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Horizontal genetic transfer in asexual fungi

Blanca R. Cortes
Florida International University

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

HORIZONTAL GENETIC TRANSFER IN ASEXUAL FUNGI

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by

Blanca R. Cortes

2000

To: Dean Arthur W. Herriott
College of Arts and Sciences

This dissertation, written by Blanca R. Cortes, and entitled Horizontal Genetic Transfer in Asexual Fungi, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Victor Apanius

Timothy M. Collins

Randy C. Ploetz

Raymond Schnell

David N. Kuhn, Major Professor

Date of Defense: July 7, 2000

The dissertation of Blanca R. Cortes is approved.

Dean Arthur W. Herriott
College of Arts and Sciences

Interim Dean Samuel S. Shapiro
Division of Graduate Studies

Florida International University, 2000

DEDICATION

To Lou and all my guiding angels, with love. I would also like to dedicate this work to my loving family for their support: my dear mother, the Casamors, the Perez and the Ortiz. A special debt of gratitude to Charlie, Carlos and Aleida. Dad, this one is also for you.

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ABSTRACT OF THE DISSERTATION

HORIZONTAL GENETIC TRANSFER IN ASEXUAL FUNGI

by

Blanca R. Cortes

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Professor David N. Kuhn, Major Professor

Four aspects of horizontal genetic transfer during heterokaryon formation were examined in the asexual pathogen *Fusarium oxysporum* f.sp. *cubense* (Foc): 1) variability based on method of heterokaryon formation 2) differences in nuclear and mitochondrial inheritance 3) the occurrence of recombination without nuclear fusion 4) the occurrence of horizontal genetic transfer between distantly related isolates. The use of non-pathogenic strains of *Fusarium oxysporum* as biocontrol agents warrants a closer examination at the reproductive life cycle of this fungus, particularly if drug resistance or pathogenicity genes can be transmitted horizontally. Experiments were divided into three phases. Phase I looked at heterokaryon formation by hyphal anastomosis and protoplast fusion. Phase II was a time course of heterokaryon formation to look at patterns of nuclear and mitochondrial inheritance. Phase III examined the genetic relatedness of the different vegetative compatibility groups using a multilocus analysis approach. Heterokaryon formation was evident within and between vegetative compatibility groups. Observation of non-parental genotypes after heterokaryon formation confirmed that, although a rare event, horizontal genetic transfer occurred during heterokaryon formation. Uniparental mitochondria inheritance was observed in heterokaryons formed either by

hyphal anastomosis or protoplast fusion. Drug resistance was expressed during heterokaryon formation, even across greater genetic distances than those distances imposed by vegetative compatibility. Phylogenies inferred from different molecular markers were incongruent at a significant level, challenging the clonal origins of Foc. Mating type genes were identified in this asexual pathogen Polymorphisms were detected within a Vegetative Compatibility Group (VCG) suggesting non-clonal inheritance and/or sexual recombination in Foc. This research was funded in part by a NIH-NIGMS (National Institutes of Health-National Institute of General Medical Sciences) Grant through the MBRS (Minority Biomedical Research Support), the Department of Biological Sciences and the Tropical Biology Program at FIU.

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CHAPTER 1: Literature review

1.1 Introduction

Asexual fungi have no known sexual cycle, are typically haploid (n) and their genome is clonally inherited from generation to generation. Yet, genetic variability has been reported in these organisms (Kistler 1997; Burdon and Silk 1997; Caten 1997; Elias and Schneider 1992; Kohn *et al.* 1991). Genetic variation in asexual fungi can be attributed to mutation, genetic drift or recombination (Burdon and Silk 1997; Milgroom 1996; McDonald *et al.* 1989; McDermott and McDonald 1993). Using *Fusarium* as a model system I investigated horizontal transfer of genetic information through recombination among asexual pathogenic fungi.

As most fungal pathogens are asexual, an understanding of the reproductive life cycle of fungi is important when considering breeding for plant resistance and for biocontrol of fungal pathogens (Kondrashov 1997; Chacko 1994), particularly if traits such as pathogenicity or drug resistance can be transmitted horizontally. Table 1 compares some advantages and disadvantages of different reproductive life cycles in fungi.

Table 1. Advantages and disadvantages of different reproductive life cycles in fungi

Reproductive Life cycle	ADVANTAGE	DISADVANTAGE
Asexual Cycle (Clonality)	• Retain favorable traits • High number of asexual spores	• Accumulation of deleterious mutations (Muller's Ratchet) • Low genetic variation within lineages
Parasexual Cycle (Mitotic Recombination)	• Retain favorable alleles while acquiring new ones • Overcome Muller's ratchet • High genetic variation through mitotic crossover	• Transmission of pathogens (mycoviruses)
Sexual Cycle (Heterothallic)	• High genetic variation through meiosis • Overcome Muller's Ratchet • Retain favorable alleles while acquiring new ones	• Locating opposing mating types • Loss of favorable alleles during chromosome reassortment • Low number of sexual spores • Sexually transmitted pathogens

A mechanism that explains potential recombination in asexual fungi is the parasexual cycle (Fig 1) and was first proposed by Pontecorvo (1953). The parasexual cycle has been defined as the horizontal transfer of genetic

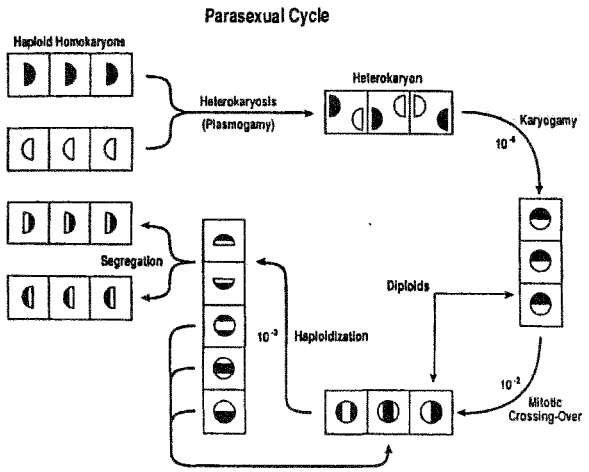


Figure 1. The parasexual cycle. From Fusarium Wilt of Banana, (Adapted from RC Ploetz 1990)

information by hyphal anastomosis without meiosis or the development of specialized sexual structures.

There are three stages to the parasexual cycle: heterokaryon formation via hyphal anastomosis, karyogamy and haploidization. A few definitions will be helpful before I expand on this subject. (1) anastomosis: fusion of two hyphae (2) septate: hyphae (filament) consisting of successive cells separated by a septum (3) conidium: specialized cell involved in asexual dispersion(4) mycelia: a mass of hyphae (5) Propagule: any kind of cell involved in the dispersal of the fungus.

Heterokaryons are cells housing more than one genetically distinct nucleus. The limiting step of the cycle is heterokaryon formation. Heterokaryon formation by anastomosis of vegetative cells occurs frequently between compatible strains, but is prevented by a self/non-self recognition system known as vegetative incompatibility. (Leslie and Zeller 1996; Leslie 1993; Glass and Kuldau 1992; Rayner 1991; Ploetz 1990; Ploetz and Correll 1988). Nonetheless, transient heterokaryon formation between incompatible or partially incompatible strains has been reported by hyphal anastomosis in some fungal systems (Kuhn *et al.* 1996; Coenen *et al.* 1994; Begueret *et al.* 1994; Rayner 1991; Garber *et al.* 1961)

Karyogamy refers to actual nuclear fusion of the distinct nuclei in a heterokaryotic cell, giving rise to somatic hybrids. Karyogamy occurs infrequently at a rate of approximately one in a million. It is during karyogamy that horizontal transfer of genetic information via mitotic crossover is thought to occur (Pontecorvo 1953).

Haploidization, via non-disjunction events, returns the cell to its original haploid state. Segregation of the genotypes present in the heterokaryon is evidenced by fast-

growing sectors from hybrid colonies. These sectors may be haploid or aneuploid and can represent the original homokaryons or altered genomes that differ from the original parental strains.

Although the parasexual cycle has been documented in asexual fungi (Ziegler *et al.* 1997; Kuhn *et al.* 1995; Daboussi and Gerlinger 1992; Garber and Ruddat 1992; Molnar *et al.* 1990; Jackson and Heale 1987; Buxton 1962, 1956), the significance of horizontal transfer through a parasexual cycle remains obscure, mainly due to lack of evidence of mitotic recombination in the field (Caten 1997, 1971; Hoekstra 1994). The advent of molecular markers has uncovered evidence of horizontal genetic transfer in different fungal systems (Bello and Paccola 1998; He *et al.* 1998; Van Diepeningen *et al.* 1998; Ziegler *et al.* 1997; Kempken 1995; Syvanen 1994; Chacko *et al.* 1994; Debets *et al.* 1994; Collins and Saville 1990). As karyogamy is a rare event and heterokaryon formation is frequently observed despite vegetative incompatibility, I propose the possibility that horizontal genetic transfer may also occur during heterokaryon formation rather than during karyogamy alone. Moreover, some evidence of transfer of mycoviruses (Milgroom personal communication) may suggest that horizontal genetic transfer may occur as early as during the initial hyphal anastomosis event, even in the absence of transient or viable heterokaryons.

1.2 Goals

Four aspects of horizontal genetic transfer during heterokaryon formation were examined with this study: (1) variability based on method of heterokaryon formation (2) differences in nuclear and mitochondrial inheritance (3) the occurrence of recombination

without nuclear fusion and (4) the occurrence of horizontal genetic transfer between distantly related isolates.

To address these goals I examined heterokaryon formation and horizontal genetic transfer in the asexual fungus *Fusarium oxysporum* by hyphal anastomosis and protoplast fusion. The genus *Fusarium* comprises many species pathogenic to plants. *F. oxysporum* is responsible for vascular wilt and crown rot of many important agricultural crops. This study uses *Fusarium oxysporum* Schlechtend,: Fr. f. sp. *cubense* (Foc) (E.F., Sm.) W.C. Snyder & H.N. Hans which causes vascular wilt of banana (Musa spp. Zingiberales;Musacea) (Figure 2).

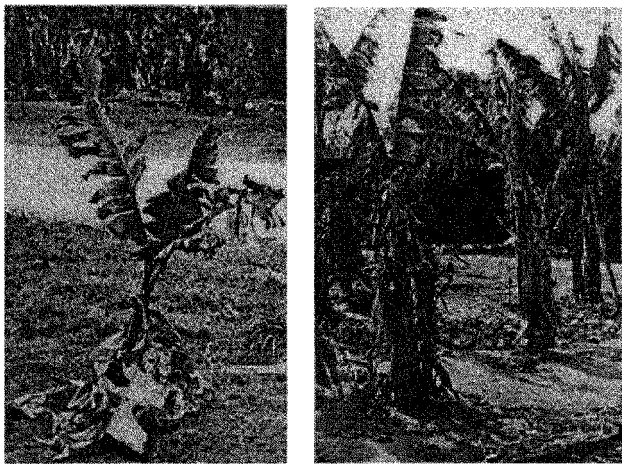


Figure 2. Vascular Wilt of Banana. A young plant of Cavendish cultivar in naturally infested soil (left) and older plants with characteristic hanging sheaths of dead leaves. From :Modern Mycology, Third Edition, J.W. Deacon, Blackwell Science Publisher

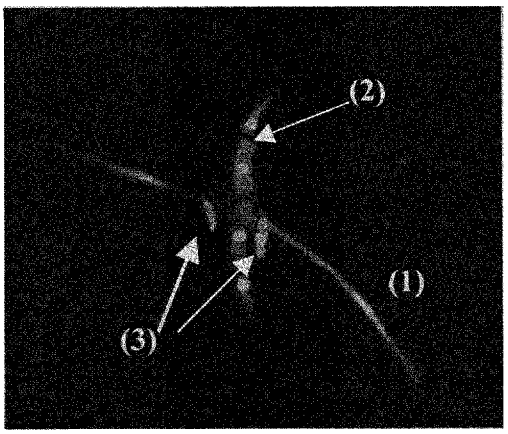


Figure 3. Propagules of *Fusarium*

Foc is an asexual, soilborne pathogen. The vegetative hyphae (Figure 3-1) of Foc is septate and produces three types of asexual spores: (1) multinucleate macroconidia (Figure 3-2), (2) uninucleate microconidia (Figure 3-3) and chlamydospores (not shown). Macroconidia are thin-walled spores produced most frequently on branched

conidiophores. Microconidia form in the aerial mycelium and are produced in false heads. Chlamydospores, on the other hand, are thick-walled spores produced through the condensation of their content (Nelson 1990). Uninucleate microconidia can facilitate the analysis of propagules from heterokaryotic cells (Maheshwari, 1999).

F. oxysporum is divided into *formae speciales* (Snyder and Hansen 1940) based on pathogenicity to the host species it affects. Pathogenicity to differential cultivars within the host species is denominated as race. Each *forma specialis* is further subdivided into vegetative compatibility groups (VCGs) based on the ability of the strains to form stable, viable heterokaryons by hyphal anastomosis (Correll 1991; Ploetz 1990; Ploetz *et al.* 1988; Puhalla 1985). Strains within the same VCGs are genetically more similar than inter-VCG or inter-forma strains (O'Donnell *et al.* 1998; Koening *et al.* 1997; Kistler 1997; Leslie 1993). This hierarchy allows the investigation of heterokaryon formation and horizontal genetic transfer at different levels of genetic similarity (Figure 4)

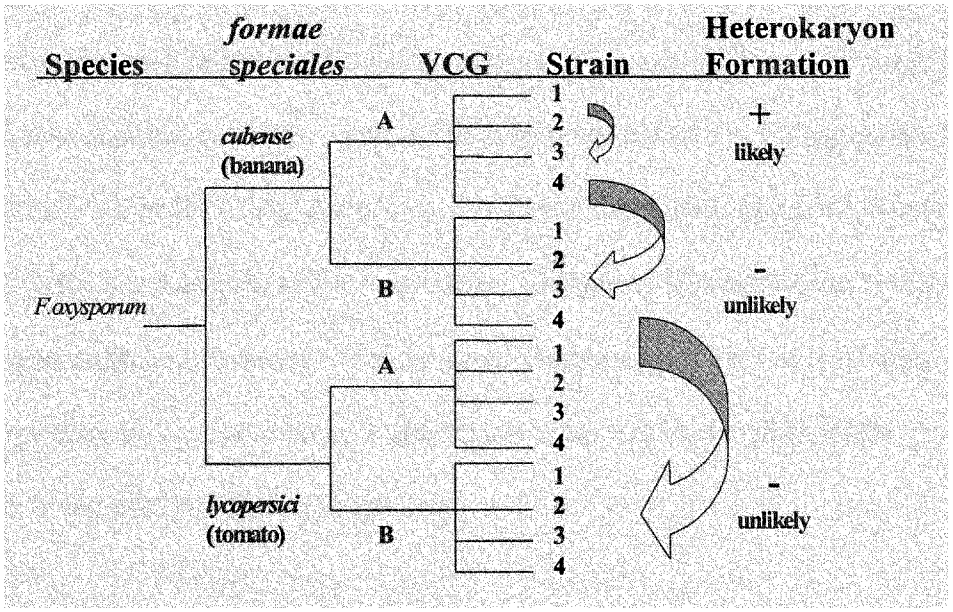


Figure 4. Levels of genetic similarity in *Fusarium oxysporum.* The species is divided into formae speciales based on the host it infects and into VCGs based on its ability to form stable heterokaryons by hyphal fusion

1.3 Experimental design

This research was divided in three phases. Phase I dealt with characterization and comparison of heterokaryon formation in Foc by hyphal anastomosis and protoplast fusion. Patterns of nuclear and mitochondrial inheritance were documented using both genetic and molecular approaches.

Phase II was a time course study of heterokaryon formation that looked at horizontal genetic transfer using auxotrophic and drug resistance markers to determine whether heterokaryon formation was sufficient or if karyogamy was also needed for horizontal genetic transfer to occur. This assay also elucidated whether biological barriers such as vegetative incompatibility or further genetic distances completely prevented horizontal genetic transfer.

Phase III addressed questions on genetic relatedness, evolutionary histories and origins in Foc by comparing and combining nuclear and mitochondrial markers in a total evidence approach (Kluge 1998, 1989) using phenetic and phylogenetic analysis. This multilocus analysis was done using the homogeneity test (a.k.a. Incongruence Length Difference test or ILD Test) featured in Paup* Version 4.0b3 for 32-Microsoft Windows © 1999 Sinauer Associates, Inc. Publishers. The aim of this test was to investigate if there was sufficient evidence to refute the strict clonality of Foc, or if recombination, whether through a parasexual or a rare sexual cycle was responsible for the genetic variation reported in this forma specialis.

CHAPTER 2: Phase I-An Investigation of Heterokaryon Formation by Protoplast Fusion and Hyphal Anastomosis

2.1 Introduction

Under controlled conditions we had previously documented that all stages of the parasexual cycle (Figure 1) occur in Foc (Cortes 1996). This study examines the process of heterokaryon formation in order to determine its role in horizontal genetic transfer.

By pairing auxotrophic strains within and between VCGs and allowing hyphal anastomosis I determined which strains were compatible (stable heterokaryon formation), partially compatible (transient heterokaryon formation) or incompatible (no heterokaryon formation). Auxotrophs are biochemical mutants unable to synthesize nutritional requirements such as amino acids or vitamins. These nutritional deficiencies provide a stringent screening assay to detect heterokaryon formation as auxotrophs, unlike prototrophs, are incapable of growth on minimal medium lacking the nutritional requirements of the mutants. Syntrophism (biochemical complementation of nutritional deficiencies) is evidenced by prototrophic growth under selective pressure (Table 2). Vegetative incompatibility can be overcome by forcing heterokaryon formation by protoplast fusion, that is, cells stripped of the cell wall with the aid of lysing enzymes that are fused under controlled conditions (van Diepeningen *et al.* 1998; Peberdy 1983). If the self/non-self recognition system is at the cell wall level, stripping the cells of a cell wall should allow cell fusion, heteroplasmy and/or organelle exchange between the strains. If on the other hand vegetative incompatibility is at the cytoplasmic level, viable heterokaryon formation should not be observed. Thus, protoplast fusion can be used to

investigate heterokaryon formation in inter-VCG pairings and between strains across further genetic distances. A few points, though, should be kept in mind when examining heterokaryon formation via protoplast fusion. It is believed that heterokaryon formation by protoplast fusion is transitory and that fused cells give rise to hybrids or diploids shortly after fusion, contrary to hyphal fusion, where the resulting heterokaryon is stable (Leslie 1996; Leslie and Zeller 1996; Stasz *et al.* 1989; Adams *et al.* 1987; Mellon 1985).

Table 2. Genetic Assay: Expected outcome

Genetic Analysis Of Microconidia Of Heterokaryons In Foc

	MMA[1]	MMA + P1 aa[2]	MMA + P2 aa[3]	CMA[4]
P1	-	+	-	+
P2	-	-	+	+
Hybrid[5]	+	+	+	+
Altered[6]		+	+	+

[1] MMA= Minimal medium agar

[2] MMA + P1aa = MMA supplemented with nutritional defficiency of parent 1

[3] MMA + P2aa = MMA supplemented with nutritional defficiency of parent 2

[4] CMA = Complete medium agar

[5] Hybrid = Prototrophic non-parental genotype.

[6] Altered = Non-parental recombinant genotype

The criterion to score balanced heterokaryon formation is the recovery of both parental genotypes on analysis of the microconidia from heterokaryons. Table 2 outlines the expected outcome of the genetic analysis. This assay resolves the heterokaryon into its individual components by replica picking (Pontecorvo 1953).

An assay developed by D'Alessio (1997) differentiates mitochondrial types in *F. oxysporum.* Primers specific for *F. oxysporum* were designed using an intergenic region of the mtDNA between the tyr-RNA gene and the cytochrome oxydase III gene. Length

differences in the PCR amplicons in general correlated to difference in vegetative compatibility groups, though some VCGs shared the same mitochondrial types. VCGs (or lineages) showing the same mitochondrial types were grouped in mitotypes. In total, 11 mitotypes were identified (Table 3).

Table 3. Foc mitochondrial haplotypes (mitotypes). Eleven mitotypes were identified using an intergenic region of the mtDNA between the tyr-RNA and the cytochrome oxydase III gene. The sequences of the VCGs and formae speciales within a mitotype are identical.

Mitotype	VCGs and Fusarium formae speciales
1	0120,0122,0129, 01211
2	0121, 01213
3	0123
4	0124, 0125, 0124/5, 0128, 01212
5	0124/5*
6	0126, 01210
7	01214
8	lycopersici
9	dianthi, melonis, pisi, raphani
10	conglutinans
11	niveum

Mitotype length differences can be distinguished on a polyacrilamide gel. These length differences were used to follow mitochondrial inheritance in pairings between vegetative compatibility groups in different mitotypes. The assay is sensitive enough to detect the presence of both mitochondrial genomes even at ratios of 1:100 (evidenced by heteroduplex bands).

The auxotrophic and mitotype genetic analysis addressed questions about patterns of nuclear and mitochondrial inheritance across greater genetic distances. Table 4 shows how the outcome of the molecular analysis of propagules from inter-mitotype pairings can unambiguously identify the mode of inheritance of mitochondrial genes.

Table 4 . Mitochondrial molecular assay: Expected Outcome in inter-mitotype pairings

Outcome	Both P1[1] & P2[2]	Only P1	Only P2	Some P1/ Some P2	Neither P1 nor P2
Heteroplasmy	+	-	-	-	-
Uniparental specific	-	+	+	-	-
Uniparental Random	-	-	-	+	-
Recombination	-	-	-	-	+

[1] P1 = Parent 1
[2] P2 = Parent 2

2.2 Materials and Methods

Phase I was divided in two parts: intra-VCG pairings (Table 6) and inter-VCG pairings (Table 7). VCG 0120, 0124 and 0126 were selected to force heterokaryon formation as they had the greater number of strains and auxotrophs within a VCG out of the 15 VCGs studied (Ploetz 1990). Wild type strains of Foc were provided from the worldwide collection of Dr. Randy C. Ploetz located at the University of Florida, Tropical Research and Education Center in Homestead, Fla. All the auxotrophic mutants used in this study (Table 5) were generated in this lab (Appendix I) following the protocols of Cortes (1996).

Table 5. Auxotrophic mutants of Foc

Species	f.sp.	VCG	Strain	Marker[a]	Race	Location	Mitotype
Oxysporum	*cubense*	0120	SA8	$M^-P^-B^R$	4	So.Africa	I
		0120	STGM1	R^-Ade^-	1	Costa Rica	I
		0124	GMB	Ade^-	1	Brazil	IV
		0124	GMB	M^-	1	Brazil	IV
		0124	GMB	K^-R^-	1	Brazil	IV
		0124	JCB1	C^-	2	Florida	IV
		0126	STB2	K^-	1	Honduras	VI
		0126	STM3	K^-R^-	1	Honduras	VI
		0126	STM3	M^-R^-	1	Honduras	VI

[a]Ade=adenine, B^R=benomyl resistant, C=cysteine, K=lys, M=methionine, P=proline, R=arginine

Each of these VCGs also represents a different mitochondrial type. Thus, these pairings encompassed intra- and inter-strain and intra-and inter-racial interactions as well as intra and inter-mitotype. Furthermore, one isolate, SA8 Met$^-$Pro$^-$Ben^R, was used as a tester strain in intra and inter VCG pairings because in addition to be genetically marked with auxotrophies, it had been genetically marked with Benomyl (DuPont) drug resistance (Appendix I). Drug resistance can be used as an unselected marker to follow genetic inheritance.

Table 6. Intra-VCG pairings

Strains	VCG	Mitotype	Inter-Strain	Inter-Race
SA8 X STGM1	0120	I	+	+
GMB X GMB	0124	IV	-	-
GMB X JCB1	0124	IV	+	+
STM3 X STB2	0126	VI	+	-

Table 7. Inter-VCG pairings

Strains	VCG	Mitotype	Inter-Strain	Inter-Race
SA8 X GMB	0120 X 0124	I x IV	+	+
SA8 X STB2	0120 X 0126	I x VI	+	+
SA8 X STM3	0120 X 0126	I x VI	+	+
GMBX STB2	0124 X 0126	IV x VI	+	-

2.3 Intra-VCG Pairings

2.3.1 Pairings by hyphal anastomosis

Auxotrophic mutants (Table 5) were used to force heterokaryon formation by hyphal anastomosis (Figure 6) in Foc by using the double-pick method (DP) and the liquid minimal medium (LMM) method as outlined in Figure 5 and described in Appendix I.

