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**Molecular Karyotypes of Pathogenic Strains of
*Fusarium Oxysporum***

H.C. Kistler, T. Katan, D. Zamir

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MOLECULAR KARYOTYPES OF PATHOGENIC STRAINS OF
FUSARIUM OXYSPORUM

Investigator's Names

Investigator's Institutions

H. C. Kistler

University of Florida

T. Katan

ARO, Bet Dagan

D. Zamir

The Hebrew University

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H. C. Kistler
Signature
Principal Investigator

Signature
Institution's Authorizing Official

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(Results of RFLP and RAPD of FOL and FORL)	

Abstract

Genetic diversity of pathogenic strains of the fungus *Fusarium oxysporum* was determined by analysis of electrophoretic karyotype, as well as by DNA variation detected by Restriction Fragment Length Polymorphisms (RFLPs) and Random Amplified Polymorphic DNAs (RAPDs). The electrophoretic karyotypes for 130 isolates of the fungus pathogenic to tomato, melon, and banana were analyzed. Electrophoretic karyotype variation, reflected in differences in apparent chromosome number and genome size, was observed even among isolates from the same host and sub specific category. Sub specific categories studied were forma specialis, vegetative compatibility group (VCG) and race. Chromosome number and genome size variation was less for isolates within the same VCG than for the collection of isolates as a whole. RFLP and RAPD analysis were performed on 62 isolates of *F. oxysporum* from tomato and melon. Phylogenetic trees were constructed from genetic diversity data. The results support the hypothesis that isolates belonging to the same VCG originate from a single ancestor compared to other isolates. The results do not support the hypothesis that all isolates belonging to the same forma specialis originate from a common ancestor. These conclusions have profound implication for breeding resistance to diseases caused by particular formae speciales of *F. oxysporum*.

Objectives

As stated in the original proposal, our objectives were:

- "I To determine the extent of variation in chromosome size and number within the species *F. oxysporum*
- II To determine if chromosome size and number vary within subspecies groupings of *F. oxysporum* such as forma specialis, VCG and physiologic race.
- III To determine the mechanisms by which chromosome variation may occur in these asexually reproducing fungi and to suggest genetic relationships among forms based on this variation".

Based on our research, published and in press, we have accomplished these goals as will be outlined in the following section. We have also extended the goals of this study to include determination of genetic diversity in *F. oxysporum* based on RFLP and RAPD analysis.

Relevant data, methodology, results and discussion

Objective I : Determine the extent of variation in chromosome size and number within the species *F. oxysporum*.

We have conducted a survey of 118 isolates of *F. oxysporum* f. sp. *cubense* (FOC) and determined chromosome number and genome size based upon electrophoretic karyotype. This is the most comprehensive investigation of electrophoretic karyotype variation for any fungus to date. The chromosome number for the 118 isolates ranged from 9 to 14 and the genome size for these same isolates ranged from 32.1 to 58.9 Mbp. The median chromosome number value was 11 with a modal value of 12. Mean genome size was 43.3 Mbp (standard deviation = 5.8; standard error = 0.54). Both length and number differences contributed to intraspecific variation in calculated genome size.

Objective II. Determine if chromosome size and number vary within subspecies groupings of *F. oxysporum* such as forma specialis, VCG and physiologic race.

Both chromosome number and genome size were strongly correlated with the subspecies grouping VCG. Isolates examined represented 11 VCGs. Genome size variation within a VCG was invariably less than that of the population as a whole as determined using ANOVA. A high R^2 value (0.721) with a low coefficient of variance was found for genome size/VCG, indicating that approximately 72% of the data is accounted for by preexisting groupings based on VCG at $P = 0.0001$. These findings strongly support the conclusion that distribution of genome size among the 11 VCGs surveyed deviates strongly from the null hypothesis and that a positive correlation exists between genome size and VCGs. Ranked mean testing of transformed genome size data produced two groups of VCGs with significantly different genome size.

Chromosome number variation within all but one VCG never exceeded ± 1 chromosome. The exception was isolates within VCG 0120 which had four possible chromosome numbers. A direct, positive linear relationship existed between chromosome number and genome size. Regression analysis yielded a R^2 of 0.995 and coefficient of variance of 7.2 at $P = 0.0001$.

Analysis of variance was used to compare mean genome size for isolates from different geographic locations. A highly significant effect of geographic location ($F = 14.5$, $P < 0.0001$) was noted. Isolates also were not randomly distributed geographically with respect to VCG. For example, the 29 isolates examined from Malawi represented only three VCGs. These VCGs were among those having the largest GS values.

Electrophoretic karyotypes obtained for six *F. oxysporum* f. sp. *lycopersici* (FOL) and six *F. oxysporum* f. sp. *melonis* (FOM) isolates also revealed considerable variation in chromosome number and size when isolates of different VCGs, races and origin were compared. Chromosome numbers ranged between 6 and 10 (or more) and in size from less than one Mb to over 5.7 Mb. Genome size was estimated at 19.5 - 32.4 Mb. These studies will be continued beyond the termination of the project.

