Population Structure of the *Fusarium graminearum* Species Complex from a Single Japanese Wheat Field Sampled in Two Consecutive Years

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ABSTRACT

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The *Fusarium graminearum* species complex (*Fg* complex) that consists of at least 11 phylogenetically distinct species contains important Fusarium head blight (FHB) pathogens of wheat and barley worldwide. We obtained members of the *Fg* complex by sampling from a 500-m² experimental wheat field in Kumamoto Prefecture, Japan in two consecutive years and assessed them for species identity and trichothecene chemotype. Haplotype diversity was estimated by using 11 variable numbers of tandem repeat (VNTR) markers. In addition to these two samples (group 03W in 2003 and group 04W in 2004), pathogen populations from seed that was harvested in Fukuoka Prefecture and planted in the experimental field in 2002 (group 02WSC) and pathogen populations from seed that was harvested in Nagasaki Prefecture and planted in 2003 (group 03WSC) were analyzed for this study. Forty-six isolates were collected in each group. Most isolates from wheat heads were classified as *F. asiaticum*; only four isolates were classified as *F. graminearum sensu stricto* (s. str.). Out of a total of 183 *Fg* complex strains, 80 isolates (44%) were of the NIV type, while 103 isolates (56%), including all four *F. graminearum* s. str. isolates, were of the 3ADON type. No 15ADON type isolate was detected in this study. Trichothecene chemotype compositions of 03W and 04W were nearly identical. High gene diversity of *F. asiaticum* was observed in all groups. Based on the observed low level of fixation index (F_{ST}) and high level of effective number of migrants (N_m), distinctive population subdivision of *F. asiaticum* was not inferred among the four groups. These results suggest that FHB in the experimental wheat field in both 2003 and 2004 was caused by a genetically similar population, which prevails in Kumamoto, Fukuoka, and Nagasaki prefectures.

Additional keywords: genetic comparison, *Gibberella zeae*, scab

In addition to causing quantitative yield losses, Fusarium head blight (FHB) of wheat and barley may result in grain contaminated with significant levels of trichothecene mycotoxins such as deoxynivalenol (DON) and the estrogenic mycotoxin, zearalenone. While FHB has been recognized as a worldwide threat to wheat and barley since the beginning of the twentieth century (18,27), recent outbreaks have been reported in the United States, Canada, Europe, and Asia (11). *Fusarium*

graminearum, a species complex consisting of at least 11 phylogenetic species, some of which are dominant in particular geographical regions (21,22,28), is the primary cause of FHB in many countries, including Japan (14,23), although many other *Fusarium* species and *Microdochium nivale* are also associated with FHB (15). Members of the *F. graminearum* species complex (*Fg* complex) infect a broad range of other plant hosts in both temperate and subtropical areas (3). One species of the *Fg* complex, *F. graminearum sensu stricto* (s. str.), is cosmopolitan, while another species, *F. asiaticum*, is widespread in Asia (8,21,22). In Japan, *F. asiaticum* and *F. graminearum* s. str. are the predominant species, although they are distributed unevenly geographically (30).

Members of the *Fg* complex are homothallic and can reproduce sexually, predominantly by selfing, but they can also out-cross (2). Genomic analyses based on sequencing suggest that out-crossing or

hybridization between different species in the *Fg* complex occurs rarely in nature (22,30), although *F. asiaticum* and *F. graminearum* s. str. have been successfully crossed in vitro (12). High genotypic diversity has been reported both for *F. graminearum* s. str. populations in the United States and *F. asiaticum* populations in eastern China, which may indicate that out-crossing within a species is frequent in nature (8,25,35,36). High genotypic diversity of *F. graminearum* s. str. was even observed from small quadrants (0.25 m²) in a Kansas and a North Dakota wheat field based on amplified fragment length polymorphism (AFLP) marker data (35).

Individual isolates of the *Fg* complex produce one of three different trichothecene profiles, 3-acetyl deoxynivalenol (3ADON), 15-acetyl deoxynivalenol (15ADON), or nivalenol (NIV) (17). The different chemotypes may affect species or population ecology, as oxygenation or acetylation is known to alter the toxicity and bioactivity of trichothecenes (1,13). Gale et al. (9) found population subdivision in *F. graminearum* s. str. in the United States that could be correlated with trichothecene chemotype.

Understanding the genetic profiles of pathogen populations may provide insights into the epidemiology and evolutionary potential of the *Fg* complex, and could lead to improved strategies for controlling this pathogen (35). Thus far, the population dynamics of *Fg* complex in a specific field is poorly understood.