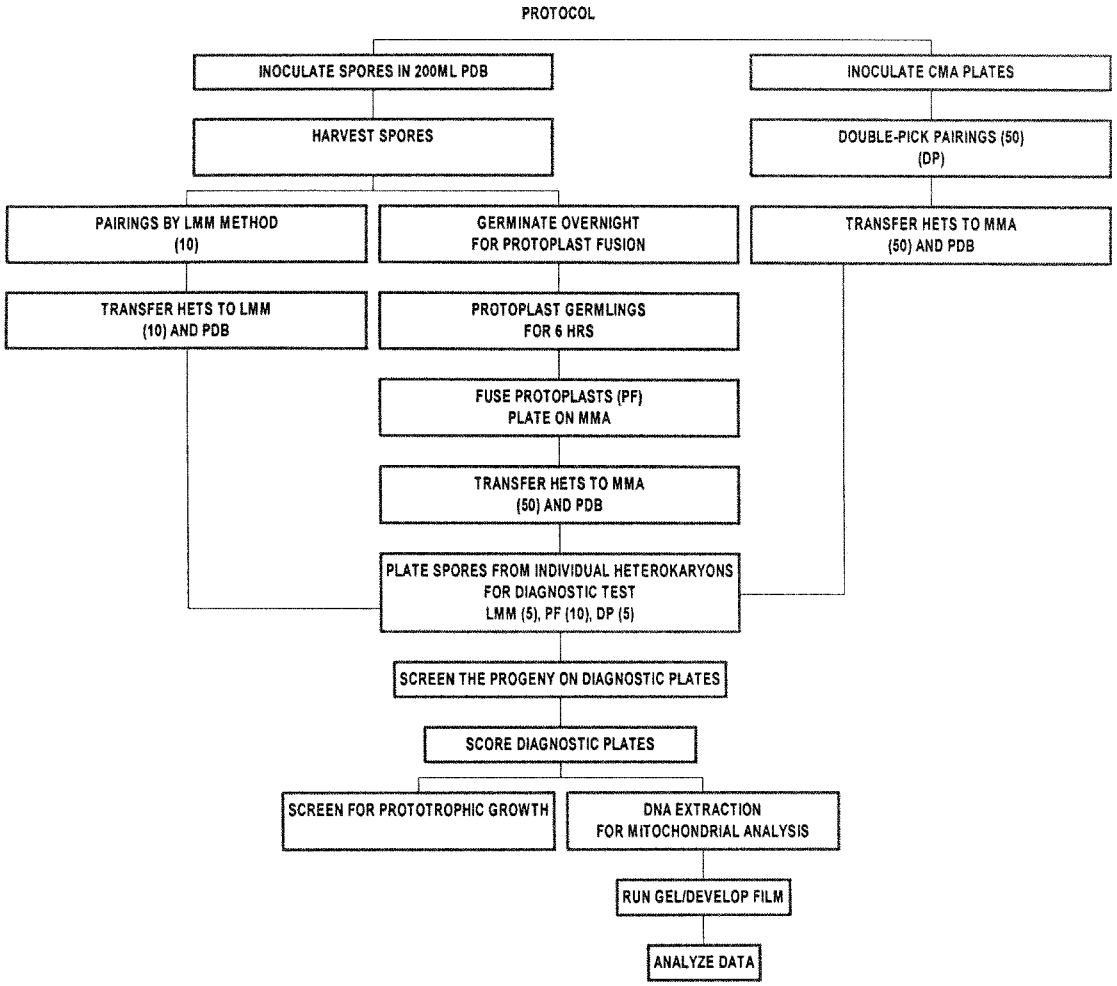


Figure 5. Phase I methods for forcing heterokaryon formation. Legend: PDB=potato dextrose broth, CMA=complete medium agar, LMM=liquid minimal medium, MMA= minimal medium agar, Hets=heterokaryons, PF=protoplast fusion, DP=Double-pick method.

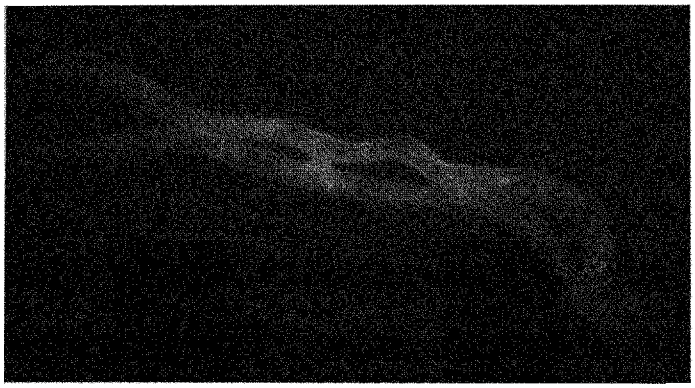


Figure 6. Hyphal Anastomosis in Foc

2.3.2 Pairings by protoplast fusion

Protoplast generation followed the protocols outlined by Boehm *et al.* (1994), optimized for the tested strains (Appendix I). Protoplast fusion (PF) was accomplished following the protocols of Peberdy (1983) and Daboussi and Gerlinger (1992).

Both, in pairings by protoplast fusion and by hyphal anastomosis, negative controls consisted in pairing the individual auxotrophic parents with themselves under the same stringency as the forced pairings between the parents. As no syntrophism is possible, the expected outcome of the controls is no growth. Intra-VCG pairings were established as positive controls. Microconidia were screened using the genetic diagnostic test (Table 2)

2.4 Results Intra VCG pairings

2.4.1 0120 x 0120 heterokaryons between SA8 $M^{-}P^{-}B^{R}$ and STGM1 $R^{-}Ade^{-}B^{S}$

2.4.1.1 Genetic Analysis

The segregation of the microconidia of the individual heterokaryons generated by hyphal fusion and protoplast fusion is shown in table 8.

Table 8. Diagnostic test of individual microconidia from intra-VCG (0120x0120) heterokaryons from pairings between SA8 $M^{-}P^{-}B^{R}$ and STGM1 $R^{-}Ade^{-}B^{S}$. Segregation of both parental phenotypes was observed on analysis of microconidia from putative heterokaryons that were transferred to selective medium for continued growth, confirming heterokaryon formation. Prototrophic colonies suggestive of hybrid formation were also recovered.

	Diagnostic test		
Hyphal Fusion	Prototrophic	Auxotrophic for Parent 1	Auxotrophic for Parent 2
HFA1122	0 (0%)	44 (88%)	6 (12%)
HFA1129	0 (0%)	43 (90%)	5 (10%)
HFA1144	0 (0%)	50 (100%)	0 (0%)
HFA1142	0 (0%)	40 (82%)	9 (18%)
HFA1148	1 (2%)	6 (12%)	43 (86%)
HFA121	1 (2%)	39 (78%)	10 (20%)
HFA122	0 (0%)	29 (62%)	18 (38%)
HFA123	0 (0%)	31 (67%)	15 (33%)
HFA124	0 (0%)	44 (88%)	6 (12%)
HFA125	1 (2%)	36 (73%)	12 (25%)
Protoplast Fusion			
PFA146	0 (0%)	40 (80%)	10 (20%)
PFA148	1 (2%)	48 (96%)	1 (2%)
PF A1412	0 (0%)	34 (68%)	16 (32%)
PF A1415	0 (0%)	45 (90%)	5 (10%)
PFA1419	5 (10%)	37 (74%)	8 (16%)
PFA1425	1 (2%)	46 (92%)	3 (6%)
PFA1428	3 (6%)	38 (76%)	9 (18%)
PFA1434	1 (2%)	46 (96%)	1 (2%)
PFA1437	1 (2%)	43 (86%)	6 (12%)
PFA1441	0 (0%)	45 (94%)	3 (6%)

2.4.1.2 Statistical Analysis

To determine if there was an association between the method of pairing (hyphal fusion (HF) vs. protoplast fusion (PF)) and the outcome (segregation of microconidia), a chi-square (χ^2) test was carried out (significant at $P \leq 0.05$), using SPSS statistical software (SPSS for Windows release 10.0 October 27, 1999). The null hypothesis was one of no association (H_o1: HF=PF). The second hypothesis, whether the microconidia from the heterokaryons were segregating in a 1:1 ratio (H_o1: P1=P2) was tested using a non-parametric χ^2 test. Once again the significance level was set at $\alpha=0.05$. The χ^2 test was applied to these and all subsequent pairings. Table 9 summarizes the results of the χ^2 test for the intra-VCG 0120 x 0120 pairings described in table 8.

Table 9. Chi-square test of intra-VCG pairings between two VCG 0120 isolates: sa8 M^- P^-Ben^R and stgm1 $R^-Ade^-Ben^S$. Significance level was set at $\alpha=0.05$.

Hypothesis	Value	df[a]	Sig.(2-sided)p-value
H_o1: HF=PF	1.250	1	0.2640*
H_o2: P1=P2	16.75	2	0.0002

[a] df=degrees of freedom ; * Not significant

2.4.1.3 Hyphal tips Analysis

Hyphal tip analysis of the 0120 x 0120 intra-VCG heterokaryons was also carried out. Blocks from putative heterokaryons growing on MMA were transferred to fresh MMA and screened for continued growth. Blocks from the transferred heterokaryons were cut and transferred to water agar for hyphal tipping (Appendix I). The tips were transferred to PDA in order to collect microconidia for a genetic diagnostic test. The

spores were serially diluted and plated on PDA. Individual cfu's were then picked into diagnostic plates (Table 2). The analysis of these hyphal tips is shown in tables 10-13.

Table 10. Genetic analysis of microconidia collected from sporulating hyphal tips from 0120 x 0120 Intra VCG pairings between sa8 $M^{-}P^{-}Ben^{R}$ and stgm1 $R^{-}Ade^{-}Ben^{S}$

Hyphal Tip Number	CFUs [a] examined	Genotype of hyphal tip		
		MP [b] (P1)[c]	**R Ade[b](P2)[c]**	**Genotype**
HT1	25/25	25 (100%)	0 (0%)	Homokaryotic
HT2	25/25	25 (100%)	0 (0%)	Homokaryotic
HT3	25/25	25 (100%)	0 (0%)	Homokaryotic
HT4	20/25	16 (80%)	4 (20%)	**Heterokaryotic**
HT5	25/25	25 (100%)	0 (0%)	Homokaryotic
HT6	25/25	25 (100%)	0 (0%)	Homokaryotic
HT7	25/25	25 (100%)	0 (0%)	Homokaryotic
HT8	25/25	25 (100%)	0 (0%)	Homokaryotic
HT9	25/25	25 (100%)	0 (0%)	Homokaryotic
HT10	13/25	6 (46%)	7 (54%)	**Heterokaryotic**
HT11	25/25	25 (100%)	0 (0%)	Homokaryotic
HT12	17/22	6 (35%)	11 (65%)	**Heterokaryotic**
HT13	25/25	25 (100%)	0 (0%)	Homokaryotic
HT14	25/25	25 (100%)	0 (0%)	Homokaryotic
HT15	25/25	25 (100%)	0 (0%)	Homokaryotic
HT16	25/25	25 (100%)	0 (0%)	Homokaryotic
HT17	21/22	5 (24%)	16 (76%)	**Heterokaryotic**
HT18	25/25	0 (0%)	25 (100%)	Homokaryotic
HT19	24/25	15 (63%)	9 (37%)	**Heterokaryotic**
HT20	25/25	19 (76%)	6 (24%)	**Heterokaryotic**
HT21	25/25	25 (100%)	0 (0%)	Homokaryotic
HT22	25/25	25 (100%)	0 (0%)	Homokaryotic
HT23	25/25	25 (100%)	0 (0%)	Homokaryotic
HT24	25/25	25 (100%)	0 (0%)	Homokaryotic
Total	570	492	78	

[a] CFUs=colony forming units

[b] A=adenine, M=methonine, P=proline, R=arginine

[c] P1=Parent 1, P2=Parent 2

Table 11. Genotype of hyphal tips from 0120 x 0120 intra-VCG Heterokaryons

	Homokaryotic	Heterokaryotic
Hyphal tips	18/24 (75%)	6/24 (25%)

In addition to the expected segregation of the individual parental genotypes, non-parental genotypes were also recovered from heterokaryotic hyphal tips. For future reference, non-parental genotypes were classified into three caterogies: (1) P1/P2 genotypes refer to colonies from uninucleate microconidia capable of growing on MMA supplemented with the nutritional requirement of the auxotrophic parents but are incapable of prototrophic growth (2) altered genotypes can only grow on CMA or PDA and (3) prototrophic genotypes can grow on unsupplemented MMA plates. The analysis of the heterokaryotic tips is presented in table 12.

Table 12. Segregation of microconidia from heterokaryotic hyphal tips of an intra-vcg heterokaryons between sa8 $M^{-}P^{-}Ben^{R}$ (P1) and stgm1$R^{-}Ade^{-}Ben^{S}$ (P2). P1/P2 genotype are spores that can grow on MMA supplemented with the nutritional requirement of P1 and P2 but are not capable of prototrophic growth. Altered only grew on PDA.

Genotype	Auxotrophic (p1)	Auxotrophic (p2)	(P1/P2)	Altered
B11T1	10 (40%)	6 (24%)	9 (36%)	0 (0%)
B17T1	12 (48%)	6 (24%)	7 (28%)	0 (0%)
B18T1	7 (29%)	2 (8%)	15 (63%)	0 (0%)
B31T1	17 (77%)	0 (0%)	0 (0%)	5 (23%)
B38T1	7 (28%)	14 (56%)	4 (16%)	0 (0%)

Table 13. Segregation of non-parental genotype microconidia from cfus from heterokaryotic hyphal tips of an intra-vcg pairing between sa8 $M^{-}P^{-}Ben^{R}$ (P1) and stgm1$R^{-}Ade^{-}Ben^{S}$ (P2). *A second round of spores were screened from cfu's showing , either the p1/p2 or altered genotypes (indented under the original cfu). Diagnostic plates of hyphal tip B18T1-14-3 (Grey) are shown in Figure 7.

Genotype	Auxotrophic (p1)	Auxotrophic (p2)	(P1/P2)	Altered
B11T1-10	0/13 (0%)	8/13 (62%)	5/13* (38%)	0 (0%)
B11T1-10-1	1/25 (4%)	24/25 (96%)	0/25 (0%)	0 (0%)
B11T1-10-2	0/23 (0%)	23/23 (100%)	0/23 (0%)	0 (0%)
B11T1-10-3	2/25 (8%)	23/25 (92%)	0/25 (0%)	0 (0%)
B11T1-12	0/25 (0%)	21/25 (84%)	4/25* (16%)	0 (0%)
B11T1-10-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B11T1-10-2	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B11T1-10-3	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B11T1-14	0/25 (0%)	21/25 (84%)	4/25 (16%)	0 (0%)
B11T1-16	11/36 (31%)	25/36 (69%)	0/36 (0%)	0 (0%)
B11T1-19	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B11T1-24	0/10 (0%)	8/10 (80%)	2/10* (20%)	0 (0%)
B11T1-24-1	0/10 (0%)	10/10 (100%)	0/10 (0%)	0 (0%)
B11T1-24-2	0/20 (0%)	19/20 (95%)	1/20* (5%)	0 (0%)
B11T1-24-2-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B17T1-5	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B17T1-8	23/25 (92%)	2/25 (8%)	0/25 (0%)	0 (0%)
B17T1-13	17/25 (68%)	8/25 (32%)	0/25 (0%)	0 (0%)
B17T1-21	30/46 (65%)	14/46 (31%)	0/46 (0%)	2* (4%)
B17T1-21-1	24/24(100%)	0/24 (0%)	0/24 (0%)	0 (0%)
B17T1-21-2	22/24 (92%)	0/24 (0%)	0/24 (0%)	2* (8%)
B17T1-21-2-1	5/5 (100%)	0/5 (0%)	0/5 (0%)	0 (0%)
B17T1-21-2-2	5/5 (100%)	0/5 (0%)	0/5 (0%)	0 (0%)
B17T1-22	14/25 (56%)	11/25(44%)	0/25 (0%)	0 (0%)
B17T1-25	9/25 (36%)	16/25(64%)	0/25 (0%)	0 (0%)
B18T1-2	2/15 (13%)	9/15 (60%)	4/15* (27%)	0 (0%)
B18T1-2-1	16/18 (89%)	0/18 (0%)	0/18 (0%)	2* (11%)
B18T1-2-1-1	24/24(100%)	0/24 (0%)	0/24 (0%)	0 (0%)
B18T1-2-1-2	0/25 (0%)	22/22 (100%)	0/22 (0%)	0 (0%)
B18T1-2-2	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-2-3	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-8	0/25 (0%)	18/25 (72%)	7/25* (28%)	0 (0%)
B18T1-8-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-10	0/25 (0%)	16/23 (70%)	7/23* (30%)	0 (0%)
B18T1-10-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)

B18T1-10-2	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-10-3	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-13	1/25 (4%)	24/25 (96%)	0/25 (0%)	0 (0%)
B18T1-14	0/10 (0%)	5/10 (50%)	5/10* (50%)	0 (0%)
B18T1-14-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-14-2	25/25(100%)	0/25 (0%)	0/25 (0%)	0 (0%)
B18T1-14-3	10/25 (40%)	9/25 (36%)	6/25* (24%)	0 (0%)
B18T1-14-3-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-14-3-2	19/25 (64%)	6/25 (36%)	0/25 (0%)	0 (0%)
B18T1-14-3-3	25/25(100%)	0/25 (0%)	0/25 (0%)	0 (0%)
B18T1-14-3-4	24/25 (96%)	1/25 (4%)	0/25 (0%)	0 (0%)
B18T1-14-3-5	18/24 (75%)	6/24 (25%)	0/24 (0%)	0 (0%)
B18T1-14-3-6	19/19(100%)	0/19 (0%)	0/19 (0%)	0 (0%)
B18T1-16	0/25 (0%)	21/25 (84%)	4/25* (16%)	0 (0%)
B18T1-16-1	0/10 (0%)	10/10 (100%)	0/10 (0%)	0 (0%)
B18T1-16-2	0/15 (0%)	15/15 (100%)	0/15 (0%)	0 (0%)
B18T1-17	7/24 (29%)	12/24 (50%)	5/24* (21%)	0 (0%)
B18T1-17-1	0/23 (0%)	23/23 (100%)	0/23 (0%)	0 (0%)
B18T1-12-2	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-21	0/25 (0%)	23/25 (92%)	2/25* (8%)	0 (0%)
B18T1-21-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-21-2	0/23 (0%)	23/23 (100%)	0/23 (0%)	0 (0%)
B18T1-23	3/15 (20%)	10/15 (67%)	2/15* (13%)	0 (0%)
B18T1-23-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-23-2	0/25 (0%)	20/25 (84%)	4/25* (16%)	0 (0%)
B18T1-23-2-1	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B18T1-23-2-2	15/24 (63%)	9/24 (37%)	0/24 (0%)	0 (0%)
B18T1-23-2-3	25/25(100%)	0/25 (0%)	0/25 (0%)	0 (0%)
B18T1-23-2-4	11/25 (44%)	12/25 (48%)	2/25 (8%)	0 (0%)
B18T1-24	3/26 (12%)	21/26 (81%)	2/26* (7%)	0 (0%)
B18T1-24-1	0/20 (0%)	20/20 (100%)	0/20 (0%)	0 (0%)
B18T1-24-2	0/25 (0%)	25/25 (100%)	0/25 (0%)	0 (0%)
B31T1	17/22 (77%)	0/22 (0%)	0/22 (0%)	5* (23%)
B18T1-31-1	24/24(100%)	0/24 (0%)	0/24 (0%)	0 (0%)
B18T1-31-2	24/24(100%)	0/24 (0%)	0/24 (0%)	0 (0%)
B18T1-31-3	24/24(100%)	0/24 (0%)	0/24 (0%)	0 (0%)
B18T1-31-4	23/24(96%)	0/24 (0%)	0/24 (0%)	1* (4%)
B18T1-31-4-1	6/6 (100%)	0/6 (0%)	0/6 (0%)	0 (0%)
B38T1-1	7/25 (28%)	14/25 (56%)	4/25* (16%)	0 (0%)
B18T1-38-1	9/25 (36%)	16/25 (64%)	0/25 (0%)	0 (0%)
B18T1-38-2	19/25 (76%)	5/25 (20%)	1/25 (4%)	0 (0%)
B18T1-38-3	6/25 (24%)	19/25 (76%)	0/25 (0%)	0 (0%)

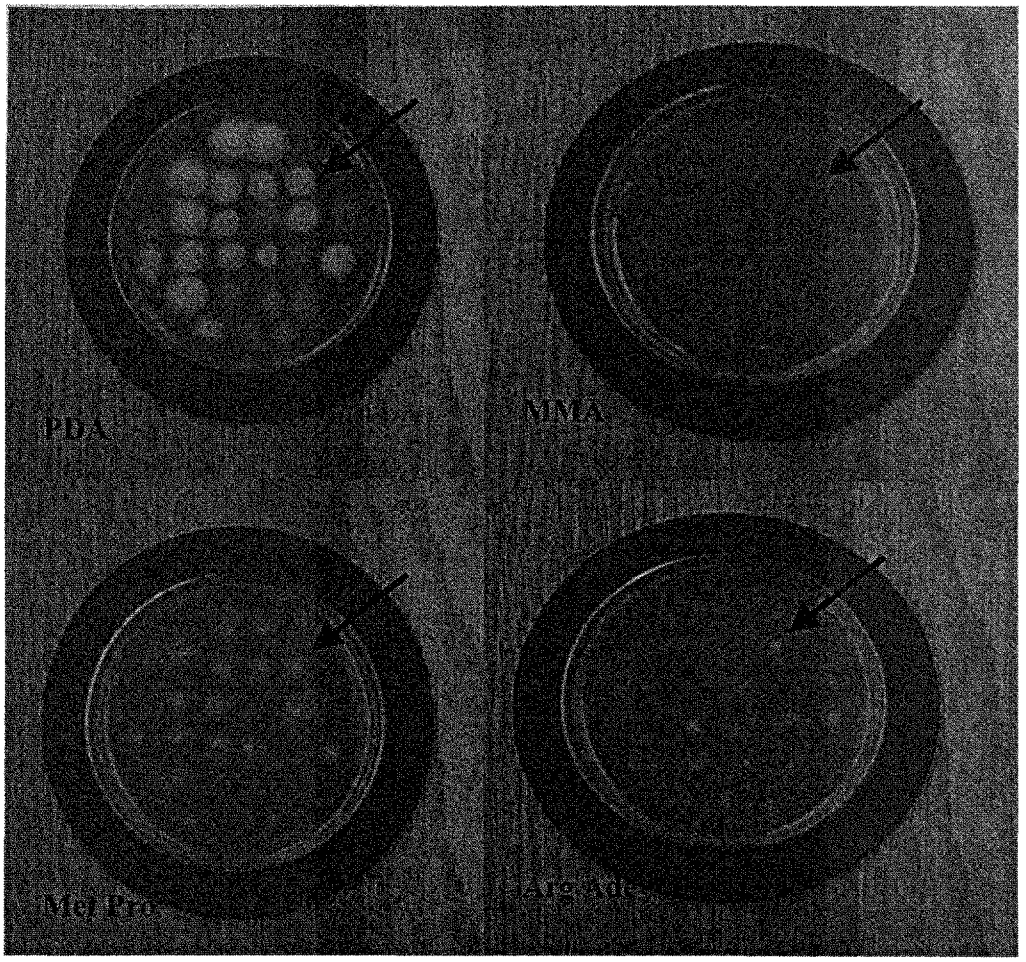


Figure 7. Genotypic segregation of microconidia from heterokaryotic hyphal tip B18T1-14-3 (Table 13). 6/25 colonies segregated into the non-parental P1/P2 genotype. P1/P2 colonies are incapable of prototrophic growth yet they can grow on MMA plates supplemented only with the nutritional requirement of either auxotrophic parent (arrows). Microconidia collected from these colonies further segregated into one parental genotype or the other (Table 13)

2.4.2 0126 x 0126 heterokaryons between stb2 K⁻ and stm3 M⁻R

2.4.2.1 Genetic Analysis

Table 14. Diagnostic test of individual microconidia from Intra-VCG Heterokaryons (0126 x 0126) from pairings between stb2 K⁻ and stm3 M⁻R⁻. Segregation of both parental phenotypes was observed on analysis of microconidia from putative heterokaryons that were transferred to selective medium for continued growth, confirming heterokaryon formation. A prototrophic colony suggestive of hybrid formation was also recovered.

	Diagnostic test		
Hyphal Fusion	Prototrophic	Auxotrophic for Parent 1	Auxotrophic for Parent 2
HFB3223	0 (0%)	40 (80%)	10 (20%)
HFB3230	0 (0%)	16 (36%)	28 (64%)
HFB3236	0 (0%)	2 (4%)	44 (96%)
HFB3240	0 (0%)	13 (28%)	33 (72%)
HFB361	1 (2%)	32 (75%)	10 (23 %)
HFB363	0 (0%)	8 (19%)	34 (81%)
HFB365	0 (0%)	12 (34%)	23 (66%)
HFB366	0 (0%)	6 (75%)	2 (25%)
HFB368	0 (0%)	25 (59%)	17 (41%)
Protoplast Fusion			
PFB3437	0 (0%)	50 (100%)	0 (0%)
PFB3424	0 (0%)	50 (100%)	0 (0%)
PFB3410	0 (0%)	50 (100%)	0 (0%)
PFB3442	0 (0%)	28 (56%)	22 (44%)
PFB3446	0 (0%)	50 (100%)	0 (0%)
PFB344	0 (0%)	49 (100%)	0 (0%)
PFB3420	0 (0%)	50 (100%)	0 (0%)
PFB3427	0 (0%)	50 (100%)	0 (0%)

2.4.2.2 Statistical Analysis

Table 15. Chi-square test of intra-VCG pairings between two VCG 0126 isolates: STB2 K⁻ and STM3 M⁻R Significance level was set at α=0.05.

Hypothesis	Value	df[a]	Sig.(2-sided)p-value
H_o1: HF=PF	3.238	1	0.072*
H_o2: P1=P2	4.2632	1	0.0389

[a] df=degrees of freedom; * Not significant

2.4.3 0124 x 0124 heterokaryons between gmb Ade- (P1) and gmb K-R- (P2)

2.4.3.1 Genetic Analysis

Table 16. Diagnostic test of individual microconidia from Intra-VCG Heterokaryons (0124x 0124) from pairings between gmb Ade- (P1) and gmb K-R- (P2) using the double pick method (Appendix I). 25 pairings were attempted. 12/25 grew and were further transferred to MMA for continued growth. Only 4 of the 12 putative hets continued to grow. In general only one parent was recovered. Segregation of both parental phenotypes was observed in one het, although at a skewed ratio. A colony with an altered genotype was detected for putative het HFC2218 and identified as K-R-Ade-.