Objective III. To determine the mechanisms by which chromosome variation may occur and to suggest genetic relationships among forms based on this variation.

Deletion, duplication, translocation, and the presence of dispensable chromosomes all have been suggested to be a source of genomic variation in

filamentous fungi. Duplication associated with aneuploidy may play a role in determining genome size in *F. oxysporum* because of the strong positive linear relationship between chromosome number and genome size (see Objective II above). Such a relationship would be expected if the presumed haploid genome of *F. oxysporum* were increasing in size either by the retention of full or partial disomes or by the accumulation of unique supernumerary chromosomes of variable size.

For objective III, we sought to determine the approximate level of genetic duplication in FOC possibly resulting from aneuploidy. The degree of genetic duplication within 14 vegetative compatibility groups of *F. oxysporum* was tested. Eight genomic clones that hybridized to single copy sequences in Southern hybridization with restriction enzyme-digested fungal DNAs were used to probe chromosomes of the fungus separated by pulsed field electrophoresis. As expected, most clones hybridized to single chromosome bands. However, two of eight "single-copy" clones hybridized to two chromosomes in some, but not all, isolates examined. These findings are at odds with the assumption that *F. oxysporum*, as an imperfect fungus allied with the Ascomycetes, is a strictly haploid organism. The level of genetic duplication however is consistent with electrophoretic karyotype analysis indicating that intraspecific differences in genome size and chromosome variation may be explained, at least in part, by persistent genetic duplication.

Unexpectedly, however, the conserved single copy clones chosen hybridized primarily only to three chromosomes. These linkages were conserved for most isolates. This non-random distribution of probes to chromosomes suggests that the chosen sequences, those hybridizing to all isolates of the fungus, are found only on a particular subset of chromosomes.

Additional Objectives: To estimate genetic relatedness and infer phylogenetic origin among isolates differing in forma specialis, VCG, race and geographic origin.

Two formae speciales of the *F. oxysporum* pathogenic on tomato were analyzed for DNA polymorphisms. FOL and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) cause tomato wilt and crown rot disease, respectively. FOM, pathogenic on melon, was used as an outgroup for comparison. The collection examined included 27 isolates of FOL, representing races 1 and 2 and the vegetative compatibility groups 0030 and 0031; 32 isolates of FORL representing 11 VCGs or subgroups, and three isolates of FOM. The isolates originated from different geographical areas, worldwide.

Of the 15 RFLP probes originating from genomic DNA of FOL, seven revealed polymorphisms in the analyzed isolates. They allowed unique characterization of different VCGs by the presence or absence of homologous sequences or by differences in banding patterns. These probes enabled, in many cases, differentiation between sub-groups within VCGs of FORL. For example, FOL probes FG213 and FG281 hybridized to isolates of VCG 0030 but not VCG 0031. In contrast, isolates collected from specific geographic areas or isolates of the same race did not show unique common patterns. In addition, a few probe-restriction enzyme combinations were found that could differentiate between the three formae speciales FOL, FORL and FOM.

All RAPD primers tested successfully amplified DNA fragments in all tested groups. Four of the primers enabled to distinguish between VCGs 0030 and 0031 of FOL. In FORL, the RAPD analysis revealed more polymorphism than the RFLP analysis and most of the isolates showed unique banding patterns. Thus a pattern unique to all isolates from a given VCG was found in only a few cases. As in the RFLP analysis, the RAPD analysis did not distinguish isolates on the basis on geographic origin or race. The RAPD analysis did not provide a distinction between the two formae speciales, FOL and FORL.

The phylogenetic trees derived from the RFLP data separated among VCGs but not between FOL and FORL. The trees derived from the RAPD data partly separate VCGs but again, not FOL and FORL. The results of this study support the hypothesis that isolates belonging to the same VCG originate from a single ancestor while VCGs belonging to one forma specialis are not necessarily more closely related than VCGs belonging to different formae speciales. The conclusion that all isolates belonging to the same VCG originate from the same ancestor supports the conclusion that new races appeared independently in each VCG.

Description of cooperation

The degree of interaction and cooperation among the American and Israel scientists throughout the entire project was excellent. On October 4, 1991 Dr. Zamir visited the University of Florida, Gainesville and delivered a seminar entitled "Classical and Molecular Genetics in a Tomato Breeding Program". At that time, Dr. Zamir reviewed the work of individuals in Dr. Kistler's lab and made suggestions for future work. The details for implementation of research goals were discussed at that time between Dr. Zamir and Dr. Kistler.

On May 14, 1992 Dr. Kistler presented a seminar at the Faculty of Agriculture, Rehovot entitled "Chromosome variability and instability in plant pathogenic *Fusarium* spp.". Dr. Kistler met with members of Dr. Zamir's laboratory and traveled to the Volcani Center to meet with Dr. Katan. Dr. Kistler presented preliminary results of the project. Dr. Katan suggested clones from previous work that would be useful to Dr. Kistler and Dr. Zamir arranged for the clones to be taken back to the U.S. by Dr. Kistler. During his visit to the Faculty of Agriculture Dr. Kistler helped to set up a CHEF apparatus and supervised the first electrophoresis run by a graduate student, Sari Kalman. Arrangements were made for Ms. Kalman to visit Dr. Kistler's lab.

