A Genetic Map of *Gibberella fujikuroi* Mating Population A (*Fusarium moniliforme*)

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ABSTRACT

We constructed a recombination-based map of the fungal plant pathogen *Gibberella fujikuroi* mating population A (asexual stage *Fusarium moniliforme*). The map is based on the segregation of 142 restriction fragment length polymorphism (RFLP) markers, two auxotrophic genes (*arg1, nic1*), mating type (*mat*A$^+$/*mat*A$^-$), female sterility (*ste1*), spore-killer (*Sk*), and a gene governing the production of the mycotoxin fumonisin B_1 (*fum1*) among 121 random ascospore progeny from a single cross. We identified 12 linkage groups corresponding to the 12 chromosome-sized DNAs previously observed in contour-clamped homogeneous electric field (CHEF) gels. Linkage groups and chromosomes were correlated via Southern blots between appropriate RFLP markers and the CHEF gels. Eleven of the 12 chromosomes are meiotically stable, but the 12th (and smallest) is subject to deletions in 3% (4/121) of the progeny. Positive chiasma interference occurred on five of the 12 chromosomes, and nine of the 12 chromosomes averaged more than one crossover per chromosome. The average kb/cM ratio in this cross is ~32.

A genetic map is a basic component of the scientific infrastructure of an experimental genetic system and serves as the underpinning for all classical genetic and many population and molecular genetic studies with the target organism. With the introduction of molecular markers such as RFLPs (restriction fragment length polymorphism) and RAPDs (random amplified polymorphic DNA), it has been possible to make maps without first obtaining the numerous morphological and biochemical markers that compose most maps in filamentous fungi. In filamentous fungi genetic maps consisting primarily of molecular markers have been constructed for the downy mildew fungus *Bremia lactucae* (HULBERT *et al.* 1988), the Southern corn leaf blight fungus *Cochliobolus heterostrophus* (TZENG *et al.* 1992), the rice blast fungus *Magnaporthe grisea* (ROMAO and HAMER 1992; SKINNER *et al.* 1993), and the commercial mushroom *Agaricus bisporus* (KERRIGAN *et al.* 1993), and these markers have been used to extend existing maps of *Neurospora crassa, Aspergillus nidulans,* and *Podospora anserina.* The existing genetic map of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*) is limited to just 12 markers on four linkage groups (PUHALLA and SPIETH 1985) and is typical of the rudimentary genetic maps available for most filamentous fungi.

The *F. moniliforme*-form species contains anamorphs of two *G. fujikuroi* mating populations (biological species), A and F (LESLIE 1991a; KLITTICH and LESLIE 1992). The A mating population is most prevalent in maize where it causes seedling blight, and stalk and root rots (SHURTLEFF 1980); losses in Kansas average 4–8% of the maize crop per year (JARDINE 1986). In addition to maize, members of the A mating population have also been recovered from bananas, figs, pine, rice, field soil, and sorghum (LESLIE 1995). *F. moniliforme* has been associated with human and animal toxicoses since it was first described in 1881 (SACCARDO 1881; CUBONI 1882). The organism has been shown to be toxic and/or carcinogenic to a variety of experimental animals including baboons, chickens, ducklings, mice, rabbits, and rats (MARASAS *et al.* 1984; LESLIE *et al.* 1996) and to cause pulmonary edema in swine and equine leukoencephalomalacia (NELSON *et al.* 1993). Consumption of contaminated grain is correlated with human esophageal cancer risk in Transkei, South Africa (RHEEDER *et al.* 1992) and in the People's Republic of China (ZHEN 1984).

Genetic interest in the organism is often associated with its economic importance, but a number of traits of intrinsic genetic interest have been identified in field isolates including spore-killer (KATHARIOU and SPIETH 1982), fumonisin biosynthetic genes (DESJARDINS *et al.* 1992, 1995), and heritable differences in spontaneous mutation frequency (KLITTICH *et al.* 1988). *G. fujikuroi* can be cultured on a defined medium, and sexual crosses mature in 3–5 weeks under laboratory conditions (KLITTICH and LESLIE 1988a), which makes it suitable for experimental studies under laboratory conditions. Four chromosomes were described from light microscopic observations of meiotic figures (HOWSON *et al.* 1963), but recently, XU *et al.* (1995) described the karyotype using contour-clamped homogeneous electric field (CHEF) gel electrophoresis and identified 12 chromosomes ranging in size from 0.7 to 10 megabase-pairs (Mb) with a total genome size of ~46 Mb.

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TABLE 1

Strains used in the RFLP mapping experiments

Strain	Origin	♀[a]	Fum. B_1[b]	Genotype[c]
A00015	UV mutant of A00102	No	High	*matA*$^+$ *Sk*^{k} *arg1 nic1 ste1*
A00102	Sorghum, San Joaquin Co., CA	Yes	High	*matA*$^+$ *Sk*^{k}
A00149	Maize, Visalia, CA	Yes	High	*matA*$^-$ *Sk*^{s}
A00488	Maize, Transkei, South Africa	No	High	*matA*$^-$
A00498	Sorghum St. George, KS	No	High	*matA*$^-$
A00549	Maize, Silver Lake, KS	Yes	High	*matA*$^-$
A00924	Spon. mutant of A00102	Yes	ND	*matA*$^+$ *Sk*^{k} *nit1*
A00959	Spon. mutant of A00102	Yes	ND	*matA*$^+$ *Sk*^{k} *nit4*
A00999	Maize, Knightstown, IN	Yes	ND	*matA*$^+$ *Sk*^{k}
A02949	Maize, Crowder, MS	Yes	ND	*matA*$^-$ *pal1*
A04516	Maize, Kathmandu, Nepal	Yes	None	*matA*$^+$ *Sk*^{s} *fum1*
A04522	Maize, Kathmandu, Nepal	Yes	None	*matA*$^+$ *Sk*^{s} *fum1*
A04643	Progeny of A00149 ♀ × A04522 ♂	Yes	None	*matA*$^-$ *Sk*^{s} *fum1*
A04644	Spon. mutant of A04643	Yes	None	*matA*$^-$ *Sk*^{s} *fum1 nit1*
A05113	Progeny of A00149 ♀ × A04516 ♂	Yes	None	*matA*$^-$ *Sk*^{s} *fum1*

[a] Female fertility: Yes, female fertile; No, female sterile.

[b] Relative level of fumonisin B_1 produced; ND, no data.

[c] Markers used: *arg1*, arginine auxotrophy; *fum1*, fumonisin biosynthesis; *matA*$^+$/*matA*$^-$, mating type; *nic1*, nicotinic acid auxotrophy; *nit1*, nitrate reductase; *nit4*, nitrate reductase molybdenum cofactor; *pal1*, perithecial pigmentation; *Sk*^{k}/*Sk*^{s}, spore-killer killer/sensitive alleles; *ste1*, female sterility.

Our objective in this study was to construct a recombinational genetic map of *G. fujikuroi* mating population A and to correlate the linkage groups that we detect with the presumptive chromosome bands detected in the electrophoretic karyotype. A preliminary report of this work has been made (Xu and Leslie 1993a).

MATERIALS AND METHODS

Strains and media: All strains (Table 1) were routinely cultured on complete media (Correll *et al.* 1987). The *arg1* and *nic1* mutations were scored by culturing them on minimal medium (Correll *et al.* 1987) supplemented with 0.5 mg/ml L-arginine·HCl or 10 μg/ml nicotinamide, respectively. Strains were preserved in 15% glycerol at −70°. All cultures were incubated at 25° with a 12 hr light/12 hr dark cycle; cool-white fluorescent (F40CW) and black lights (F40/BLB) were used for illumination.

Sexual crosses were made on carrot agar, and random ascospore progeny were separated by micromanipulation as described by Klittich and Leslie (1988a). Ascospores were collected such that each of the progeny could be assumed to have originated from a separate meiotic event (Leslie 1991b). Progeny and parental strains are available from the Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas. The complete genotype of each of the 121 progeny strains will be published elsewhere (Xu and Leslie 1996).

