

## Molecular Standardization of Mating Type Terminology in the *Gibberella fujikuroi* Species Complex†

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**Mating type in the *Gibberella fujikuroi* species complex is controlled by a single locus with two alleles and is usually identified following sexual crosses with standard, female-fertile tester isolates. The mating type alleles have been arbitrarily designated “+” and “–” within each biological species, and the nomenclature is tied to the standard tester strains. We developed a pair of PCR primers that can be used to amplify a unique fragment of one of the mating type alleles (*MAT-2*) from at least seven of the biological species in this species complex. Based on the amplification pattern, we propose a replacement for the existing, arbitrary +/- terminology that is presently in use. The new terminology is based on DNA sequence similarities between the mating type allele fragments from the biological species of the *G. fujikuroi* species complex and the corresponding fragments from other filamentous ascomycetes.**

*Gibberella fujikuroi* is a complex of at least eight different mating populations (biological species) that are reproductively isolated and are denoted by letters (e.g., *G. fujikuroi* mating population A). Recently, some of these mating populations have been given *Gibberella* species names that parallel the names of their *Fusarium* anamorphs, and additional anamorphs have been identified (4, 19, 22, 34). These species have a worldwide distribution and have been recovered from hosts as diverse as banana, fig, maize, mango, pine, rice, sorghum, and sugarcane (26). These species also produce a variety of secondary metabolites and mycotoxins, such as fumonisins (28, 29, 41), gibberellic acid (43), moniliformin (28), fusaric acid (3), beauvericin (30), and fusaproliferin (37), that can contaminate animal feed and human food.

Mating type in filamentous ascomycetes is usually dimictic, with one locus and two functional alleles (9), often designated *MAT-1* and *MAT-2* following the convention proposed by Yoder et al. (51). The alleles are idiomorphs, which means that they share no significant sequence similarity but do map to the same position on homologous chromosomes (5). Mating type genes have been cloned and sequenced from several filamentous ascomycetes, including *Neurospora crassa* (11), *Podospira anserina* (7), *Cochliobolus heterostrophus* (45), and *Magnaporthe grisea* (17). Although there is little *MAT* sequence similarity across broad evolutionary distances (44), there is enough sequence similarity in the HMG-box region of the *MAT-2* allele to permit identification of this region by PCR methods.

Mating type has not been well-characterized at the molecular level in any of the mating populations (biological species) of the *G. fujikuroi* species complex. Mating type in these fungi is dimictic and is usually denoted by a base letter (A through H) that specifies the mating population and a superscript “+” or “–” that specifies the mating type. The + and – designations were arbitrarily assigned by the researchers who described the mating populations, and the + allele from one mating popu-

lation may or may not be the same at the molecular level as the + allele from another.

Arie et al. (2) designed degenerate PCR primers that could be used to amplify the HMG box of the *MAT-2* region of various ascomycetes, including representative species of the genera *Cryphonectria*, *Gaeuemannomyces*, *Mycosphaerella*, *Nectria*, *Neurospora*, *Pyrenophora*, and *Setosphaeria* and 18 species of the genus *Cochliobolus*. Our objectives in this study were (i) to determine if the primer sets developed by Arie et al. (2) could be used to amplify the *MAT-2* HMG box from strains in the different mating populations of the *G. fujikuroi* species complex, (ii) to develop PCR primers that are specific for the *MAT-2* allele in this species complex, and (iii) to replace the arbitrary +/- terminology presently in use with terminology that is consistent with known molecular characteristics and parallels the terminology used for other filamentous ascomycetes.

(A preliminary report of the results has been presented previously [18].)

### MATERIALS AND METHODS

**Strains.** Except for the progeny in the mapping population used by Xu and Leslie (48, 49) to construct a genetic map for *G. fujikuroi* mating population A, all of the strains which we used are listed in Table 1. Strains were routinely cultured on minimal medium (6), if they had no auxotrophic requirements, or on complete medium (6), if additional nutritional supplements were needed. All incubations were performed at 25°C under a 12 h of light-12 h of darkness diurnal cycle. Strains were preserved for long-term storage as spore suspensions in a glycerol-water mixture (15:85) frozen at –70°C.