During August, 1992, Ms. Kalman visited Dr. Kistler's lab in Gainesville to gain more experience in pulse-field gel electrophoresis, especially in separating large, chromosome-sized DNAs. By bringing chromosome preparations from Israel, Ms. Kalman was able to complete two electrophoresis runs during her visit and obtained the first separation of chromosomes >2 Mb in *F. oxysporum* f. sp. *lycopersici*. During her visit, Ms. Kalman also brought single copy DNA clones from Dr. Katan's lab for use in study of genetic duplication in FOC. These clones, from FOL, were used to probe Southern blots of electrophoretic karyotypes from FOC and formed the basis of the study published in Current Genetics (Kistler, et al., 1995).

Evaluation of the research achievements with respect to the aims of the original research proposal and emphasizing the benefit to agriculture.

Plant diseases caused by *Fusarium oxysporum* are important to a wide variety of crops including tomato, melon and banana. The most common approaches for disease control of these pathogens is use of resistant plant varieties and pre-plant soil fumigation. Methyl bromide currently is the major soil fumigant used for control of diseases caused by soil-borne *Fusarium oxysporum*. With the signing of the Montreal Protocol in 1992, the production and use of methyl bromide will be sharply curtailed and, in the US, completely eliminated by the year 2001. Alternative methods for disease control must be developed. Knowledge of the genetic makeup of these soil pathogens will assist in breeding crops resistant to the pathogens. This is especially critical knowledge since FOL, FORL and FOC appear to consist of two or more genetically distinct lineages. These different lineages must be taken into account when developing resistant varieties of plants.

List of all publications resulting from the research project

Kistler, H.C. and V.P.W. Miao. 1992. New modes of genetic change in filamentous fungi. *Annu. Rev. Phytopathol.* 30:131-152.

Boehm, E.W.A., R.C. Ploetz and H.C. Kistler. 1994. Statistical analysis of electrophoretic karyotype variation among vegetative compatibility groups of *Fusarium oxysporum* f. sp. *cubense*. *Molecular Plant-Microbe Interactions* 7:196-207.

Kistler, H.C., U. Benny, E.W.A. Boehm, and T. Katan. 1995. Genetic duplication in *Fusarium oxysporum*. *Current Genetics* (In press; accepted for publication 1/25/95).

Two serious diseases of tomato are caused by different formae speciales of Fusarium oxysporum: F.o. lycopersici (FOL) causes a wilt disease and F.o. radicle-lycopersici (FORL) causes the crown-and-root rot disease. The diseases occur in all tomato growing regions of the world.

In FOL three physiologic races are known, of which races 1 and 2 occur worldwide. The major vegetative compatibility group (VCG) of FOL (VCG 0030) has a worldwide distribution and contains isolates of all three races. Two minor VCGs (0031 and 0032) have more limited geographic distribution and contain isolates of races 1 and 2. In addition, many isolates have been found that constitute single member VCGs. A genetic study of a collection of FOL, applying cluster analysis of RFLP phenotypes generated with genomic clones from an isolate of FOL, had revealed that isolates are genetically more similar to other isolates in the same VCG than to those in other VCGs; isolates in VCG 0032 were more similar to isolates in VCG 0030 than those in VCG 0031, and single-member VCGs could be interpreted as mutational derivatives of multiple-member VCGs. Less variation was observed between isolates of the same VCG than between those of different VCGs that belonged to the same race or shared a common geographic origin.

In FORL, no physiologic races are known but seven VCGs (0090-0097) have been identified that may vary in their geographic distribution. The genetic relatedness among isolates of FORL or between FOL and FORL is not known.

In this study, the RFLP and RAPD-PCR methods were used to estimate the genetic relatedness between isolates of FOL and FORL on the basis of f.sp., race, VCG and geographic origin. In particular we asked if isolates of each f.sp. are more similar to each other than to those of the other f.sp., and if strains of the tomato pathogens are more closely related to each other than to strains of the non-tomato pathogen F.o. melonis (FOM, pathogenic to muskmelon) that were used as an outgroup in this study.