Mating type, female sterility and spore killer: All progeny were used as males in crosses with standard tester strains, A00149 (*matA*$^-$) and A00999 (*matA*$^+$), of opposite mating type (Leslie 1991a). Fertile crosses produced abundant perithecia with oozing ascospores within 4 weeks of fertilization. These crosses were used to diagnose mating type, with the progeny's mating type being the opposite of that of the tester with which it formed a fertile cross. Asci from these crosses were examined to determine the *Sk* phenotype. All crosses were made at least twice.

Female fertility was assayed using the progeny as the female parent in crosses with the appropriate tester strain as the male parent. These crosses also were made at least twice. A00015 was female sterile and carried a mutation of unknown origin at a locus arbitrarily designated *ste1*. If a progeny was fertile as a male, but sterile as a female, then it was assumed to carry the female sterility allele of *ste1*.

Spore killer is a meiotic drive phenomenon controlled by a nuclear gene or a tightly linked gene complex that has been described in several filamentous fungi including *N. sitophila, N. intermedia, G. fujikuroi,* and *P. anserina;* see Raju (1994) for a recent review of this area and pictures of this phenotype in *G. fujikuroi.* One of the parents of the mapping population, A00015, is derived from strain F80 of Kathariou and Spieth (1982) and carries the killer allele *Sk*^{k}, while the other parent, A04643, carries the spore-killer sensitive allele, *Sk*^{s} (Desjardins *et al.* 1992). The spore killer phenotype of the progeny was scored by crushing 2-week-old perithecia in lacto-phenol cotton blue dye (Tuite 1969) and counting the number of ascospores/ascus. In homozygous crosses all eight spores normally mature, but in heterozygous crosses *Sk*^{s} spores usually do not mature but instead degenerate. The number of ascospores/ascus was determined in at least five asci from each perithecium examined.

Fumonisin assays: Cultures were inoculated on cracked corn and incubated for 4 weeks as previously described (Leslie *et al.* 1992a,b). Ten grams of the fermented corn culture was extracted with 25 ml of acetonitrile:H_2O (1:1) for 3 hr with occasional shaking (Plattner *et al.* 1990, 1992). The extraction suspensions were filtered through Whatman #2 filter paper, and aliquots were stored at −20°.

The amount of fumonisins present was determined using TLC and HPLC analyses. TLC analyses (Ackermann 1991; Plattner *et al.* 1992) were made using standard fumonisin B_1 (Sigma, St. Louis, MO, 2 μl 1 mg/ml) and fumonisin samples (3 μl) from the progeny cultures, which were spotted onto silica gel 60 plates (Merck #5719). The TLC plates were developed with acetonitrile:distilled water (85:15) and then sprayed with *p*-anisaldehyde (Gelderblom *et al.* 1988). Violet

fumonisin spots were observed after heating the TLC plates at 130–140° for 5–10 min. The presence of fumonisin B_1 was determined by comparing the color and R_f value of sample spots with the fumonisin B_1 standard. The lower detection limit of this test is 300–400 ppm. When TLC results were equivocal, fumonisins were assayed by R. D. PLATTNER (USDA-NCAUR, Peoria, IL) using fluorescence HPLC (SHEPHARD *et al.* 1990). The lower detection limit of the HPLC assay is 5–10 ppm for fumonisin B_1.

DNA manipulations: Gibberella DNA was isolated in mini-preps and in large scale purifications. For mini-preps, fresh mycelium was collected from 3-day-old liquid cultures and extracted following the general protocol of GARBER and YODER (1983) with a few modifications (XU 1994). In large scale preparations, mycelia were collected from 500 ml liquid cultures, ground in liquid nitrogen, and extracted following the general protocol of ZOLAN and PUKKILA (1986) using 2% (w/v) CTAB (cetyltrimethylammonium bromide) with a few modifications (XU 1994).

RAPD markers: PCR was performed as described by WILLIAMS *et al.* (1990). Random 10-mers (B primer kit) were purchased from Operon Technologies Inc. (Alameda, CA). PCR reactions contained 0.25 unit of *Taq* polymerase (Promega, Madison, WI), 1× *Taq* buffer (Promega), 0.1 mM each of dATP, dGTP, dCTP and dTTP (USB, Cleveland, OH), 25 ng of genomic DNA and 0.2 μM primer. The amplification reactions were incubated in a Perkin-Elmer-Cetus DNA Thermal Cycler for 45 cycles of 1 min at 94°, 1 min at 36°, and 2 min at 72°.

Construction of random genomic clones: A00102 genomic DNA was digested with *Pst*I or *Eco*RI, extracted with phenol:chloroform (1:1) and precipitated with ethanol. The digested genomic DNA was ligated with *Pst*I- or *Eco*RI-digested pUC18 (YANISCH-PERRON *et al.* 1985) and transformed into *Escherichia coli* DH5α competent cells (GIBCO-BRL, Gaithersburg, MD) following standard methods (MANIATIS *et al.* 1982; AUSUBEL *et al.* 1989). Some of the digested DNA was run through a 10–40% sucrose gradient to enrich for DNA fragments ~1 kb in size before ligating with pUC18. All the probes used for mapping originated in one of these two plasmid libraries and are available from the Fungal Genetics Stock Center.

Plasmid DNAs were isolated by the alkaline lysis method (BIRNBOIM and DOLY 1984) and digested with restriction enzymes under conditions specified by the manufacturer. DNA restriction fragments were isolated from agarose gels with GeneClean II kit (BIO-101, La Jolla, CA).

Southern blotting and hybridization were carried out according to standard methods (MANIATIS *et al.* 1982). The membranes were treated with 120 mjoules of UV-light (254 nm) using a Stratalinker 2400 (Stratagene) and further baked at 80° for 1–2 hr. Probe DNAs were labeled with [α-^{32}P]-dCTP by random-hexamer priming (FEINBERG and VOGELSTEIN 1983). Free nucleotides were removed by gel filtration through Sephadex G-50. All other DNA manipulations not specifically mentioned were carried out as described by MANIATIS *et al.* (1982) or AUSUBEL *et al.* (1989).

CHEF gels: CHEF gels and marker hybridizations were made as described by XU *et al.* (1995).

RFLP data analysis: Data were analyzed as F_2 backcross data with the Mapmaker program (LANDER *et al.* 1987). One parental phenotype was treated as the "homozygote for the recurrent parent genotype", and the other parental type was treated as the "heterozygote" as defined by Mapmaker. This arrangement is necessary if Mapmaker's diploid algorithims are to be used to analyze haploid intercrosses. We have written a program to analyze haploid segregation (XU and LESLIE 1993b), but the results from this program are nearly identical to those from Mapmaker, and Mapmaker is more widely available, more user-friendly and better documented than our program is.

The LOD (logarithm of the odds ratio) first proposed by HALDANE and SMITH (1947) was used as a pairwise test for linkage with a minimum LOD of 4. This criterion is stricter than the commonly used linkage criterion LOD ≥ 3 that was first proposed by MORTON (1955).

Vegetative compatibility group (VCG) tests: Vegetative compatibility is a multigenic trait. Strains that are identical at all vegetative incompatibility (*vic*) loci can form a stable heterokaryon and are said to be in the same vegetative compatibility group (LESLIE 1993). VCG tests were made on minimal medium in 24-well hybridoma plates as previously described (KLITTICH and LESLIE 1988b). All auxotrophic progeny were tested for vegetative compatibility with nitrate nonutilizing (*nit*) mutants of both parents (KLITTICH and LESLIE 1988a). Strains A04644 (*nit1*), A00924 (*nit1*), and A00959 (*nit4*) were used to do the VCG tests (Table 1). The pairing between *nit* mutants A00959 and A00924 served as a positive control. VCG tests were repeated twice.

RESULTS

RFLP markers

Random genomic clones construction: A total of 797 *Eco*RI clones and 998 *Pst*I clones were picked and hybridized in colony blots with *Eco*RI-digested A00102 genomic DNA to identify multiple copy clones. Colonies with strong signals were designated high- or medium-copy probes depending on the strength of the signal. All other clones (>95% of the total) were considered low-copy number probes.