	Diagnostic test		
Hyphal Fusion	Prototrophic/ non-parental	Auxotrophic for Parent 1	Auxotrophic for Parent 2
HFC222	0 (0%)	0 (0%)	25 (100%)
HFC229	0 (0%)	0 (0%)	25 (100%)
HFC2210	0 (0%)	1 (4%)	24 (96%)
HFC2217	0 (0%)	0 (0%)	25 (100%)
HFC2218*	1 (4%)	0 (0%)	24 (96%)

2.4.4 0124 x 0124 pairings between gmb K^-R^- (P1) and jcb1 Cys^- (P2)

No heterokaryon formation was detected for these pairings

2.4.5 0124 x 0124 pairings between gmb Ade- (P1) and jcb1 Cys^- (P2)

No heterokaryon formation was detected for these pairings

2.4.6 0124 x 0124 pairings between gmb Ade- (P1) and gmb M^- (P2)

No heterokaryon formation was detected for these pairings

2.5 Results Inter-VCG Pairings

2.5.1 0120 x 0126 heterokaryons between sa8 M$^-$P$^-$Ben^R and stm3 K$^-$R$^-$Ben^S

2.5.1.1 Genetic Analysis

Table 17. Heterokaryon formation by hyphal anastomosis and protoplast fusion of inter-VCG pairings between sa8 M$^-$P$^-$Ben^R and stm3 K$^-$R$^-$Ben^S. Putative heterokaryons were transferred to MMA. Spores were collected from colonies that continued to grow under selective pressure.

	Diagnostic test		
Hyphal Fusion	Prototrophic	Auxotrophic for Parent 1	Auxotrophic for Parent 2
HFA521	0 (0%)	14 (58%)	10 (42%)
HFA522	0 (0%)	25 (100%)	0 (0%)
HFA523	0 (0%)	23 (96%)	1 (4%)
HFA524	0 (0%)	25 (100%)	0 (0%)
HFA525	0 (0%)	25 (100%)	0 (0%)
HFA5248	0 (0%)	0 (0%)	48 (100%)
Protoplast Fusion			
PFA541	0 (0%)	48 (100%)	0 (0%)
PFA542	0 (0%)	48 (100%)	0 (0%)
PFA545	0 (0%)	48 (100%)	0 (0%)
PFA5415	0 (0%)	31 (100%)	0 (0%)
PFA5416	0 (0%)	48 (100%)	0 (0%)
PFA5418	0 (0%)	48 (100%)	0 (0%)
PFA5420	0 (0%)	48 (100%)	0 (0%)
PFA5425	0 (0%)	7 (42%)	10 (58%)
PFA5431	0 (0%)	48 (100%)	0 (0%)
PFA5440	0 (0%)	48 (100%)	0 (0%)

2.5.1.2 Statistical Analysis

Table 18. Chi-square test of inter-VCG pairings between VCG 0120 and VCG 0126 isolates: sa8 $M^{-}P^{-}Ben^{R}$ and stm3 $K^{-}R^{-}Ben^{S}$. Significance level was set at α=0.05.

Hypothesis	Value	df[a]	Sig.(2-sided)p-value
$H_0$1: HF=PF	0.152	1	0.696 *
$H_0$2: P1=P2	12.2500	1	0.0005

[a] df=degrees of freedom ; * Not significant

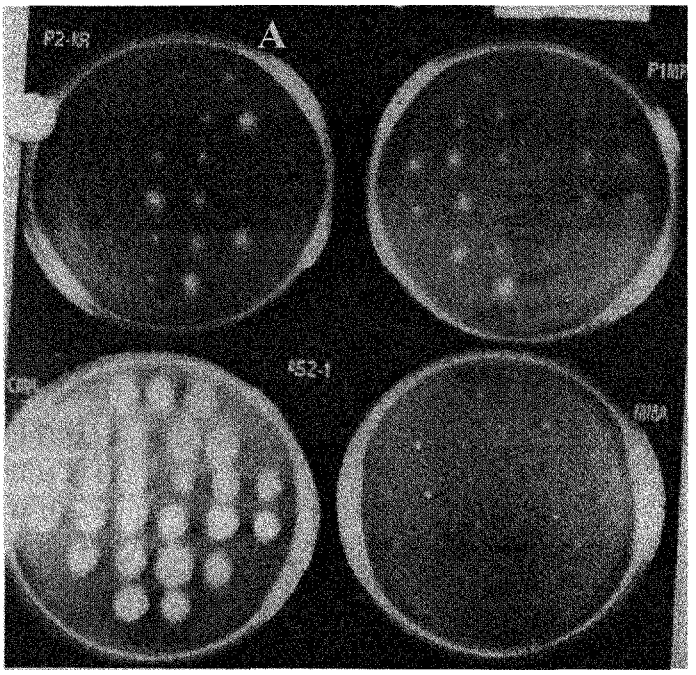


Figure 8 A Diagnostic Plates of Heterokaryon HFA521 (Table 17) top left MMA + KR, top right MMA +MP, Bottom left CMA, bottom right MMA

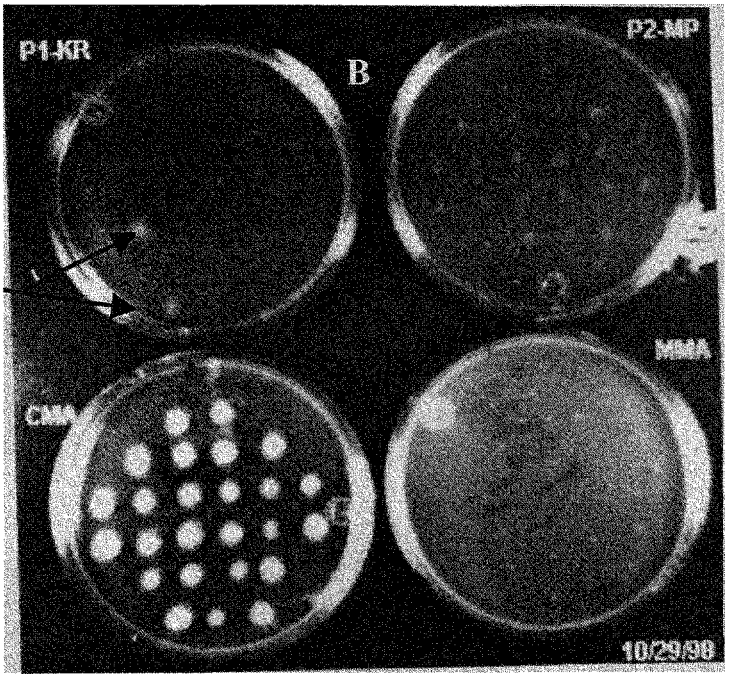


Figure 8 B
Diagnostic Plates of Heterokaryon HFA523 (Table 17). Top left MMA + KR, top right MMA +MP, Bottom left CMA, bottom right MMA

2.5.2 0120 x 0126 heterokaryons between sa8 $M^-P^-Ben^R$ and stb2 K^- Ben^S

2.5.2.1 Genetic Analysis

Table 19. Heterokaryon formation **by** hyphal anastomosis and protoplast fusion on an inter-VCG pairing between sa8 $M^-P^-Ben^R$ and stb2 K^- Ben^S. Putative heterokaryons were transferred to MMA. Spores were collected from colonies that continued to grow under selective pressure.

	Diagnostic test		
Hyphal Fusion	Prototrophic	Auxotrophic for Parent 1	Auxotrophic for Parent 2
HFB5211	0 (0%)	25 (100%)	0 (0%)
HFB5215	0 (0%)	3 (12%)	22 (88%)
HFB5237	0 (0%)	0 (0%)	25 (100%)
HFB5246	0 (0%)	2 (8%)	23 (92%)
HFB5248	0 (0%)	0 (0%)	25 (100%)
HFB561	0 (0%)	2 (5%)	40 (95%)
HFB562	0 (0%)	2 (100%)	0 (0%)
HFB563	0 (0%)	1 (11%)	8 (89%)
HFB564	0 (0%)	0 (0%)	2 (100%)
HFB566	1 (2%)	3 (7%)	40 (91%)
Protoplast Fusion			
PFB5412	0 (0%)	0 (0%)	48 (100%)
PFB5416	0 (0%)	0 (0%)	48 (100%)
PFB5419	0 (0%)	0 (0%)	48 (100%)
PFB5424	0 (0%)	0 (0%)	48 (100%)
PFB5425	0 (0%)	1 (2%)	47 (98%)
PFB5430	0 (0%)	0 (0%)	48 (100%)
PFB5436	0 (0%)	0 (0%)	48 (100%)
PFB5439	0 (0%)	0 (0%)	48 (100%)
PFB5443	0 (0%)	0 (0%)	48 (100%)
PFB5446	0 (0%)	0 (0%)	48 (100%)

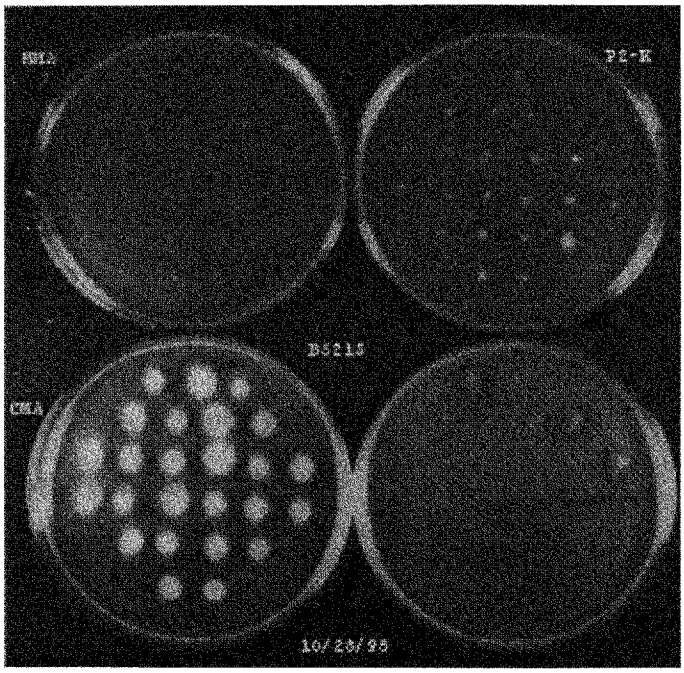


Figure 9 A. Diagnostic plates of heterokaryon HFB5215 (Table 19). Top left MMA, top right MMA + K, bottom left CMA, bottom right, MMA + MP

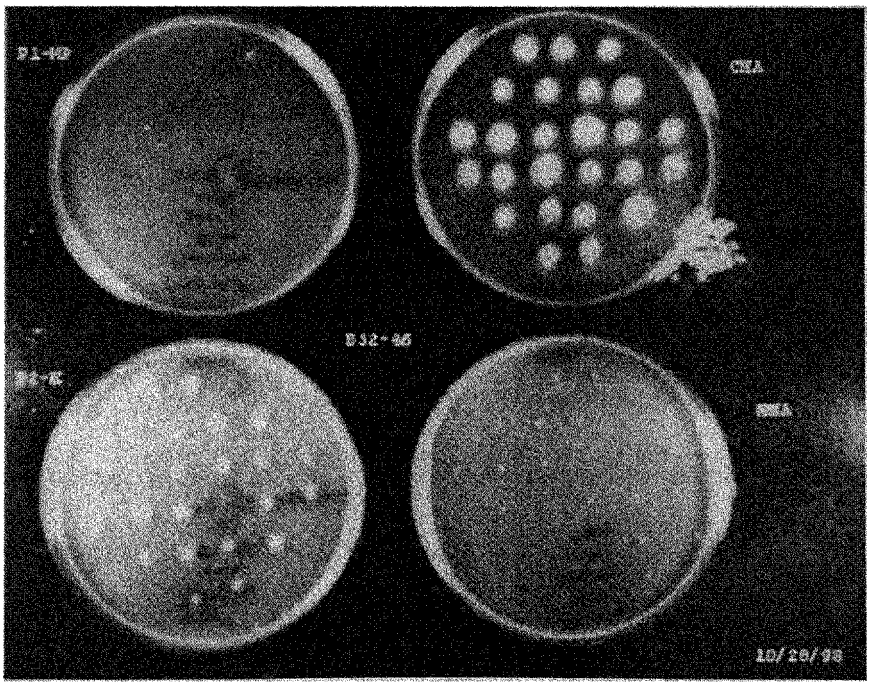


Figure 9 B. Diagnostic plates of heterokaryon HFB5246 (Table 19). Top left MMA + MP, top right CMA , bottom left MMA + K, bottom right, MMA

2.5.2.2 Statistical Analysis

Table 20. Chi-square test of inter-VCG pairings between VCG 0120 and VCG 0126 isolates: sa8 $M^{-}P^{-}Ben^{R}$ and stb2 $K^{-}Ben^{S}$. Significance level was set at α=0.05.

Hypothesis	Value	df[a]	Sig.(2-sided)p-value
H_o1: HF=PF	2.2222	1	0.136 *
H_o2: P1=P2	12.80	1	0.001

[a] df=degrees of freedom ; * Not significant

2.5.2.3 Molecular analysis

Propagules from inter-VCG/inter-mitotype heterokaryons (tables 17 and 19) were cultured in PDB. DNA extraction and PCR amplification procedures for mitotype determination were done following the protocols of D'Alessio (1997).

After seven days, heterokaryons were transferred into 1 ml of PDB in Eppendorf tubes. Forty-eight to seventy-two hours later, DNA was isolated from mycelium and amplified in the presence of ^{32}P to assay for mitochondrial type (Figure 10).
HFA and PFA samples refer to heterokaryons between sa8 $M^{-}P^{-}Ben^{R}$ and stm3 $K^{-}R^{-}Ben^{S}$ by hyphal fusion (HF) and protoplast fusion (PF). HFB and PFB samples refer to heterokaryons between sa8 $M^{-}P^{-}Ben^{R}$ and stb2 $K^{-}Ben^{S}$. In these seven-day heterokaryons, we observed the survival of only one mitochondrial type. All heterokaryons between sa8 $M^{-}P^{-}Ben^{R}$ and stm3 $K^{-}R^{-}Ben^{S}$ had mitotype I, all heterokaryons between sa8 $M^{-}P^{-}Ben^{R}$ and stb2 $K^{-}Ben^{S}$ had mitotype VI but there was no evidence of heteroplasmy even in heterokaryons formed by protoplast fusion (Table 21).

In the inter-VCG pairings between sa8 $M^{-}P^{-}Ben^{R}$ and stb2 $K^{-}Ben^{S}$, though, we

found evidence of recombination of nuclear and mitochondrial genomes both on HF and PF heterokaryons.

Table21. Genotypic segregation of microconidia and mitotype from heterokaryons between VCG 0120 and VCG 0126 where both parents were recovered (Tables 17&19)

Code	Prototrophs (MMA)	Parent 1 VCG0120	Parent 2 VCG0126	Parent 1 and Parent 2	Mitotype
HFB5246	0/25	2/25	23/25	0/25	0126
HFB5215	0/25	3/25	22/25	0/25	0126
HFB5211	0/25	25/25	0/25	0/25	0126*
HFB563	0/9	1/9	8/9	0/9	0126
HFB561	0/48	2/48	40/48	6/48	0126
PFB5425	0/48	47/48	0/48	1/48	0126*
HFB566	1/48	3/48	40/48	4/48	0126
HFA521	0/25	14/25	10/25	1/25	0120
HFA523	0/25	23/25	1/25	1/25	0120
PFA5425	0/18	7/18	10/18	1/18	0120

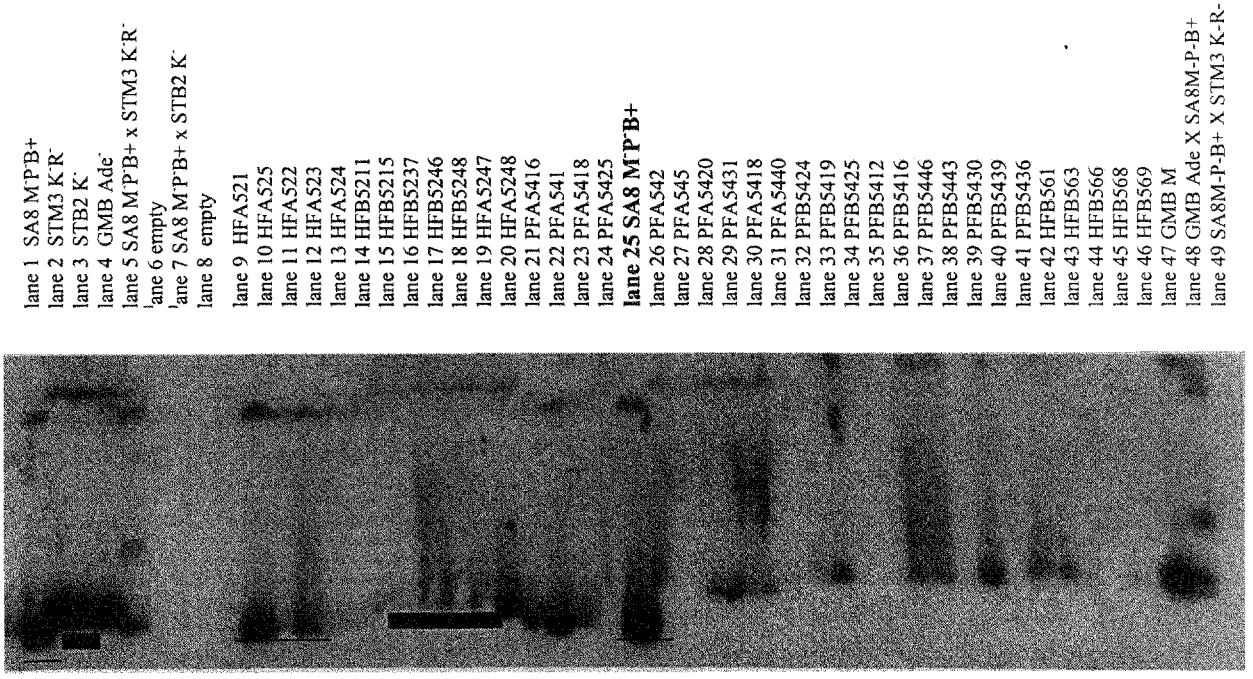


Figure 10. Autoradiograph of a 5% PAGE of amplified DNA from heterokaryons between VCG 0120 and VCG 0126 to determine mitotype. Lanes 1-8: controls and parental mitotypes. Lanes 9-20: heterokaryons formed by hyphal anastomosis. Lanes 21-24, 26-41 : heterokaryons formed by protoplast fusion. Lanes 42-46: heterokaryons formed by hyphal anastomosis. The bars under the heterokaryons (figure 10) correspond to the mitotype based on the controls. The thinner bar represents mitotype I, the thicker bar represents mitotype VI. There was no evidence of heteroplasmy (controls lanes 5 and 49).

2.5.3 0120 x 0124 heterokaryons between sa8 $M^-P^-Ben^R$ and GMB Ade^-

No heterokaryon formation was detected for sa8 $M^-P^-Ben^R$ and GMB Ade^- by either method of fusion.

2.5.4 0124 x 0126 heterokaryons between stb2 K^- and gmb M^-

2.5.4.1 Genetic Analysis

Table 22. Diagnostic test of individual microconidia from Inter-VCG Heterokaryons (0124x 0126) from pairings between GMB M^- (P1) and STB2 K^- (P2) using the double pick method. 25 pairings were attempted. 3/25 grew and were further transferred to MMA for continued growth. Segregation of both parental phenotypes was observed. Altered genotypes were recovered (Figure 11). After one more generation the altered genotypes segregated into the parental strain.

	Diagnostic test		
Hyphal Fusion	Auxotrophic for Parent 1	Auxotrophic for Parent 2	Altered
HFD622	11 (46%)	13 (54%)	0 (0%)
HFD629	6 (26%)	16 (70%)	1 (4%)
HFD6213	6 (29%)	12 (57%)	3 (14%)

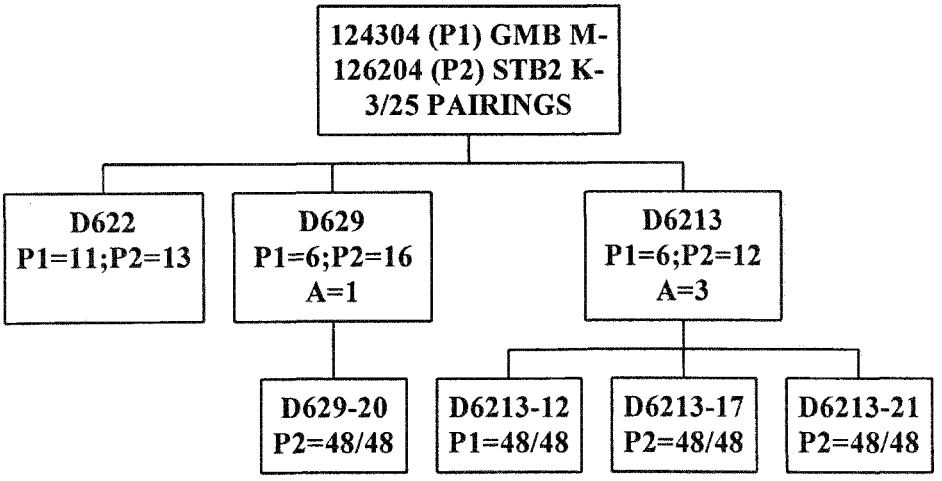


Figure 11. Segregation of altered genotypes in pairing between gmb M^- (0124) and stb2 K^- (0126). Non-parental genotypes were identified after the first generation of spores. A second generation of spores resolved the non-parental types into one parental type or the other

2.6 Summary of Heterokaryon formation

Tables 23 and 24 summarize the interactions observed in all the pairings. Stable recombinant genotypes are presented in Table 23 under the heading NP.

Table 23. Summary of heterokaryon formation and analysis of the progeny of the heterokaryons examined during Phase I. The first subset deals with intra-VCG pairings while the second subset summarizes Inter-VCG pairings. No heterokaryon formation was evident in Hets examined showing "0"

Markers P1:P2[a]	Hets[b]	Cfus[c]	Genotype P1	Genotype P2	NP[d]	Percent NP	Pairing
$M^-P^-B^R$:$R^-Ade^-B^S$	25	1105	854	229	22	1.99	0120 x 0120
M^-R^-: K^-	18	805	578	134	1	0.12	0126 x 0126
K^-R^-: Ade^-	4	125	1	123	1	0.80	0124 x 0124
K^-R^-: C^-	0	0	0	0	0	0.00	0124 x 0124
Ade^-: M^-	0	0	0	0	0	0.00	0124 x 0124
Ade^-: C^-	0	0	0	0	0	0.00	0124 x 0124
SUB-TOTAL	**47**	**2035**	**1436**	**575**	**24**	**1.18**	
$M^-P^-B^R$: K^-R^-	16	613	532	79	0	0.00	0120 x 0126
$M^-P^-B^R$: K^- *	20	704	39	664	1	0.14	0120 x 0126
$M^-P^-B^R$: Ade^-	0	0	0	0	0	0.00	0120 x 0124
M^- : K^-	3	256	71	185	0	0.00	0124 x 0126
SUB-TOTAL	**39**	**1573**	**642**	**928**	**1**	**0.06**	
TOTAL	**86**	**3608**	**2078**	**1503**	**25**	**0.69**	

[a] P1:P2=Parent 1 x Parent 2; markers: Ade=Adenine, BR=benomyl resistant, C=cysteine, K=Lysine, M=Methionine, P=Proline, R=Arginine

[b] hets=Number of individual heterokaryons examined

c Cfus=Colony forming units (number of individual spores examined)

[d] NP=Non-parental genotypes (Three non-parental genotypes were recovered: Altered, prototrophic and P1/P2)

* In addition to recombination of the nuclear genome, 72/704 cfus showed nuclear and mitochondrial recombination, i.e., the nuclear genotype of Parent 1 with the mitotype of parent two

Recovery of these non-parental genotypes occur at a rate of 1 in 80 to 1 in 1500, three to four orders of magnitude different than recovery of non-parental genotypes recovered through karyogamy (estimated at 1 in a million)

Table 24. Summary of heterokaryon formation. During stable heterokaryon formation all putative heterokaryons continued to grow when transferred to fresh unsupplemented MMA plates. In transient heterokaryon formation, not all the putative heterokaryons continued to grow when transferred to MMA and the number of heterokaryons that remained heterokaryotic diminished from transfer to transfer.

Pairing	Hyphal Fusion	Protoplast Fusion
0120 x 0120	Stable	Stable
0124 x 0124	Transient	Transient
0126 x 0126	Stable	Stable
0120 x 0124	None	None
0120 x 0126	Transient	Transient
0124 x 0126	Transient	N/D

2.7 Discussion

Hyphal anastomosis is a widespread occurrence in fungi. It turns a two-dimensional structure into a three-dimensional network, thus increasing cytoplasmic flow throughout the colony. The vegetative hyphae in Foc is septate and is believed to be homokaryotic, that is, only one type of nucleus is found in each cell. Although hyphal fusion can occur between hyphal cells of the same strain, genetic diversity can only be achieved when fusion occurs between genetically distinct homokaryotic cells. This is the basis of heterokaryon formation. While evidence of heterokaryon formation in the field is very scarce, under controlled conditions we have been able to observe heterokaryon formation. One possibility why heterokaryons have not been observed in nature might be the selection pressure when strains are being isolated from the soil or infected plants. Heterokaryon formation may be stable where the heterokaryotic colony can be transferred to selective media repeatedly without evidence of segregation into the individual components (parental strains), or it might be transient where it no longer remains prototrophic after repeated transfers. Yet, as the cytoplasmic stream is a very dynamic process, this short association may be all the fused cells need to exchange genes or cytoplasmic organelles, thus, a transfer of genetic information (Milgroom, personal communication).

This study was undertaken to see if horizontal genetic transfer was possible during heterokaryon formation, especially under transient heterokaryon formation. Phase I dealt with characterization of heterokaryon formation among and between strains in Foc. The following observations were made on analysis of all the attempted pairings.