The 62 monoconidial isolates used in this study are listed in Table 1. FOL was represented by 27 isolates of VCGs 0030 and 0031, including races 1 and 2 from diverse geographic origins. FORL was represented by 32 isolates from seven VCGs (a total of 11 VCGs and subgroups) from several countries. FOM was represented by three isolates (race 0, VCG 0135). The genomic clones (Table 2) used as probes for RFLP were derived from strain FOL-0-1078 (race 2, VCG 0030) and had been used previously (Elias et al 1993). Three restriction enzymes were used: Eco RI, Eco RV, DraI. The nine oligonucleotides used for RAPD-PCR were the following 10-mer primers from Operon (kit OPL): L1, L2, L3, L4, L7, L10, L11, L12, L13. For each isolate, the banding pattern obtained from RFLP and RAPD were analyzed. Each band was recorded as 1 (present) or 0 (absent) and the data were processed using EXCEL and PAUP 3.1 computer programs. Phylogenetic "most parsimonious" consensus trees were constructed, using the semistrict option, for each f.sp. separately and for all three f.sp. combined. Electrophoretic karyotyping of FOL was done on a limited scale only (due to technical problems related to a defective CHEF-DRII apparatus supplied by BIORAD, and unsatisfactory service by the supplier and its representative).

Chromosomes embedded in LMP agarose were separated by electrophoresis for 144 hours (6 days) using the following conditions: 0.5 TAE, 60V, pulse time 1000-3200 sec. Yeast chromosomes of *S. pombe* and *H. wingei* served as DNA size markers.

RFLP patterns were generated for the 62 isolates, using three restriction enzymes and 15 probes. Examples of autoradiograms obtained from Southern blots of genomic DNA cut with *EcoRV* and hybridized to three probes are shown in Fig. 1, and the *DraI*/FG 261 combination is shown in Fig. 2. Copy number per genome of each isolate, of sequences homologous to each probe, was estimated from the minimal number of bands labelled by that probe (Table 3): these numbers ranged from 0 (no labelling) to over 5 (repetitive, e.g. FG 163). Table 4 shows that copy numbers could not differentiate between ff.sp. but in a few cases VCGs within f.sp. could be partially distinguished. An arbitrary serial number was assigned to each RFLP banding pattern (Table 5). four probes enabled the distinction between FOL and FORL with at least one enzyme, and 10 probes differentiated the tomato pathogens from FOM. No single enzyme probe combination generated a pattern specific for a f.sp.

Phylogenetic trees were constructed using various sets of RFLP data obtained with the three enzymes. Fig. 3 demonstrates separation between FOL VCGs 0030 and 0031 but not between races 1 and 2; FOM isolates are also grouped separately although, with *EcoRV*, FOL VCG 0031 seems to be related more closely to FOM than to FOL VCG 0030. Fig. 4 shows that RFLP could not distinguish the sub-groups within VCGs of FORL; furthermore, some VCGs were clustered into larger groups (A=VCGs 0090, 0091, 0092, 0096; B=VCGs 0094, 0097). FOM was separated also from FORL. When all three ff.sp. were included in the same trees (Fig. 5) VCGs or their clusters remained as units but their relative positions depended on the enzyme used; thus, using *DraI*, FOL VCGs 0030 and 0031 shared a common branch separated from FOM, whereas using *EcoRV* FOL VCG 0031 and FOM shared a common branch separated from FOL VCG 0030. From the consensus trees, the minimal and maximal numbers of genetic changes between isolates of the same or different VCGs were calculated (Table 6): within VCGs the numbers ranged from 0-13 whereas between VCGs it was generally higher. Variable results were obtained with different enzymes, and changes between VCGs within ff.sp. were not always fewer than changes between ff.sp.

Genomic DNA was amplified by the PCR technique using nine primers and the amplified fragments were resolved by electrophoresis (Fig. 6) to give RAPD patterns. A total of 49 unambiguous and consistent bands were recorded and comparisons of the RAPD patterns were based on the assumption that bands of the same size represent similar genomic sequences. Each banding pattern was given an arbitrary serial number. The RAPD patterns obtained for the various VCGs are summarized in Table 7. The patterns did not distinguish between FOL and FORL or between subgroups within VCGs of FORL, but with some primers FOM was distinguished from the tomato pathogens and VCGs could also be partially differentiated. In FOL, no banding pattern was race specific (Table 8). Phylogenetic trees were constructed using various sets of RAPD data. Fig. 7 demonstrates that the distance found between FOL and FORL was not larger than between VCGs of these ff.sp., and that their distances to FOM were not always larger than among themselves.