The objective of this study was to assess the genetic diversity of species, trichothecene chemotypes, and VNTR haplotypes in *Fg* complex populations in seed lots in a 500-m² field in Kumamoto Prefecture, Japan and ensuing diseased wheat heads in two consecutive years.

MATERIALS AND METHODS

Isolates. A collection of 184 isolates of *Fusarium* spp. was established from seeds before planting and from symptomatic wheat heads after maturity at an experimental field (10 × 50 m) of the National Agricultural Research Center for Kyushu Okinawa Region (KONRC; north latitude:

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32.879, east longitude: 130.742), Kumamoto Prefecture, Japan (Fig. 1; supplementary table). Wheat seeds of the variety Chikugo Izumi that were harvested from fields in Fukuoka Prefecture (Fig. 1) were planted in the experimental field in fall 2002 and matured in spring 2003. Wheat seeds harvested from fields in Nagasaki Prefecture (Fig. 1) were planted at the same experimental field in fall 2003 and matured in spring 2004. To isolate *F. graminearum*, symptomatic seed was sterilized by washing sequentially with 70% ethanol, 1% sodium hypochlorite, and sterile distilled water and placed on *F. graminearum*-selective medium modified from Komada's medium (32). Subsequent colonies were subcultured on synthetic nutrient agar (SNA). Conidia produced on SNA were spread on water agar, and a single conidium was isolated. Colonies showing typical morphologies for *F. graminearum* on potato sucrose agar (PSA), such as red pigmentation and fast growth, were used in this study. Isolates were maintained on potato dextrose agar (PDA) and kept at 6°C for short-term storage and at -80°C in 50% glycerol for long-term storage. Forty-six isolates were obtained from each group in this study. The fungal populations isolated from seed planted in the experimental field in 2002 and 2003 were referred to as groups 02WSC and 03WSC, respectively. Populations from diseased wheat heads at the experimental field in 2003 and 2004 were referred to as groups 03W and 04W, respectively. Diseased wheat heads in each of the 12 rows were collected at an interval of 10 m from the field edge (Fig. 1), and strains from wheat heads were named by location. For example, 03W5-10-1 referred to an isolate belonging to group 03W from the fifth row of the site, 10 m away from the field edge and first in the site's wheat head serial numbers. Seeds were planted in lines without any specific spacing.

FHB in the experimental field was severe in 2003, and all 46 isolates were obtained from different sites in the field. In 2004, one to four isolates were obtained from single sites in the field due to mild FHB occurrence. All isolates in both years were obtained from different wheat heads and about 10% of symptomatic seeds from each bulk harvest.

Genomic DNA extraction and PCR-based assessment of species and trichothecene type. Genomic DNA was extracted from 3- to 4-day-old mycelium (2 to 3 cm diameter) cultured in potato dextrose broth (PDB) as previously described (30). The final DNA pellet was dissolved in 200 µl of water, and DNA yields were estimated based on comparison to DNA of known concentrations by agarose gel electrophoresis. All isolates were subjected to *Fg* complex-specific PCR with the UBC85F410 and UBC85R410 primer pair (24) and diagnostic PCR-restriction frag-

ment length polymorphism (RFLP) for the distinction of *F. asiaticum* and *F. graminearum* s. str. with the primer pair H3dStyI and H3R1 and *StyI* or *EcoRV* as restriction enzyme (30). A multiplex PCR based on *Tri12* sequences (30,33) was performed to determine the trichothecene type of each isolate.

Generation of VNTR data. Eleven VNTR markers were used for isolate genotyping. Ten VNTR markers were developed previously (29). One additional VNTR marker, HK1003, developed by Gale et al. (7), was included. The forward primer for HK1003 is: 5'-ATCATATAG CACAGAACGGCG-3', the reverse primer is: 5'-TTACTCCAGCTTCACCGCAT-3'. The VNTR motif in the *F. graminearum* s. str. NRRL 31084 for this locus is nine repeats of GAATGGTAT followed by eight repeats of GAATGGTTC (Table 1). All forward primers were labeled with either fluorescent substance 6-FAM, VIC, NED, or PET to detect DNA fragments by ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Table 1). The total volume of the PCR reaction mixture was 10 µl, containing 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5 mM MgCl₂,

0.001% (wt/vol) gelatin, 200 µM dNTP, 0.1–1.0 µM of each of forward and reverse primer, 0.025 units *Taq* polymerase, and 1 ng/µl genomic DNA. PCR was performed in an iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following cycling parameters: 94°C for 2 min, 25 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min. PCR products with different fluorescent labeled primers were mixed and diluted 20-fold in water. Then, a 2-µl sample was mixed with 8 µl of HiDi Formamide including 0.25 µl of 500 LIZ size standard (Applied Biosystems). Samples were loaded onto an ABI 3100 Genetic Analyzer with 50 cm capillary and POP 6 polymer (Applied Biosystems). The data were processed using the genotyping software Gene Mapper (Applied Biosystems). Allele types were represented according to detected fragment size compared to a 500 LIZ standard.

