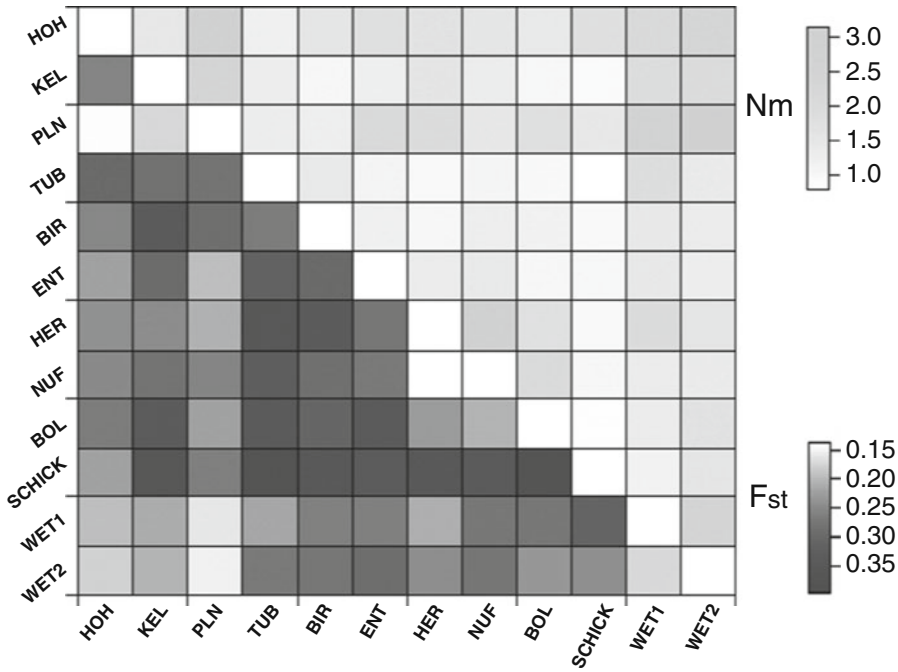

Fig. 1 Black-white contrast of pairwise estimated values of gene flow (Nm, above diagonal) and pairwise differentiation (F_{st}, below diagonal) of 12 *F. graminearum* s.s. populations[a] sampled from South and North of Germany using 19 microsatellite loci. [a]For abbreviation of the populations please refer to Table 1

2008; Suga et al. 2008; Ward et al. 2008). We detected all three chemotypes in German populations of *F. graminearum* s.s., sometimes even together in a small sample of 30 wheat heads, *e.g.* in WET1 or in HOH. Before this study, a very limited number of 3-ADON isolates has been reported in Europe, consisting only of three out of 219 Swedish isolates (1.4%), four out of 101 British isolates (4%, Chandler et al. 2003; Jennings et al. 2004, respectively) and four out of 19 isolates of *F. graminearum* s.s. collected from Italy (21%, Gale et al. 2007). However, in Germany the 3-ADON types occur in appreciable frequencies (6.8%) according to our study. Only four of our *F. graminearum* s.s. isolates were classified as NIV type. Their occurrence in Germany has been previously reported (Miedaner et al. 2000), but the new study illustrates that their frequency is not comparable to the situation in UK where 25% occurred (Jennings et al. 2004). It might be worthwhile to re-analyze the chemotype composition of *F. graminearum* s.s. in Germany in time intervals to determine whether a shift in chemotypes will occur.

SSR data revealed high genetic diversity within populations as shown by the high average number of alleles and moderate to high Shannon indices ranging from 0.53 in SCHICK to 0.96 in NUF. Only a few haplotypes were identified more than once in the same population, similar high genetic diversity in *F. graminearum* populations has been reported from several other regions around the world (Miedaner et al. 2001; Gale et al. 2007; Karugia et al. 2009; Zhang et al. 2010a). Analyzing the populations by AMOVA showed that variation within populations (71%) was indeed considerably higher than between populations (29%). A similar proportion of among *vs.* within population variance was reported from Chinese *F.*

Table 3 Hierarchical analysis of molecular variance (AMOVA) partitioning the genetic diversity among and within 12 *F. graminearum s.s.* populations collected from South and North Germany

Source of variation	d.f.[a]	Sum of squares	Variance components	Percentage of variance (%)
Among populations	11	429.52	1.56	28.82
Within populations	300	1013.05	3.86	71.17
Total	311	1442.57	5.42	

[a] Degree of freedom

asiaticum populations (Zhang et al. 2010a). Karugia et al. (2009) considered all isolates of *F. asiaticum* from two locations and 2 years in Japan as part of a geographically larger population without noticeable sub-structuring which is different from the structuring patterns found in China (Zhang et al. 2010a).