High copy number probes and rDNA: Forty-two probes that appeared to hybridize to sequences present in the genome in multiple copies were identified via *in situ* colony hybridization and screened for polymorphisms between A00015 and A04643; no reliable polymorphisms were detected. The hybridization band patterns of these probes were very similar, but not identical in *Bam*HI, *Eco*RI, *Eco*RV, *Pst*I and *Sal*I digests of genomic DNA, and all hybridized to chromosome *2* on Southern blots of CHEF gels. From previous work (XU *et al.* 1995), the rDNA region is known to be on chromosome *2* and we concluded that all of these clones were from this region. This hybridization pattern is indirect evidence that *F. moniliforme* has very few large repetitive DNA sequences and that most of these sequences are rDNA. We also amplified the 18S and 28S rDNA regions using PCR with standard primers for this region (VILGALYS and HESTER 1990; WHITE *et al.* 1990) and used the amplification products to probe Southern blots of CHEF gels and genomic DNA. The hybridization patterns were similar to those of high copy probes confirming that these probes were presumptive rDNA clones.

We attempted to place the rDNA gene cluster on the genetic map. The 18S, 28S, ITS, and IGS rDNA sequences were amplified and used to probe genomic blots of A00015 and A04643; no length polymorphisms were detected. PCR-amplified IGS DNAs were digested with *Eco*RI, *Bam*HI, *Pst*I, *Hind*III, *Eco*RV and *Sal*I, but

no polymorphisms were detected. Thus, the rDNA regions of these two strains are very similar, and we could not position the rDNA cluster on our genetic map.

Medium copy number probes: Clones with somewhat weaker signals in the colony blot hybridizations were classified as medium copy probes and hybridized to several bands each. Three of these probes (RFLP1, RFLP37 and RFLP41) detected polymorphisms at single bands between A00015 and A04643 and are included in the genetic map.

Two other probes, 7E1 and 7E33, were interesting even though they did not detect any polymorphisms between the two parental strains. Both probes had a single intense hybridization band on Southern blots of genomic DNA, but neither hybridized to a transcribed sequence on a Northern blot. Both probes hybridized with genomic digests of strains representing the six mating populations within *G. fujikuroi,* and the resulting patterns could be used to distinguish the A and F mating populations from the other four mating populations and might form the basis for a diagnostic identification protocol. Probe 7E1 hybridized to every chromosomal band on CHEF gel Southern blots, suggesting that this probe might contain centromeric or telomeric sequences.

Low copy number RFLP patterns and their genetic backgrounds: The RFLPs used had three basic banding patterns. The first was a single band that was present in two different sizes that corresponded to two different alleles; 103/142 probes had this pattern. The second type (12/142 probes) had the presence and absence of a band as the two allelic forms. Some of these probes detected simple deletions, *e.g.,* 7E77 that hybridizes to A00015 but not to A04643, while others, *e.g.,* 5E34, have hybridizing sequences in both A04643 and A00015 but have a second copy in only one of the two strains. The third type (27/142 probes) had a probe that hybridized with more than one band per strain, but only one of the bands was polymorphic.

RFLP screening of potential parents: Genomic digests of eight strains (Table 1), A00102, A00149, A00488, A00498, A00549, A02949, A04643 and A05113, using eight restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Pst*I, *Sal*I, *Sma*I, and *Xba*I, were used to identify parents that differed at the most RFLPs. A04643 and A02949 had more RFLPs with A00102 than the other strains in this preliminary screen and were selected for further examination. [A00015 was derived from A00102 via UV mutagenesis and carried two markers (*nic1* and *arg1*) that had been used in previous mapping studies.]

Southern blots of genomic DNA of A00015, A02949 and A04643 digested with one of five restriction enzymes were screened with 311 low-copy number clones from the *Eco*RI library and 186 clones from the *Pst*I library (Table 2). Approximately 32% of the probes from the *Eco*RI library and 27% of those from the *Pst*I library identified RFLPs between A00015 and A04643,

TABLE 2

Frequency of RFLP probes in libraries based on genomic digests with *Bam*HI, *Eco*RI, *Eco*RV, *Pst*I, and *Sal*I

Strains	Library		Total[a]
	*Eco*RI[a]	*Pst*I[a]	
A00015/A04643			
*Bam*HI	38/58	25/32	63/90
*Eco*RI	32/57	21/38	53/95
*Eco*RV	47/57	25/30	72/87
*Pst*I	38/60	13/26	51/86
*Sal*I	41/54	13/19	54/73
Total	98/311	51/186	149/497
A00015/A02949			
*Bam*HI	17/55	11/27	28/82
*Eco*RI	18/51	13/30	31/81
*Eco*RV	23/52	13/20	36/72
*Pst*I	26/58	11/25	37/82
*Sal*I	22/47	4/16	26/63
Total	73/311	31/175	104/486
Common	63/311	30/186	93/497
Total	108/311	52/186	160/497

[a] Number of polymorphic probes/total number of probes tested.

and nearly 60% of the possible probe-restriction enzyme combinations were tested. The highest level of polymorphism was seen in the *Eco*RV digests, where >80% of the probes tested were polymorphic, while the lowest levels of polymorphism were in the *Eco*RI and *Pst*I digests where fewer than 60% of the probes tested were polymorphic. Probes and genomic digests used in map construction are listed in Table 3, but this list does not include all of the probes that detected polymorphisms. The percentage of probes that were polymorphic was slightly lower for A00015 and A02949 (Table 2). Of the 149 probes detecting RFLPs between A00015 and A04643, 62% also detected polymorphisms between A00015 and A02949. Of the 160 total RFLPs detected, 92% detected polymorphisms between A00015 and A04643. We selected A04643 and A00015 as the parents of the mapping population because of the higher level of DNA polymorphism and the difference in fumonisin production.

Cross and progeny isolation: The cross from which the mapping population was derived was fertile with ascospores oozing out of the perithecium ~2 weeks after fertilization; A04643 was the female parent and A00015 the male parent. From seven perithecia we recovered a total of 123 random ascospores; spore viability of the separated ascospores was >80%. Two of the progeny were discarded because of the presence of both parental phenotypes for most of the RFLP probes.

Other markers

Auxotrophs: Two auxotrophic markers were mapped, one for arginine (*arg1*) and the other for nicotinic acid

TABLE 3

Genetic markers used for map construction

Marker[a]	LG[b]	Chromosome(s)[c]	Segregation[d]	A-04643[e]	A-02949[f]
Morphological/biochemical markers					
arg1	3	ND	60:56		
fum1	1	ND	54:46		
matA	6	ND	68:48		
nic1	5	ND	37:83***		
Sk	5	ND	1:116***		
ste1	3	ND	38:62*		
RAPD markers					
B11	3	ND	56:50		
B12	6	ND	71:50		
RFLP markers					
RFLP1[1]	5	ND	4:117***	***V***	
RFLP37[2]	9	ND	56:61	***B***	
RFLP41[2]	2	*2*	62:42	***S***	
1E	4	*4/5*	56:59	e***P******S***	EPS
5E	U	*11*	62:57	BE***P***	BEP
7E	4	*4/5*	53:68	Ep***V***	ePV
10E	8	*8/9*	66:54	Be***V***	bEP
11E	3	*3*	58:60	be***P***	bEP
14E	9	*8/9*	58:61	BE***P***	bep
22E	8	*8/9*	64:57	bep***S***	beps
23E[1]	9	*8/9*	57:61	be***P***	bep
32E	4	*4/5*	56:65	e***P***	eP
37E[2]	9	*8/9*	58:63	***B***eP	beP
38E	2	*2*	69:52	bep***V***	BeP
39E[2]	4	*4/5; 8/9*	60:61	BE***P***	BEP
73E[2]	6	*6*	74:46*	***B***EpSV	bEpsv
75E	4	*4/5*	57:63	***B***Ep	bep
78E	1	*1*	58:63	be***P***	bep
86E	U	*10*	54:67	BEp***S***	bepS
87E	2	*2*	50:71	b***E***p	bep
P7	5	*5*	54:67	B***E***p	bEp
P9[1]	7	*7*	54:67	***B***EP	Bep
P13	10	*10*	63:57	BE***P***	BEP
P15	10	*10*	63:57	be***P***	bEP
P18	8	*8*	64:55	beP***S***	bePs
P19	2	ND	52:69	BE***P***	BEP
P20	2	*2*	57:64	beP***V***	bePV
P22	8	*8*	64:57	bep***V***	bEpV
P25	10	*10*	60:61	Bep***V***	BepV
P34[2]	6	*6*	61:51	***B***EP	BEP
P37[2]	8	*7/8/9*	55:63	B***E***p	BEp
P39	9	*8/9*	62:59	***E***p	Ep
P49[2]	2	*2*	71:49*	EP***V***	ePV
P51	2	*2*	66:55	Be***P***V	BeP
5E5[1]	4	*4/5*	50:69	***B***ePSV	bepsv
5E14[2]	4	*4/5*	56:63	***B***	B
5E23	5	*4/5*	34:83***	be***P***sv	bepsv
5E32	12	*12*	59:56	BE***P***SV	bePsv
5E34[1,2]	5	*2; 4/5*	53:67	BE***P***SV	BEPSV
5E36	5	*4/5*	49:72*	EpS***V***	EPSV
5E43	11	*11*	48:67	b***S***v	bsv
5E49[2]	1	*1*	66:55	b***S***v	Bsv
5E50	1	*1*	57:53	beps***V***	bepsV
5E51[1]	8	*8/9*	56:65	BEPSV	bepsv
5E56	U	*4/5*	61:55	B***E***PSV	bepSV
5E57	1	*1*	63:55	b***E***psv	bEpsv
5E60[2]	3	*1/2/3*	57:64	***B***epsv	bepsv
5E63	3	*3*	58:63	eps***V***	epsV
5E68[2]	8	*8/9*	65:56	beps***V***	bepsV