Population genetic analyses. Most population genetic measures were computed using the software program Arlequin 2.000 (S. Schneider, D. Roessli, and L. Excoffier, Laboratoire de Génétique et Biométrie, Université de Genève, Switzer-

Fig. 1. A, Prefectures associated with collection of *Fusarium graminearum* species complex in Japan are shown in gray. The experimental field of the National Agricultural Research Center for Kyushu Okinawa Region (KONRC; north latitude: 32.879, east longitude: 130.742), Kumamoto Prefecture, is indicated by a circle. Seeds for planting in the experimental field were obtained from the Fukuoka and Nagasaki prefectures. B, Layout of experimental field. Isolates with same haplotype were obtained from 04W group at sites indicated by "a" (04W2-10-1 and 04W2-10-2), "b" (04W4-20-2 and 04W4-30-1), and "c" (04W6-10-1 and 04W6-10-3).

land). Analysis of molecular variance (AMOVA) was applied to partition the genetic diversity into, among, and within population components (5). The fixation index (F_{ST}) and effective number of migrants (N_m) were used to infer genetic differentiation between populations. The P value for linkage disequilibrium (26) that was developed from Fisher's exact probability test and implemented in Arlequin were obtained with the following conditions on the Markov chain length: 100,000; dememorization: 1,000. The P value for linkage disequilibrium was also computed using GenePop version 3.3 (M. Raymond and F. Rousset, Laboratoire de Génétique et Environnement, Université Montpellier, France) with the following Markov chain conditions: dememorization: 1,000; batches: 100; iterations per batch: 1,000. Nei's gene diversity (19) for each VNTR marker was computed using PopGene32 (34).

RESULTS

Species composition. Forty-six isolates were obtained for each group (03W, 04W, 02WSC, and 03WSC). *Fg* complex specific-PCR (24) was performed prior to the diagnostic PCR-RFLP test for *F. graminearum* s. str. and *F. asiaticum* (30). A DNA fragment of 332 bp specific to the *Fg*

complex (24) was amplified for all isolates except for DNA from isolate 04W10-10-3, which did not amplify. The diagnostic PCR-RFLP test indicated that most isolates (179 out of 184) were *F. asiaticum* (Table 2). Only four isolates, all from wheat heads, were *F. graminearum* s. str. (Table 2). Again, no amplification was observed for DNA of the 04W10-10-3 isolate. We concluded that this isolate may not be a member of *Fg* species complex and removed it from further analyses.

Trichothecene type composition. Out of 183 tested *Fg* complex isolates, 80 isolates (44%) were of the NIV type as determined by the multiplex PCR (30,33), while 103 isolates (56%) and all four *F. graminearum* s. str. isolates were of the 3ADON type (Table 2). No 15ADON type isolate was detected in this study. In the head groups and in both years (03W and 04W), more NIV type isolates than 3ADON type were detected, in similar proportions in both years (Table 2).

Population analyses. Alleles for 11 VNTR markers were obtained for 183 isolates. All VNTR markers displayed polymorphisms among isolates (Table 1). The number of alleles (4 to 24) and the gene diversity in the whole sample (19) (0.15 to 0.94) were highly variable among

markers. HK1003 had the highest number of alleles and gene diversity among 11 VNTR markers (Table 1; supplementary table). Based on five VNTR markers, one of the alleles was specific to *F. graminearum* s. str. isolates. All but six out of 183 isolates represented unique VNTR haplotypes. The six isolates with shared haplotypes were all from the 04W group. Two pairs of isolates (04W2-10-1 and 04W2-10-2, 04W6-10-1 and 04W6-10-3) were isolated from different heads at the same position in the field, but the pair 04W4-20-2 and 04W4-30-1 were isolated from field locations 10 m apart (Fig. 1). In addition to the wheat head populations 03W and 04W, the seed populations 02WSC and 03WSC were included for population level analyses in order to compare the two genetic structures of these pathogen populations.