Interestingly the two populations collected at the same location in 2 years (WET1, WET2) had a higher gene flow (Nm=2.46) than most of the other pairs of populations. Pairwise differentiation (F_{st}=0.17) was among the lowest values of this study, but still statistically significant (P<0.01). Both values, however, highly differed from those of Karugia et al. (2009) who also sampled one wheat field in two consecutive years. They found Nm>100 and a non-significant F_{st} value (0.005) for *F. asiaticum* in Japan. Our results illustrate that haplotypes between both years were already different. This might be caused by rearrangement of alleles during the time between 2006 and 2008, and/or a high genetic variation within the location, e.g. maintained by sexual reproduction with outcrossing, and subsequent sampling effects. New haplotypes can arise by: (i) sexual recombination among existing haplotypes transferred from the previous year, (ii) new immigrants and/or (iii) the recombination of (i) and (ii). This is rather probable, because the wheat crop at WET was directly sown into maize stubble with no tilling and perithecia frequently occur on maize (Sutton 1982; Windels and Kommedahl 1984). It has been found that genetic diversity of isolates sampled from maize is even higher than for those sampled from wheat (Naef and Défago 2006). Recently, Chen and Zhou (2009) firstly confirmed sexual crossing of *F. graminearum* s.s. in the field experimentally with outcrossing rates of 6–20%.

Levels of pairwise comparison of gene flow (Nm) were large enough (0.76–3.16) that random genetic drift in these populations should play a minor role only. Values of gene flow were not correlated with geographic distance, for example a relatively high gene flow (Nm=2.35) occurred between the most geographically distant populations HOH/WET2. This could be explained by commercial seed or plant materials exchange stimulating gene flow (migration). Even infrequent migrants can re-arrange the genetic structure of the local population when they are genetically distinct and competitive enough (Hartl and Clark 2007). That allows establishing a new population expected to be genetically closer to the population where the migrant comes from. Differences among two neighbouring populations reflects the time required for the alleles to diffuse across the distance that separate them (Zeller et al. 2004). It should be noted that we have sampled all isolates from visually infected ears, *i.e.* they were aggressive. A similar situation was reported from Canada where high genetic similarity occurred between some populations sampled from geographically distant areas (Guo et al. 2008). In a large population sample of 1,106 isolates of *F. asiaticum* a similar mismatch between geographical distance and genetic differentiation was reported from China (Zhang et al. 2010a). In contrast, Schmale et al. (2006) found a significant negative correlation (r=−0.59, P<0.01) between genetic identity and geographical distance of analyzed populations about 3,000 km apart. There are no data on the occurrence of airborne ascospores in Europe that may play a major role of gene flow in USA (Schmale et al. 2006). However, the major wind direction in Europe is from west to east and not from north to south. *F. graminearum* s.s. populations in Germany have in our study rather low gene flow and higher fixation values compared to older population studies of *F. graminearum* senso lato (F_{st}<0.1, Miedaner et al. 2008). More recently, Guo et al. (2008) reported F_{st} values ranging from 0.03 up to 0.76 from Canada, thus fitting well with our F_{st} values (0.14–0.40). Newer US studies (e.g. Gale et al. 2011) also claim a higher population substructuring than reported by Zeller et al. (2004) with F_{st} values up to 0.5 between pairs of populations. Natural gene flow between northern and southern Germany might be restricted by the forested uplands of central and southern Germany that are in sharp contrast to the flat and low-lying lands of north Germany. As it is well documented that *F. graminearum* has a higher sexual reproduction rate in maize compared to wheat (Sutton 1982), the high acreage of maize in Germany, together with a narrow cropping system with alternating maize and wheat growing might support sexual recombination with outcrossing maintaining a high diversity within populations. Mostly, seed is multiplied regionally and the acreages of individual wheat varieties differ between North and South Germany.

Finally, it can be concluded that FHB disease in Germany is caused to a great extent by populations of *F.*

graminearum s.s. The dominating chemotype in these populations is 15-ADON. Further work is needed to examine if this domination of 15-ADON will change over the time. Isolates within the same population of *F. graminearum* s.s. in Germany are genetically highly diverse, the variation among populations is restricted.

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References

Agrios, G. N. (2004). *Plant pathology* (5th ed., 922 pp.). San Diego, USA: Elsevier Press.