2.7.1 On methods of fusion

Chi-square tests were conducted to determine if there were significant differences between the method of pairing used (hyphal anastomosis [HF] versus protoplast fusion [PF]) and the segregation of microconidia from the examined intra- and inter-VCG heterokaryons. The null hypothesis is one of no association between the method and the outcome, that is, $H_0$1:HF=PF. Contrary to *Gibberella Fujikoroi*, the sexual state of *Fusarium moniliforme* (Leslie 1997) there were no significant differences ($p>0.05$) between heterokaryons formed by hyphal anastomosis or protoplast fusion and the outcome of segregation of propagules from the heterokaryons. This indicates that the segregation of propagules from a heterokaryon is not dependent on the method used, but rather on the interaction of the parental strains used for the pairing, regardless of whether they belonged to the same VCG or not.

2.7.2 On heterokaryon formation

Differences were observed in the ability of coinoculated strains to form heterokaryons. For instance, intra strain heterokaryons were formed quite readily in VCG 0120 and in VCG 0126. Heterokaryons were stable and remained prototrophic through several rounds of transfers to selective media. In both VCGs all of the heterokaryons transferred continued to grow. However, 3 / 4 attempts at intra VCG pairings between strains and between isolates within a strain in VCG 0124 failed and the number of heterokaryons that continued to grow on subsequent transfers, failed to recover all the transferred colonies.

Transient inter-VCG heterokaryon formation was documented in pairings between strains in VCG 0126 and VCG 0120 and VCG 0126 and VCG 0124. These results suggest that the barriers imposed by the self/non-self recognition mechanism are not stringent enough to prevent vegetative heterokaryon formation.

As Leslie (1997) points out, though, in *Fusarium* spp., regulation of vegetative incompatibility (*vic* loci) is allelic and vegetatively compatible strains are identical at each locus in the *vic* gene. Furthermore, it is believed that a single change in the *vic* locus can make two compatible strains incompatible (Ploetz, 1990, Leslie 1997, Glass and Kuldau 1992). Our auxotrophic mutants were generated by UV mutagenesis. However, only scorable mutations can be detected. If allelic changes to the *vic* loci resulted from the mutagenesis, they might go unnoticed due to inadequate screening techniques for changes other than biochemical deficiencies or morphological changes. Yet, if the recognition system must be present in both strains, the fact that pairings are possible using wild type testers (Cortes 1996) where one strain is unmutagenized, will argue the fact that inter-VCG heterokaryon in Foc is solely due to changes in the *vic* loci due to mutagenesis.

One difference between intra-VCG pairings and inter-VCG pairings (except for VCG 0124) is that the number of putative heterokaryotic colonies that continue to grow under selective pressure is diluted from transfer to transfer (i.e. the number of heterokaryons diminished with the transfers). Transient heterokaryons, though, are a good target to examine horizontal transfer without karyogamy.

There were no differences in heterokaryons formed between different strains or races when compared to within strains or races pairings.

2.7.3 On heterokaryotic hyphal tips

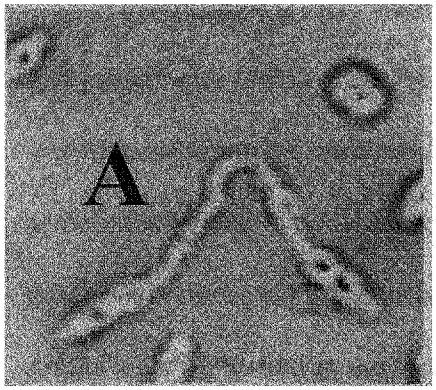
We know from the literature that there are two types of heterokaryons, one that forms and continues to reform to sustain prototrophic growth of the colony and a second type where the two nuclei divide and can travel through the length of the hypha (Chacko et al. 1994, Glass and Kuldau 1992). In septate, filamentous fungi, although they are not coenocytic (aseptate) the cytoplasmic continuity is guaranteed by open pores in the septa, big enough to allow nuclear and cytoplasmic movement through the hyphal tube. In *Gibberella* heterokaryons are confined to the anastomosed cell. Thus, it is not surprising to find heterokaryons forming only in the area of fusion, away from the tips upon transfer of propagules under selective pressure (Puhalla 1985). Moreover, the hyphal tips of these colonies would be expected to be homokaryotic.

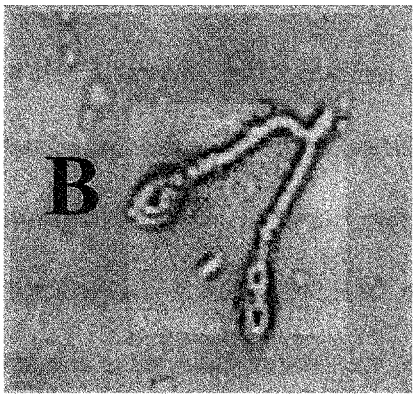
I believe, though, that both types of heterokaryon formation are expressed in Foc. Where the heterokaryon is confined to the anastomosed cell, microconidia segregate from the heterokaryotic cell into propagules representing both individual parental genotypes and homokaryotic hyphal tips. Where the nuclei divide in the heterokaryotic cell and travel between cells, one can expect propagules expressing either parental genotypes within a cell and on occasion heterokaryotic hyphal tips.

Figure 12. Multinucleate hyphal cells. Individual cells within the hyphal tube can harbor more than one nucleous per cell. As many as eight nuclei per cell have been documented in Foc

Contrary to the belief that the hyphal cells in Foc are uninucleate, microscopic analysis of germinated microconidia has revealed that anywhere between one and eight nuclei can coexist in a hyphal cell (Figure 12). Evidence supporting the hypothesis of nuclear division and migration in Foc, implicating that hyphal tips of heterokaryons can be heterokaryotic, was provided by hyphal tip analysis of an intra-VCG pairing between two strains in VCG 0120. Recovery of both auxotrophic parental genotypes was achieved from single spores from individual hyphal tips of the heterokaryons. Although most of the hyphal tips tested were homokaryotic segregating into one parent or the other, repeated experiments showed that heterokaryotic hyphal tips were also be recovered. (Tables 11 and 12). Therefore, considering the physiological factors differentiating the strains, and the multi-nucleate hyphal cells, perhaps syntrophism is controlled by the contribution of the nuclei controlled by the ratio in which the parental nuclei are present in the cell.

One oddity observed during hyphal anastomosis, could also explain heterokaryotic hyphal tips. Tip to tip hyphal anastomosis was observed in which the two tips fused, only to continue as one hyphal tip (Figure 13 A-C). If the fusion was between genetically distinct hyphal tips, one could expect heterokaryotic hyphal tips to result from that fusion event.





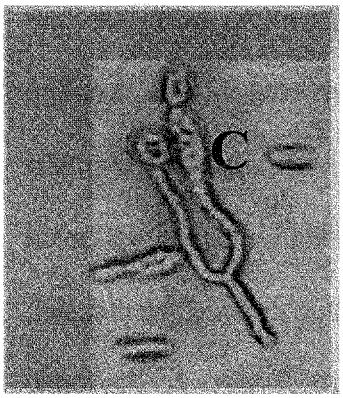


Figure 13 Tip to tip hyphal fusion from two germinated microconidia (A). A small bud forms from the fusion site (B) and the two hypha continue to grow as one (C)

2.7.4 On segregation of microconidia

A segregation ratio of the parental genotypes of 1:1 was not observed, regardless of whether intra-VCG or inter-VCG heterokaryons were examined. In all cases, the null hypothesis of no difference (H_o2: P1-P2=0) was statistically challenged using a non-parametric chi-square test with a significance level set at α=0.05 Tables 10, 17 and 22). What could account for the preferential recovery of one parental genotype over the other?

Physiological differences were observed from strain to strain such as sporulation and growth rate (own results, Ploetz 1990). Under the same nutrition, temperature and growth conditions the number of harvested spores from strain to strain differed sometimes by one to two orders of magnitude, even within the same VCG. Viability and growth-rate under similar nutrient conditions were also different. Slow and fast growing strains were identified in the tested VCGs. Although care was taken to co-inoculate the pairings with equal number of spores, if two physiologically different strains were paired, it is not difficult to believe that segregation of the propagules would be skewed, even if we started with equal number of spores. Moreover, the genetic diagnostic test is done by replica picking (Appendix I). Thus, differences in the growth rate between the strains would select fast growing strains over slow growing strains, hence the skewed ratio. On multiple occasions only one parental genotype was recovered even though the heterokaryon continued to grow under selection pressure after several transfers. One possible explanation could be low to no viability of the spores of the second parental genotype. A cross-feeding possibility was discarded from experiments where the auxotrophic strains were inoculated on selective medium (MMA) but were physically separated by membranes such as dialysis tubing or sterile cellophane sheets. These membranes allowed diffusion of nutrients into the media but prevented physical contact between the co-inoculated spores. No heterokaryon formation was observed under these conditions. Thus we can conclude that hyphal anastomosis, even if transient, is required for heterokaryon formation to occur.

2.7.5 On altered genotypes

Occasionally, segregation of microconidia from heterokaryons gave rise to propagules with non-parental genotypes. Non-parental genotypes included P1/P2 genotypes, altered genotypes and prototrophic genotypes.

P1/P2 microconidia grew under selective media supplemented with the nutritional requirements of either parent and on unselective media (CMA or PDA), but failed to grow prototrophically. Altered microconidia could only grow on unselective media and prototrophs were monoconidia capable of growth in all the plates.

Microconidia recovered showing different non-parental genotypes were further analyzed. Of over 3000 mitospores analyzed a recovery rate of non-parental genotypes can be estimated at 1 in 140 (Table 23). In general, altered genotypes represent a transition stage since one to two generations of microconidia usually resolves the colony into one parent or the other. P1/P2 genotypes always resolved into one parent or the other given enough generations, usually between two or three. Prototrophic genotypes are less common and, just as the altered genotypes, after several generations they resolve into the parental components. However, both parental genotypes as well as prototrophic microconidia can be recovered from a single colony, which leads me to believe that prototrophic microconidia may represent a transient stage before hybrid formation as propagules from heterokaryons generally resolve into one parent or the other. Although a rare event, the estimated rate of recovery cannot rule-out the possibility that horizontal transfer occurs during heterokaryon formation.

2.7.6 On mitochondrial inheritance

Mitochondrial analysis was done on heterokaryons of 0120 x 0126 Inter-VCG pairings (Tables 21, Figure 10). As observed by D'Alessio (1997) during hybrid

formation, mitochondrial inheritance in these heterokaryons was also uniparental. We still can't predict, though, which mitochondria will be inherited or the mechanism by which one mitochondrial type disappears. Even where the nuclear encoded VCG barriers have been removed (i.e. heterokaryons formed by protoplast fusion), only one mitochondrial genome survives. We speculate that the cellular mechanism to remove one mitochondrial genome does not rely on a physical mechanism such as migration into a cell. It is interesting to note, though, that in at least two cases where only one parental genotype was recovered, the identified mitotype belonged to the mitochondrial type of the missing parental genotype (*Table 21). There was no evidence of heteroplasmy in the examined heterokaryons even when fused by protoplast fusion. We know, though, that the preferential recovery of one mitotype can be documented within the first week after the anastomosis event. In other systems, such as in *Neurospora tetrasperma,* uniparental inheritance is observed as early as three days after hyphal fusion.

2.7.7 On horizontal genetic transfer

Forty-seven intra-VCG and thirty-nine inter-VCG individual heterokaryons with over 3000 propagules were examined during Phase I (Table 23). Of those, 24/2035 (or about 1 in 80) propagules of intra-VCG heterokaryons and 1 in 1573 propagules of inter-VCG heterokaryons showed recombination at the nuclear genome level. Mixing of nuclear and mitochondrial genome was observed in inter-VCG pairings at 72 in 1573 (about 1 in 20). Overall, combining intra and inter vcg pairings, the recovery rate of non-parental genotypes was estimated at about 1 in 140 propagules. Compared to a karyogamy event, which is estimated at 1 in a million, one can postulate that horizontal genetic transfer can occur during heterokaryon formation without karyogamy.

Mitochondrial exchange appears to be more stable than nuclear horizontal transfer during the heterokaryotic stage.

Since the genetic and molecular analysis were done on seven-day heterokaryons, and in lieu of the evidence that horizontal genetic transfer could occur during heterokaryon formation, there was a need for a more stringent assay to determine (1) how long after coinoculation can horizontal transfer be detected? and, (2) since uniparental mitochondrial inheritance was observed, when was the second mitotype lost?

This assay, should allow the analysis and scoring of heterokaryon formation on a daily basis. As there is no bearing on the outcome of heterokaryon formation from the method used, if equal number of microconidia from auxotrophic parents were co-inoculated in Liquid Minimal Media, daily aliquots could be collected for diagnostic analysis. Thus Phase II was born: A time course of heterokaryon formation: In search of horizontal transfer in asexual fungi.

CHAPTER 3 Phase II: Time course analysis of heterokaryon formation

3.1 Introduction

The main goal of Phase II was the analysis and scoring of heterokaryon formation on a daily basis, in order to (1) establish a time frame of heterokaryon formation and (2) to evaluate if horizontal genetic transfer can occur without karyogamy. Table 25 contrasts and compares heterokaryosis and karyogamy (Cortes 1996). Based on this profile, the time course was established for one week.

Table 25. Comparison of heterokaryosis vs. karyogamy

Description	Heterokaryosis	Karyogamy
Rate of Formation	1 in 10^2	1 in 10^6
Inoculum needed	$>10^5$ of each parent	$<10^4$ of each parent
Prototrophic growth under selective pressure	Yes	Yes
Morphology under unselective pressure	Stable colonies	Unstable with segregants (Sectors)
Time first evidenced	1-3 days	7-10 days
Nuclear fusion	No	Yes
Segregation of microconidia	Mostly auxotrophic parental types	Mostly non-parental prototrophic types

3.2 Materials and Methods

Auxotrophic strains marked with drug resistance were used to follow horizontal genetic transfer. Figure 14 is a cartoon of the protocol used during the time course.

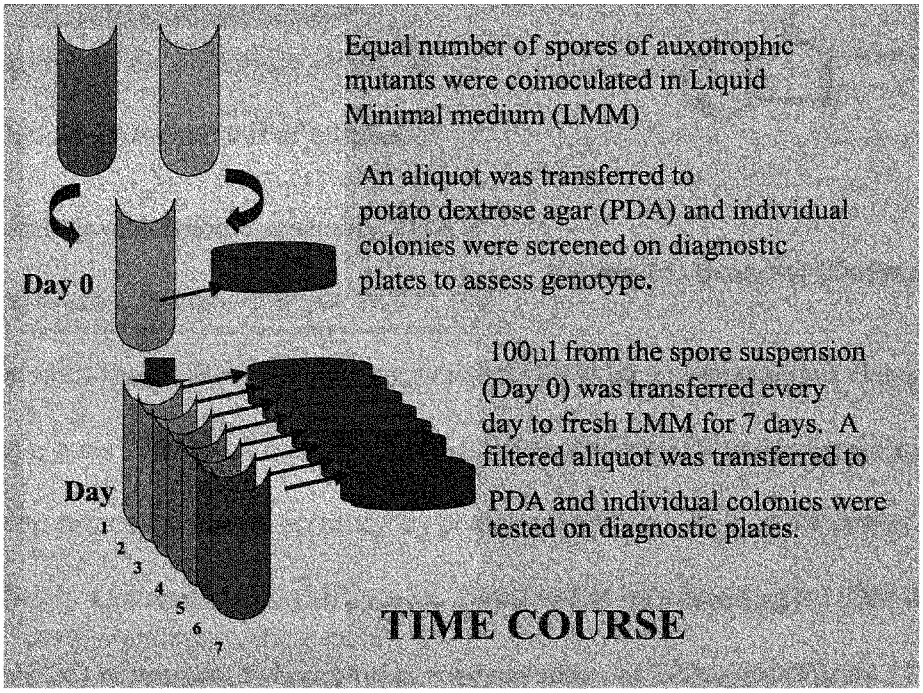


Figure 14. Time course of heterokaryon formation

Two drug resistance markers were used during this study: benomyl (Figure 15) and hygromycin (Figure 16).

Benomyl (DuPont) is a benzimidazole fungicide used in agricultural fields (Davidse 1986). It binds to the beta subunit of tubulin, preventing microtubule assembly, such as in the mitotic spindle, thus preventing cell division in fungi. Benomyl resistance is conferred by a conformation change in the β-tubulin gene. Benomyl resistance can be induced using UV mutagenesis and expression of drug resistance can be screened by positive selection . In *F. oxysporum*, Minimum Inhibitory Concentrations (MIC) were calculated at less than 2μg/ml. Our tester strain (Table 26) is resistant to benomyl at concentrations of 100μg/ml (Figure 17).

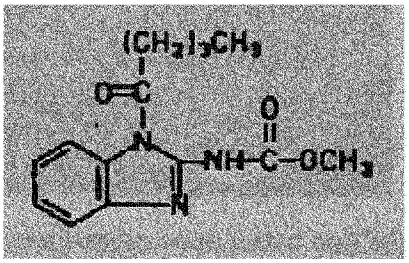


Figure 15. Benomyl (DuPont)

Table 26. Pairings under non-selective drug pressure

Pairings	Strains[a]	Markers[b]	Morphology
cubense x cubense	Sa8 (P1)	$M^-P^-B^R$	Aerial-white
	Stgm1 (P2)	$R^-Ade^-B^S$	Pionnotal-pink
cubense x pisi	Fop 247 (P1)	R^-H^R	Pionnotal-purple
	Stgm1 (P2)	$R^-Ade^-H^S$	Pionnotal-pink
	Fop 247 (P1)	R^-H^R	Pionnotal-purple
	Gmb (P2)	M^-H^S	Aerial-white
	Fop 247 (P1)	R^-H^R	Pionnotal-purple
	Sa8 (P2)	$M^-P^-B^R H^s$	Aerial-white

[a] P1=Parent 1, P2=Parent 2

[b] Ade=adenine, B^R=benomyl resistant, B^S=benomyl sensitive, H^R=hygromycin resistant, H^S=hygromycin sensitive, M=methionine, P=proline, R=arginine

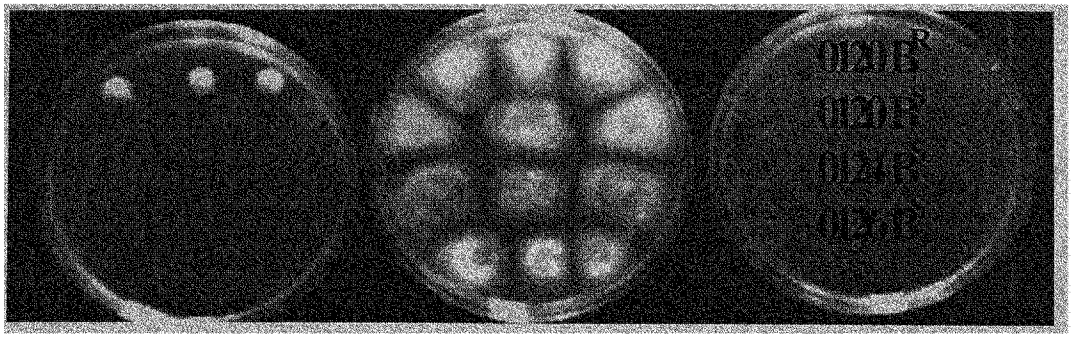


Figure 17. Drug sensitive auxotrophs and the drug resistant strain were tested on CMA + Benomyl @ 100µg/ml (CMB100 [left]), PDA (center) and MMA (right). Only the Benomyl resistant strain grows in the CMB100 plate. No growth is detected in the MMA plate.

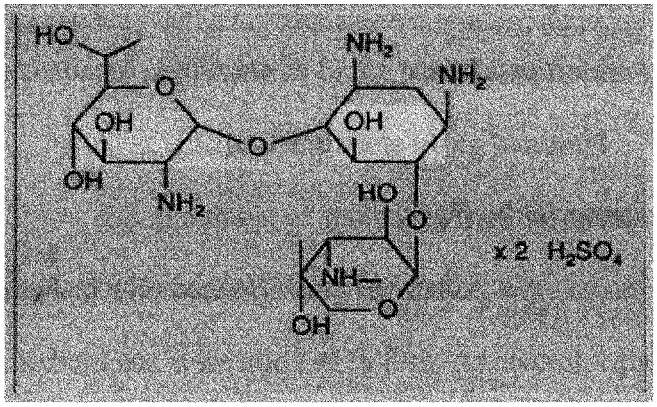


Figure 16. Hygromycin B (Sigma H7772)

The second marker, commonly used in transformation systems, is hygromycin resistance. Hygromycin is isolated from *Streptomyces hygroscopicus.* It works by inhibiting protein synthesis by inducing misreading of the m-RNA template. The bacterial hygromycin phosphotransferase gene (*hph* gene) confers resistance to hygromycin B by phosphorilation of the 7"-hydroxyl group in the destomic acid ring. This study used a strain transformed with pHRC, a construct derived from pDH25, and cloned into a pUC19 vector. (Kistler and Benny 1988). MIC for hygromycin B in *F. oxysporum* was calculated at 30µg/ml. The *Fusarium oxysporum* hygromycin-transformed strain (Table 26), (courtesy of H. Corby Kistler, USDA, University of Minnesota) is resistant to hygromycin B at levels between 100 and 300

μg/ml and was used to force heterokaryon formation across barriers beyond vegetative compatibility.

The combination of drug resistance and auxotrophic genetic markers allowed the analysis of heterokaryon formation under selective and non-selective drug pressure.

3.2.1 Pairings under non-selective drug pressure

Under non-selected drug pressure, two auxotrophic mutants, (one drug-resistant and one drug-sensitive) were paired under nutritional selective pressure in the absence of the drug on liquid minimal medium (LMM, Difco) [minimal medium missing the nutritional requirements of the paired auxotrophic parental strains](Table 26).

3.2.1.1 Pairings using benomyl

Equal number of spores (10^6) of the auxotrophic parental strains, one strain marked with benomyl drug resistance, were coinoculated in one ml of liquid minimal medium and were allowed to fuse. As control, equal number of spores of the individual parental strains was paired with themselves under the same stringent conditions as the complementary parental strains. Daily aliquots were sampled to assess the genotype of propagules from the pairings. Although multiple heterokaryons could be formed within a tube, each tube was scores a single heterokaryon. Pairings were done in triplicate and screened for a week.

The screening consisted in daily plating of serially diluted propagules on potato dextrose (PDA-Difco) plates. Emerging colonies were replica picked into four diagnostic plates, minimal medium agar (MMA). MMA supplemented with the nutritional requirements of the individual parental strains and PDA plates. The first two diagnostic

plates identify the recovery of parental genotypes. The last two plates were used to identify the recovery of non-parental genotypes and as controls.

Putative heterokaryons were transferred to fresh LMM to look for continued growth. Propagules of transferred heterokaryons were further transferred to MMA plates. Spores were collected and screened for the presence of the drug resistance marker in drug-sensitive strains by plating 10^6 spores in MMA supplemented with the nutritional requirement of the drug-sensitive strain plus benomyl.

In addition, radial picks and blocks from the transferred heterokaryons growing on the MMA plates were replica picked to minimal medium (MMA), MMA plus benomyl and Potato dextrose agar (PDA) plates. These propagules were screened for the expression of drug resistance in heterokaryotic colonies.

3.2.1.2 Pairings using hygromycin

One million spores of two auxotrophic mutant strains, one strain transformed with the *hph* gene, were coinoculated in liquid minimal medium. Ten individual pairings were attempted. The tubes were screened on a daily basis for growth. After a week, propagules from heterokaryons were transferred to fresh LMM and screened for continued growth. Propagules from heterokaryons that continued to grow were further transferred to MMA and MMA supplemented with Hygromycin B (MMH100) at concentrations of 100 μg/ml . Plugs from heterokaryons that continued to grow were transferred weekly to MMA for two months. The final week of the transfer, plugs were also transferred to MMH100. Propagules from all the heterokaryons were collected and screened on diagnostic plates as above (See section 3.2.1.1).

3.2.2 Pairings under selective drug pressure

Previously I had forced heterokaryon formation by pairing wild type tester strains against wild type strains (Cortes 1996) (Table 27). Under selective drug stress (Leslie 1993), drug-resistant auxotrophic mutants are paired with drug-sensitive wild type strains or drug-sensitive auxotrophic mutants on MMA augmented with the drug or antibiotic being selected for. Auxotrophic mutants are incapable of growth in MMA and wild type strains are unable to grow on the selective media supplemented with the drug above its minimum inhibitory concentrations (MIC).