**Sexual crossing protocol.** Crosses were made on carrot agar (20) by using standard tester strains (Table 1) as the female parents. Fertile crosses produced perithecia that exuded a cirrhous of ascospores 2 to 4 weeks after fertilization.

**DNA manipulation.** DNA was isolated by a cetyltrimethylammonium bromide (CTAB) procedure modified from the procedure of Murray and Thompson (32). Briefly, a frozen sample (approximately 1.5 to 2 g [wet weight]) was ground to a fine powder under liquid nitrogen with a mortar and pestle. Then we added 8 ml of hot (65°C) 2% CTAB buffer (2% CTAB, 100 mM Tris-HCl [pH 8.0], 20 mM EDTA, 1.4 M NaCl). We incubated the sample at 65°C for 30 min, extracted the preparation once with an equal volume of chloroform-isoamyl alcohol (24:1), removed the aqueous layer, and precipitated the crude nucleic acids with an equal volume of 2-propanol. We dissolved the pellet in 1 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.4) and extracted it sequentially with equal volumes of phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1). The last extract (approximately 600 µl) was treated with 1.5 µl of RNase (2 mg/ml), and the remaining nucleic acids were precipitated with either 2.5 volumes of 95% ethanol or 1 volume of 2-propanol. The resulting pellet was washed twice with 70% ethanol, dried briefly, and then resuspended in 50 to 100 µl of TE buffer. We estimated final DNA concentrations by using

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TABLE 1. *Fusarium* strains used in this study