Chandler, E. A., Duncan, R. S., Thomsett, M. A., & Nicholson, P. (2003). Development of PCR assays to Tri7 and Tri13 trichothecene biosynthetic genes and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. *Physiological and Molecular Plant Pathology, 62*, 355–367.

Chen, Y., & Zhou, M. G. (2009). Sexual recombination of carbendazim resistance in *Fusarium graminearum* under field conditions. *Pest Management Science, 65*, 398–403.

Demeke, T., Clear, R. M., Patrick, S. K., & Gaba, D. (2004). Species-specific PCR –based assays for the detection of Fusarium species and a comparison with the whole seed agar plate method and trichothecene analysis. *International Journal of Food Microbiology, 103*, 271–284.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver. 3.5: a new series of perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources, 10*, 564–567.

Gale, L. R., Bryant, J. D., Calvo, S., Giese, H., Katan, T., O'Donnell, K., et al. (2005). Chromosome complement of the fungal plant pathogen *Fusarium graminearum* based on genetic and physical mapping and cytological observations. *Genetics, 171*, 985–1001.

Gale, L. R., Ward, T. J., Balmas, V., & Kistler, H. C. (2007). Population subdivision of *Fusarium graminearum* sensu stricto in the Upper Midwestern United States. *Phytopathology, 97*, 1434–1439.

Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W., et al. (2011). Nivalenol type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. *Phytopathology, 101*, 124–134.

Giraud, T., Fournier, E., Vautrin, D., Solignac, M., Vercken, E., Bakan, B., et al. (2002). Isolation of eight polymorphic microsatellite loci using an enrichment protocol, in the phytopathogenic fungus *Fusarium culmorum*. *Molecular Ecology Notes, 2*, 121–123.

Guo, X. W., Fernando, W. G. D., & Seow-Brock, H. Y. (2008). Population structure chemotype diversity and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. *Plant Disease, 92*, 756–762.

Hartl, D. L., & Clark, A. G. (2007). *Principles of population genetics* (4th ed., 652 pp.). Minnesota, USA: Sinauer Associates Press.

Hedrick, P. W. (2000). *Genetics of populations* (2nd ed.). Basic Book: Boston.

Hoffman, C. S., & Winston, F. (1987). A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. *Gene, 57*, 267–272.

Jennings, P., Coates, M. E., Walsh, K., Turner, J. A., & Nicholson, P. (2004). Determination of deoxynivalenol and nivalenol producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. *Plant Pathology, 53*, 643–652.

Karugia, G. W., Suga, H., Gale, L. R., Nakajima, T., Tomimura, K., & Hyakumachi, M. (2009). Population structure of *Fusarium graminearum* species complex from a single Japanese wheat field sampled in two consecutive years. *Plant Disease, 93*, 170–174.

Knoll, S., Mulfinger, S., Niessen, L., & Vogel, F. (2002). Rapid preparation of Fusarium DNA from cereals for diagnostic PCR using sonification and an extraction kit. *Plant Pathology, 51*, 728–734.

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual* (388 pp.). Lowa, USA: Blackwell publishing.

Lowe, A., Harris, S., & Ashton, P. (2005). *Ecological genetics. Design, analysis, and application* (344 pp.). Minnesota, USA: Blackwell Publishing.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research, 27*, 209–220.

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology, 40*, 349–379.

McDonald, M. V., & Chapman, R. (1997). The incidence of *Fusarium moniliforme* on maize from Central America, Africa and Asia during 1992–1995. *Plant Pathology, 46*, 112–125.

Miedaner, T., Reinbrecht, C., & Schilling, A. G. (2000). Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight. *Journal of Plant Disease and Production, 107*, 124–134.

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., & Geiger, H. H. (2001). Effects of genotype and genotype x environment on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. *Plant Breeding, 120*, 97–105.

Miedaner, T., Cumagun, C. J. R., & Chakraborty, S. (2008). Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. *Journal of Phytopathology, 156*, 129–139.

Miller, J. D., Greenhalgh, R., Wang, Y. Z., & Lu, M. (1991). Trichothecene chemotypes of there Fusarium species. *Mycologia, 82*, 121–130.

Naef, A., & Défago, G. (2006). Population structure of plant-pathogenic *Fusarium* species in overwintered stalk resi-

dues from Bt-transformed and non-transformed maize crops. *European Journal of Plant Pathology, 116*, 129–143.

Nelson, P. E., Toussoun, T. A., & Marasas, W. F. O. (1983). *Fusarium species: an illustrated manual for identification* (193 pp.). Pennsylvania, USA: University Park, Pennsylvania State University.