Table 27. Isolates of *Fusarium* used under selective drug pressure

Species	f.sp.	VCG	UV	race	mt[a]	origin[b]	Source[c]	ID[d]	isolate	marker[e]
oxysporum	*cubense*	0120	3	4	I	SA		wtt1	sa8	$M^{-}P^{-}B^{R}$
			3	4	I	SA		wtt2	sa8	$M^{-}R^{-}B^{R}$
			3	4	I	SA		wtt3	sa8	$M^{-}Y^{-}B^{R}$
			3	1	I	Hon		wtt4	sth1	$R^{-}Ade^{-}B^{R}$
			2	4	I	Au		wtt5	22425	$R^{-}B^{R}$
			3	4	I	Au		wtt6	22425	$L^{-}M^{-}B^{R}$
			0	?	I	Mal	RCP		15638	WT
			0	4	I	Au	RCP		22425	WT
			0	4	I	Au	RCP		22425	Nit1
			0	4	I	Au	RCP		22425	Nit3
			0	1	I	Hon	RCP		34661	WT
			0	4	I	Au	RCP		a2	WT
			0	4	I	CI	RCP		adj1	WT
			0	4	I	SA	RCP		sa3	WT
			0	4	I	SA	RCP		sa8	WT
			0	1	I	Hon	RCP		stc2	WT
			0	1	I	CR	RCP		stgm1	WT
			0	1	I	CR	RCP		stgm2	WT
			0	1	I	Hon	RCP		sth1	WT
			2	1	I	CR			stgm1	$R^{-}Ade^{-}B^{S}$
		0121	0	4	II	Tw	RCP		t3	WT
			0	4	II	Tw	RCP		gm	WT
		0122	0	4	I	Ph	RCP		ph2	WT

		0	4	I	Ph	RCP		pw5	WT
		0	2	I	Ph	RCP		saba	WT
	0123	0	1	III	Ph	RCP		davao	WT
		0	1	III	Tw	RCP		f9129	WT
	0124	2	1	IV	Br		wtt7	gmb	K^-B^R
		1	1	IV	Br			gmb	M^-
		0	1	IV	Br	RCP		gmb	WT
		2	2	IV	Fl-USA		wtt8	jcb1	C^-B^R
		0	2	IV	Fl-USA	RCP		jcb1	WT
		0	2	IV	Fl-USA	RCP		b2	WT
		0	2	IV	Hon	RCP		blug	WT
		0	1	IV	Br	RCP		maca	WT
		0	2	IV	Jam	RCP		s?	WT
		0	2	IV	Hon	RCP		std1	WT
		0	2	IV	Hon	RCP		std2	WT
		0	1	IV	Ni	RCP		stn1	WT
	0124/5	0	?	IV	EA	RCP		ea1	WT
		0	?	IV	EA	RCP		ea25	WT
		0	?	V	EA	RCP		ea23	WT
	0125	0	1	IV	Au	RCP		8610	WT
		0	1	IV	Au	RCP		8625	WT
	0126	3	1	VI	Hon		wtt9	stb2	$K^-R^-B^R$
		0	1	VI	Hon	RCP		stb2	WT
		0	1	VI	Hon	RCP		stb3	WT
		0	1	VI	Hon	RCP		stm1	WT
		0	1	VI	Hon	RCP		stm3	WT
	0128	0	2	IV	Au	RCP		22994	WT
		0	2	IV	Au	RCP		a47	WT
	0129	0	4	I	Au	RCP		n5331	WT
		0	1	I	Au	RCP		0-1221	WT
	01210	0	1	VI	Fl-USA	RCP		jc14	WT
		0	1	VI	Cu	RCP		f1cuban	WT
	01211	0	1	I	Au	RCP		13721	WT
		0	?	I	Au	RCP		sh3142	WT
	01212	0	?	IV	Tz	RCP		stnp1	WT
		0	?	IV	Tz	RCP		stnp4	WT
	01213	0	?	II	Indo	RCP		stsum2	WT
	01214	0	?	VII	Mw	RCP		mw2	WT
		0	?	VII	Mw	RCP		mw41	WT
lycopercisi	Fol	0	2	VIII	Fl-USA	HCK		73	WT

			0	1	VIII	It	HCK		R1sc626	WT
			0	3	VIII	Fl-USA	HCK		R3sc761	WT
	pisi	Fop	2	5	IX	Wa-USA		wtt10	247	$M^{-}R^{-}H^{R}$
			1	5	IX	Wa-USA			247	$R^{-}H^{R}$
			0	5	IX	Wa-USA	HCK		247	WT
	raphani	0102	0	?	IX	Wi-USA	HCK		699	WT
	conglutinans	0101	0	1	IX	Jap	HCK		777	WT
Solani		mp V	0	?	N/A	?	HCK		s66	WT
	pisi	mp VI	0	?	N/A	?	HCK		77137	WT

[a] mt=Mitochondrial haplotype (a.k.a. mitotypes)

[b] Origin: Au=Australia, Br=Brazil, CI=Canary Islands, CR=Costa Rica, Cu=Cuba EA=East Africa, Fl-USA=Florida, Hon=Honduras, Indo=Indonesia, It=Italy, Jam=Jamaica, Jap=Japan, Mal=Malasia, Mw=Malawi, Ni=Nicaragua, SA=South Africa, Tw=Taiwan, Tz=Tanzania, Wa-USA=Washington, Wi=Wisconsin

[c] Wild type strains of *Fusarium oxysporum* f.sp. *cubense* were obtained from the worldwide collection of Dr. Randy C. Ploetz (RCP) located at the University of Florida, Tropical Research and Education Center in Homestead, Fla. Formae Speciales (f.sp.) wild types and species other than *oxysporum* were provided by Dr. H. Corby Kistler (HCK) USDA, University of Minnesota

[d] WTT=Wild type testers

[e] Markers: Ade=Adenine, B^{R}=Benomyl resistant, B^{S} =Benomyl sensitive, C=cysteine, H^{R}=Hygromycin resistant, K=Lysine, L=Leucine, M=methionine, P=Proline, R=Arginine, WT=Wild type, Y=Tyrosine

The results of 3000 pairings using wild type testers were recapped in Table 35.

3.3 Results

3.3.1 Pairings under non-selective drug pressure: A time course of heterokaryon formation

Heterokaryon formation was evident in all three trials. Propagules were collected and diagnosed. A flow-chart of the time course is represented in Figure 18.

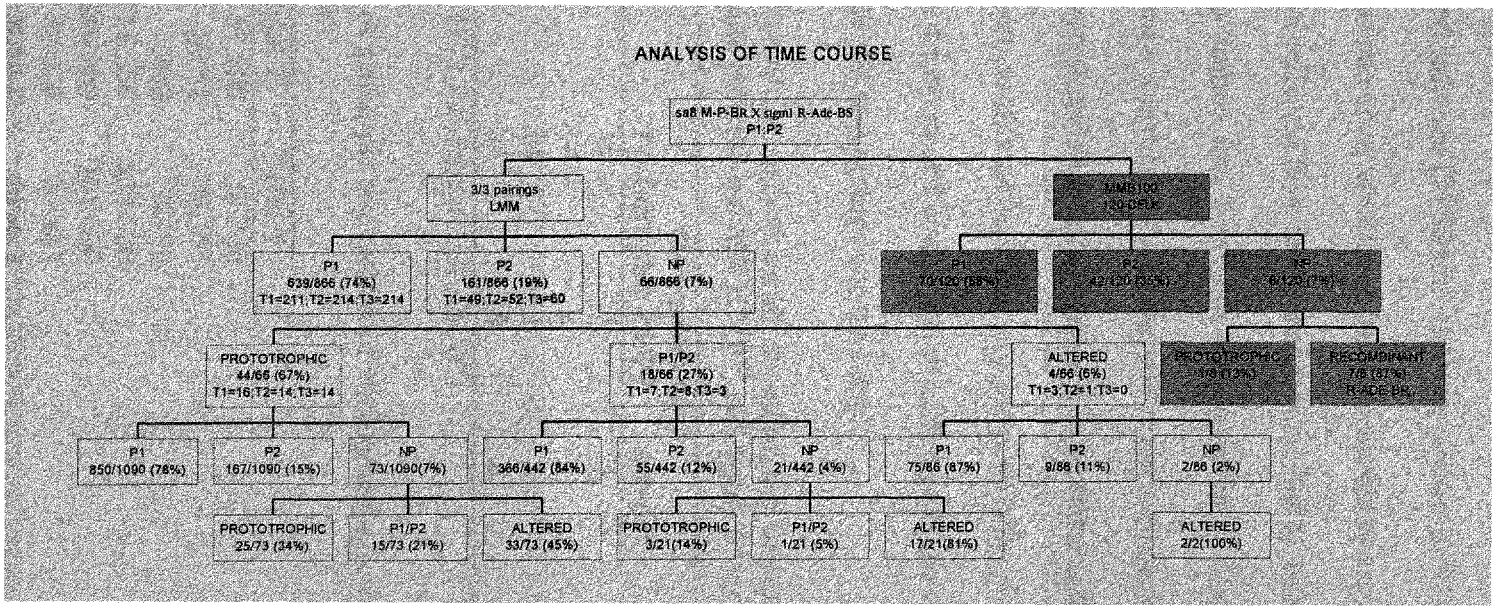


Figure 18. Flow chart of the time course of heterokaryon formation

Table 28 summarizes the diagnosis of microconidia from heterokaryons using the time course by trial. A chi-square (χ^2) test was carried out (significance level set at α=0.05%),using SPSS statistical software (SPSS for Windows release 10.0 October 1999). The null hypothesis was one of no association between the heterokaryons and the segregation of the microconidia collected from the heterokaryons, that is, there was no difference in the heterokaryons formed, thus the results were reproduced in all three trials (H_o: T1=T2=T3). Table 29 recaps statistical information of the three trials. A p-value of 0.515 failed to reject the null hypothesis supporting that there is no difference between the trials. These results were also confirmed by a one-way anova test ($F_{2,12}$=0.000, p-value=1.000). Table 30 pools the result of the segregation of microconidia from the heterokaryons of the three trials and brakes them down by day.

Table 28. Genotypic segregation of microconidia from the individual heterokaryons under non-selective drug pressure between sa8 $M^-P^-B^R$ (P1) and stgm1 $R^-Ade^-B^S$ (P2)

	Parental[a]		Non-parental			
	P1	**P2**	**Prototrophic P1/P2**		**Altered**	**Total**
Het[b] 1	211	49	16	7	3	**286**
Het 2	214	52	14	8	1	**289**
Het 3	214	60	14	3	0	**291**
Total	639	**161**	**44**	**18**	**4**	**866**

[a] Parental: P1=Parent 1 ($M^-P^-B^R$); P2=Parent 2 ($R^-Ade^-B^S$)

[b] Het = heterokaryon

Table 29. Statistical analysis of genotypic segregation of microconidia from triplicate trials of heterokaryon formation

Statistic	Trial 1	Trial 2	Trial 3
Mean	57.200	57.800	58.200
Std Dev	87.859	89.52	90.367
Minimum	3.000	1.000	0.000
Maximum	211.000	214.000	214.000
Sum	286.000	289.000	291.000
SE Mean	39.291	40.034	40.413
Variance	7719.2	8014.2	8166.2

Table 30. Genotypic segregation of microconidia of heterokaryons under the time course by day (Pooled results)

		D0	D1	D2	D3	D4	D5	D6	Total
Parental	**Parent 1**	137	128	126	89	91	42	26	639 (74%)
	Parent 2	4	10	16	44	35	32	20	161 (18.5%)
Non-Parental	**Prototrophic**	0	1	1	11	15	1	15	44 (5%)
	P1/P2	0	1	1	0	3	0	13	18 (2%)
	Altered	0	3	0	0	0	0	1	4 (0.5%)
	Total	**141**	**143**	**144**	**144**	**144**	**75**	**75**	866 (100%)

Non-parental genotypes were recovered as early as one day after inoculation. To determine how stable were these genotypes, individual spores from the colonies showing non-parental genotypes were collected and screened using genetic diagnostic tests (Table 2). The results are presented in Table 31 and summarized in Table 32.

Table 31. Analysis of cfus with non-parental genotypes

			Genotypic segregation of Microconidia					
	CFU	**Genotype**	**MMA**	**MP(P1)**	**Rade(P2)**	**P1/P2**	**Altered**	**PDA**
Day 1	1-19	Altered	0	25	0	0	0	25
	1-26	Altered	0	25	0	0	0	25
	1-30	MMA	2	17	6	0	0	25
	1-34	Altered	0	25	0	0	0	25
	3-48	P1/P2	0	25	0	0	0	25
Day 2	1-13	P1/P2	0	25	0	0	0	25
	1-45	MMA	1	24	0	0	0	25
Day 3	1-1	MMA	1	21	0	3	0	25
	1-8	MMA	4	5	15	0	0	24
	1-43	MMA	3	21	1	0	0	25
	2-41	MMA	0	23	0	0	2	25
	3-9	MMA	1	19	1	0	4	25
	3-13	MMA	0	25	0	0	0	25
	3-17	MMA	0	22	1	0	2	25
	3-21	MMA	0	24	0	0	1	25
	3-33	MMA	1	14	9	0	0	24
	3-43	MMA	0	25	0	0	0	25
	3-48	MMA	0	22	0	1	1	24
Day 4	1-5	MMA	0	17	0	1	6	24

	1-9	MMA	0	22	2	1	0	25
	1-21	MMA	0	18	3	0	2	23
	1-35	MMA	0	22	0	2	0	24
	2-6	P1/P2	0	11	1	0	11	23
	2-8	MMA	1	21	3	0	0	25
	2-12	P1/P2	0	10	7	1	4	22
	2-17	MMA	1	19	5	0	0	25
	2-18	MMA	0	25	0	0	0	25
	2-19	MMA	0	25	0	0	0	25
	2-21	MMA	0	25	0	0	0	25
	2-38	MMA	1	19	3	1	1	25
	2-42	P1/P2	0	25	0	0	0	25
	2-45	MMA	0	25	0	0	0	25
	3-15	MMA	0	17	6	1	1	25
	3-17	MMA	2	17	3	0	3	25
	3-25	MMA	0	24	1	0	0	25
	3-46	MMA	0	24	1	0	0	25
Day 5	1-11	MMA	1	19	5	0	0	25
Day 6	1-1	P1/P2	2	5	17	0	0	24
	1-4	MMA	0	24	1	0	0	25
	1-6	P1/P2	0	25	0	0	0	25
	1-9	P1/P2	1	21	3	0	0	25
	1-15	MMA	1	15	9	0	0	25
	1-16	MMA	1	20	4	0	0	25
	1-20	P1/P2	0	23	1	0	0	24
	1-21	P1/P2	0	23	2	0	0	25
	1-26	P1/P2	0	25	0	0	0	25
	2-6	MMA	1	7	16	0	0	24
	2-7	P1/P2	0	25	0	0	0	25
	2-8	MMA	0	17	8	0	0	25
	2-9	Altered	0	0	9	0	2	11
	2-10	MMA	0	1	21	0	2	24
	2-11	MMA	0	20	0	0	5	25
	2-12	P1/P2	0	25	0	0	0	25
	2-14	MMA	0	21	2	2	0	25
	2-15	P1/P2	0	25	0	0	0	25
	2-17	P1/P2	0	25	0	0	0	25
	2-23	P1/P2	0	24	0	1	0	25
	2-25	MMA	0	24	0	0	1	25
	3-1	MMA	0	0	25	0	0	25
	3-2	MMA	0	25	0	0	0	25
	3-3	MMA	1	18	5	0	0	24
	3-4	MMA	2	19	4	0	0	25
	3-5	MMA	0	19	4	0	2	25
	3-13	P1/P2	0	11	12	0	2	25

3-16	P1/P2	0	13	12	0	0	25
3-23	MMA	0	19	3	2	0	24
	Totals	**28**	**1291**	**231**	**16**	**52**	**1618**

TABLE 32. Summary of segregation of microconidia from 66/866 individual colonies showing non-parental genotypes from the pooled results of heterokaryon formation between sa8 $M^{-}P^{-}B^{R}$ (P1) and stgm1 $R^{-}Ade^{-}B^{S}$ (P2)

			Parental		Non-Parental			
Non-parental Genotype	cfu[a]	Screened spores[b]	P1[c]	P2[d]	Proto-trophic[e]	P1/P2[f]	Altered[g]	Total
Prototrophic	44/66 (67%)	1090/1100	850/1090 (78%)	167/1090 (15%)	25/1090 2%	15/1090 1%	33/1090 4%	1090/1090 (100%)
P1/P2	18/66 (27%)	442/450	366/442 (83%)	55/442 (12%)	3/442 0.7%	1/442 0.3%	17/442 4%	442/442 (100%)
Altered	4/66 (6%)	86/100	75/86 (88%)	9/86 (10%)	0/86 0.0%	0/86 0.0%	2/86 2%	86/86 (100%)
Total	66/66 (100%)	1618/1650 (98%)	1291/1618 (80%)	231/1618 (14%)	**28/1618 2%**	**16/1618 1%**	**52/1618 3%**	1618/1618 (100%)

[a] colony forming units with non-parental genotype (66/866)
[b] 25 spores were collected and analyzed per cfu. Some were lost to contamination
[c] Spores showing the parental genotype of Parent 1
[d] Spores showing the parental genotype of Parent 2
[e] Spores showing the non-parental prototrophic genotype
[f] Spores showing the non-parental P1/P2 genotype
[g] Spores showing the non-parental altered genotype

In general, non-parental genotypes were unstable and segregated into one parent or the other. Nonetheless, a small percentage of recombinants (6%) were recovered.

3.3.2 Drug resistance as an unselected marker

3.3.2.1 Intra-forma species pairings

Benomyl resistance was expressed in heterokaryons paired under non-selective drug stress when propagules were transferred to MMB100 (Figure 19).

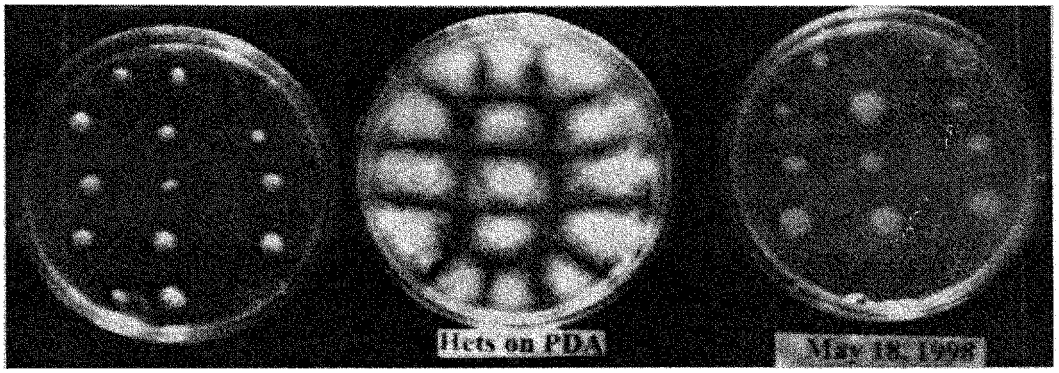


Figure 19. Heterokaryons express Benomyl resistance on minimal medium agar plates augmented with benomyl at a concentration of 100 µg/ml (left). Potato dextrose agar plates (center) and minimal medium agar plates (right) were used as controls.

One hundred and twenty individual spores were collected from these heterokaryons and were screened on diagnostic plates. An additional plate was added to the regular diagnostic plates to test for drug-sensitive spores that may have acquired benomyl resistance. The segregation of these spores is shown in Table 33 and Figure 18 (dark boxes in the flow-chart).

Table 33. Segregation of microconidia of heterokaryons expressing benomyl resistance. Both parental types were recovered as well as colonies showing non-parental genotypes. 7/120 spores analyzed had the nuclear genotype of the benomyl sensitive parent but were now benomyl resistant. One out of 120 was prototrophic but remained benomyl sensitive.

	Parental[a]		Non-Parental		
	P1	**P2**	**Recombinant**	**Prototrophic**	**Total**
Sa8 (P1) X Stgm1 (P2)	70 (58%)	42 (35%)	7 (6%)	1 (1%)	120

[a] Parental: P1=Parent 1 ($M^-P^-B^R$); P2=Parent 2 ($R^-Ade^-B^S$)

Hygromycin resistance was also expressed in heterokaryons paired under non-selective drug stress.

3.3.2.2 Inter-forma species pairings

A. Fop 247 R^-H^R x Foc gmb M^-

Ten out of ten pairings between Fop 247 R^-H^R x Foc gmb M^- coinoculated in LMM produced putative heterokaryons. As these pairings involved inter-forma interactions, each putative heterokaryon was transferred to fresh liquid minimal media for evidence of continued growth. These heterokaryons were transferred four times to determine stability of the heterokaryon for a period of two months. Ten out of the ten continued to grow upon transferring propagules. In addition, twice during these transfers propagules were transferred to MMH100 to examine the expression of drug resistance in the heterokaryon. Spores were collected and screened using normal diagnostic test (Table 2). The results are summarized in Table 34.

Table 34. Segregation of microconidia from heterokaryons between Fop 247 $R^{-}H^{R}$ (P1) x Foc gmb $M^{-}H^{S}$. Preferential recovery of the Hygromycin sensitive parent was observed as well as prototrophic colonies that failed to segregate in the parental components. As these colonies were hygromycin sensitive, reversion of the hygromycin resistant parent was ruled-out. Two colonies, though, sectored and blocks from these sectors continued to grow on MMH100 plates.

Heterokaryon	Growth on MMH100[a]	$R^{-}H^{R}$ genotype (P1)	$M^{-}H^{S}$ genotype (P2)	non-parental genotype
LFPC461	No		25	
LFPC462*	Yes			25
LFPC462-S	Yes			25*
LFPC463	No			25
LFPC464	Yes		25	
LFPC465	No		25	
LFPC466	No		25	
LFPC467	Yes			25
LFPC468	Yes			25
LFPC469	No		25	
LFPC4610*	Yes		25	
LFPC4610-S	Yes			25*

[a] MMH100 = minimal media plus hygromycin at concentration of 100μg/ml

* Heterokaryons marked with * sectored. Blocks transferred from these sectors also grew on MMH100.

B. Fop 247 $R^{-}H^{R}$ x Foc stgm1 WT

In addition to pairings under non-selective drug stress several pairings using the hygromycin transformed strain in *F.o.pisi* (Fop 247 $R^{-}H^{R}$) and a wild type strain of *F.o.cubense* (Foc stgm1 WT)were carried out on potato dextrose agar (PDA) plates, in the absence of drug or nutritional pressure (Figure 20). Blocks from each of the parental strains were allowed to grow towards each other and picks and blocks from the interaction zone were transferred to the diagnostic plates: minimal medium, minimal medium plus hygromycin and PDA plates.

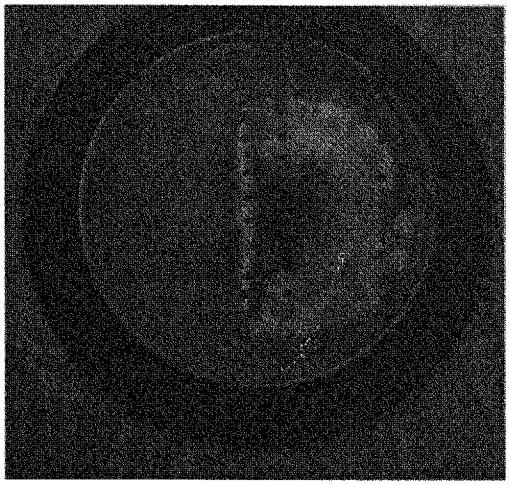


Figure 20. Wild type STGM1(right side) was also paired against Fo *pisi* $R^{-}Hyg^{R}$ (left side), under non-selective pressure. Picks along the interaction zone, as well as one cm away from the interaction zone were transferred to MMHyg100 (MMH100) to assay for heterokaryon formation and/or drug transfer. 3/30 transferred continued to grow on MMH100.

Three out of thirty propagules grew on MMH100 plates. The colonies were pionnotal. Propagules were collected from each of the three colonies. However, only the hygromycin sensitive strain was recovered.

C. Other pairings

The same non-selective method was attempted between Fop 247 $R^{-}H^{R}$ x Foc gmb M^{-}, but under no selection we did not observe heterokaryon formation between these two strains.

In addition, both methods, the nutritional selection stress and the non-selection pairings were attempted between Fop 247 $R^{-}H^{R}$ and sa8 $M^{-}P^{-}B^{R}$. No heterokaryon formation was observed.

3.3.3 Pairings under selective drug pressure

The analysis of 3000 pairings by hyphal anastomosis is recapped in Table 35. Some pairings were lost to contamination so the final count was 2458 heterokaryons. Recall from the introduction that in order for a heterokaryon to form under selective pressure it must express drug resistance since the auxotrophic tester cannot grow in MMA supplemented with the drug and the wild types cannot survive at levels above their MICs. For benomyl resistance MIC were estimated at 2μg/ml. For hygromycin, MICs were estimated at 30 μg/ml. Pairings under benomyl selection were carried out in plates supplemented with benomyl at a concentration of 100μg/ml (MMB100). Hygromycin plates were plated at a concentration of 100μg/ml (MMH100). Neither the wild type testers nor the wild types grew on their own under this strong selection. Syntrophism of the auxotrophic markers and drug resistance was evident by the prototrophic growth of the colonies growing under the same stringency as the controls.

Ten auxotrophic mutants were used as Wild type testers (WTT) (Leslie 1993) using benomyl resistance (all F.o. *cubense*) and hygromycin resistance (F.o. *pisi*) as dominant selective markers (Table 27). These 10 WTT were paired against 59 wild type strains. Fifty-one wild type strains were from species *oxysporum*, forma specialis *cubense*, six wild type strains while *oxysporum*, belonged to four different formae speciales and two wild types belonged to a different species. Thus, these pairings encompassed inter strain, inter racial, inter-vcg, inter-forma and inter-specific

interactions. The analysis of three thousand pairings is recorded in Table 35. Some pairings were lost to contamination. All the pairings were done using the double-pick method.

Of the 485 possible intra-VCG heterokaryons forced under drug selective pressure, only 264 (54%) were formed.

Drug-resistant heterokaryons were also evident in all inter-VCG pairings. The rate of Inter-VCG heterokaryon formation ranged from 6% in VCG 0120 to 64% in VCG 0124/5. The overall rate of inter-VCG heterokaryons was 749 out of 1640 possible pairings (46%).

In summary, roughly half of all heterokaryons that were possible within forma species *cubense* were formed, or 1 out of every two pairings attempted.

Inter-forma heterokaryons were also formed by hyphal anastomosis. 45 out of 250 (18%) heterokaryons were successful for an estimated rate of 1 out of every five attempted pairings.

Inter-specific pairings between *oxysporum* and *solani* also expressed drug resistance in 12/82 possible pairings for an estimated rate of 1 out of every 6 attempted pairings.