Strain <sup>a</sup>	Mating type		Origin	Supplier <sup>d</sup>	Other designation(s) <sup>e</sup>	Reference(s) <sup>f</sup>
	Previous studies <sup>b</sup>	This study <sup>c</sup>				
<i>G. fujikuroi</i> mating population A						
A-00015	<i>matA</i> <sup>+</sup>	<i>MATA-2</i>	Laboratory cross	JEP	FGSC 6895	48
A-00149 <sup>g</sup>	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Sorghum, California	PTS	M-3125, FGSC 7600	4, 16, 47, 48, 50
A-00488	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Transkei, South Africa	WFOM	M-1325, MRC-826	16, 47, 48, 50
A-00501	<i>matA</i> <sup>+</sup>	<i>MATA-2</i>	Maize, Kansas	JFL	M-6461	16, 50
A-00552	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Kansas	JFL	M-6467	16, 50
A-00606	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Kansas	JFL	M-6468	16, 50
A-00679	<i>matA</i> <sup>+</sup>	<i>MATA-2</i>	Maize, Kansas	JFL	M-5124	16, 50
A-00999 <sup>g</sup>	<i>matA</i> <sup>+</sup>	<i>MATA-2</i>	Maize, Indiana	JFL	M-3703, FGSC 7603, ATCC 201261	4, 16, 48, 50
A-01501	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Illinois	JFL		
A-01660	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Beijing, People's Republic of China	JFL	M5430	16, 50
A-04367	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Maize, Assyut, Egypt	JFL		16, 50
A-04643	<i>matA</i> <sup>-</sup>	<i>MATA-1</i>	Laboratory cross	JFL	FGSC 8078	47, 48
<i>G. fujikuroi</i> mating population B						
B-00278	<i>matB</i> <sup>+</sup>	<i>MATB-2</i>	Sugarcane, Taiwan	PTS	M-3127, FGSC 7608, ATCC 201262	16, 47, 50
B-00281	<i>matB</i> <sup>-</sup>	<i>MATB-1</i>	Sugarcane, Taiwan	PTS	M-3128, FGSC 7609, ATCC 201263	16, 47, 50
B-01722	<i>matB</i> <sup>-</sup>	<i>MATB-1</i>	Sorghum, The Philippines	JFL	M-5476	16, 47, 50
B-03852 <sup>g</sup>	<i>matB</i> <sup>+</sup>	<i>MATB-2</i>	Laboratory cross	JFL	M-6865, FGSC 7610, ATCC 201264	4, 16, 50
B-03853 <sup>g</sup>	<i>matB</i> <sup>-</sup>	<i>MATB-1</i>	Laboratory cross	JFL	M-6866, FGSC 7611, ATCC 201265	4, 16, 50
<i>G. fujikuroi</i> mating population C						
C-01993 <sup>g</sup>	<i>matC</i> <sup>+</sup>	<i>MATC-1</i>	Rice, Taiwan	EGK	M-1148	16, 50
C-01995 <sup>g</sup>	<i>matC</i> <sup>-</sup>	<i>MATC-2</i>	Rice, Taiwan	EGK	M-1150	16, 50
<i>G. fujikuroi</i> mating population D						
D-00502	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Maize, Kansas	JFL	M-6471, FGSC 7612, ATCC 201266	16, 47, 50
D-00526	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Maize, Kansas	JFL	M-6480	16, 50
D-00550	<i>matD</i> <sup>-</sup>	<i>MATD-1</i>	Maize, Kansas	JFL	M-6482	16, 50
D-00637	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Maize, Kansas	JFL	M-6484	16, 50
D-00666	<i>matD</i> <sup>-</sup>	<i>MATD-1</i>	Maize, Kansas	JFL	M-5123	16, 47, 50
D-00720	<i>matD</i> <sup>-</sup>	<i>MATD-1</i>	Sorghum, Kansas	JFL	M-6485	16, 47, 50
D-00741	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Shattercane, Kansas	JFL	M-6492	16, 50
D-00753	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Sorghum, Kansas	JFL	M-6495	
D-00875	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Sorghum, Kansas	JFL	M-5128	16, 50
D-02945	<i>matD</i> <sup>-</sup>	<i>MATD-1</i>	Sorghum, Mississippi	JFL	M-3793, FGSC 7613, ATCC 201267	16, 47, 50
D-04853 <sup>g</sup>	<i>matD</i> <sup>+</sup>	<i>MATD-2</i>	Laboratory cross	JFL	FGSC 7614, ATCC 201268	4
D-04854 <sup>g</sup>	<i>matD</i> <sup>-</sup>	<i>MATD-1</i>	Laboratory cross	JFL	FGSC 7615, ATCC 201269	4
<i>G. fujikuroi</i> mating population E						
E-00434	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Kansas	JFL	M-6496	16, 47, 50
E-00505	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Kansas	JFL	M-6497	
E-00507	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Kansas	JFL	M-5119	16, 47, 50