Table 1. isolates of FOL, FORL and FOM listed by code, VCG, race and origin

f.sp.	Code	Isolate	VCG	Race	Origin
FOL	BO	ATCC 34298	0030	1	קליפורניה-CA
	BN	ATCC 15643	0030	1	יוון-GR
	BZ	FOL-MT	0030	1	ישראל-IS
	CC	FOL-650	0030	1	ישראל-IS
	CH	FOL-1(ATCC 66044)	0030	1	ישראל-IS
	BO	FOL ITAM 6	0030	1	איטליה-IT
	BR	LEV 697 B	0030	1	ניו זילנד-NZ
	BS	LEV 1642	0030	1	ניו זילנד-NZ
	BW	FUS 37	0030	1	ניו זילנד-NZ
	BX	66125	0030	1	ניו זילנד-NZ
	BY	1391 F5	0030	1	פלורידה-FL
	CG	O-1078	0030	1	ישראל-IS
	BT	FOL-KB	0030	1	ישראל-IS
	BU	FOL-355 AA	0030	1	ישראל-IS
	BV	FOL-W 842	0030	1	ישראל-IS
	CA	FOL-W 345 M	0030	1	ישראל-IS
	CB	FOL-KA	0030	1	ישראל-IS
	CC	FOL-W841D(ATCC76535)	0030	1	ישראל-IS
	CE	FOL-F611T	0030	1	ישראל-IS
	CF	FOL-W1004D	0030	1	ישראל-IS
	CJ	FOL-1295(ATCC 66047)	0030	1	הולנד-NL
	CI	WCS 362	0030	1	דרום אפריקה-SA
	BP	ATCC 46933	0030	1	בלגיה-BE
	CN	01109 B	0031	1	לואיזיאנה-LA
	CL	B FOL 51	0031	1	קליפורניה-CA
	CK	DAV 139	0031	1	אוחיון-OH
	CM	OSU 451 B	0031	1	אוחיון-OH
FORL	AA	0-1090	0090 I	-	קנדה-CN
	AB	C 63 F	0090 I	-	ישראל-IS
	AC	DJV 78	0090 I	-	כרתים-CR
	AD	C 809 L	0090 II	-	ישראל-IS
	AE	C 608	0090 II	-	ישראל-IS
	AF	C 415	0090 II	-	ישראל-IS
	AG	C 696 A	0090 III	-	ישראל-IS
	AH	GS 711 b	0090 III	-	ישראל-IS
	AI	C 825 A	0090 III	-	ישראל-IS
	AJ	C 732	0091	-	ישראל-IS
	AK	C 56 Q	0091	-	ישראל-IS
	AL	SH 391 A	0091	-	אנגליה-UK
	AM	UK 9 B	0091 II	-	אוחיון-OH
	AN	OSU 471 C	0091 II	-	ישראל-IS
	AO	C 424	0091 II	-	ישראל-IS
	AP	C 832	0092	-	ישראל-IS
	AQ	C 609	0092	-	ישראל-IS
	AR	C 856 C	0092	-	ישראל-IS
	AS	C 201	0093	-	ישראל-IS
	AT	C 202	0093	-	ישראל-IS
	AU	C 204	0093	-	בלגיה-BE
	AV	O1152	0094 I	-	בלגיה-BE
	AW	O1166	0094 I	-	אנגליה-UK
	AX	UK 30	0094 I	-	אנגליה-UK
	AY	149 74 R	0094 II	-	אנגליה-UK
	AZ	UK 2-E	0094 II	-	אנגליה-UK
	BA	FU 90 5 d	0094 II	-	הולנד-NL
	BB	C 304 A	0096	-	ישראל-IS
	BC	C 622 A	0096	-	ישראל-IS
	BD	C 651	0096	-	ישראל-IS
	BE	01163 D	0097	-	בלגיה-BE
	BF	01169 C/(2)	0097	-	בלגיה-BE
FOM	CO	FOM318	0135	0	ישראל-IS
	CP	FOM637	0135	0	ישראל-IS
	CO	FOM714	0135	1	ישראל-IS

Table 2. Genomic clones of FOL isolate 0-1078 used as probes (Elias et al, 1993)

Clone	bp	Clone	bp	Clone	bp
FG 4	650	FG 103	1,000	FG 213	950
FG 25	1,100	FG 155	600	FG 261	1,500
FG 30	550	FG 163	2,000	FG 278	1,300
FG 34	800	FG 177	500	FG 281	2,000
FG 43	2,000	FG 236	2,000	FG 302	1,400

Fig. 1 RFLP patterns of DNA of isolates of FOL and FORL digested with *Eco* RV and hybridized to probes (a) FG213, (b) FG34, and (c) FG155.

Fig.2. RFLP Patterns of DNA of isolates of FOL and FORL digested with Dra I and hybridized to probe FG261.