Table 35. Summary of heterokaryon formation under drug selective pressure

				Intra VCG						Inter VCG		
Species	**Fsp**	**VCG**	**# WTs**	**Pairings**	**%**	**Observed**	**Possible**	**# wtt**	**%**	**Observed**	**Possible**	**# wtt**
Oxysporum	*cubense*	0120	13	598	39%	233	390	6	6%	38	208	4
		0121	2	82	0%			0	43%	35	82	10
		0122	3	123	0%			0	46%	56	123	10
		0123	2	82	0%			0	27%	22	82	10
		0124	9	349	6%	21	80	2	58%	202	269	8
		0124/5	3	123	0%			0	64%	79	123	10
		0125	2	81	0%			0	42%	34	81	10
		0126	4	154	6%	10	15	1	35%	54	139	9
		0128	2	82	0%			0	29%	24	82	10
		0129	2	82	0%			0	38%	31	82	10
		01210	2	82	0%			0	41%	34	82	10
		01211	2	82	0%			0	59%	48	82	10
		01212	2	82	0%			0	51%	42	82	10
		01213	1	41	0%			0	44%	18	41	10
		01214	2	82	0%			0	39%	32	82	10
		Subtotal	**51**	**2125**	**54%**	**264**	**485**		**46%**	**749**	**1640**	
	lycopersici	Fol	3	123	0%			0	28%	34	123	10
	pisi	Fop	1	41	0%	0	1	1	15%	6	40	9
	raphani	For	1	46	0%			0	9%	4	46	10
	conglutinans	Focong	1	41	0%			0	2%	1	41	10
	Other fsp	**subtotal**	**6**	**251**	**0%**	**0**	**1**		**15%**	**45**	**250**	
solani			2	82	0%			0	15%	12	82	
		Total	**59**	**2458**	**54%**	**264**	**486**		**33%**	**806**	**1972**	

3.4 Discussion

3.4.1 On heterokaryon formation

There were no statistically significant differences between the outcome of the three trials conducted under the time course (p-value >0.05). The results of the three trials were, therefore, pooled for the analysis of heterokaryon formation.

Heterokaryon formation, evidenced by recovery of parental and non-parental genotypes was detected one day after coinoculation of equal number of spores in liquid minimal medium. The non-parental genotypes were unstable and segregated into one parental genotype or the other. Prototrophic propagules mainly segregated into both parental genotypes, with occasional segregation into prototrophic spores. Prototrophic propagules recovered during heterokaryon formation could represent a transition state into hybrid formation, which is usually observed 7-14 days after coinoculation of spores. Propagules of hybrids, though, segregate mainly into prototrophic cfus that are stable under selective pressure (Table 26). These propagules, contrary to heterokaryotic cells, sector readily once removed from selective stress. Nonetheless, even if transient, approximately 6% of the propagules analyzed showed non-parental genotypes. That is, 1 out of every 17 propagules is different from the original parental strains. As this rate is lower than the estimated rate of karyogamy of 1 in a million, these results suggest that horizontal genetic transfer is not dependent on nuclear fusion to occur.

Heterokaryon formation in inter-forma pairings was achieved by hyphal anastomosis even though we failed to recover the drug resistant strain on analysis of the propagules from these heterokaryons. We attribute the preferential recovery of one parental strain over the other on marked physiological differences between the paired

strains rather than on the possibility that no heterokaryons were formed. Reversion of the hygromycin resistant strain was ruled-out as we mainly recovered the auxotrophic hygromycin sensitive strain, and occasionally prototrophic spores.

The length of time (two months) that we maintained prototrophic colonies under strong nutritional selection could have selected for hybrid formation, One can postulate that propagules from these heterokaryons may represent break-down products of hybrids, usually seen after nuclear fusion, rather than nuclear segregation of a heterokaryotic cell.

3.4.2 On drug resistance

Benomyl resistance was expressed in heterokaryons paired under non-selective drug stress. These heterokaryons were stable. Recombinant genotypes were only seen in a limited number of drug resistant propagules (7/120) that represented the benomyl-sensitive auxotrophic mutant strain that had acquired benomyl resistance. Nonetheless, as Glass and Kuldau (1992) point out, "if heterokaryosis were a factor in natural populations of pathogens, the possibility that non-pathogenic strains could complement one another to virulence or drug resistance in a heterokaryon would be a significant consideration".

A more compelling evidence of expression of drug resistance in heterokaryons comes from the pairing of a hygromycin sensitive strain with a hygromycin resistant strain under non-selective drug stress. Propagules of transferred heterokaryons, continued to grow aerially under strong selective pressure and although there was preferential recovery of one parental strain over the other, the pairings were effected by hyphal anastomosis between two different forma speciales, *cubense* and *pisi*. Therefore, expression of drug resistance can also be observed across greater genetic distances.

These results were also evident in inter-forma and inter-specific pairings under strong drug-selective pressure where 18% of the heterokaryons, 1 out of every 5 pairings, expressed drug resistance. Recombinant genotypes were not recovered from these heterokaryons.

Although expression of drug resistance during heterokaryon formation may not promote genetic change, it may be viewed as a possible mechanism by which slower adaptable strains may survive longer in hostile environments. If genes such as pathogenicity genes can also be expressed during transient associations, it may also provide a mechanism to increase host range without the stress of host resistance. Something to consider when using fungal non-pathogenic isolates as bio-control agents.

CHAPTER 4 Phase III: Different Evolutionary Histories Inferred From Multilocus Analysis In *Fusarium oxysporum* f.sp. *cubense*: Is Foc strictly clonal?

4.1 Introduction

Asexual fungi have no known sexual cycle; they reproduce vegetatively and are usually identical except for rare mutational events. The inherited genome is linked and, should reflect similar evolutionary histories (Kohli and Kohn 1998, Kohn 1995, Anderson and Kohn 1995). Thus, analysis of individual genes or multilocus gene analysis should be congruent and show little or no conflict. But what happens when the analysis of multilocus genes in asexual organisms supports conflicting phylogenies?

There are several schools of thought that address the problems of disparate data sets when inferring a phylogenetic hypothesis. Followers of the conditional combination approach advocate partitioning of data sets in the phylogenetic analysis with *a priori* comparison of tree topologies (Dubuisson *et al.* 1998, de Queiroz *et al.* 1995, Bull *et al.* 1993). Data sets that are not significantly incongruent are then combined. A second approach, the taxonomic congruence approach, supports the idea that conflicting data sets should be analyzed individually and should not be combined in a simultaneous analysis (Miyamoto and Fitch 1995). The third point of view supports the total evidence approach in which conflicting data sets are combined regardless of conflicting signals (Carbone *et al.* 1999, Kluge 1998). The total evidence approach will be examined using a multilocus analysis in the asexual, phytopathogen *Fusarium oxysporum* f.sp. *cubense.*

Fusarium oxysporum Schlechtend,: Fr. f. sp. *cubense* (Foc) (E.F.,Sm.), W.C. Snyder & H.N. Hans, is the causal agent of vascular wilt of banana, also known as Panama disease. Although asexual, genetic diversity has been identified in Foc

evidenced by RFLP (Koenig *et al.* 1997) and RAPD analysis (Bentley *et al.* 1995), electrophoretic karyotype (Boehm *et al.* 1994) and Vegetative Compatibility Groups (VCGs) (Ploetz 1990). While mutation, selection and recombination are possible mechanisms that can generate genetic variability in asexual fungi (Burdon and Silk 1997), we propose horizontal genetic transfer via recombination as a source of the observed genetic diversity in Foc.

In clonal populations different molecular markers should yield trees with no conflicting topologies. If genetic exchange has occurred, the topology from individual genes will show conflict and incongruence (Anderson and Kohn 1995, Burt *et al.* 1994).

A priori examination of individual phylogenetic and phenetic trees constructed using different molecular markers in Foc reflects conflicting tree topologies.

Strong incongruence between partitions may suggest different evolutionary histories or recombination over a clonal or monophyletic origin (Bull *et al.* 1993). The assumptions for combining data sets are that the same tree is being reconstructed in all the studies and the chosen method is appropriate for the individual data sets.

4.2 Materials and methods

There are several programs designed to determine the degree of incongruence in conflicting data sets (Taylor *et. al.* 1999, Cunningham 1997, Rodrigo *et al.* 1993) such as the Incongruence Length Difference (ILD) test which calculates the difference between the number of steps required by individual and combined analysis. The ILD compares the Mickevich and Farris (1981) index to a null distribution based on multiple randomizations. Although the size of the randomized partitions is the same as the original partitions, the randomized partitions represent a mixture of characters from each

partition. Conflict among data sets is reflected in topological disagreements among the fundamental cladograms (Miyamoto and Fitch 1995). One advantage of the ILD over other commonly used methods is that both sequence data and binary files can be combined in the same analysis.

Using the ILD Test featured in Paup* Version 4.0b3 for 32-Microsoft Windows © 1999 Smisonian Institution, Sinauer Associates, Inc. Publishers (Swofford 1998), multiple data sets were combined in a simultaneous analysis. Table 36 lists the different data sets used in this study. The data sets are comprised of one mitochondrial marker (mitotypes) and five nuclear markers.

Table 36. Multiple data sets used in this study

Molecular Marker	Number of Taxa	Source
RFLP	172	Koenig *et al.* 1997
RAPD	54	Bentley *et al.* 1995
Karyotype	118	Boehm *et al.* 1994
Mitotypes*	58	D'Alessio 1998
Allozymes	52	Own results
Mating Types*	111	Hemmings and Kuhn Personal communication

* Sequence data

4.2.1. Molecular Markers

4.2.1.1 RFLPs

The data set for the RFLPs was obtained directly from the author. This data set was obtained using anonymous, single-copy restriction RFLPs. 19 loci were represented and allelic variations in banding patterns were scored as character states.

4.2.1.2. RAPD

This data set was obtained directly from the literature. The binary file for the RAPD data scores the absence or presence of a band by (0,1).

4.2.1.3. Electrophoretic karyotype

To generate the data necessary for this partition the electrophoretic mobility of the chromosomes of 118 different strains of Foc was analyzed and scored. A data matrix was constructed where each chromosome is an individual character and the absence or presence of a particular band is scored as (0,1) respectively. 79 differences in electrophoretic mobility were scored.

4.2.1.4. MtDNA

The data set for the mitotypes was obtained directly from the author. A 576 bp intergenic region of the mitochondrial DNA was sequenced. Eleven mitotypes were identified. Each strain within a VCG had identical sequence. However, several VCGs shared a mitotype.

4.2.1.5. Mating type genes

The author provided this data set. There are two mating type genes present in Foc: Mat 1 and Mat 2. Presence-absence of mating type genes has been scored in over 100 strains. However, as both idiomorphs were sequenced, the sequence data was chosen over the binary data for this partition.

4.2.1.6. Allozymes

Ten different enzymes were analyzed representing housekeeping genes using allozyme analysis (own results-Appendix I). Seventeen loci were identified. A data

matrix was constructed where each individual loci represents the characters and the allele variability represents character states.

As the number of taxa varied from data set to data set (Table 36 and Appendix II), a subset of the taxa common to most data sets was selected for the combined analysis. These isolates are listed in Table 37. Isolates CS 85-4 (fon854 f.sp. *niveum*), SC626 (folR3 f.sp. *lycopersici* and SC761 (folR1 f.sp. *lycopersici*) were used as outgroups. Trees were rooted using isolate CS 85-4.

Table 37. Taxa Subset common to most data sets used in this study

ISOLATE	FSP	VCG	RACE	ORIGIN	Source	MT	mt-dna	allo	rflp	rapd	Mat	EK
CS 85-4	niveum	0082	?	Florida	HCR	XI	x	x	x		x	x
STGM1	cubense	0120	1	Costa Rica	RCP	I	x	x	x	x	x	x
GM	cubense	0121	4	Taiwan	RCP	II	x	x	x	x	x	x
JC14	cubense	01210	1	Florida	RCP	VI	x	x	x	x	x	x
1372-1	cubense	01211	?	?	RCP	I	x	x	x	x	x	
SH3142	cubense	01211	?	Australia	RCP	I	x	x	x		x	x
STNP1	cubense	01212	?	Tanzania	RCP	IV	x	x	x	x	x	x
STNP4	cubense	01212	?	Tanzania	RCP	IV	x	x	x	x	x	x
MW 2	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
MW 40	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
MW 41	cubense	01214	Mbufu	Malawi	RCP	VII	x	x	x		x	x
MW 89	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
SABA	cubense	0122	2	Philippines	RCP	I	x	x	x	x	x	x
DAVAO	cubense	0123	1?	Philippines	RCP	III	x	x	x	x	x	x
F9129	cubense	0123	1	Taiwan	RCP	III	x	x	x	x	x	x
MACA	cubense	0124	1	Brazil	RCP	IV	x	x	x		x	x
MW 52	cubense	0124	Sukali	Malawi	RCP	IV	x	x	x		x	x
STD2	cubense	0124	1	Honduras	RCP	IV	x	x	x	x	x	x
STN2	cubense	0124	?	Nicaragua	RCP	IV	x	x	x	x	x	x
8611	cubense	0125	1	Australia	RCP	IV	x	x	x	x	x	x
STB2	cubense	0126	1	Honduras	RCP	VI	x	x	x	x	x	x
STM3	cubense	0126	1	Honduras	RCP	VI	x	x	x	x	x	x
A47	cubense	0128	2?	Comores	RCP	IV	x	x	x		x	x
0-1221	cubense	0129	1	Australia	RCP	I	x	x	x		x	x
SC626	Lycopersici	Folr1	1	Italy	HCR	VIII	x	x	x			
SC761	Lycopersici	Folr3	3	Florida	HCR	VIII	x	x	x			

4.2.2 Phylogenetic Analysis

To assess if the incongruence in tree topologies was an artifact of the algorithm used, individual trees for the different molecular markers were constructed for the taxa subset using a distance algorithm (Neighbor-Joining [NJ]), cluster analysis (Unweighted Pair-Group Method with Arithmetic Mean [UPGMA]) and Maximum parsimony analysis (50% majority rule consensus of heuristic search) using Paup* Version 4.0b3

4.3 Results

A nexus file for the subset was generated combining all the markers and trees were generated using NJ, UPGMA and parsimony analysis. These trees were then compared to the trees from the individual molecular markers. Tree statistics for the most parsimonious trees (MPTs) are presented in Table 38. Figures 21-23 show the results of the three analyses. Nodal support for the combined data set was determined through bootstrap analysis as implemented in Paup* and decay indices (Bremer support) were calculated using TreeRot v.2 (Sorenson 1999). The decay index is enclosed in parenthesis above the bootstrap value (Figure 24).

To assess the individual contribution of a particular data set to node support, parsimony trees were generated removing a partition at a time. The trees are shown in Figure 25 and the statistical analysis of the trees is presented in Table 39.

To investigate whether a particular taxon was responsible for the conflict, an agreement subtree was constructed in Paup* from the 50% majority rule consensus trees of all the individual data sets (Figure 26).

For the ILD test, each molecular marker was identified as a partition. 1000 replicates using heuristic search were carried out to test for congruence among data sets.

The individual partitions were compared against each other (Figure 27) and the mitochondrial data set was compared against the nuclear data sets (Table 40). To test for congruence between the different partitions a one-to-one comparison of each data partition was also done (Table 41). To determine the contribution of a particular data set to the results, the ILD test was done on the combined data sets removing a partition at a time. P-values are tabulated in Tables 42.

To test if there was a difference in the evolutionary history of the mitochondrial genome versus the nuclear genome, dendrograms were generated using PopGene Version 1.31. (A joint project development by Francis C. Yeh and Rong-cai Yang, University of Alberta and Tim Boyle, Centre for International Forestry Research for Windows 95, 08 and NT users [32-bit version]) (Figure 28). PopGene uses Nei's genetic distance (Nei 1972, 1973, 1978) to construct the dendrograms. These trees were imported to GeneTree (win32) 1.01 copyright © Roderick DM Page 1998 where the tree based on the mitochondrial sequence data was compared against the combined nuclear gene trees. A similar analysis was generated using maximum parsimony. Figure 29 compares 50% majority rule consensus trees of the mitochondrial haplotypes versus the combined nuclear markers.

GeneTree (Page 1999) was used to compare a pathogen tree based on host-parasite interaction (Figure 30) and dendrograms were generated for the individual markers (not shown) and the combined data sets. Figure 31 show a tanglegram of the organism tree versus the combined data set.

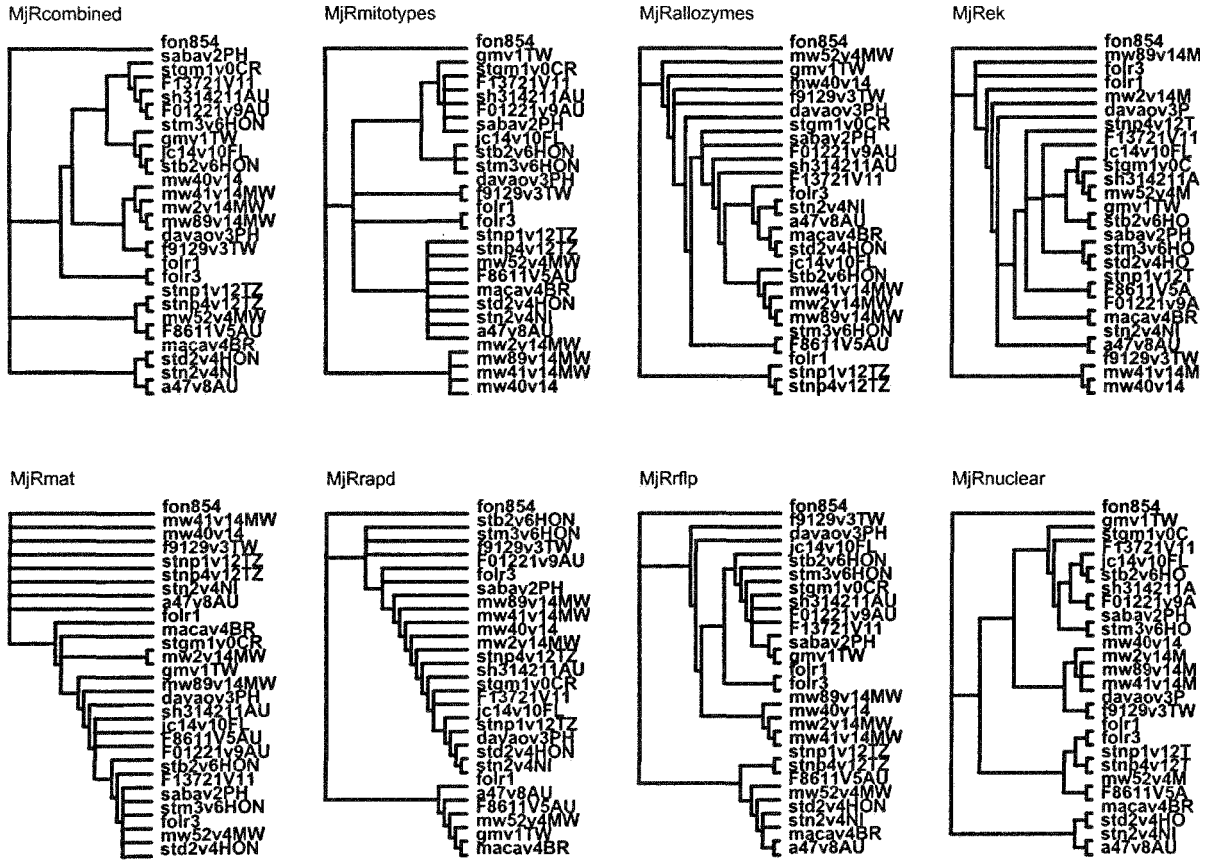


Figure 21. 50% Majority rule consensus tree of MPTs using 100 replicates for the heuristic search (Paup* 4.02b). Trees were rooted using fon854 as an outgroup. Isolates are identified as follows: name-vcg-origin. Thus sabav2PH is strain Saba from VCG 0122 found in Philippines. V0 to v14 represent VCG 0120 to VCG 01214. Origins are as follows: AU = Australia, BR = Brazil, CR = Costa Rica, HON = Honduras, Fl = Florida, NI = Nicaragua, MW = Malawi, PH = Philippines, TW = Taiwan, TZ = Tanzania

Table 38. Tree statistics for 50% majority rule consensus tree of MPTs Generated from individual Data Partitions

Partitions	NC[a]	PIC[b]	CI[c]	HI[d]	RI[e]	RC[f]	TL[g]	NT[h]
Mitotype	576	13	0.9524	0.0476	0.9844	0.9375	42	13
Allozyme	17	14	0.4717	0.5283	0.7021	0.3312	53	100
RFLP	19	13	0.8286	0.1714	0.8788	0.7281	70	100
RAPD	29	18	0.8000	0.2000	0.8286	0.6629	30	100
EK	79	33	0.5000	0.5000	0.4792	0.2396	100	100
Mat Type	467	14	1.0000	0.0000	1.0000	1.0000	20	100
All	1187	127	0.5622	0.4378	0.6472	0.3638	386	100

[a] NC = Number of characters
[b] PIC = Parsimony-informative characters
[c] CI = Consistency Index
[d] HI = Homoplasy index
[e] RI = Retention Index
[f] RC = Rescaled Retention Index
[g] TL = Tree Length
[h] NT = Number of trees

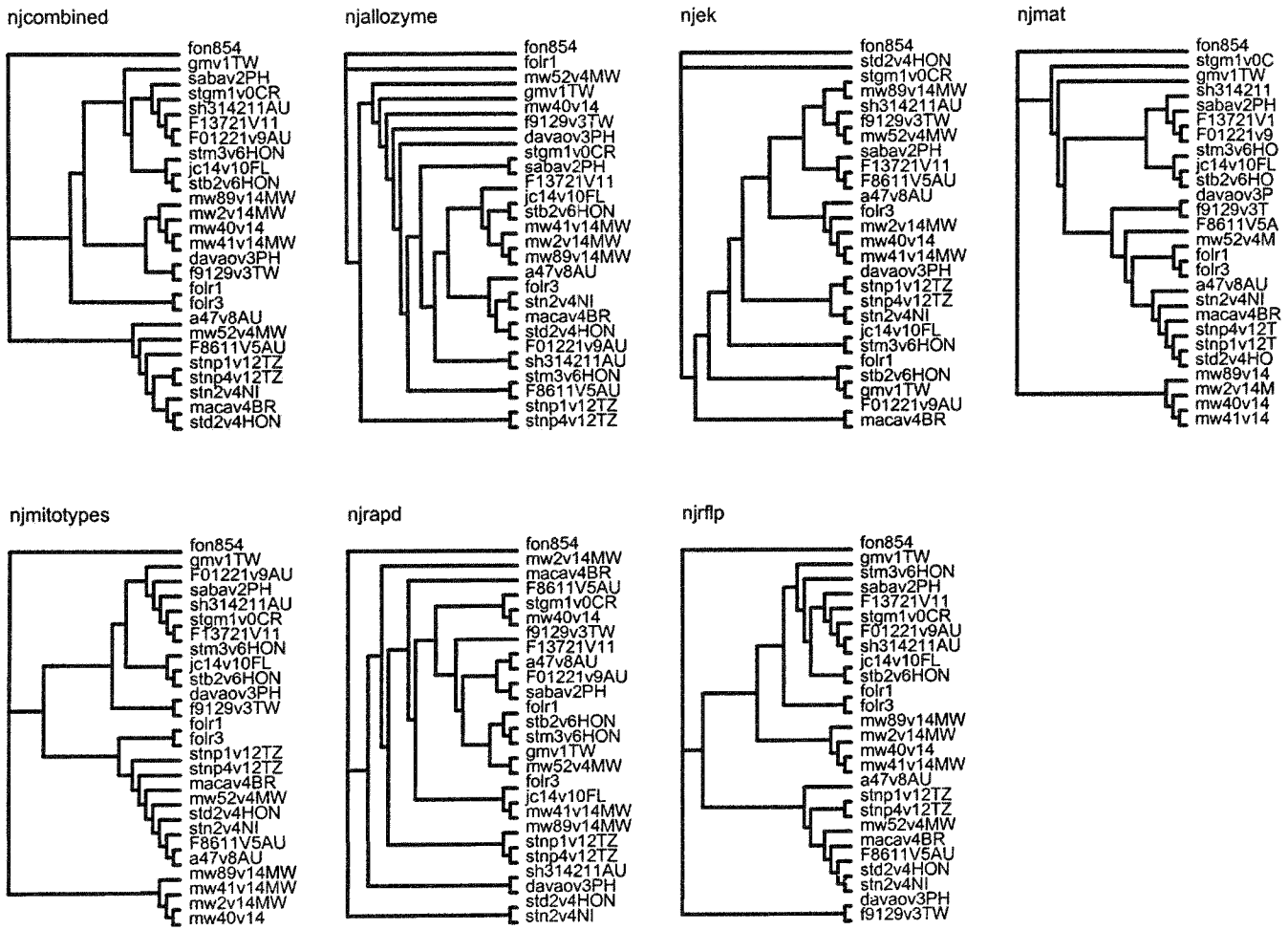


Figure 22. Distance analysis using the Neighbour-Joining algorithm

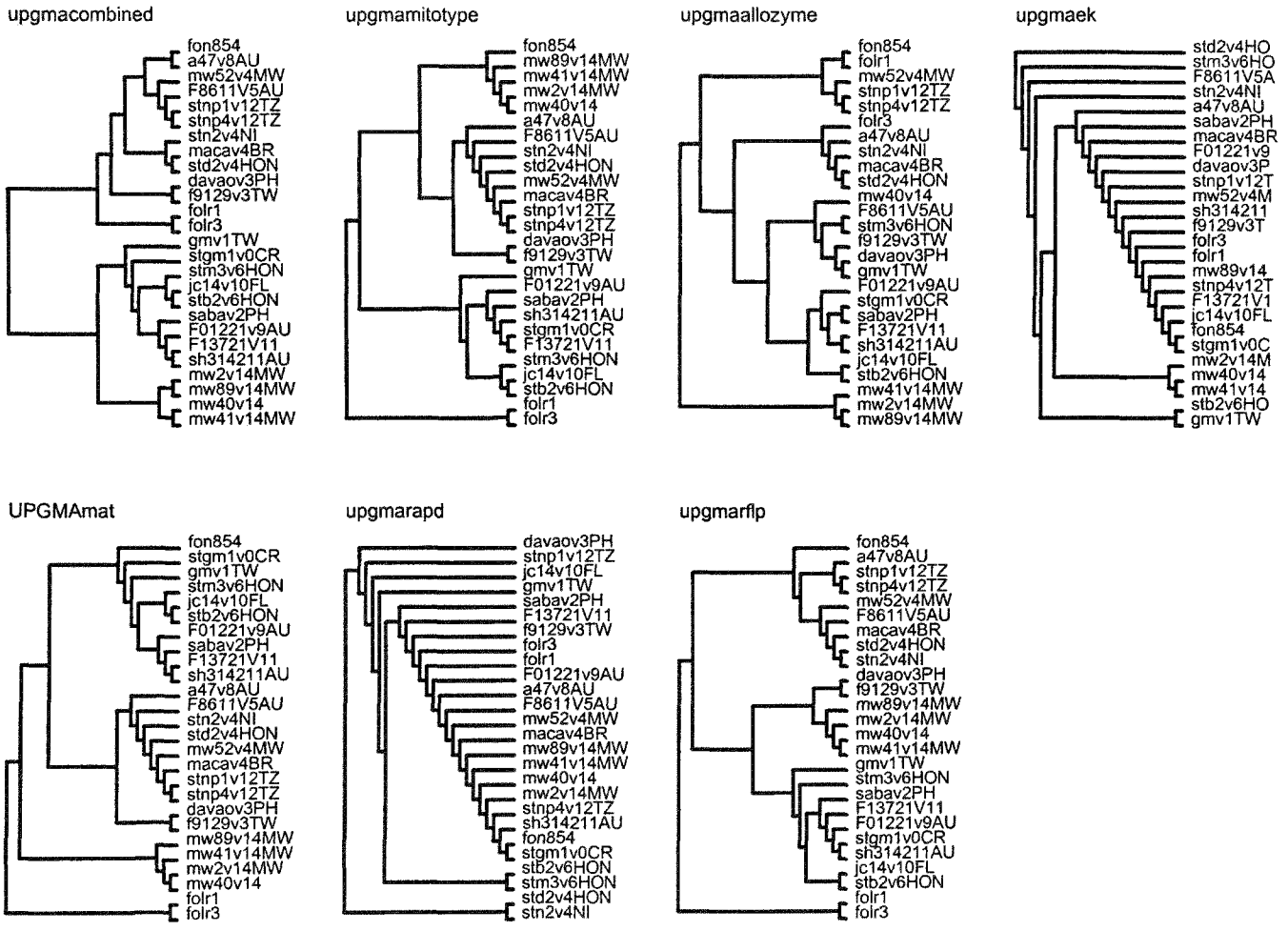


Figure 23. Cluster analysis using UPGMA (Paup*4.0b3)