Continued on following page

TABLE 1. Continued

Strain <sup>a</sup>	Mating type		Origin	Supplier <sup>d</sup>	Other designation(s) <sup>e</sup>	Reference(s) <sup>f</sup>
	Previous studies <sup>b</sup>	This study <sup>c</sup>				
E-00520	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Kansas	JFL	M-6498	16, 50
E-00551	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Kansas	JFL	M-6501	16, 47, 50
E-00990 <sup>g</sup>	<i>matE</i> <sup>-</sup>	<i>MATE-1</i>	Maize, Illinois	JFL	M-3696, FGSC 7616, ATCC 201270	4, 16, 34, 47, 50
E-02192 <sup>g</sup>	<i>matE</i> <sup>+</sup>	<i>MATE-2</i>	Maize, Illinois	JFL	M-3693, FGSC 7617, ATCC 201271	4, 16, 47, 50
<i>G. fujikuroi</i> mating population F						
F-00728	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Sorghum, Kansas	JFL	M-5598, MRC 5713	16, 22, 47, 50
F-00779	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Sorghum, Arkansas	JFL	MRC 6477	21, 22
F-00921	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Sorghum, Kansas	JFL	M-5132, MRC 5708	21, 22, 50
F-00965	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Sorghum, Kansas	JFL	M-5134, MRC 5707	16, 22, 47, 50
F-01054	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Sorghum, Kansas	JFL	M-5594, MRC 5709	16, 22, 50
F-01087	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Sorghum, Kansas	JFL	M-5595, MRC 5710	16, 22, 50
F-01106	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Sorghum, Kansas	JFL	M-5589, MRC-5703	16, 22, 50
F-01137	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Sorghum, Kansas	JFL	M-5596	16, 22, 50
F-01183	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Sorghum, Kansas	JFL	M-5590, MRC 5704	16, 22, 50
F-01321	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Sorghum, Kansas	JFL	M-5591, MRC 5705	16, 22, 50
F-04093 <sup>g</sup>	<i>matF</i> <sup>-</sup>	<i>MATF-2</i>	Laboratory cross	JFL	M-6563, FGSC 7056, ATCC 200521	4, 21, 22, 34
F-04094 <sup>g</sup>	<i>matF</i> <sup>+</sup>	<i>MATF-1</i>	Laboratory cross	JFL	M-6564, FGSC 7057, ATCC 200522	21, 22
<i>G. fujikuroi</i> mating population G						
G-05111 <sup>g</sup>	<i>matG</i> <sup>+</sup>	<i>MATG-1</i>	Laboratory cross	PEN	M-7492, ATCC 201272	16, 19
G-05112 <sup>g</sup>	<i>matG</i> <sup>-</sup>	<i>MATG-2</i>	Laboratory cross	PEN	M-7491, ATCC 201273	16, 19
<i>G. zeae</i>						
Z-10588	Homo-thallic		<i>Andropogon gerardii</i> , Kansas	KAZ		
Z-10628	Homo-thallic		<i>Andropogon scoparius</i> , Kansas	KAZ		
<i>F. oxysporum</i>						
O-02332		<i>MAT-2</i>	Banana, Malaysia	BS		
O-02520		<i>MAT-1</i> (?)	Celery, California	JEP	O-1	6
O-02523		<i>MAT-1</i> (?)	Chrysanthemum, Florida	PEN	O-734	6
O-02527		<i>MAT-1</i> (?)	Tomato, Florida	PEN	O-1078	6
O-02529		<i>MAT-1</i> (?)	Watermelon, Florida	PEN	O-1087	6
O-02530		<i>MAT-2</i>	Tomato, Ontario, Canada	PEN	O-1090	6
O-02533		<i>MAT-2</i>	Cotton, Mississippi	PEN	O-1139	6
O-02534		<i>MAT-2</i>	Banana, Queensland, Australia	PEN	O-1222	6

<sup>a</sup> Kansas State University Strain Collection designation.

<sup>b</sup> Mating type described in references 19, 21, 22, 24, and 26.

<sup>c</sup> Strains that have only a *MAT-1* or *MAT-2* designation have not been proven to be fertile in a sexual cross.

<sup>d</sup> BS, Baharuddin Salleh; JEP, John E. Puhalla; JFL, John F. Leslie; KAZ, Kurt A. Zeller; PEN, Paul E. Nelson; PTS, Philip T. Spieth; WFOM, Walter F. O. Marasas. <sup>e</sup> ATCC, American Type Culture Collection, Manassas, Va.; FGSC, Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City; M- and O-, Fusarium Research Center, Pennsylvania State University, University Park; MRC, PROMEC, Medical Research Council, Tygerberg, South Africa.

<sup>f</sup> References cited by Yan et al. (50) are not included.

<sup>g</sup> Standard strain for the mating type.

*Hind*III-digested bacteriophage λ DNA and an Applied Biosystems model IS-1000 digital imaging system (Alpha Inotech Corp., San Leandro, Calif.); samples and sample dilutions were electrophoresed in 1% agarose gels.