Table 3. Estimated copy number of each probe in isolates of FOL, FORL and FOM

Code	VCG	Race	Ort	FG4	FG25	FG30	FG34	FG43	FG103	FG155	FG163	FG177	FG213	FG236	FG261	FG278	FG281	FG302
FOL	BO	0030	1	CA	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	BN	0030	1	GR	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	BZ	0030	1	IS	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	CC	0030	1	IS	1	1	1	3	3	1	1	5 <	1	1	1	1	2	1
	CH	0030	1	IS	1	0	-	2	2	1	1	5 <	1	1	1	1	2	1
	BQ	0030	1	IT	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	BR	0030	1	NZ	1	1	1	2	3	1	1	5 <	1	1	1	1	2	1
	BS	0030	1	NZ	1	1	1	3	3	1	1	5 <	1	1	1	1	2	1
	BW	0030	1	NZ	1	0	1	4	3	1	1	5 <	1	1	1	1	2	1
	BX	0030	1	NZ	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	BY	0030	1	NZ	1	1	1	4	2	1	1	5 <	1	2	1	1	2	1
	CG	0030	2	FL	1	1	1	4	3	1	1	5 <	1	1	1	1	2	1
	BT	0030	2	IS	1	1	1	3	3	1	1	5 <	1	1	1	1	2	1
	BU	0030	2	IS	1	1	1	3	3	1	1	5 <	1	1	1	1	2	1
	BV	0030	2	IS	1	1	1	3	3	1	1	5 <	1	1	1	1	2	1
	CA	0030	2	IS	1	1	1	4	3	1	1	5 <	1	2	1	1	2	1
	CB	0030	2	IS	1	1	1	3	3	1	1	5 <	1	2	1	1	2	1
	CD	0030	2	IS	1	1	1	3	2	1	1	5 <	1	2	1	1	2	1
	CE	0030	2	IS	1	1	1	4	2	1	1	5 <	1	2	1	1	2	1
	CF	0030	2	IS	1	0	1	4	3	1	1	5 <	1	2	1	1	2	1
	CI	0030	2	IS	1	0	1	4	3	1	1	5 <	1	2	1	1	2	1
	BP	0030	2	NZ	1	1	1	4	3	1	1	5 <	1	1	1	1	0	1
	CN	0031	1	SA	1	0	1	2	1	1	1	5 <	1	0	1	1	0	1
	CL	0031	1	BE	1	0	1	2	1	1	1	5 <	1	0	1	1	0	1
	CK	0031	2	LA	1	0	1	2	1	1	1	5 <	1	0	1	1	0	1
	QM	0031	2	CA	1	0	1	2	1	1	1	5 <	1	0	1	1	0	1
				OH	1	0	1	2	1	1	1	5 <	1	0	1	1	0	1
FORL	AA	0090 I	1	CN	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AB	0090 I	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AC	0090 I	1	CR	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AD	0090 II	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AE	0090 II	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AF	0090 II	1	IS	1	0	1	2	3	1	1	5 <	1	1	1	2	1	0
	AG	0090 III	1	IS	1	0	1	2	3	1	1	5 <	1	1	1	2	1	0
	AH	0090 III	1	IS	1	0	1	2	3	1	1	5 <	1	1	1	2	1	0
	AI	0090 III	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AJ	0091 I	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AK	0091 I	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AL	0091 I	1	UK	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AM	0091 II	1	OH	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AN	0091 II	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AO	0091 II	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AP	0092	1	IS	1	0	1	2	2	1	1	5 <	1	1	1	2	1	0
	AQ	0092	1	IS	1	0	1	2	2	1	1	5 <	1	0	1	1	1	1
	AR	0092	1	IS	1	0	1	2	4	1	1	5 <	1	0	1	1	1	1
	AS	0093	1	IS	1	0	1	2	4	1	1	5 <	1	0	1	1	1	0
	AT	0093	1	IS	1	0	1	2	4	1	1	5 <	1	1	1	2	1	0
	AU	0093	1	IS	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	AV	0094 I	1	BE	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	AW	0094 I	1	BE	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	AX	0094 I	1	UK	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	AY	0094 II	1	UK	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	AZ	0094 II	1	UK	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	BA	0094 II	1	NL	1	0	1	1	2	1	1	5 <	1	1	1	2	1	0
	BB	0096	1	IS	1	0	1	2	1	1	1	5 <	1	1	1	2	1	0
	BC	0096	1	IS	1	0	1	2	1	1	1	5 <	1	1	1	2	1	0
	BD	0096	1	IS	1	0	1	1	1	1	1	5 <	1	1	1	2	1	0
	BE	0097	1	BE	1	0	1	1	1	1	1	5 <	1	1	1	2	1	0
	BF	0097	1	BE	1	0	1	1	1	1	1	5 <	1	1	1	2	1	0
FOM	CO	O135	0	IS	1	0	1	2	2	1	1	5 <	1	0	1	1	1	1
	CP	O135	0	IS	1	0	1	2	2	1	1	5 <	1	0	1	1	1	1
	CQ	O135	0	IS	1	0	1	2	2	1	1	5 <	1	0	1	1	1	1

-, Copy number not known

Table 4. Copy number of each probe arranged by VCGs

VCG		FG4	FG25	FG30	FG34	FG43	FG103	FG155	FG163	FG177	FG213	FG236	FG261	FG278	FG281	FG302
FOL	OO30	1	1(0)	1	2-4	2,3	1	1	5<	1	1,2	1	1	1	2	1
	OO31	1	0	1	1,2	1	1	1	5<	1	0	1	1	1	0	1
FORL	0090 I	1	0	1	2	2	1	1	5<	1	1	1	2	1	0	1
	0090 II	1	0	1	2	2	1	1	5<	1	1	1	2	1	0	1
	0090 III	1	0	1	2	3	1	1	5<	1	1	1	2	1	0	1
	0091 I	1	0	1	2	2	1	1	5<	1	1	1	2	1	0	1
	0091 II	1	0	1	2	2	1	1	5<	1	1	1	2	1	0	1
	0092	1	0	1	2	2	1	1	5<	1	1	1	2	1	1	1
	0093	1	0	1	2	4	1	1	5<	1	0	1	1	1	0	1
	0094 I	1	0	1	1	2	1	1	5<	1	1	1	2	1	0	1
	0094 II	1	0	1	1	2	1	1	5<	1	1	1	2	1	0	1
	0096	1	0	1	2	1	1	1	5<	1	1	1	2	1	0	1
	0097	1	0	1	1	1	1	1	5<	1	1	1	2	1	0	1
FOM	O135	1	0	1	2	2	1	1	5<	1	0	1	1	1	1	1