For further analyses, only VNTR data of 179 isolates of *F. asiaticum* were used since most isolates detected in this study were *F. asiaticum*, and limited gene flow between *F. asiaticum* and *F. graminearum* s. str. strains was presumed due to the presence of species-specific alleles in *F. graminearum* s. str. isolates (30). The average number of pairwise differences between isolates (5.717 to 6.275) and gene diversity (0.518 to 0.570) were similar for the four groups (Table 3). The majority (99.28%) of the variance was caused by variation among isolates within populations. Low levels of pairwise F_{ST} (0.002 to 0.016) and a high level of effective number of migrants (N_m) (29.852 to 301.847) between groups were observed (Table 4). Based on a previous report (9) that population subdivision in *F. graminearum* s. str. in the midwestern United States was correlated with trichothecene type, we classified 179 *F. asiaticum* isolates into NIV ($n = 80$) and 3ADON ($n = 99$) populations for additional analysis. F_{ST} (0.060) and N_m (7.883) values suggested that population subdivision may be present between NIV and 3ADON trichothecene types, but it was not distinctive. Due to the lack of population subdivision, we considered all 179 isolates of *F. asiaticum* as part of a geographically larger population in this study, and the data were combined to assess the level of linkage disequilibrium between loci pairs. Significant linkage

Table 1. Observed number of alleles and gene diversity in Japanese populations of the *Fusarium graminearum* species complex^a

VNTR marker ^b	Fluorescent label ^c	Location ^d	No of alleles	Gene diversity ^e
HK1043	6-FAM	Ch1/Ct52/41,839	4 ^f	0.15 (0.12)
HK913	VIC	Ch1/Ct73/664	13 ^f	0.65 (0.63)
HK917	PET	Ch1/Ct82/2,471	16 ^f	0.81 (0.81)
HK957	NED	Ch1/Ct91/16,055	23	0.88 (0.88)
HK1003	PET	Ch1/Ct437/6,986	24	0.94 (0.94)
HK965	PET	Ch2/Ct154/51,671	15	0.83 (0.82)
HK967	VIC	Ch2/Ct154/53,868	5	0.28 (0.27)
HK1059	NED	Ch3/Ct196/164,228	6	0.66 (0.66)
HK977	NED	Ch3/Ct208/47,696	4 ^f	0.18 (0.15)
HK630	6-FAM	Ch4/Ct371/112,325	6 ^f	0.33 (0.30)
HK1073	6-FAM	Ch4/Ct398/70,812	8	0.53 (0.52)
Mean			10.36	0.57 (0.55)

^a Results based on analysis of 179 isolates of *F. asiaticum* and four isolates of *F. graminearum* s. str.

^b VNTR = variable numbers of tandem repeat. HK1003 was initially described in Gale et al. (7), all others are from Suga et al. (29).

^c Forward primer was fluorescent-labeled to obtain data by use of a 3100 Genetic Analyzer.

^d Marker location in the genome was indicated by Chromosome No./Contig No./Position in contig.

^e Nei's gene diversity (h) (9) calculated in PopGene32 (34). Values in parentheses are for the 179 isolates of *F. asiaticum* only.

^f One allele was specific to isolates(s) of *F. graminearum* s. str.

Table 2. Species and trichothecene type composition for four Japanese populations of the *Fusarium graminearum* species complex

Group	Species ^a				<i>Fusarium graminearum</i> s. str.				Total
	NIV ^b	3ADON	15ADON	Subtotal	NIV	3ADON	15ADON	Subtotal	
02WSC	16	30	0	46	0	0	0	0	46
03W	26	18	0	44	0	2	0	2	46
03WSC	13	33	0	46	0	0	0	0	46
04W	25	18	0	43	0	2	0	2	45
Total	80	99	0	179	0	4	0	4	183

^a Species determined by diagnostic polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) for *F. asiaticum* and *F. graminearum* s. str. (30).

^b Trichothecene chemotype was determined by chemotype-specific multiplex PCR (30,33). Number of isolates is indicated.

disequilibrium ($P < 0.05$) was detected in 10 (18%) and 12 (22%) out of 55 combinations by Arlequin ver. 2.000 and GenePop ver. 3.3, respectively (Table 5).

DISCUSSION

Although population genetic studies of the *Fg* complex have previously been reported in the United States and eastern China (8,9,25,35,36), population dynamics of this fungal complex remains poorly understood in other countries. We assessed genetic profiles of the *Fg* complex from infested seed lots and from a wheat field in Kumamoto, Japan in two consecutive years. Efficacy and adequacy of 11 VNTR markers (7,29) for population level analyses of the *Fg* complex were also confirmed in this study.