TABLE 3

Continued

Marker[a]	LG[b]	Chromosome(s)[c]	Segregation[d]	A-04643[e]	A-02949[f]
RFLP markers					
6E1	6	*6*	70:51	***V***	v
6E7[1]	7	*7*	41:79***	***B***EPSV	bepsv
6E10[1,2]	8	*8/9*	60:56	B***E***PSV	BEPSV
6E11[2]	3	*3*	57:64	be***S***	BEs
6E14	3	*3*	58:63	***S***	S
6E15[2]	2	*2*	62:58	***S***	s
6E18	7	*7*	45:68*	***V***	v
6E27[2]	1	*1*	61:56	***E***	e
6E28	1	*1*	66:55	***V***	V
6E31[2]	10	*2;10*	60:61	BE***P***VS	BEPSV
6E37	10	*10*	59:60	***V***	v
6E46	8	*8/9*	67:54	s***V***	Sv
6E48	1	*1*	44:44	***B***	b
6E49	6	*6*	63:58	S***V***	Sv
6E51	1	*1*	62:55	***B***SV	bSV
6E58	6	*6*	50:44	p***V***	Pv
6E62	9	*8/9*	58:63	Be***P***SV	bepsv
6E66	5	*5*	38:83***	***B***ps	bPS
6E67[1]	U	*1*	64:55	B***P***SV	BPSV
6E68	5	*4/5*	19:101***	e***P***	eP
6E73[2]	7	ND	57:64	bep***V***	bepV
6E74[2]	U	*11*	58:63	BE***P***V	bEPV
6E75	6	*6*	71:49	bep***S***v	bepsv
6E78	11	*11*	56:65	be***P***Sv	bepsv
6E79	10	*10*	55:66	bePS***V***	bePSV
6E81[2]	1	*1*	64:54	***B***pvs	bpsv
6E85[2]	7	*2;7*	54:62	***S***	s
6E88	12	*12*	59:55	BepSV	BepSV
7E5	4	*4/5*	54:67	***S***	S
7E12	4	*4/5*	56:65	B***P***V	BPV
7E14	2	*2*	70:50	B***E***V	BeV
7E16	10	*10*	43:43	***V***	v
7E19	12	*12*	52:63	***E***PS	EPS
7E20	11	ND	54:67	B***V***	bV
7E24	6	*6*	65:55	p***S***V	PSV
7E25	3	*3*	59:61	***E***	E
7E26[1,2]	7	*1;7*	42:79***	bP***V***	Bpv
7E27[2]	6	*6;8/9*	67:52	EPS***V***	EPSV
7E28	8	*8*	57:61	***S***	s
7E29	9	*8/9*	62:59	E***P***	EP
7E31	7	ND	53:66	***V***	v
7E31BB	4	ND	56:64	***V***	v
7E32	3	*3*	54:65	***E***	E
7E35	4	*4*	52:66	***P***	P
7E37	7	*7*	41:78***	***B***S	BS
7E38[2]	U	*7/8/9*	60:56	***S***V	SV
7E41	9	*8/9*	58:54	***B***PS	bps
7E47S[2]	U	*8/9*	62:59	***V***	v
7E48	8	*8/9*	64:54	***B***	B
7E49[2]	5	*4/5*	45:76**	P***S***	pS
7E51	7	*7*	56:65	***B***P	bp
7E52	3	ND	59:62	***V***	v
7E58[2]	U	*1*	64:57	***B***EPSV	bepsV
7E60L[1]	8	*8*	63:58	BEP***V***	bepv
7E69[2]	7	ND	50:71	***V***	v
7E77[1]	7	*7*	57:64	***B***EPSV	bepsv
7E80	2	*2*	59:62	b***P***	Bp

TABLE 3

Continued

Marker[a]	LG[b]	Chromosome(s)[c]	Segregation[d]	A-04643[e]	A-02949[f]
RFLP markers					
10P12	10	ND	47:45	EB***V***	
10P16	U	*3;11*	59:56	***E***	E
10P18	2	*2*	65:56	BE***S***V	
10P19	11	*10*	48:59	BE***V***	
10P23	2	*2*	56:54	E***V***	EV
10P25S	10	*10*	60:61	***B***V	BV
10P33^2	2	*1;2;6;10*	61:47	B***V***	
11P10^2	2	*2;8*	55:65	***B***esv	besv
11P18	5	*4/5*	11:105***	E***S***	s
11P25	U	*1*	64:57	Be***S***V	beSV
11P26^2	2	*2;3*	58:62	be***S***V	besv
11P28	U	*3*	66:54	***P***V	P
11P30^2	7	*2;7*	55:62	bEp***V***	bEpV
11P34	6	*6*	70:50	be***P***	beP
11P43	12	*12*	59:56	BE***P***SV	bEpSV
11P45^2	8	*8*	66:54	Bep***V***	bepV
12P5^1	4	*4/5*	53:66	***B***EPSV	bepsv
12P6	8	ND	53:64	epS***V***	ePSV
12P18	10	ND	54:66	Bes***V***	besV
12P25^2	4	*4/5*	56:65	***B***epsv	Bepsv
12P27^2	6	*6*	77:43**	***B***epsV	bepsV
12P28	10	*10*	58:60	***B***pV	bpv
12P34	9	*9*	60:61	***B***epsv	Bepsv
12P38S^2	3	*2;3*	56:61	***S***	s
12P38L	6	*6*	70:50	***S***	s
12P59	3	*3*	63:57	***B***epsv	BEPsv
15P3	1	ND	58:51	***V***	
15P12	10	ND	52:64	***E***	
15P18	10	ND	57:53	***V***	
15P22	2	ND	64:47	***V***	
15P26	3	ND	55:55	***V***	
15P37	4	ND	56:64	***S***	
15P38	1	ND	62:58	***B***	
15P43	10	*10*	47:55	***S***	
15P44^2	U	ND	51:44	***E***S	
15P45	9	*8/9*	58:55	***B***E	

[a] Morphological and biochemical markers are defined in the legend of Table 1. RFLP loci are one locus with two alternate bands unless otherwise marked; markers denoted with superscript 1 have presence/absence alleles; markers denoted with superscript 2 hybridize with two (or more) bands, but a single band with two alternative alleles is polymorphic in the cross.