(0) Four out of 23 isolates of VCG 0030 were not labeled by probe FG25.

Table 5. RFLP patterns observed in different VCGs of FOL, FORL and FOM. DNA was cut with restriction enzymes, electrophoresed, blotted and hybridized to radioactively-labelled FG probes. Each pattern is represented by a serial number that has no other meaning.

radioactively labeled										
has no other meaning.										
FG	4	25	30	34			43		103	155
	D R I R V	D R I R V	D R I R V	D	R I	R V	D	R I	D R I R V	D R I R V
VCG (N)										
<u>FOL</u>										
0030 (23)	1 1 1	1,0 1,0 1,0	1 1 1	5,6,7 8,9,10 11,13	4,6,7 8,9,10	5,6,8,9,12 13,14,15, 17,18,20	4	4,7,8 10,11 12	1(2) 1 1 (4)	2 1 1
0031 (4)	1 1 1	0 0 0	1 1 1	13,14	1,6	11,19	5	3,5	2 3 1	1 1 2
<u>FORL</u>										
0090 I (3)	1 1 1	0 0 0	1 1 1	1	1	1	1	1	1 1 1	1 1 1
0090 II (3)	1 1 1	0 0 0	1 1 1	1	1	1	2	2	1 1 1	1 1 1
0090 III (3)	1 1 1	0 0 0	1 1 1	1	1	1	3	1	1 1 1	1 1 1
0091 I (3)	1 1 1	0 0 0	1 1 1	1	1	1	3	1	1 1 1	1 1 1
0091 II (3)	1 1 1	0 0 0	1 1 1	1	1	1	1	1	1 1 1	1 1 1
0092 (3)	1 1 1	0 0 0	1 1 1	2	2	2	2	1	1 1 1	1 1 1
0093 (3)	1 1 1	0 0 0	1 1 1	3	3	3	3	1	2 2 1	1 1 2
0094 I (3)	1 1 1	0 0 0	1 1 1	3	3	3	3	1	2 2 1	1 1 2
0094 II (3)	1 1 1	0 0 0	1 1 1	1	1	1	2	2	1 1 1	1 1 1
0096 (3)	1 1 1	0 0 0	1 1 1	3	3	4	3	3	2 2 1	2 1 1
0097 (2)	1 1 1	0 0 0	1 1 1							
<u>FOM</u>										
0135 (3)	1 1 1	0 0 0	1 1 1	15,16 17	14	23,24	6	13,14	3 4 2	1 2 2

FG	163	177	213	236	261	278	281	302
	D R I	D R I R V	D R I R V	D R I R V	D R I R V	D R I R V	D R I R V	D R I R V
VCG (N)								
FOL								
0030 (23)	5,6,9 2,3 10,11 4,8	1 1 1	3,4 3,4 5[1] 5	1 1 1	5 4[6] 5	1 1 1	- 2 3	1 1 5
0031 (4)	7,8,9 7	1 1 2	0 0 0	1 1 1	4 5,4 6	1 1 1	- 0 0	1,2 1 3,7,8
FORL								
0090 I (3)	1 -	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	1 1 1
0090 II (3)	1 -	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	1 1 1
0090 III (3)	1 -	1 1,2 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	2 2 2
0091 I (3)	- -	1 1,3,4 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	2 2 2
0091 II (3)	- -	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	1 1 1
0092 (3)	- -	1 1 1	1 1 1	1 1 1	1,2 1,2 1,2	1 1 1	1 1 1	1 1 1
0093 (3)	2 1	1 1 1	0 0 0	1 1 1	2,3 2,3 2,3	1 1 1	0 0 0	3 1 3
0094 I (3)	- -	1 1 2	2 2 2	1 1 1	3 3 3	1 1 1	0 0 0	3 1 3
0094 II (3)	- -	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	0 0 0	1 1 1
0096 (3)	1,3 -	1 1 1	1 1 1	1 1 1	4 1 4	1 1 1	0 0 0	1 1 3
0097 (2)	4 -	2 1 1	2 2 1	1 1 1				
FOM								
0135 (3)	12 -	1 2 2	0 0 0	2 1 1	6 7 7	2 2 1	2 - 4	- 3 6

(N) Number of isolates in each VCG.

0, No labelling.

Information not available.