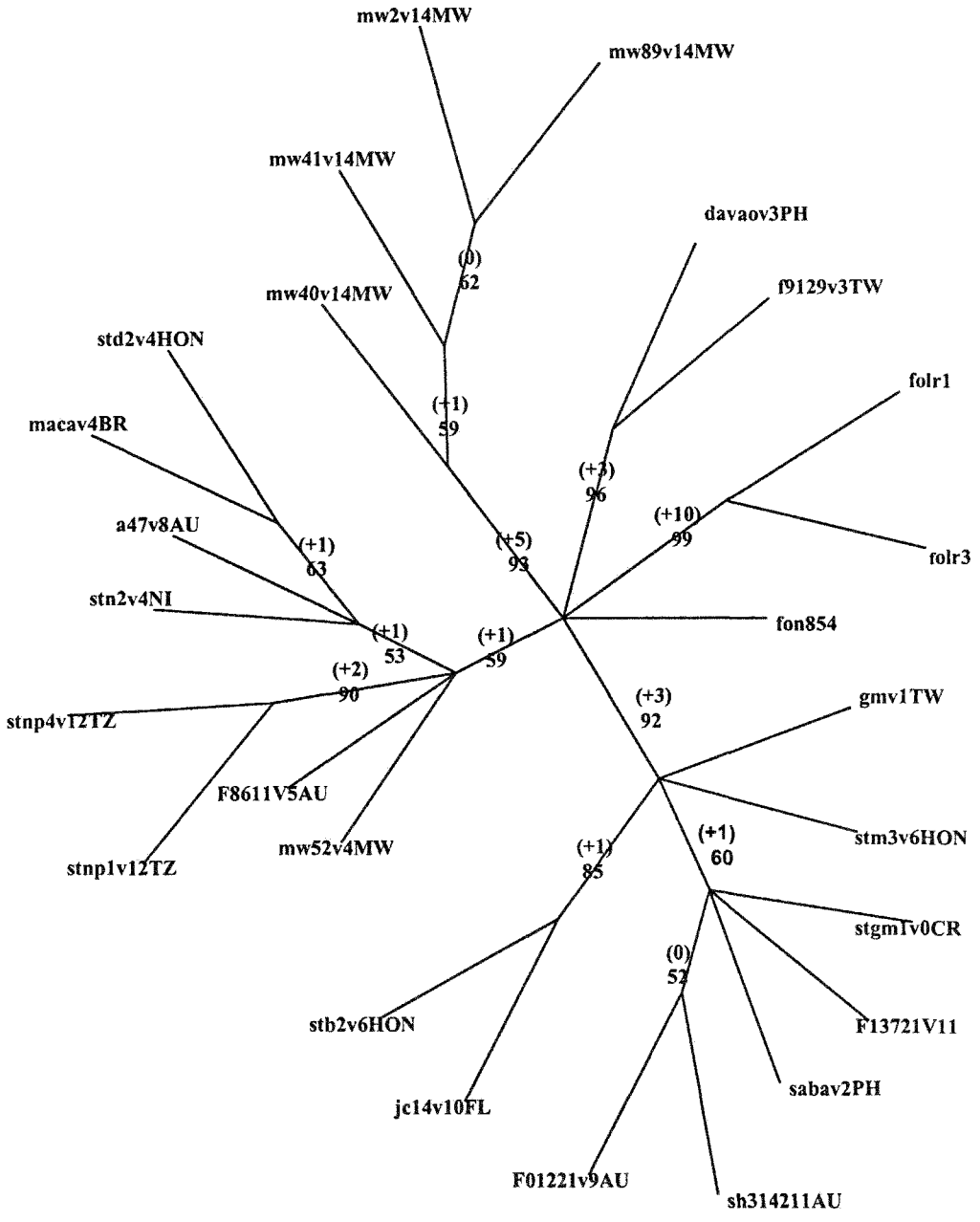


Figure 24. Bootstrap 50% majority rule consensus tree of the combined data set was generated using Paup* 4.03b (Swofford 1999). Bremer support indices (in parenthesis) were calculated using TreeRot (Sorenson 1999)

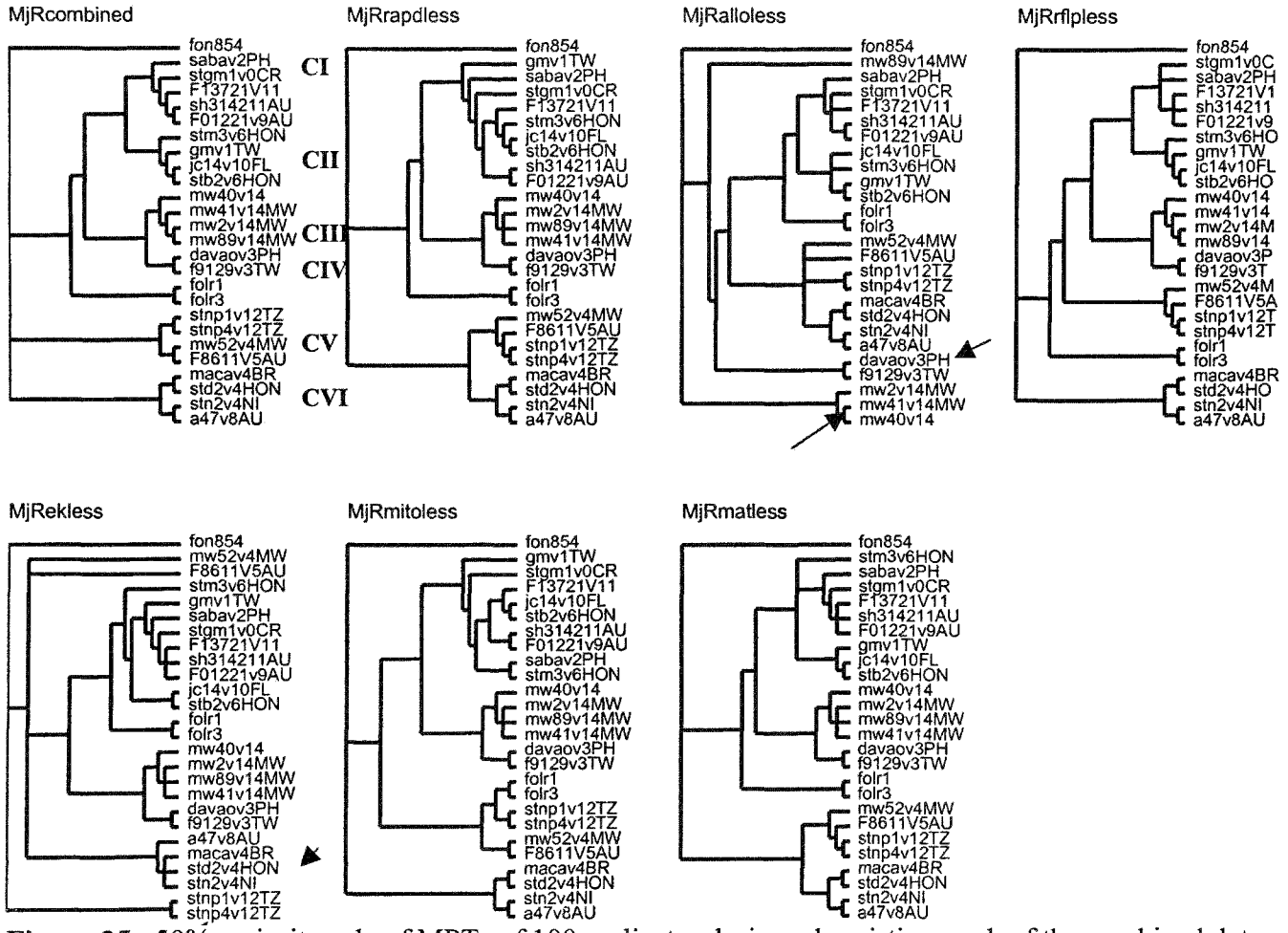


Figure 25. 50% majority rule of MPTs of 100 replicates during a heuristic search of the combined data set, removing one partition at a time. In general, terminal taxa within a clade remained the same, although the resolution of the clade varied depending on the partition removed. Regardless of the partition removed, all tree topologies were different.

Table 39. Tree statistics of 50% majority rule consensus tree of MPTs Generated from the combined analysis after removal of a partition at a time. The removed partition is highlighted in bold letters. The last set represents the combined data set without removal.

Partitions	NC	PIC	CI	HI	RI	RC	TL	NT
MatvsMitovsEKvs RFLPvsAllovs**RAPD**	1158	109	0.5794	0.4206	0.6836	0.3961	340	46
MatvsMitovsEKvs RFLPvs**Allo**vsRAPD	1170	113	0.5534	0.4466	0.6482	0.3587	356	500
MatvsMitovsEKvs **RFLP**vsAllovsRAPD	1168	114	0.5710	0.4290	0.6726	0.3840	345	9
MatvsMitovs**EK**vs RFLPvsAllovsRAPD	1108	94	0.5597	0.4403	0.6571	0.3677	352	240
Matvs**Mito**vsEKvs RFLPvsAllovsRAPD	611	92	0.5534	0.4466	0.6482	0.3587	356	2
MatvsMitovsEKvs RFLPvsAllovsRAPD	720	113	0.5777	0.4223	0.6814	0.3937	341	234
MatvsMitovsEKvs RFLPvsAllovsRAPD	1187	127	0.5622	0.4378	0.6472	0.3638	386	100+

[a] NC = Number of characters
[b] PIC = Parsimony-informative characters
[c] CI = Consistency Index
[d] HI = Homoplasy index
[e] RI = Retention Index
[f] RC = Rescaled Retention Index
[g] TL = Tree Length
[h] NT = Number of trees

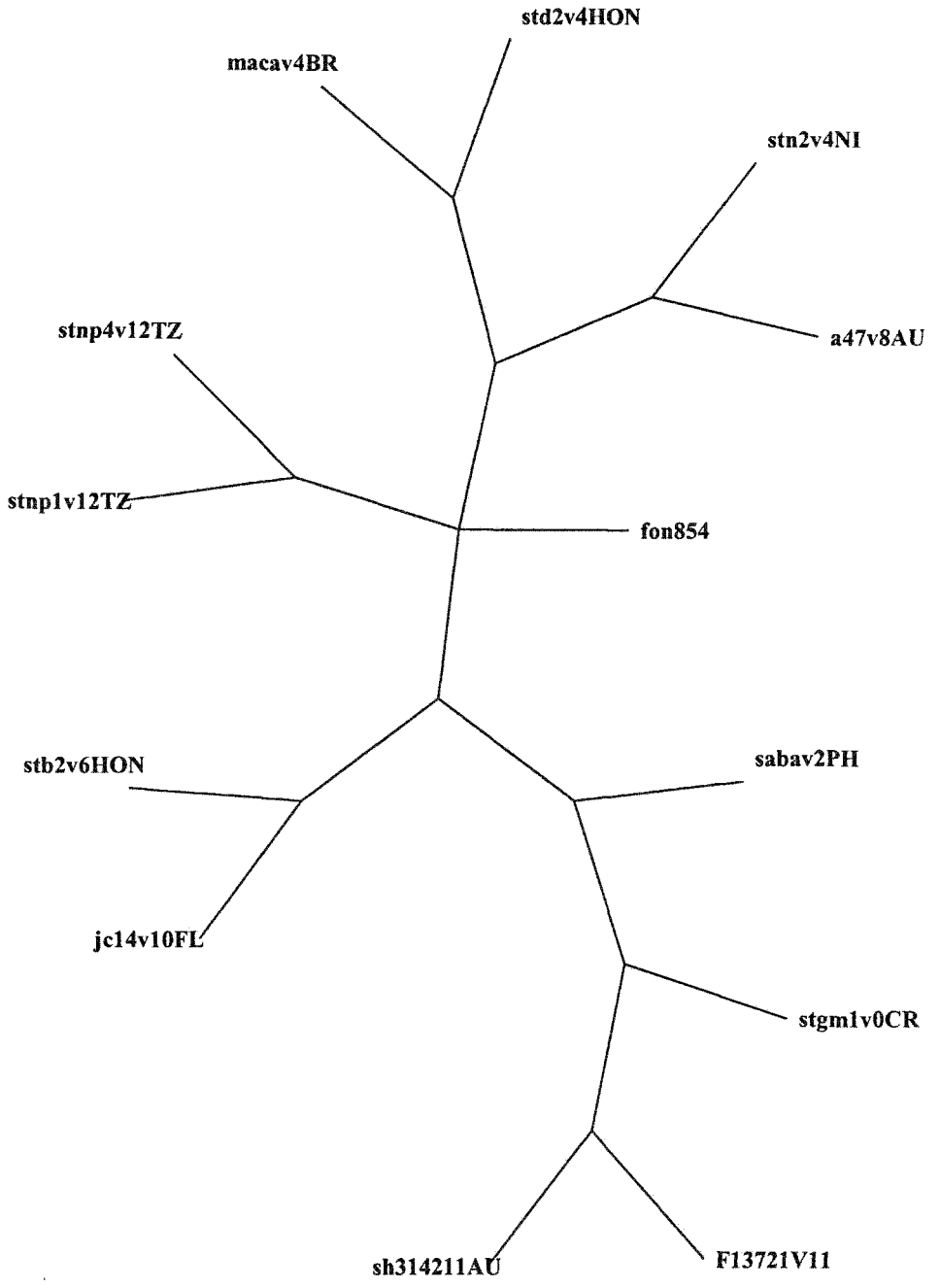


Figure 26. Agreement subtree of the 50% majority rule consensus tree of the individual partitions. The following taxa (13/26) were removed: gmv1tw, davaov3ph, f9129v3tw, mw52v4mw, f8611v5au, stm3v6hon, f01221v9au, mw89v14mw, mw2v14mw, mw40v14mw, mw41v14mw, folr11 and folr3.

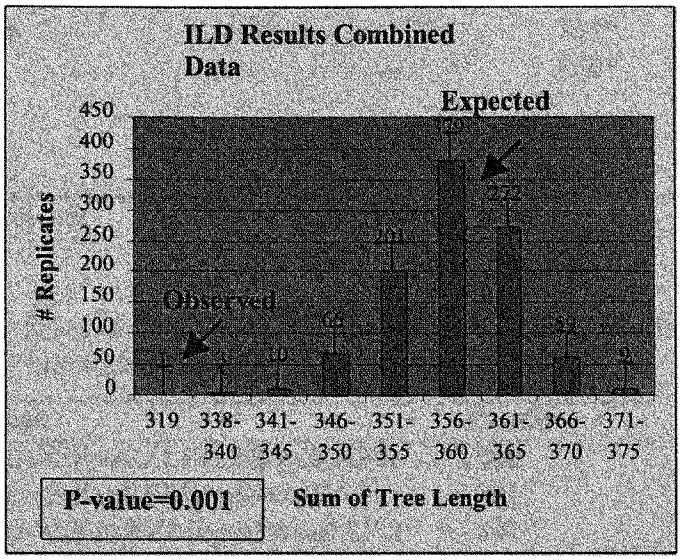


Figure 27. ILD results for the combined data set. In clonal populations, since the genome is effectively linked, the observed data set should generate trees equal in length to those generated by randomization due to similar evolutionary history. A P-value of 0.001 rejects the null hypothesis of congruence between the different processed partitions.

Table 40. P-values from ILD tests among data partitions

Comparison	P-value
Mat[a] vsMito[b] vsEK[c] vsRFLP[d] vsAllo[e] vsRAPD[f] (Combined)	0.001
MatvsEKvsRFLPvsAllovsRAPD (Nuclear)	0.001
Mitochondrial[g] vs Nuclear	0.9020*

[a] Mat = Mating type genes
[b] Mito = Mitochondrial haplotype (a.k.a. mitotypes)
[c] EK = electrophoretic karyotype
[d] RFLP = Restriction Fragment Length Polymorphism
[e] Allo = allozymes
[f] RAPD = Random Amplified Polymorphic DNA
[g] Mitochondrial: Mito = mitotypes
*not significant

Table 41. P-values from ILD tests between data partitions. (Significant level α=0.05)

	Mitotype	**Allozymes**	**RFLP**	**RAPD**	**EK**	**Mat Types**
Mitotypes	-	.001	.093*	.005	.006	.001
Allozymes		-	.002	.768*	.010	.002
RFLP			-	.836*	.038	.001
RAPD				-	.993*	.857*
EK					-	.308*
Mat Types						-

* not significant

Table 42. P-values from ILD tests resulting from the removal of a partition The removed partition is highlighted in bold letters.

Comparison	**P-value**
MatvsMitovsEKvsRFLPvsAllovs**RAPD**	.001
MatvsMitovsEKvsRFLPvs**Allo**vsRAPD	.001
MatvsMitovsEKvs**RFLP**vsAllovsRAPD	.001
MatvsMitovs**EK**vsRFLPvsAllovsRAPD	.001
MatvsMitovsEKvs RFLPvsAllovsRAPD	.001
MatvsMitovsEKvsRFLPvsAllovsRAPD	.001

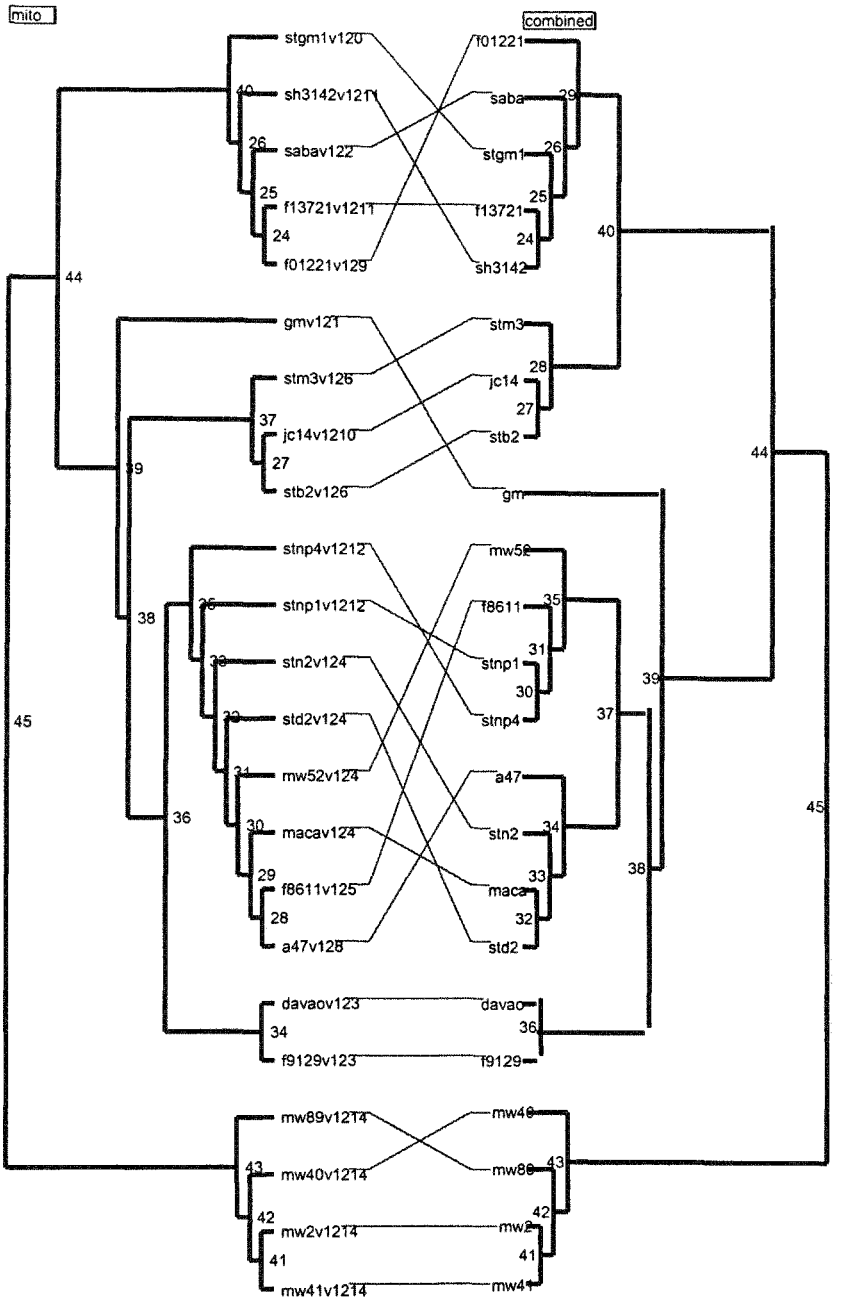


Figure 28. Tanglegram of mitotypes vs combined nuclear genome in Foc. The individual dendrograms were generated in PopGene using Nei's genetic distance.

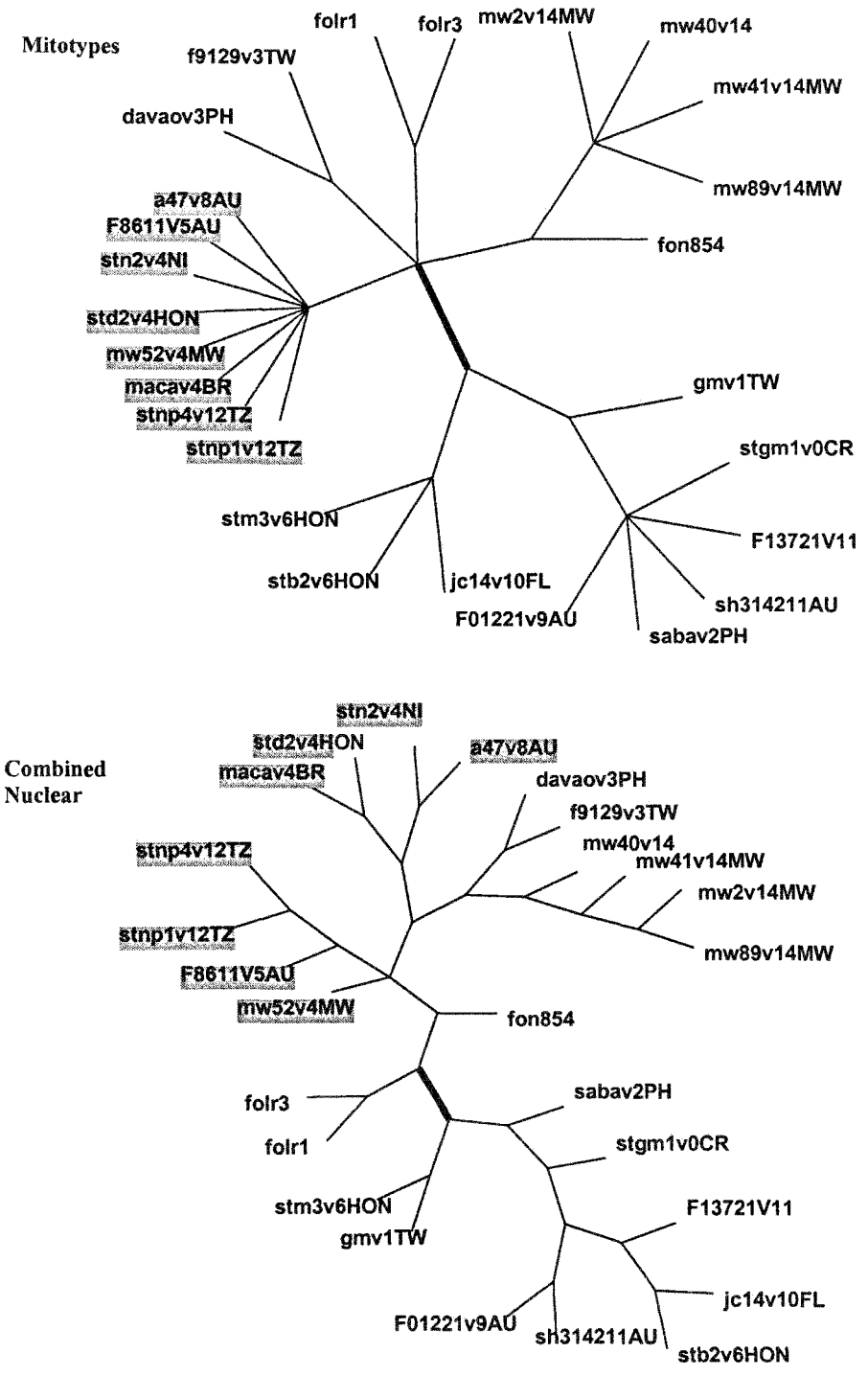


Figure 29. 50% majority rule consensus trees comparing mitochondrial and combined nuclear markers. This result shows different evolutionary histories for the mitochondrial and the nuclear genomes.