Nicholson, P., Simpson, D. R., Wilson, G., Rezanoor, H. N., Lees, A. K., Parry, D. W., et al. (1998). Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiological and Molecular Plant Pathology, 53*, 17–37.

Nicholson, P., Chandler, E., Draeger, R. C., Gosman, N. E., Dimpson, D. R., Thomsett, M., et al. (2003). Molecular tools to study epidemiology and toxicology of fusarium head blight of cereals. *European Journal of Plant Pathology, 109*, 691–703.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., & Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics and Biology, 41*, 600–623.

O'Donnell, K., Ward, T. J., Aberra, D., Kistler, H. C., Aoki, T., Orwig, N., et al. (2008). Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. *Fungal Genetics and Biology, 45*, 1514–1522.

Peakall, R., & Smouse, P. E. (2006). Genalex 6: genetic analysis in Excel. Population genetics software for teaching and research. *Molecular Ecology Notes, 6*, 288–295.

Schmale, D. G., Leslie, J. F., Zeller, K. A., Saleh, A. A., Shields, E. J., & Bergstrom, G. C. (2006). Genetic structure of atmospheric populations of *Gibberella zeae*. *Phytopathology, 96*, 1021–1026.

Shaner, G. (2003). Epidemiology of Fusarium Head Blight of small grain cereals in North America. (pp. 84–119). In K. J. Leonard, & W. R. Bushnell (Eds.), *Fusarium Head Blight of Wheat and Barley* (512 pp.). St. Paul, Minnesota, USA: APS Press.

Stack, R. W. (2003). History of Fusarium head blight with emphasis on North America. (pp. 1–34). In K. J. Leonard, & W. R. Bushnell (Eds.), *Fusarium Head Blight of Wheat and Barley* (512 pp.). St. Paul, Minnesota, USA: APS Press.

Suga, H., Gale, L. R., & Kistler, H. C. (2004). Development of VNTR markers for two *Fusarium graminearum* clade spcies. *Molecular and Ecology Notes, 4*, 468–470.

Suga, H., Karugia, G. W., Ward, T., Gale, L. R., Tominura, K., Nakajima, T., et al. (2008). Molecular characterization of the *Fusarium graminearum* species complex in Japan. *Phytopathology, 98*, 159–166.

Sutton, J. C. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Canadian Journal of Plant Pathology, 4*, 195–209.

Ward, T. J., Bielawski, J. P., Kistler, H. C., Sullivan, E., & O'Donnell, K. (2002). Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 9278–9283.

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., et al. (2008). An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genetics and Biology, 45*, 473–484.

Windels, C. E., & Kommedahl, T. (1984). Late season colonization and survival of *Fusarium graminearum* group II in cornstalks in Minnesota. *Plant Disease, 68*, 791–793.

Xu, X. M., Nicholson, P., Thomsett, M. A., Simpson, D., Cooke, B. M., Doohan, F. M., et al. (2008). Relationship between the fungal complex causing Fusarium head blight of wheat and environmental conditions. *Phytopathology, 98*, 69–78.

Yang, L., Van der Lee, T., Yang, X., Yu, D., & Waalwijk, C. (2008). Fusarium populations on Chinese barley show a dramatic gradient in mycotoxin profiles. *Phytopathology, 98*, 719–727.

Yli-Mattila, T., Gagkeva, T., Ward, T., Aoki, T., Kistler, H. C., & O'Donnell, K. (2009). A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from Russian Far East. *Mycologia, 101*, 841–852.

Zeller, K. A., Bowden, R. L., & Leslie, J. F. (2003). Diversity of epidemic populations of *Gibberella zeae* from small quadrants in Kansas and North Dakota. *Phytopathology, 93*, 874–880.

Zeller, K. A., Bowden, R. L., & Leslie, J. F. (2004). Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. *Molecular Ecology, 13*, 563–571.

Zhang, Z., Zhang, H., Van der Lee, T., Chen, W. Q., Arens, P., Xu, J., et al. (2010). Geographical substructure of *Fusarium asiaticum* isolates collected from barley in China. *European Journal of Plant Pathology, 127*, 239–248.

Zhang, H., Zhang, Z., Van der Lee, T., Xu, J., Yang, L., Yu, D., et al. (2010). Population genetic analyses of *Fusarium asiaticum* populations from barley suggest a recent shift favoring 3ADON producers in southern China. *Phytopathology, 100*, 328–336.