Fig. 3. Semistrict consensus trees for isolates of FOL and FOM, calculated from the RFLP data using restriction enzymes DraI, Eco RI and Eco RV.

Fig. 5. Semistrict consensus trees for isolates of FOL, FORL and FOM, calculated from the RFLP data using restriction enzymes DraI, EcoRI and EcoRV.

Table 6. Minimal and maximal numbers of changes (a) within and (b) between VCGs of FOL, FORL and FOM calculated from the RFLP data

a

VCG (N)		Dra I	Eco RI	Eco RV
FOL	0030 (23)	0-11	0-13	0-9
	0031 (4)	1-6	1-6	1-5
FORL	0090 I (3)	0-1	0	0
	0090 II (3)	0	0	0-2
	0090 III (3)	0	0-4	0
	0091 I (3)	0	0-1	0
	0091 II (3)	0	0	0
	0092 (3)	0	0-5	0
	0093 (3)	0	0	0
	0094 I (3)	0	0	0
	0094 II (3)	0	0	0-3
	0096 (3)	0-1	0	0-5
	0097 (2)	0	0	0
	A* (21)	0-7	0-8	0-5
	B* (8)	0-10	0-3	0-7
FOM	0135 (3)	2	0-1	0-1

(N) Number of isolates in each VCG.

*A, Twenty one isolates of VCGs 0090, 0091, 0092 and 0096.

*B, Eight isolates of VCGs 0094 and 0097.

b

VCGs	Dra I	Eco RI	Eco RV
0030-0031	14-21	19-28	22-30
0030-A	23-33	15-26	9-17
0030-0093	25-31	15-21	14-19
0030-0094	17-23	23-29	20-28
0030-0097	13-19	24-30	24-29
0030-FOM	33-41	33-40	29-35
0031-A	23-33	15-26	9-17
0031-0093	39-48	10-18	17-23
0031-0094	33-38	10-13	2-8
0031-0097	29-34	11-14	6-9
0031-FOM	49-56	20-24	7-11
0093-A	10-14	10-15	9-12
0094-A	12-16	16-21	15-21
0097-A	16-20	17-22	19-22
FOM-A	26-32	24-30	24-28
93-94	13	18	14-17
93-97	18	19	18
93-FOM	28-30	30-31	23-24
94-97	10	3	4-7
94-FOM	22-24	20-21	9-14
97-FOM	26-28	21-22	11-14

Fig. 6. Banding pattern obtained from PCR amplification of DNA of isolates of FOL and FOM using primer L12.

Table 7. RAPD patterns generated with isolates of different VCGs of FOL, FORL and FOM. Each pattern is represented by a serial number that has no other meaning

VCG (N)	L1	L2	L3	L4	L7	L10	L11	L12	L13
FOL									
OO30 (23)	2	1	5[6]	1	1,2,3	[7],[9],11,[12],13	3	3[4]	1
0031 (4)	2	3	5	5,4	9,12	7,9	4	4	1
FORL									
CO90 I (3)	1	1	1	1	1,2	1,2	1,2	1	1
CO90 II (3)	1	1	1	1	1,2,3	2,3	3	1,2	1
CO90 III (3)	1	1	1,2	1	1,2	1	2	1	1
CO91 I (3)	1	1	1,3	1,2	2,3	4,5	3	3,4	1
CO91 II (3)	1	1	1	2	3	6	3	4	1
CO92 (3)	1	1	1	1	2	1,2	2,3	1	1
CO93 (3)	2	1	3,4	1,3	4,5,6	7,8,9	3	4	1
OO94 I (3)	2	1	3	4	7,8	7,9	4	4	1
OO94 II (3)	2	1	3,5	4	9,10	7,9	4	4	1
OO96 (3)	1	1	1	1	2,11	1,10	2	1	1
OO97 (2)	2	1	3	4	10	3	4	5	1
FOM									
O135 (3)	3	4	7,8	6	6	11,14	4	6	1

(N) Number of isolates in each VCG.

[] Pattern obtained with one isolate out of 23.

Table 8. RAPD banding patterns generated with isolates of the two physiologic races of FOL

Race (N)	L1	L2	L3	L4	L7	L10	L11	L12	L13
1 (11)	2	1,3	5	1,5	1,2,3,9	7,9,11	3,4	3,4	1
2 (12)	2	1,3	5,6	1,5	1,2,3,13	7,9,11,12,13	3,4	3,4	1

(N) Number of isolates in each race.

Electrophoretic karyotypes obtained for FOL isolates revealed considerable variation in chromosome number and size (Fig. 8) when isolates of different VCGs, races and origin were compared. Chromosome numbers ranged between 6-10 (or more) in sizes from less than 1Mb to over 5.7 Mb (the largest size marker). Genome size was estimated at 19.5-32.4 Mb. This study is being continued beyond the termination of the project

Fig. 8. Electrophoretic karyotype of six isolates of FOL. Left lane: DNA size markers (SP, H: *S. pombe* and *H. wingei*)

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