Sample collection sites for the four groups were all located in the Kyushu area (Fig. 1). Previous studies of the *Fg* complex on wheat or barley revealed the presence and differential geographical distribution of *F. asiaticum* and *F. graminearum* s. str. in Japan (21,30). The prevalence of *F. asiaticum* in all samples in this study confirmed the dominance of *F. asiaticum* in southern Japan, including Kyushu (30). Although the previous study did not detect any *F. graminearum* s. str. isolates from Kyushu, *F. graminearum* s. str. was detected at low frequency in this study. The co-existence of both *F. asiaticum* and *F. graminearum* s. str. provides a potential for introgression (hybridization) in the field. Progeny from crosses between these two species have been obtained previously in

vitro (12). However, the presence of alleles specific to isolates of *F. graminearum* s. str. (Table 1) suggests these two species live sympatrically, supporting results from a previous study in Tohoku, Japan where both species co-occur. Similarly, no apparent hybrids were detected in that study (30). Nevertheless, we currently cannot rule out occasional or low levels of hybridization between these species.

NIV type strains of *F. graminearum* s. str. have not been detected in Japan (30), but global surveys previously identified all three trichothecene types (NIV, 3ADON, 15ADON) in both *F. asiaticum* and *F. graminearum* s. str. (30,33). In this study, *F. asiaticum* isolates were either NIV or 3ADON types, and all four *F. graminearum* s. str. isolates were the 3ADON type (Table 2). Population subdivisions correlated with 15ADON and 3ADON trichothecene types in *F. graminearum* s. str. were detected in the midwestern United States (9). We hypothesized that Japanese population subdivisions of *F. asiaticum* may be correlated with NIV or 3ADON. Although no clear correlation between haplotypes and chemotypes was found, organization of isolates based on trichothecene types nevertheless resulted in higher levels of population subdivision ($F_{ST} = 0.060$) and in lower number of migrants ($N_m = 7.883$) compared to other groupings. More intensive sampling from a wider area is probably needed for further insight.

Most studies have demonstrated high genotypic diversity in collections of *F.*

graminearum (8). Zeller et al. (35) analyzed *F. graminearum* from diseased wheat heads in small (0.25 m²) quadrants in Kansas and North Dakota by AFLP. Among 253 tested isolates, 169 were of a unique haplotype. High genotypic diversity of *F. asiaticum* was also observed in this study. VNTR data indicated that 88 out of 91 isolates (including the four *F. graminearum* s. str. isolates) from the 500-m² wheat field (03W and 04W) and all 92 isolates from seeds (02WSC and 03WSC) were of a unique haplotype. Identical haplotypes, which were apparently clonal, have been recovered from neighboring plants with low frequency (8,35). Three pairs of isolates that shared VNTR haplotypes were detected in the 04W sample but not in the 03W sample. Different sampling strategies used in 03W and 04W may have been influential. For 03W, all isolates were obtained from different sites in the field, but due to the mild FHB occurrence in 2004, one to four isolates were obtained from single sites in the 04W sample. However, our results did not contradict the hypothesis that most infections are initiated by genotypically diverse spores, followed by limited secondary infection (8,35). Occurrence of the same haplotypes in different heads can be explained by secondary spread of asexual conidia, a local primary inoculum source, or asexual conidia and/or ascospores derived from selfing.

Of 55 paired combinations between the 11 VNTR markers, 10 (18%) or 12 (22%) marker pairs were in linkage disequilibrium. Of 78 possible combinations between 13 AFLP loci, four locus pairs (5%) in the Kansas and eight locus pairs (10%) in the North Dakota population were in

Table 3. Mean number of pairwise differences and gene diversity (H) across 11 variable numbers of tandem repeat (VNTR) markers in four Japanese populations of *Fusarium asiaticum*

Group ^a	No. of isolates ^b	Pairwise differences	
		and SD ^c	H and SD ^d
02WSC	46	5.717 (2.789)	0.518 (0.281)
03W	44	6.275 (3.035)	0.570 (0.306)
03WSC	46	6.169 (2.987)	0.561 (0.301)
04W	43	6.213 (3.010)	0.565 (0.304)
Total	179	6.123 (2.923)	0.577 (0.294)

^a 02WSC were from seeds harvested at Fukuoka Prefecture and planted in the experimental fields in Kumamoto Prefecture in 2002. 03WSC isolates were from seeds harvested at Nagasaki Prefecture and planted in 2003. 03W and 04W isolates were from diseased wheat heads in the experimental field in 2003 and 2004, respectively.