Plasmid isolation, restriction digestion, ligation, bacterial transformation, and Southern hybridization were performed by using standard procedures (38). Agarose gels containing the amplification products were capillary blotted onto a

Hybond N+ membrane (Amersham International, Amersham, United Kingdom) and probed with DNA fragments labeled with [ $\alpha$ -<sup>32</sup>P]dCTP. Stringent conditions were used for Southern hybridization; i.e., the membranes were washed first in 2× SSC–0.1% sodium dodecyl sulfate at 25°C for 20 min and then two times in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C for 20 min (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

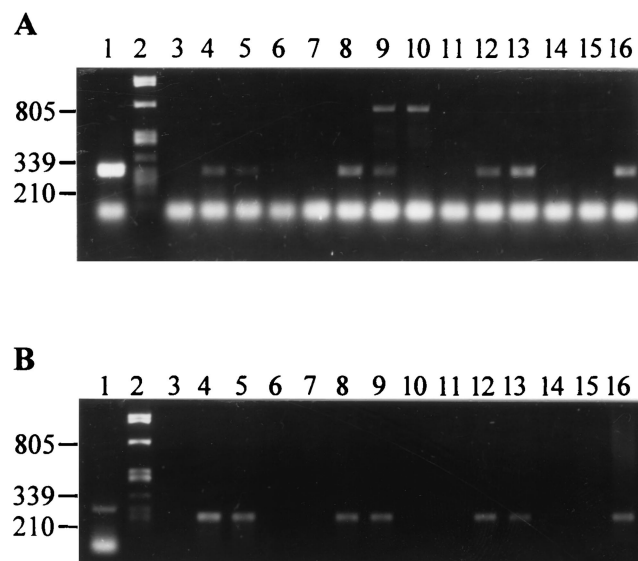


FIG. 1. (A) PCR amplification of a putative HMG-box sequence from mating type testers of the *G. fujikuroi* species complex with degenerate primers NcHMG1 and NcHMG2. Lane 1, *N. crassa* FGSC 4066; lane 2,  $\lambda$  phage DNA digested by *Pst*I; lanes 3 through 16, *G. fujikuroi* A-00149, A-00999, B-03852, B-03853, C-01993, C-01995, D-04853, D-04854, E-00990, E-02192, F-04093, F-04094, G-05111, and G-05112, respectively. (B) PCR amplification of the HMG box with *Gibberella*-specific primers GfHMG1 and GfHMG2. Lanes 2 to 16 are amplification products from the same source as in panel A. Lane 1, HMG-box sequence amplified from *G. fujikuroi* A-00999 by PCR with the NcHMG1 and NcHMG2 primers.

PCR products amplified from *G. fujikuroi* A-00999 were extracted from an agarose gel with QIAEX II (Qiagen Inc., Chatsworth, Calif.) by following the manufacturer's instructions. Purified DNA fragments were blunt-end ligated into the *Eco*RV site of plasmid pBluescript KS II+ (Stratagene, La Jolla, Calif.).

**Amplification conditions.** We used degenerate oligonucleotide primers NcHMG1 and NcHMG2 (2) to amplify the putative HMG-box from the *G. fujikuroi* standard mating type tester strains. The PCR mixtures (50  $\mu$ l) contained 1 $\times$  PCR buffer (Promega, Madison, Wis.), 20 ng of fungal DNA, 2.5 mM MgCl<sub>2</sub>, each deoxynucleoside triphosphate (Promega) at a concentration of 0.25 mM, each degenerate primer at a concentration of 2  $\mu$ M, and 1 U of *Taq* DNA polymerase (Promega). For amplification we used the following program: initial denaturation at 95°C for 3 min, followed by 30 cycles consisting of 1 min at 94°C, 30 s at 55°C, and 1 min at 72°C and a final elongation incubation step consisting of 72°C for 10 min. PCR amplification products were separated by electrophoresis in 1.5% (wt/vol) agarose gels, stained with ethidium bromide, and visualized with UV light.