[b] Linkage group as determined in this study. Linkage group numbers correspond with physical chromosomes with the same number. U indicates no linkage was observed with markers on any of the defined linkage groups.

[c] Chromosome: band on CHEF gel (Xu *et al.* 1995); numbers separated by a semicolon indicate clear hybridization to more than one chromosome; numbers separated by a / indicate hybridization to a band that contains more than one chromosome. ND, no data or ambiguous data.

[d] Segregation data are given in the form A04643:A00015 alleles; when the sum is less than 121, then the remaining progeny either were not scored or were ambiguous. Details of segregations for each marker can be found in Xu and Leslie (1996). Segregation ratios were tested for significant difference from 1:1 with a chi-square test using the Yate's correction factor; significant differences are noted as follows: *5%, **1%, ***0.1%.

[e] Genomic restriction digests used to detect polymorphisms between A-00015 and A-04643. Uppercase letter indicates polymorphisms were detected; lowercase letter indicates no polymorphism was detected; bold-italic indicates the genomic digest used in assigning the alleles for this mapping experiment. B/b, *Bam*HI, E/e, *Eco*RI; P/p, *Pst*I; S/s, *Sal*I; V/v, *Eco*RV.

[f] Genomic restriction digests used to detect polymorphisms between A-00015 and A-02949. Uppercase letter indicates polymorphisms were detected; lowercase letter indicates no polymorphism was detected. B/b, *Bam*HI; E/e, *Eco*RI; P/p, *Pst*I; S/s, *Sal*I; V/v, *Eco*RV.

(*nic1*); these markers were first described by PUHALLA and SPIETH (1983, 1985). The *arg1* locus mapped to chromosome *3* and segregated in a 1:1 manner. The *nic1* locus mapped to chromosome *5*. but the segregation was significantly different from 1:1, probably due to linkage to *Sk*.

Mating type: All of the progeny crossed with one of the two mating type tester strains in crosses where the standard tester served as the female parent and the individual progeny served as the male parent. Mating type segregated in a 1:1 manner and was mapped to chromosome *6*.

Female sterility: Perithecia are produced by a hermaphroditic strain that is serving as the female parent in a cross (CHAISRISOOK and LESLIE 1990). One of the parental strains, A00015, is female sterile. This strain is an auxotrophic mutant derived from A00102 by Dr. J. E. PUHALLA that has been propagated in the laboratory for many generations, and its female fertility may have degenerated, or female sterility may have been introduced coincidentally with the auxotrophic mutations. When female sterility was scored in the mapping population, the female sterility locus (*ste1*) mapped to linkage group 3. Segregation of this trait was not 1:1 (38 fertile:62 sterile), but 21 progeny were not scored for this trait. These progeny were preferentially of the A04643 type at the next closest marker *15P26* (XU and LESLIE 1996). If the alleles at *15P26* are used to predict the fertility/sterility of these 21 progeny, then the segregation ratio becomes 51 fertile:68 sterile with two progeny not being scored for both traits. This ratio is not significantly different from 1:1 ($P > 0.10$), so while it is possible that a second female-sterility locus could be segregating in this cross, we think that it is unlikely.

Spore killer: Normal asci of *G. fujikuroi* contain eight viable spores. However, when a cross is heterozygous for spore killer ($Sk^k \times Sk^s$), four of the eight spores receive the Sk^s nucleus, and abort and degenerate leaving only the four Sk^k spores to mature normally. A few exceptional Sk^s progeny may escape killing in heterozygous asci (KATHARIOU and SPIETH 1982). The perithecia from which the progeny in this mapping population were selected generally contained four viable and four aborted spores in each ascus, indicating that this cross is heterozygous for *Sk*, as expected from previous work with the parental strains (KATHARIOU and SPIETH 1982; DESJARDINS *et al.* 1992).

To score *Sk*, we made test crosses between the members of the mapping population and either A00149 (Sk^s $matA^-$) or A00999 (Sk^k $matA^+$), depending on mating type. Asci from crosses of A00999 (Sk^k) × A04643 (Sk^s), A00149 (Sk^s) × A00015 (Sk^k), and A00149 (Sk^s) × all $matA^+$ members of the mapping population (putative Sk^k) all had four ascospores/ascus. In crosses between A00999 (Sk^k) and all of the $matA^-$ members of the mapping population (putative Sk^k), except number 117, there were eight ascospores/ascus. Thus A00015 and all of the progeny except progeny number 117 carry the Sk^k allele, and A04643 carries the Sk^s allele. When progeny number 117 was crossed with A00999, asci with four spores were observed instead of the expected eight spores. This phenomenon was repeatable and progeny number 117 presumably escaped the spore killer effect. In this cross, the Sk^s escape frequency was 0.8%, which is lower than 5% escape rate previously reported (KATHARIOU and SPIETH 1982). The *Sk* locus maps to linkage group 5, and the other loci on this chromosome have skewed segregation ratios, with those of the A00015 (Sk^k) parent in excess. The distortion is apparent for all of the markers on this chromosome, but decreases in severity as the distance from the *Sk* locus increases (Figure 1).

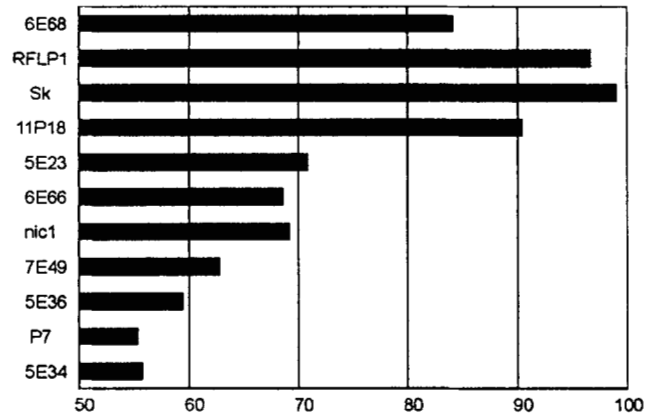

FIGURE 1.—Histogram showing the proportion of A00015 alleles among progeny for markers on linkage group 5, which carries the segregation distorting locus *Sk*. Loci are arranged in the order in which they occur on the linkage group.

Fumonisin assays: All progeny were grown on cracked corn to assess fumonisin production. By the end of the 28-day incubation, most of the progeny had produced a purple pigment in amounts sufficient to turn the entire culture from light violet to a dark purple. The fumonisin phenotype segregated 46 producing:54 nonproducing and was scored simply as presence or absence (background level) of fumonisins in the culture medium, as expected for the *fum1* locus (DESJARDINS *et al.* 1992, 1995).

RAPD markers: We examined fifteen 10-mers from the B-primer kit manufactured by Operon for the identification of RAPD polymorphisms. Two of the 10-mers, B-11 (GTAGACCCGT) and B-12 (CCTTGACGCA), resulted in presence/absence polymorphisms with respect to bands between 600 and 800 bp in size. The *B-11* polymorphism segregated 56:50 and mapped on linkage group 3, while the *B-12* polymorphism segregated 71:50 and mapped on linkage group 6. Additional minor bands also were present but were not analyzed.

Vegetative compatibility group (VCG) tests: The 102 auxotrophic members of the mapping population were tested for vegetative compatibility with *nit* mutants derived in the same VCG as either of the parental strains.

None of the progeny tested were vegetatively compatible with either of these strains; the number of VCGs present in the mapping population was not determined. These data are insufficient to determine the exact number of *v*egetative *i*ncompatibility (*vic*) loci that are segregating in this cross, but the minimum number of segregating *vic* loci can be estimated. If **n** *vic* loci are segregating, then $2/2^n$ possible VCGs will be parental types. The probability of not getting a parental type is $(1 - (1/2)^{n-1})^{102}$. For five, six, seven and eight segregating *vic* loci in this cross these probabilities are as follows: 0.1, 3.9, 20.1, and 44.9%. Thus it is likely that at least seven or eight *vic* loci are heterozygous in this cross. This estimate could be biased if a *vic* locus is linked to *Sk*, since all but one of the progeny is Sk^k, or to either of the auxotrophic markers in the cross since only auxotrophic progeny were tested for vegetative compatibility.