^b *F. asiaticum* isolates.

^c Mean number of pairwise differences and standard deviation (SD) calculated using Arlequin version 2.000 (S. Schneider, D. Roessli, and L. Excoffier, Laboratoire de Génétique et Biométrie, Université de Genève, Switzerland).

^d Average gene diversity over loci and SD (in parentheses) was determined according to Nei (20) and Tajima (31).

Table 4. Pairwise F_{ST} ^a (above diagonal) and effective number of migrants (N_m)^b (below diagonal) between populations of *Fusarium asiaticum*

	02WSC	03W	03WSC	04W
02WSC	...	0.009	0.003	0.016
03W	57.230	...	0.002	0.005
03WSC	198.838	301.847	...	0.009
04W	29.852	100.503	53.025	...

^a F_{ST} values were calculated using Arlequin version 2.000 (S. Schneider, D. Roessli, and L. Excoffier, Laboratoire de Génétique et Biométrie, Université de Genève, Switzerland).

^b Effective number of migrants (N_m) was calculated using Arlequin version 2.000 that implements a concept developed by Nei (20).

Table 5. Observed significant linkage disequilibrium ($P < 0.05$) between 11 variable numbers of tandem repeat (VNTR) markers in 179 isolates of *Fusarium asiaticum*

Marker pair	P value	
	Arlequin ^a	GenePop ^b
HK913, HK917	0.000	0.000
HK913, HK957	0.000	0.000
HK913, HK965	0.001	0.000
HK917, HK1003	0.009	0.047
HK917, HK1059	0.007	0.010
HK957, HK965	0.000	0.002
HK1003, HK630	0.032	0.029
HK967, HK1059	0.022	0.011
HK977, HK630	0.014	0.016
HK1059, HK1073	0.001	0.001
HK967, HK1073	0.056 ^c	0.048
HK1043, HK917	0.078 ^c	0.047

^a In Arlequin version 2.000 (S. Schneider, D. Roessli, and L. Excoffier, Laboratoire de Génétique et Biométrie, Université de Genève, Switzerland), a test by Slatkin (26) based on Fisher's exact probability test is implemented.

^b In GenePop version 3.3 (M. Raymond and F. Rousset, Laboratoire de Génétique et Environnement, Université Montpellier, France) Fisher's exact probability test is implemented.

^c Not significant by Arlequin version 2.000 but significant by GenePop version 3.3.

linkage disequilibrium in a previous study (35). Low levels of linkage disequilibrium may be interpreted as evidence for frequent out-crossing. However, it might also be attained by maintenance of high genetic diversity in large populations. Gale et al. (8) found linkage disequilibrium ($P < 0.05$) on 13 locus pairs (36%) of 36 possible combinations between six RFLP loci in an eastern China population. Marker position is a conceivable cause of linkage disequilibrium, although markers HK965 and HK967 are only separated from each other by 2,200 bp according to the genome database of *F. graminearum* s. str. (Table 1). Surprisingly, significant linkage disequilibrium between these two markers was not found in this study ($P = 0.425$ in Arlequin, $P = 0.329$ in GenePop). In the 2.2-kb α_1 -antitrypsin locus in humans, linkage disequilibrium was low between alleles at three RFLPs (4). Alternatively, the genome construction of marker regions in *F. asiaticum* may be different from that of *F. graminearum* s. str.

Actual frequencies of intraspecies out-crossing in nature remain unclear. Genetic profile analyses of ascospores derived from perithecia collected from fields could give more direct evidence of the prevalence of intraspecies out-crossing.

FHB may be initiated by regional inoculum. However, the contribution of spores transported long-distance to FHB epidemics has also been discussed (6,16,25). Gilbert et al. (10) showed that *Fusarium*-infested seed was not a major cause of FHB in the crop year it was planted. Schmale et al. (25) suggested that FHB inoculum may originate from multiple locations over large geographic areas. For *F. asiaticum*, the contribution of the seed population to FHB in this study could not be attained because distinctive population subdivisions between the groups were not detected (Table 4). However, the lack of distinctive population subdivisions among the four groups at least indicated that FHB in both 2003 and 2004 was caused by a genetically homogeneous population that prevails in Kumamoto, Fukuoka, and Nagasaki prefectures.

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