We designed two *Gibberella*-specific primers, GfHMG1 (5'-ACCGTAAGGAGCGTACCATT-3') and GfHMG2 (5'-GGGGTACTGTCCGCGATGTT-3'), based on the sequence of the 262-bp putative HMG-box amplification product obtained from *G. fujikuroi* A-00999 (EMBL accession no. AJ 131527). These specific primers were used to confirm the results obtained from amplifications performed with the degenerate primers, to identify the mating types of previously untested field isolates, and to confirm mating type assignments made by classical genetic analysis of the progeny of laboratory crosses. When the specific primers replaced the degenerate primers, we altered the amplification protocol by increasing the annealing temperature from 55 to 60°C and by reducing the primer concentration from 2 to 0.1  $\mu$ M.

**Sequencing protocol.** DNA was sequenced with a Sequenase kit (version 2.0; United States Biochemicals, Cleveland, Ohio). Nucleotide sequences were compared by using the GCG software package (8).

## RESULTS

We used two degenerate oligonucleotide primers, NcHMG1 and NcHMG2 (2), to amplify the *MAT-2* HMG box from the tester pairs of mating populations A through G of the *G. fujikuroi* species complex (Fig. 1A). All of the strains produced a bright band consisting of unincorporated primers, and the two *G. fujikuroi* mating population D strains each produced an additional amplification product of approximately 800 bp. As

the 800-bp amplification product was found in strains of both mating types, this amplification product is not specifically associated with either mating type idiomorph. Another PCR product, which was approximately 260 bp long, was clearly amplified from one member of each of the tester pairs used for mating populations A, C, E, F, and G but not from their counterparts. A similar amplification product was obtained from the representatives of mating populations B and D, but in each case the band was less intense than the bands obtained for the other five mating populations.

We cloned and sequenced the 262-bp fragment amplified from *G. fujikuroi* A-00999. The nucleotide sequence of this fragment, including the intron, was 58 and 62% similar to the nucleotide sequences of the HMG boxes in *Neurospora crassa mt a* (39) and *Podospora anserina FPR1* (7), respectively. The *G. fujikuroi* mating population A sequence also contains a putative intron (12) at a conserved site between bp 92 and 138.

We used the nucleotide sequence of the putative *MAT-2* HMG box from strain A-00999 to design two primers to be used to amplify the homologous fragment from any *MAT-2* strain of the *G. fujikuroi* species complex. Primer GfHMG1 corresponds to bases 29 to 49 at the 5' end of the HMG box, and primer GfHMG2 corresponds to bases 223 to 252 at the 3' end of the HMG box. When we used this pair of primers to amplify PCR products from genomic DNAs of the seven pairs of mating type testers, a single 213-bp PCR product was generated from only one of the mating type tester strains for each mating population (Fig. 1B). We identified PCR amplification products obtained from GfHMG1 and GfHMG2 that corresponded to the *MAT-2* allele in strains representing *matA*<sup>+</sup>, *matB*<sup>+</sup>, *matC*<sup>-</sup>, *matD*<sup>+</sup>, *matE*<sup>+</sup>, *matF*<sup>-</sup>, and *matG*<sup>-</sup>. We probed Southern blots of these PCR amplification preparations with the 262-bp HMG fragment cloned from A-00999 and observed strong hybridization signals under high-stringency conditions (data not shown). No hybridization was observed when the PCR product amplified from *N. crassa* was probed under the same conditions (data not shown).