Map construction and linkage group analyses: One hundred and fifty markers were scored on the 121 members of this mapping population; the genotype of each of these strains will be published elsewhere (XU and LESLIE 1996). One hundred and thirty-five markers were grouped into 12 linkage groups (Figure 2) with Mapmaker (LANDER *et al.* 1987) by selecting a minimum LOD of 4.0 and a maximum recombination frequency of 30%. The Kosambi mapping function was used to adjust recombination frequencies to cM. These linkage groups were correlated with the electrophoretic karyotype (XU *et al.* 1995) by hybridizing Southern blots of CHEF gels with many of the RFLP probes (Table 3). Chromosome bands corresponding to each linkage group were identified, and the linkage groups were given the same number as the corresponding chromosome.

Some additional markers can be placed in linkage groups if the stringency of the association required from members of the group is relaxed. Linkage group 9 has four markers (*7E29, 15P45, 7E41* and *23E*) that form two groups that are distal to the other six markers in this linkage group. If the recombination frequency parameter is relaxed from 30 to 35%, then these markers are all associated with this linkage group. Since all four of the probes also hybridize to the chromosome *8/9* doublet on the CHEF gels, we have placed these markers at opposite ends of linkage group 9. Similarly, probes *7E47S, 7E38* and *6E67,* which are tightly linked to one another, were placed on one end of linkage group 1. These probes are all linked to probe *78E* ($P < 0.05$ for independence).

Some markers can be associated with linkage groups only on the basis of their hybridization with bands from the CHEF gel blots. Probes *5E* and *6E74* are linked to one another and both hybridized to chromosome *11* on CHEF gel Southern blots. In Figure 2 they are shown as part of linkage group 11, but their placement distal to *6E78* is arbitrary, and a distance of 50 cM has been used in determining the total map size. Markers *11P25* and *15P44* are linked (4.3% recombination) and probably belong on chromosome *1* distal of 6E51, since 11P25 hybridizes to chromosome *1*.

There are five markers that cannot be unambiguously associated with any of the linkage groups: *86E, 5E56, 7E58, 10P16,* and *11P28.* Of these five markers, two, *7E58* and *10P16,* were linked (7.8% recombination) but do not hybridize to the same chromosome on CHEF gel Southern blots and thus cannot be localized further without additional data. Markers *11P28* and *86E* appear to be associated with chromosomes *3* and *10,* respectively, based on CHEF gel data, but no linkage data confirm these assignments. Marker *5E56* hybridizes to the chromosome *4/5* doublet band and cannot be definitively associated with either of these chromosomes. These data are evidence that gaps remain in our map. If these unlinked markers are excluded, then the total map length is ~1450 cM, and the average ratio of physical distance to map distance is 32 kb/cM (Table 4). If the gaps are included at 50 cM/gap, then the total map distance is 1700 cM, and the average ratio of physical distance to map distance is ~27 kb/cM.

Of the 150 markers scored, 16 did not segregate in a 1:1 manner at the 5% significance level (7.5 expected), and 12 did not segregate 1:1 at the 1% significance level (1.5 expected). The apparent aberrant segregation of *P49* (linkage group 2) at the 5% level is probably due to chance, since some errors would be expected with a sample of 150 markers. The remaining aberrant segregations are associated with linkage groups 5, 6 and 7. Nine of the markers mapped to chromosome *5* and the segregation distortion is attributable to linkage to *Sk*. On linkage group 5, only markers *P7* and *5E34* segregate in a manner that is consistent with a 1:1 segregation, and their ratios are still skewed to the A00015 allele (Figure 1).

The remaining six markers with unusual segregation ratios are located on either linkage group 6 or 7. On linkage group 6, the aberrant segregation involves two loosely linked markers, *73E* and *12P27,* and in both cases there is an excess of A04643 parental types. On linkage group 7, aberrant segregation can be found for the tightly linked markers *6E7, 7E26* and *7E37,* and marker *6E18,* which is loosely linked to this cluster. Segregation for the linked marker *7E69* is also skewed, but the ratio is not quite statistically significant at the 5% level. On this linkage group the A00015 alleles predominate.

We also determined the number of cross-over events for each chromosome (Table 4). The average number of cross-overs per chromosome ranged from 0.116 for chromosome *12* to 1.686 for chromosome *2*. For chromosomes *3, 11* and *12* less than half of the progeny had a cross-over. We tested the distribution of cross-overs to determine if the number of chromosomes with zero, one, two, or three or more cross-overs was ran-

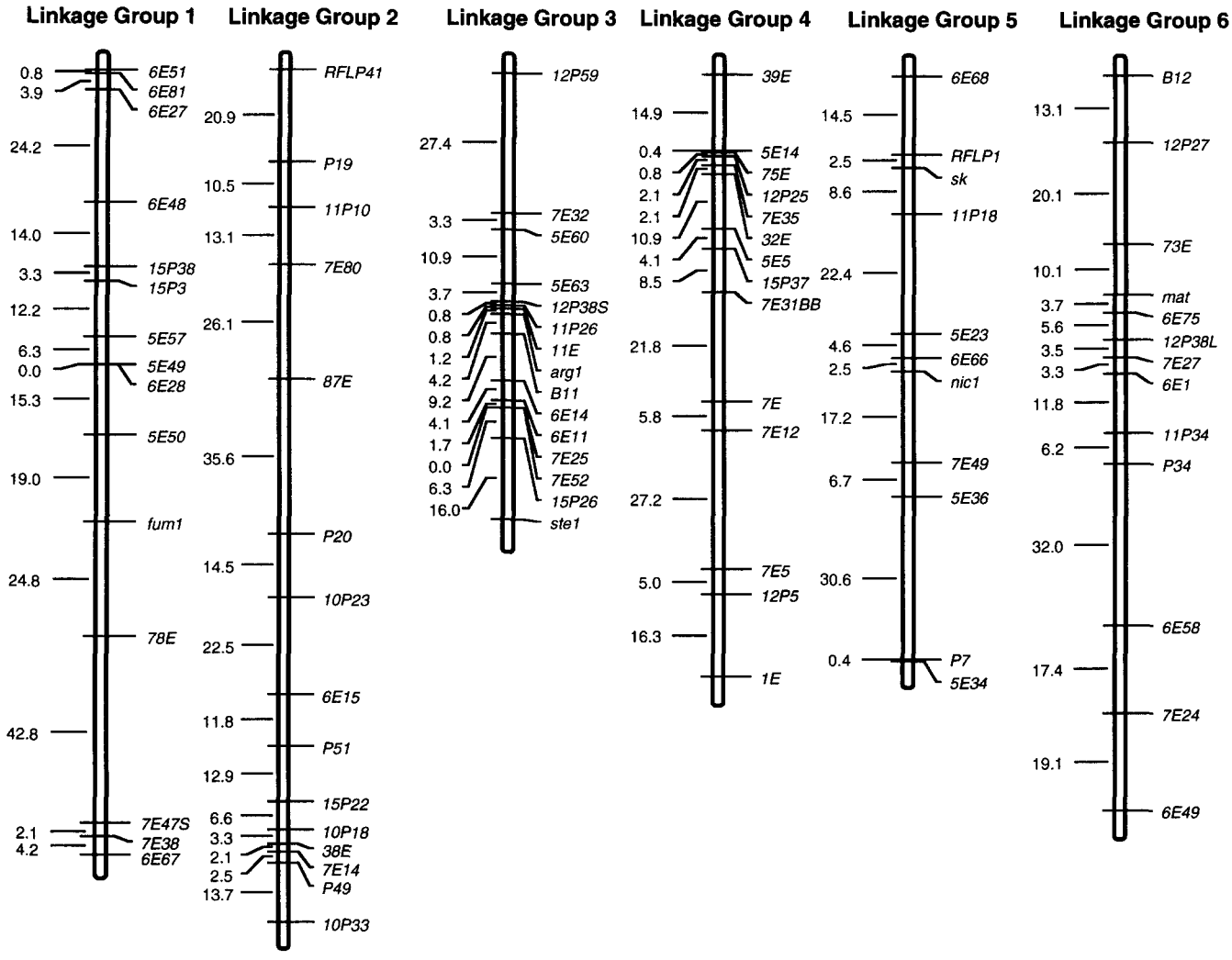

FIGURE 2.—Genetic map of *G. fujikuroi* mating population A (*F. moniliforme*). Linkage groups are numbered according to the molecular size of the corresponding chromosome on a CHEF gel (XU *et al.* 1995). The largest chromosome is number *1*. Chromosome *11* is composed of two linkage groups separated by a double line. The markers in both linkage groups hybridize to chromosome *11*. Locus names are defined in Table 3.

domly distributed. The distribution was not significantly different from random for chromosomes *1, 3, 4, 7, 10, 11* and *12* but was significantly different for chromosomes *2, 5, 6, 8* and *9*. When the distribution was significantly different from random, then the number of chromosomes with zero or three or more cross-overs is smaller than expected and the number of chromosomes with one and, occasionally, two cross-overs is larger than expected. The departure from random provides evidence that chiasma interference is positive.