Based on amplification patterns such as those shown in Fig. 1, we assigned *MAT-1* and *MAT-2* allele designations to the

TABLE 2. Correspondence between previous mating type terminology and revised mating type terminology for the *G. fujikuroi* species complex

Species <sup>a</sup>	<i>G. fujikuroi</i> mating population	Mating type designation	
		Previous studies	This study
<i>Fusarium moniliforme</i> ( <i>Fusarium verticillioides</i> ) ( <i>Gibberella moniliformis</i> )	A	<i>matA</i> <sup>+</sup> <i>matA</i> <sup>-</sup>	<i>MATA-2</i> <i>MATA-1</i>
<i>Fusarium subglutinans</i> ( <i>Fusarium sacchari</i> )	B	<i>matB</i> <sup>+</sup> <i>matB</i> <sup>-</sup>	<i>MATB-2</i> <i>MATB-1</i>
<i>Fusarium fujikuroi</i>	C	<i>matC</i> <sup>+</sup> <i>matC</i> <sup>-</sup>	<i>MATC-1</i> <i>MATC-2</i>
<i>Fusarium proliferatum</i>	D	<i>matD</i> <sup>+</sup> <i>matD</i> <sup>-</sup>	<i>MATD-2</i> <i>MATD-1</i>
<i>Fusarium subglutinans</i> ( <i>Gibberella subglutinans</i> )	E	<i>matE</i> <sup>+</sup> <i>matE</i> <sup>-</sup>	<i>MATE-2</i> <i>MATE-1</i>
<i>Fusarium thapsinum</i> , <i>Gibberella thapsina</i>	F	<i>matF</i> <sup>+</sup> <i>matF</i> <sup>-</sup>	<i>MATF-1</i> <i>MATF-2</i>
<i>Fusarium nygamai</i> , <i>Gibberella nygamai</i>	G	<i>matG</i> <sup>+</sup> <i>matG</i> <sup>-</sup>	<i>MATG-1</i> <i>MATG-2</i>

<sup>a</sup> Anamorph name (*Fusarium* species), followed by teleomorph name (*Gibberella* species) if other than a mating population of *G. fujikuroi*. The names in parentheses are synonyms.



known mating types (Table 2). Independent blind tests of the diagnostic ability of GfHMG1 and GfHMG2 were conducted in both Gödöllő, Hungary, and Manhattan, Kans. We examined 128 progeny (49 *matA*<sup>+</sup> progeny and 79 *matA*<sup>-</sup> progeny) from the mapping population of Xu and Leslie (48, 49), the 14 standard testers representing the seven *G. fujikuroi* mating populations, and 48 field strains representing five of the seven mating populations, *Fusarium oxysporum*, and *Gibberella zeae* (Table 1). For these tests, the strains were coded such that we knew the mating population to which a strain belonged but did not know its mating type. The results of the PCR amplifications were identical to the results of the sexual crosses with standard strains. Thus, the PCR results were predictive of the crossing results, and the crossing results were predictive of the PCR results. Four of the eight *F. oxysporum* strains examined had a PCR amplification product indicative of the *MAT-2* allele. No PCR amplification products were observed with either *G. zeae* strain.

### DISCUSSION

We sequenced and analyzed a 262-bp fragment resulting from PCR amplification of A-00999 DNA by using the NcHMG1 and NcHMG2 primers (2) and then designed two new primers, GfHMG1 and GfHMG2, based on the sequence obtained. Three lines of evidence suggest that the 262-bp fragment is part of one of the *MAT* alleles of *G. fujikuroi*. First, the fragment shares significant sequence similarity with *N. crassa mt a-1* and *P. anserina FPR1*. Second, in the 52 field strains belonging to the seven different mating populations examined, the fragment is consistently associated with only one mating type within a mating population. Finally, the presence of the amplified fragment cosegregated with the *matA*<sup>+</sup> mating type and the absence of the fragment cosegregated with the *matA*<sup>-</sup> mating type for 128 progeny of the mapping population previously used to construct a *G. fujikuroi* genetic map (48, 49). On the basis of these classical genetic mapping data, the amplified fragment maps with 95% certainty to a 2.3-map unit region that includes *MAT* and is unlikely to map more than 1 map unit from *MAT* if it is not coincident with it.

Based on the genetic mapping data and the correlations observed with mating type in the field strains, we propose that the current nomenclature (e.g., *matA*<sup>+</sup> and *matA*<sup>-</sup>) be replaced with new terminology (e.g., *MATA-1* and *MATA-2*) (Table 2). The new terminology includes the mating population with which an allele is associated because of the multiple biological species in the *G. fujikuroi* species complex and the potential for significant differences in the sequences associated with different biological species. Such differences might be exploited to design primers that are specific for both mating population and mating type. If a single species is identified by name (e.g., *Gibberella moniliformis* instead of *G. fujikuroi* mating population A), then the letter in the gene symbol for the mating population is not needed and may be deleted.

We scored *MAT-1* as the absence of the *MAT-2* amplification product. The accuracy of scoring could be increased if primers were available to amplify a specific fragment from *MAT-1* as well, since at the moment, anything that results in no amplification automatically leads to a diagnosis of *MAT-1*. Development of a system in which positive results are used to identify both *MAT* alleles (40) is probably essential for studying the molecular basis of homothallism in *G. zeae* (33) and *G. fujikuroi* mating population B (4).

The lack of amplification in the *G. zeae* strains may have several causes. First, the HMG box-containing *MAT* allele may be absent, as it is in some homothallic *Neurospora* species (10, 11). Alternatively, the sequence may be present but divergent

enough from the sequences of strains in the *G. fujikuroi* species complex that amplification does not occur. Finally, it is possible that some *G. zeae* strains have *MAT-1* and some have *MAT-2* and that the two strains which we examined had only *MAT-1*. Based on preliminary reports (52), it appears that some *G. zeae* strains have coding sequences from both *MAT-1* and *MAT-2*. Presumably, the region corresponding to either GfHMG1 or GfHMG2 was altered in the fusion process, which resulted in no amplification in a PCR when this primer pair was used.

We examined both of the strains (B-03852 and B-03853) that Britz et al. (4) identified as occasionally homothallic. One of these strains (B-03852) clearly yielded a *MAT-2* PCR amplification product, while the other strain did not. Thus, the homothallism in these strains cannot be due to mating type switching, as has been observed in some yeasts and filamentous fungi (13–15, 31, 36, 42). Molecular scoring of mating types should reduce the amount of effort required to screen field populations for sexual fertility and should increase the efficiency of the process through which new mating populations are identified by 50 to 75%, since only strains known to differ in mating type need to be crossed.

The availability of molecular diagnostic tools for mating type also may enable workers to analyze purportedly asexual fungi, including *Fusarium oxysporum* and 12 of the 13 new *Fusarium* taxa recently described by Nirenberg and O'Donnell (34) and Nirenberg et al. (35). There is circumstantial evidence that sexual reproduction occurs in *F. oxysporum*, and this evidence is in the form of high levels of diversity with respect to the multilocus vegetative compatibility trait (1, 23, 25, 46), especially in populations of putatively nonpathogenic strains. As Leslie and Klein (27) have shown, sexual reproduction need not be frequent to play an important role in the maintenance and generation of genotypic diversity in field populations of fungi. The availability of mating type data should make it easier to identify potentially cross-fertile strains that can be used to test some of these hypotheses.

In conclusion, our work shows that one of the *MAT* idiomorphs of *G. fujikuroi* contains an HMG fragment that is similar to a mating type idiomorph found in other model and economically important filamentous ascomycetes. We anticipate that the similarities which have been observed mean that we will be able to apply much of the information obtained from studies of other filamentous ascomycetes, such as *Neurospora*, *Podospora*, and *Cochliobolus*, to members of the *G. fujikuroi* species complex without any major changes. By standardizing the nomenclature from the present *A*<sup>+</sup>/*A*<sup>-</sup> form to the *MAT-1*/*MAT-2* form, we hope to improve communication between researchers who study filamentous fungi and to establish a terminology in which all of the mating types are named based on molecular structure rather than the arbitrary choices of numerous researchers.

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