Chromosome *12*: Four probes (5E32, 6E88, 7E19 and 11P43) hybridize to the smallest chromosome band on CHEF gel Southern blots and map to linkage group 12. When the mapping population was scored for these markers, four of the progeny (34, 64, 118 and 121) had lost the sequences associated with markers *5E32, 6E88* and *11P43*. With respect to marker *7E19*, progeny 34, 118 and 121 had no hybridizing sequences while progeny number 64 appeared to have alleles that had originated from both parents. Chromosome *12* was missing in CHEF gel preparations of progeny 34, 118 and 121, and was slightly larger than the parent for progeny 64.

DISCUSSION

Properties of the map: We have constructed a genetic map of *G. fujikuroi* mating population A (*F. moniliforme*) consisting of 12 linkage groups that have been correlated with chromosomes resolved via CHEF gel electrophoresis. Linkage groups and chromosomes share the same number and are numbered on the basis of physical size, with the largest chromosome being *1* and the smallest *12*. The electrophoretic karyotype (XU *et al.* 1995) contained two bands (chromosomes *4/5* and *8/9*) that were assumed to be doublets based on staining intensity. The recombination data we present here are consistent with this 12-chromosome interpretation of the CHEF gel data. The map we have developed

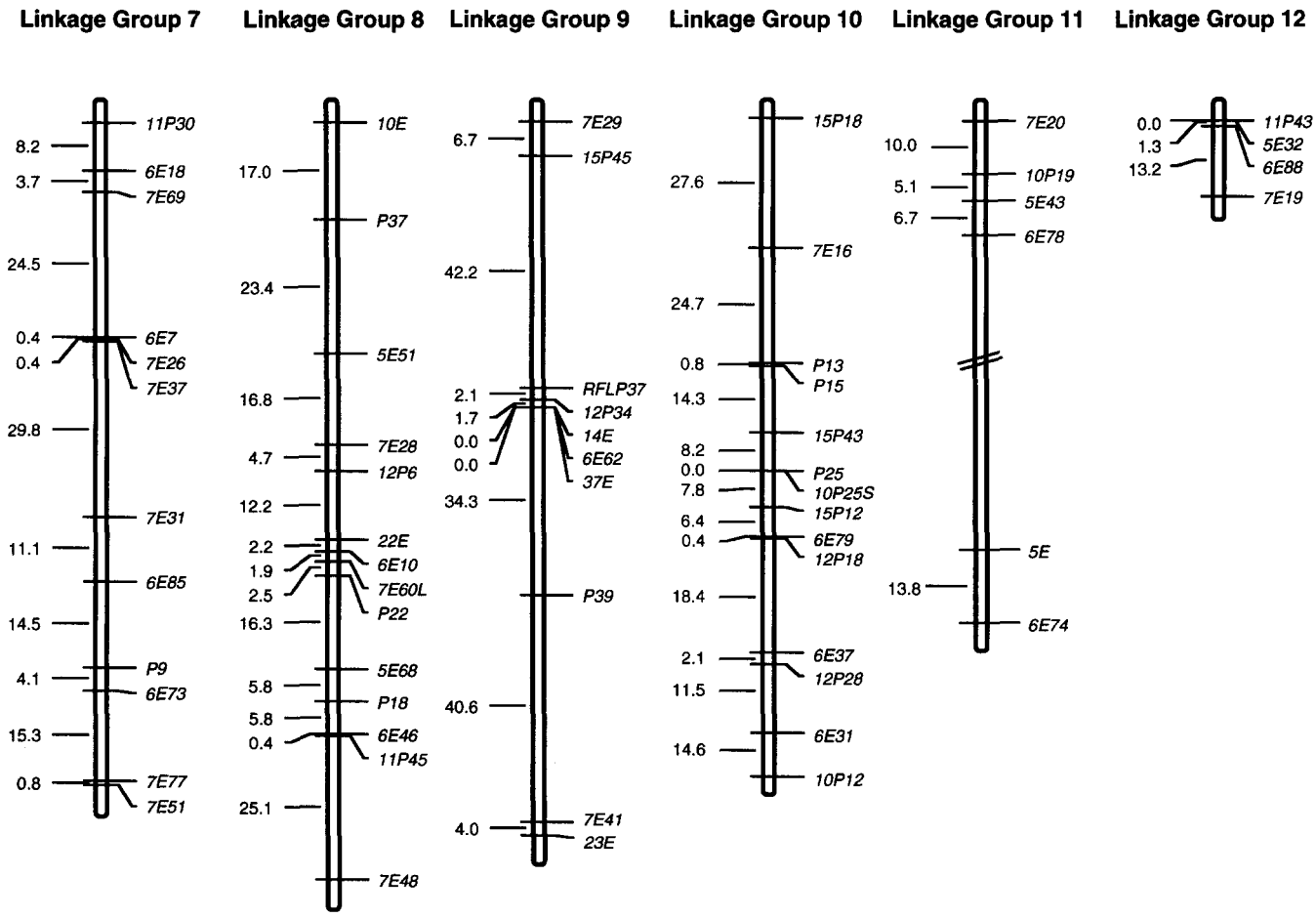

FIGURE 2.—*Continued*

can be used to assign cloned genes or DNA probes to chromosomes and, as the initial step in cloning efforts that require chromosome walking, to map quantitative trait loci, to compare genome organization of other species within the genus, to construct and evaluate isogenic lines, and as a source for markers in population genetic studies.

Our map is based on the segregation of 150 biochemical, molecular and morphological markers in a mapping population with 121 members that originated as ascospore progeny of two unrelated parents. The most recent previous map of this organism (PUHALLA and SPIETH 1983, 1985) contained four linkage groups and was based on the segregation of 15 morphological and biochemical markers in a series of mapping crosses. Their linkage group I contains *nic1* and corresponds to our chromosome *5;* their linkage group II contains *arg1* and corresponds to our chromosome *3.* We cannot orient our map directionally with respect to theirs, however, because we have only one marker in common on each of the two linkage groups. PUHALLA and SPIETH's linkage groups III and IV cannot yet be correlated with our chromosomes since we have not included any of the four markers they used to identify these linkage groups in our crosses. PUHALLA and SPIETH also had data on mating type segregation but could not detect linkage between *matA* and any other markers, making it unlikely that either linkage group III or IV corresponds to our present chromosome *6.*

The physical and recombinational sizes of the *G. fujikuroi* chromosomes are not well correlated (Table 4). Averaged over the entire genome the kb/cM ratio is ~32, but ranges from 58 kb/cM for chromosome *1* to 16 kb/cM for chromosome *10.* The genetic map still has some gaps, however, and as additional markers are incorporated into these gaps the total genetic length of the map will increase and reduce the kb/cM ratio. With the exceptions of chromosomes *3, 11* and *12,* the amount of recombination per kb of DNA generally increases as the chromosomes get smaller.

We also examined the number of cross-overs per chromosome. For seven of the 12 linkage groups, the distribution of the number of cross-overs per chromosome was not significantly different from random. For the other five chromosomes, there was generally an excess of single or double cross-over types and a shortage of chromosomes with no cross-overs or with three or more cross-overs. This result indicates that positive chiasma interference occurs in *G. fujikuroi.* The maximum number of cross-overs per chromosome that we

TABLE 4

Distribution of cross-over events for each chromosome

Chromosome			Estimated no. of cross-over events per chromosome							
No.	Mbp[a]	cM[b]	None	1	2	3	4	5	6	Avg.
1	10	173	29	47	31	10	4	0	0	1.36
2	6.5	196	11	48	42	10	9	0	1	1.69
3	4.9	90	63	40	12	6	0	0	0	0.68
4	4.1	120	32	56	26	5	2	0	0	1.08
5	4.0	110	34	52	32	3	0	0	0	1.03
6	3.6	146	25	58	30	5	0	1	2	1.24
7	3.0	113	35	58	22	5	1	0	0	1.00
8	2.6	134	24	59	28	7	3	0	0	1.22
9	2.5	132	33	52	31	5	0	0	0	1.07
10	2.2	137	41	56	19	3	2	0	0	0.92
11	2.0	86	104	16	1	0	0	0	0	0.15
12	0.7	15	107	14	0	0	0	0	0	0.12
Total	46.1	1452								

[a] Size of chromosome in megabase pairs (XU *et al.* 1995).
[b] Size of linkage group in cM (Figure 2).

observed was six, and only four chromosomes had five or more cross-overs on them. Chromosomes *3, 11* and *12* all averaged less than one crossover per bivalent and are of interest because it is hypothesized that at least one exchange per tetrad is required for proper meiotic disjunction (DARLINGTON 1937; GRELL 1962, 1976; LOPEZ-LEON *et al.* 1991; ZETKA and ROSE 1992; HAWLEY *et al.* 1993a,b). The relative deficiency in crossovers for chromosome *11* might be due to undetected crossovers that would be identified if additional markers were available to link the two different groups of markers that are known to hybridize to this chromosome. Chromosomes *3* and *12* do not have any obvious gaps in their linkage maps although there could be segments missing at one or both of the ends of these chromosomes. If these chromosomes fail to form crossovers at a sufficently high rate, then they would probably segregate through "distributive pairing" at meiosis, which usually occurs on the basis of size. Chromosomes *3* and *12* are probably sufficently different in size (4.9 and 0.7 Mb, respectively) that they are unlikely to pair with one another on the basis of size. A lack of proper meiotic disjunction could explain the loss of chromosome *12* in some of the progeny. A similar loss of chromosome *3* also might occur but would be lethal if it carried essential genes, which chromosome *12* evidently does not.

The only evidence for a reciprocal translocation involves markers *7E58* and *10P16,* which are genetically linked (7.8% recombination) but hybridize to different chromosomes (*1,* and *3* and *11,* respectively) on CHEF gel blots. If such a translocation exists, then it must be relatively small since there is no difference in chromosome size based on CHEF gel analyses and neither *7E58* nor *10P16* were genetically linked to other markers on the chromosomes to which these markers hybridized.

The distribution of markers on the map is not uniform. At present the map spans 1450 cM, but additional map expansion to 1700, or greater, seems likely given the unlinked markers that still have not been associated with any particular portion of the map. If there are other gaps that have not been detected, or if the known gaps prove to be larger than we now estimate, then this map size could increase even further. In regions of particular interest, *e.g.,* near *fum1* on chromosome *1,* the characterization of this mapping population through bulked-segregant analysis (MICHELMORE *et al.* 1991) might identify markers more tightly linked than those that are presently available. At the other extreme, clusters of markers are prominent on chromosomes *3* and *4* and somewhat less prominent on chromosome *6.* The cluster on chromosome *6* may be of use as a starting point from which *matA* can be cloned.

Spore killer: Fungal spore killers cause a meiotic drive phenomenon and are best described in *Neurospora* spp. but are also known in *G. fujikuroi* and *Cochliobolus heterostrophus* (see TURNER and PERKINS 1991; RAJU 1994). In both Neurospora and in Cochliobolus differences in the *Sk* phenotype are associated with chromosome abnormalities; a large recombination block in Neurospora (CAMPBELL and TURNER 1987) and an insertion and a translocation in Cochliobolus (TZENG *et al.* 1992). In Neurospora, *Sk-2* and *Sk-3* from *N. intermedia* have been introgressed into *N. crassa* and mapped to a region that is tightly linked to the centromere of linkage group III.

The *G. fujikuroi* mapping population we analyzed consists only of random ascospore progeny, so we could not identify centromere locations, which requires tetrad data. We do not have any evidence to suggest that heterozygosity for *Sk* in our mapping population is associated with a chromosome rearrangement or recombina-

tion block. Based on our genetic map, probe RFLP1 could be used to initiate a chromosome walk to clone the *Sk* gene and to begin the molecular characterization of this phenomenon. At a distance of 2.5 cM, the *Sk* locus should be no more than 80–100 kb away. This target is a particularly attractive one since no *Sk* gene has yet been cloned from any fungus.

Fumonisins: Fumonisins are a family of structurally closely related metabolites, of which fumonisin B_1 is the most prominent (NELSON *et al.* 1993). The *fum1* locus has been previously described, and the available mutants produce fumonisin at or below background levels (DESJARDINS *et al.* 1992, 1995). Recently another locus, *fum4,* which also maps to this region has been identified (PLATTNER *et al.* 1996). Thus it appears that there may be a cluster of genes involved in the production of fumonisins that map to this portion of chromosome *1.* Cloning the genes in this region via map-based cloning techniques is an important scientific and economic objective. Unfortunately, the closest markers presently flanking *fum1, 5E50* and *78E* are 19 and 24.8 cM away, respectively. If the ratio of 58 kb/cM for chromosome *1* is correct for this region, then these two markers are both more than a megabase away from the desired region. Filling in the gaps in this region at least partially will be necessary before the *fum* genes in this region will be accessible to map-based cloning strategies.

Chromosome *12*: Chromosome *12* appears to be a dispensable chromosome since the four progeny in which it has been partially or completely deleted have no obviously abnormal phenotype. Our Southern blot hybridizations indicate that this material is completely missing and that it has not simply been transferred to another chromosome in the genome. Progeny number 64 is particularly interesting since it appears to have lost one portion of chromosome *12* and duplicated another portion of it. The mechanism underlying this apparently gross chromosome rearrangement is unknown. Dispensable chromosomes have been reported in other fungi including *Nectria haematococca* (MIAO *et al.* 1991) and *C. heterostrophus* (TZENG *et al.* 1992). In *N. haematococca* this chromosome has been shown to carry determinants that are important in determining the pathogenic capability of the organism.

Map utilization: This map is the first comprehensive genetic map for any member of the genus Fusarium. Members of this genus include species that cause billions of dollars of crop damage per year as plant pathogens and produce secondary metabolites that are important for the commercial fermentation industry, such as gibberellic acid and zearalenone (HIDY *et al.* 1977; CERDA-OLMEDO *et al.* 1994), and mycotoxins such as T-2, nivalenol, and fumonisins that are major hazards in food and feedstuffs worldwide (MARASAS *et al.* 1984; NELSON *et al.* 1993). One use for this map will be to compare the closely related biological species in the *G. fujikuroi* species complex; the probes used could also form the basis of similar maps for these organisms.

Even though this genetic map is not saturated and has some gaps, it can be used to locate other unmapped genes and to map additional markers. Uncloned classical markers in *G. fujikuroi* are primarily in the same genetic background as the A00015 parent (PUHALLA and SPIETH 1983, 1985) and could be incorporated into this map through the analysis of progeny between A04643 and a strain carrying the uncloned classical marker. This genetic map is especially useful for mapping genetically under-studied traits, such as vegetative compatibility and quantitative trait loci that have a multigenic basis and require a comprehensive genetic map before they can be studied. Because the low content of repetitive DNA and the relatively small average ratio of kb/cM, map-based cloning of other interesting genes, such as *matA* and *Sk,* is a feasible strategy since there are closely linked markers that map near these loci. In the short term, most of our work with this map will focus on filling gaps in crucial areas, such as near *fum1,* and on placing centromeres.

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