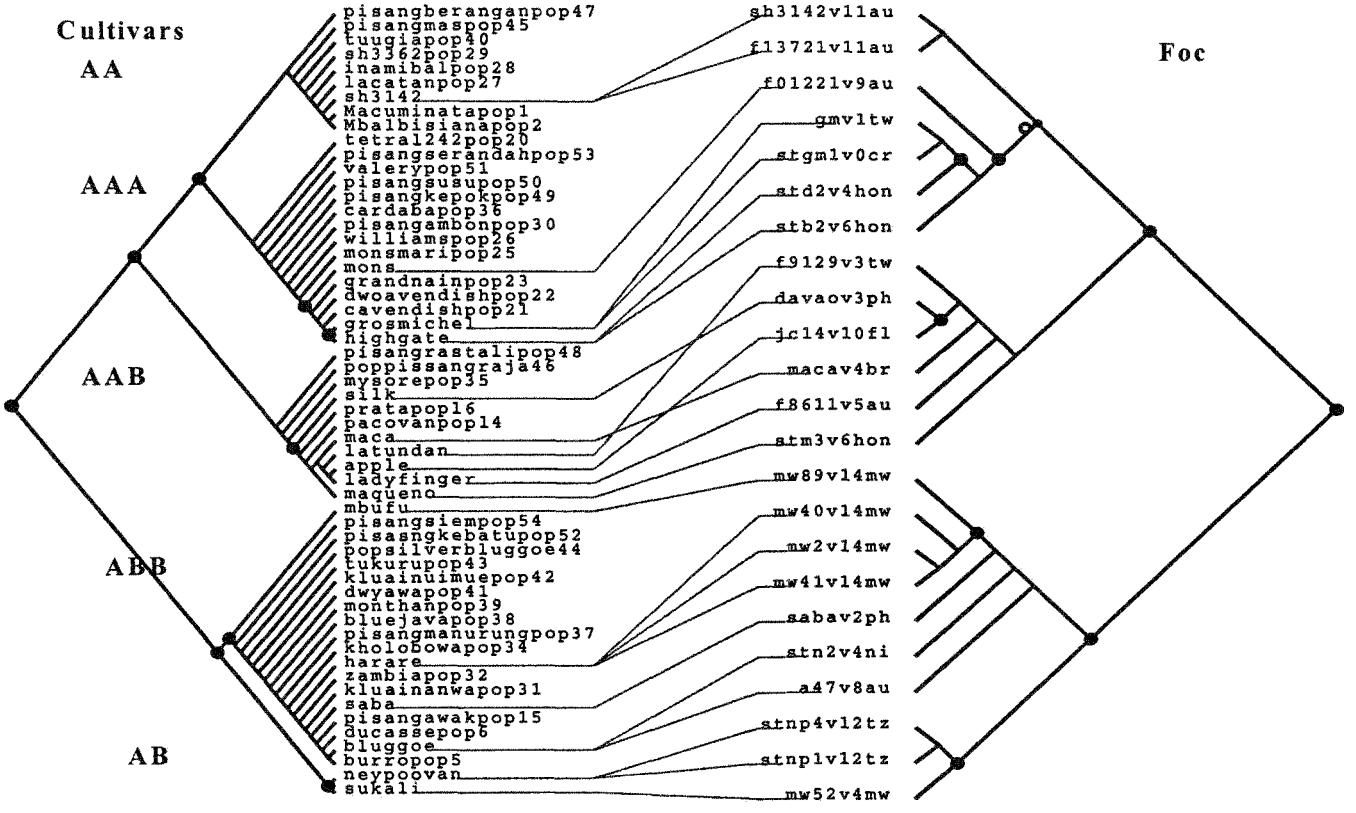


Figure 30. Tanglegram comparing Banana Cultivars (Host) vs Foc (Pathogen) using TreeMap for Windows version 1.0a ©1995, Roderic D.M. Page

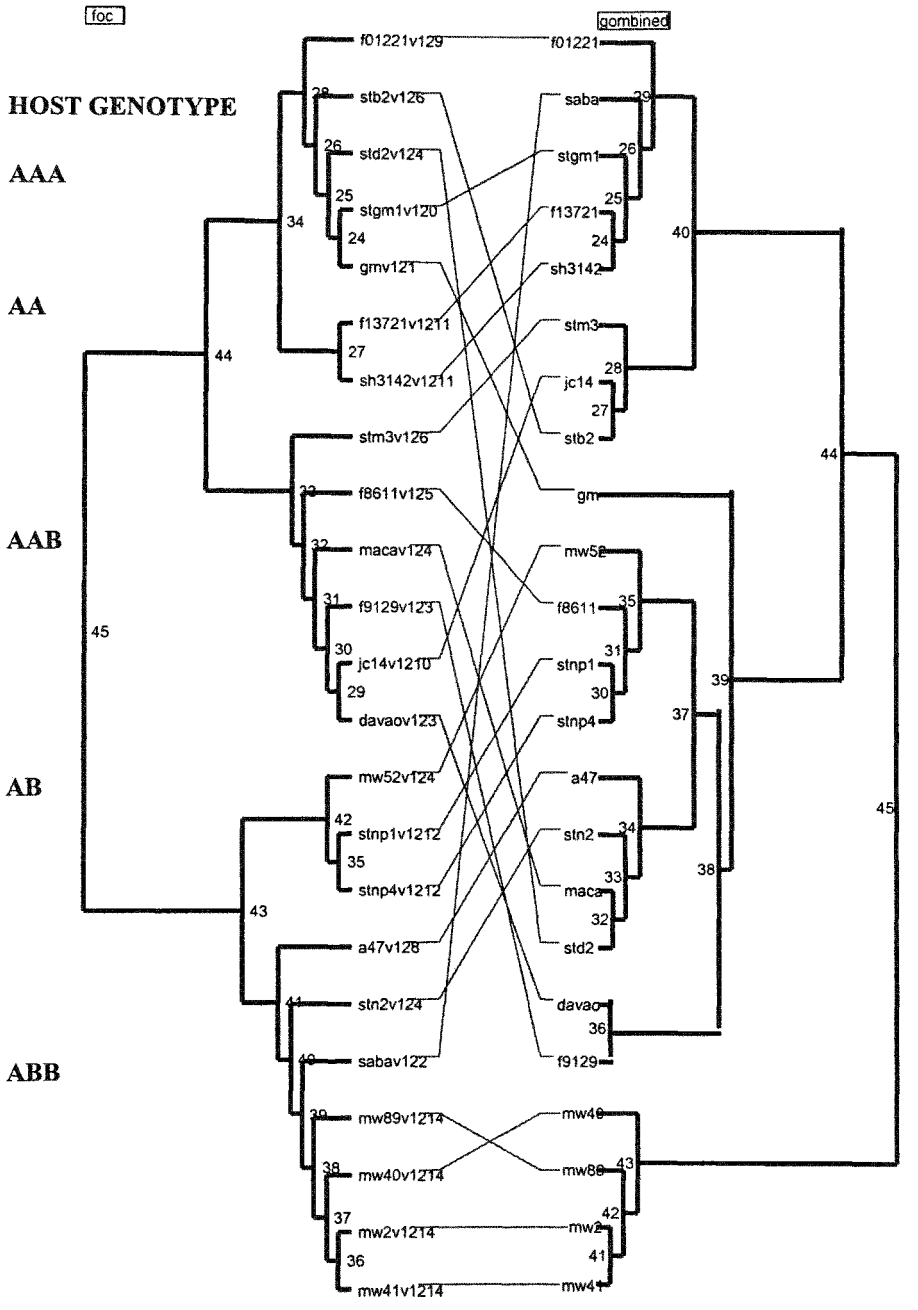


Figure 31. Tanglegram of Foc organismal tree based on host (*M. acuminata* hybrids or *M. acuminata Colla x M. balbisiana* hybrids) vs the combined multilocus tree.

Table 43. Distribution of the two major clades observed in the Multilocus analysis of Foc for the original data sets (Table 36)

VCG	MITO	ALLO	RFLP	RAPD	EK	MAT	Combined
0120	A	A	A	A	A	A	A
0121	A	A	A*	A	A	A	A
0122	A	A	A	A	A	A	A
0123	B	A	A**	B	A	B	B
0124	B	B	B	B	B	B	B
0125	B	B	B	B	B	B	B
0124/5	B	B	B	B	N/A	A/B	N/A
0126	A	A	A	A	N/A	A	A
0128	B	A	B	N/A	N/A	B	B
0129	A	A	A	A	A	A	A
01210	A	A	A	A	B	A	A
01211	A	A	A	A	N/A	A	A
01212	B	A	B	A	N/A	B	B
01213	A	A	A*	N/A	A	B	N/A
01214	B	A	A	N/A	B	B/C	A
01215	A	A	A	N/A	N/A	B	N/A
0120/ 01215	A	A	A	N/A	N/A	N/A	N/A

*One isolate in each of these VCGs (GM VCG 0121 and ES2-1 VCG 01213) could not be placed into any of the 72 identified haplotypes. GM also has a unique sequence for the MAT2 gene.

**Members of the same VCG were classified in different haplotypes.

4.4 Discussion

4.4.1 Phylogenetic analysis

Fusarium oxysporum fsp cubense is an asexual phytopathogen. Individual phylogenetic and phenetic analysis using different molecular markers had previously identified several clonal lineages in this *forma specialis* (Kistler 1997, O'Donnell 1998). However, when these molecular markers are combined in a simultaneous analysis, rather than contributing to the robustness of the phylogeny conflicting signals emerged. For instance, when the individual trees inferred from the different molecular markers were compared, there were inconsistencies in the topology of the trees, regardless of the algorithm used. Under the assumption of clonality for asexual pathogens, as the genome is linked, a multilocus analysis should yield congruent trees (Milgroom 1996). Moreover, conflicting phylogenies are often construed as evidence of genetic transfer (Tibayrenc 1996).

One commonality emerged, though, from the combined analysis, namely a dichotomy splitting the strains of Foc into two main phylogenetic lineages or branches (Table 43). The conflict between the data sets came from the distribution of terminal branches within the clades in these two branches.

The bifurcation, though, is an interesting phenomenon. Ploetz and Pegg (1997) found a parallel between the evolution of banana at the center of origin, the native distribution of the pathogen within this area and the Wallace line. It was proposed that the Wallace line, as redefined by Wallace in 1910, correlates with the eastern limits of the pathogen in the Indo-Malayan region. With one exception (VCG 01214), distribution of

the pathogen in areas outside the demarcation of the Wallace line can be attributed to anthropogenic intervention. Domesticated bananas are hybrids of two diploid species: *M. acuminata* Colla (AA) and *M. balbisiana* (BB). Cultivars are either hybrids of *M. acuminata* Colla or *M. acuminata* Colla x *M. balbisiana* (Ploetz and Pegg 1997, Osuji *et al.* 1997, Stover 1990). Examination of the dendrogram based on Nei's genetic distances (Figure 30) comparing the host (banana cultivars) to the pathogen (Foc) showed that the bifurcation was present in both dendrograms, and there appears to be good correspondence between the host and the pathogen trees. Comparison of this organismal tree to trees generated by combining the different molecular markers clustered isolates from the VCG 0120-01215 complex, mainly affecting AA hybrids of *M. acuminata* in one branch and those in the VCG 0124-0125-0128-01220 complex, mainly affecting *M. acuminata x M. balbisiana* hybrids, in the other branch, just as Boehm's (1994) and Pegg's (1993) had previously observed. VCGs 0121, 022, 0126, 0129, 01210 and 01211 clustered within the VCG 0120 complex branch while VCGs 01212 and VCG 0123 clustered within the VCG 0124 complex branch. VCG 01214 formed a sister clade with the dichotomy in the phenetic approach. This result suggests a different evolutionary origin for VCG 01214. As Gordon and Martin (1997) indicated, the association of multiple VCGs within the same clonal lineage suggests that VCGs arised secondarily through somatic processes. Inconsistencies in the topology of the combined molecular markers and the organismal tree also support the hypothesis of different evolutionary histories between the organismal tree and the gene tree. However, in clonal populations shouldn't the gene tree mirror the organism tree? (Tibayrenc 1996).

The independent origin for VCG 01214 from Malawi was also supported from the analysis of the individual data sets. A deletion, unique for Malawi isolates from VCG 01214 but not Malawi isolates from VCG 0124 was detected using the mitochondrial marker (mitotypes, D'Alessio 1997). There were no apparent differences between the strains, though, within this VCG under the mitochondrial analysis. RAPD data was not available for this VCG. A unique loci, *pgm-2* was also detected for VCG 01214 with the allozyme multilocus analysis (own results).

Nonetheless, even within this VCG there were strains showing autopomorphic states. MW2v14mw and MW7v14mw have three chromosome bands with different electrophoretic mobility than the corresponding chromosome in the other tested strains of VCG 01214 (Boehm *et al.* 1994). Different mating types were also evident within this VCG suggestive of recombination.

An agreement subtree generated to identify conflicting taxa pruned 13/26 tested strains from the combined trees. The conflicting taxa fell within the following VCGs: 0121, 0126, 0129, 0123, 0124, 0125 and 01214 (Figure 26). Generation of agreement subtrees from 50% majority rule consensus tree of two partitions at a time failed to produce concordant agreement subtrees in any combination of makers. This incongruence was supported in the ILD Test.

4.4.2 ILD Results

How significant is the conflict observed on *a priori* examination of the individual data sets? The ILD test has been used reliably to test for congruence between and within multiple data sets (Baker *et al.* 1998, Flynn and Nedbal 1998, Gatesy *et al.* 1999,

Huelsenbeck *et al.* 1996, Remsen and De Salles1998, O'Donnell 1998, O'Donnell 1999, Taylor 1999). One thousand replicates, using maximum parsimony were done for the individual partitions and for the combined data sets (mitochondrial and nuclear data) using the ILD test. The combined analysis rejected the null hypothesis of homogeneity within data partitions (p-value = 0.001) (Table 40). Figure 27 summarizes the results. If Foc is strictly clonal, the observed trees and the trees generated by randomization would have the same length owed to similar evolutionary histories between the genes (Taylor, 1999). If different evolutionary histories are involved, the observed tree may be smaller than the randomized trees, thus rejecting the null hypothesis of congruence.

Incongruence was also evident within the nuclear data sets (Table 40 p-value = 0.001) and between most of the individual data sets (Table 41).

One interesting result, though, is the comparison of the mitochondrial data set against the combined nuclear markers. A p-value of 0.9020 failed to reject the null hypothesis of congruence. This last result could suggest one of two things: first, similar evolutionary history for the mitochondrial and the nuclear genome or it could also mean that the phylogenetic signal is muffled by the different evolutionary histories of the nuclear genome. Clades showing homoplasy in the mitochondrial tree were fully resolved in the combined molecular tree (Figure 29). The fact that several VCGs share a mitotype (Gordon and Martin 1997), even when different evolutionary origins are suggested within a clade (Figure 21), strongly suggests recent outcrossing and recombination.

Removing individual partitions one at a time did not improve the conflicting signals in the combined analysis (Table 42). P-values of 0.001 in each case rejected the

null hypothesis of homogeneity. Maximum parsimony analysis removing one partition at a time also failed to yield trees with congruent topologies. In general significant changes were observed in the more basal branches. The terminal taxa within the clade were rather uniform across the data sets, however, the resolution of the clade varied depending on the examined gene(s). Bootstrap values showing nodal support ranged from 52% to 99% and decay indices ranged from +1 to +10.

The multilocus analysis using the ILD test opened a series of questions regarding the use of different data sets (sequence vs binary files) in a combined analysis and the possible effect of weights imposed on the individual partitions when combining the data sets, particularly when the test rejects the null hypothesis of homogeneity of the partitions. The importance of homogeneity of taxa became evident when different partitions were compared not sharing all the members of a data set. In an effort to homogenize the taxa set, missing data symbols were placed in the partitions where a particular molecular marker was not tested. The ILD test was conducted and the results were compared against a truly homogeneous taxa subset where most of the molecular markers were tested for. The large taxa set with the missing data had to be stopped due to space and time constraints. On the other hand, the results obtained from the homogeneous taxa subset were consistent with phenetic and phylogenetic analysis conducted for the subtest. Something to keep in mind when comparing different partitions.

4.5 Conclusions

Different evolutionary origins can be inferred from this multilocus analysis dividing the forma species into basically two big clades in Foc, encompassing most of the

studied VCGs. In addition, molecular and physiological evidence supports the idea of a different evolutionary origin for the isolates of VCG 01214.

Different evolutionary histories between the organism tree and the gene trees can be inferred from phylogenetic and phenetic trees generated during the simultaneous multilocus analysis as well as from the statistical analysis through the ILD test. Thus, we cannot exclude the possibility of recombination in this asexual fungus.

A recent reexamination of two lineages previously identified as clonal through the RFLP data, FOC I and VIII, (Taylor *et al.* 1999) failed to reject the null hypothesis of incongruence postulated by the permutation Test (p-value =0.85), suggesting recombination. Incongruence was also evident in a phylogenetic analysis comparing sequenced DNA and RAPD data in *Fusarium oxysporum* (O'Donnell *et al.* 1999).

A caveat, though, these tests can only suggest recombination but cannot discern how, when or how frequent recombination is occurring.

Genetic variation via mutation can lead to meltdown in clonal populations by the accumulation of deleterious mutations (Muller's ratchet). Recombination can stop the effects of the ratchet without the loss of favorable alleles. Thus, horizontal genetic transfer through somatic recombination is one possibility.

A second possibility that cannot be ruled out is the idea of cryptic sex. The fact that mating type genes were identified in this asexual fungus opens the question of recombination via an occasional sexual cycle. Moreover, the fact that all possible combinations of mating type genes occur in lineages that were considered clonal lineages (VCG 0120-0215 complex) suggests that these strains could be having sex (personal communication). Another VCG where all possible combinations of the two mating types

occur is in VCG 01214. In addition, the incidence of VCGs that have been discovered that do not fall within an identified VCG (Ploetz 1998a, 1998b, Nasir 1999) and the increase in VCG complexes (Bentley 1998) also points out to the possibility of cryptic sex.

One idea emerges, though, that the regulation of vegetative and sexual genes is independent of one another in Foc as sexually incompatible strains (sharing the same mating type) are still capable of forming viable, stable heterokaryons (own results). How can we tell if recombination is due to sexual or somatic origin? If recombination occurs between strains devoid of mating type genes, or sharing the same mating type genes, the possibility of somatic recombination cannot be discarded, although an occasional sexual cycle or somatic recombination via a parasexual cycle might be indistinguishable in nature.

As Milgroom (1996) postulated, a criteria to detect recombination may be summarized in four basic points: the presence of sexual structures, evidence of genotypic variation, all possible recombinant genotypes are present for pairs of polymorphic loci and different regions of the genome may have different evolutionary histories.

The first alternative is beyond the scope of this paper. However, genetic variability has been established in Foc from the analysis of individual molecular markers (Kistler 1997, Ploetz 1990, O'Donnell 1998). The multilocus simultaneous analysis (this work), provided evidence of inconsistencies within and between different molecular markers representing different parts of the genome, suggestive of different evolutionary origins or histories that may be attributed to recombination. Last, but not least, all

recombinant genotypes are present within one VCG, evidenced by Mating Type analysis (personal communication).

Regardless of whether recombination occurs via somatic means or through an occasional sexual cycle, the multilocus analysis of Foc challenges its strictly clonal reproductive history. The possibility of horizontal genetic transfer in asexual fungi should be taken into consideration when using non-pathogenic strains as biocontrol agents or when breeding new cultivars for resistance.

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APPENDIX I: PROTOCOLS

A. Media Preparation

I. Complete media (CMA) (per liter)

Modified Czapeks dox: 20 g Dextrose, 3 g Yeast Extract (Difco), 3 g Peptone (Difco), 3 g Casein Hydrosylate (Difco), 2 g Sodium Nitrate ($NaN0_3$), .5 g Magnesium Sulfate ($MgS0_4$), .5 g Potassium Chloride (KCl), 20 g bacto-agar(Difco), 10 ml Potassium Phosphate (K_2HP0_4) [.1g/ml] and 1 ml Ferrous Sulfate ($FeS0_4$) [.01g/ml]. Autoclave for 20 minutes in the liquid cycle.

CM top agar uses the same recipe except half the amount of the bacto-agar.

II. Minimal Medium Agar (MMA) (per liter)

30g Sucrose, 2 g Sodium Nitrate ($NaN0_3$), .5 g Magnesium Sulfate ($MgS0_4$), .5 g Potassium Chloride (KCl), 15 g bacto-agar (Difco), 10 ml Potassium Phosphate [.1g/ml], 1 ml Ferrous Sulfate ($FeS0_4$) [.01g/ml], 100 1 trace elements. Autoclave for 20 minutes in the liquid cycle.

MM top agar uses the same recipe except half the amount of the bacto-agar.

Liquid MM is as above without the addition of the bacto-agar.

III. Potato Dextrose Broth (PDB) (per liter)

24 g Potato Dextrose Broth (Difco) in 1L distilled water.

For Potato Dextrose Agar (PDA) plates, as above plus 15g /L bacto-agar. Autoclave for 20 minutes in the liquid cycle.

IV. Benomyl stock solution (Dupont) (for MMB/CMB plate preparation)

A stock solution was prepared by diluting 1g of 50% benolate/benomyl (Dupont) in 100 ml of chloroform for a final concentration of 10mg/ml. The stock solution was then filtered sterilized. MMB and CMB plates are prepared as CMA/MMA above.

Benomyl was added at the working concentration before autoclaving. Benomyl plates were kept at 4°C for storage.

V. Hygromycin B (Sigma H7772) stock solution (for MMH/PDH plates)

A stock solution was prepared by diluting 1g of Hygromycin B in 10ml of sterile distilled water for a final concentration of 100mg/ml. The stock solution was filtered sterilized. MMH and PDH plates are prepared as PDA/MMA above. Hygromycin was added to the media at the working concentration after autoclaving after allowing the temperature to go down to 42°C. Hygromycin plates were kept @ 4°C for storage.

B. Protocols

I. UV Mutagenesis

A. Generation of auxotrophic mutants

Inoculate the wild type strains from cultures obtained from single spored isolates. Grow culture on PDA. Transfer a $1mm^2$ block from the edge of a growing colony to 200 ml PDB (Difco, Detroit, MI)) and allow to sporulate 4 days at 125 rpm (Lab-line G25 Incubator shaker, New Brunswick Scientific, NJ) @ 30°C. Harvest spores through 4 layers of sterile miracloth (Calbiochem, San Diego, Ca), spin @ 7000 rpm for 10 minutes to pellet in a Sorvall GSA rotor (Sorvall RC5B, Dupont). Decant and wash spores in 10 ml sterile distilled water. Transfer to 50ml sterile centrifuge tube. Centrifuge at 3000 rpm for 5 mins in a table top centrifuge (IEC Centra Cl2, MA) Wash spores in 10 ml sterile distilled water and centrifuge at 3000 rpm for 5 minutes. Resuspend pellet in 10 ml sterile distilled water.

A spore count was done, using 10ul of the microconidia suspension using the hemocytometer (Fisher Scientific). One million spores/ml were placed into a sterile 9 cm petri dish. 10 ml distilled water was added to form a monolayer. The uncovered plate was then placed in a UV Crosslinker (Fisher Scientific) and the microconidia were mutagenized at 20 mjoules/cm2 to achieve 90-95% kill. Following the ultraviolet treatment, the spores were serially diluted and plated using 100 µl of the desired dilution in 3 ml CMA top agar to get 100 colonies/plate. Plates were stored in the dark to prevent photoreactivation. After 48 hours 1000 colonies were picked into a 50 square grid on a 9 cm MMA and CMA plate. Auxotrophs grew on CMA but not on MMA. Putative auxotrophs were isolated and purified through a process of three single sporings. To

determine the nutritional deficiencies of the auxotroph, an auxanography test was performed using the Holliday method (Holliday 1956).

Double auxotrophs were made by collecting spores from single mutants. The same protocol was followed. Double auxotrophs grew on CMA but not on MMA supplemented with the nutritional requirement of the single auxotroph. Putative auxotrophs were isolated and purified through a process of three single sporings. To determine the nutritional deficiencies of the auxotroph, an auxanography test was performed using the Holliday method (Holliday 1956), ammended by supplementing all diagnostic plates with the nutritional requirement of the single mutant.

Reversion tests were done on single and double auxotrophs. Cultures were grown in 200 ml PDB shaking @ 180 rpm for four days. The spores were harvested by filtering through sterile miracloth (Calbiochem) to remove mycelial growth. The filtrate was centrifuged at 3000 rpm for five minutes and the resulting pellet washed with 10 ml sterile distilled water. The procedure was repeated twice. The final spore pellet was resuspended in 1 ml sterile distilled water and the number of spores determined using a hemocytometer (Fisher Scientific). The entire spore suspension was plated in MM top agar on a MMA plate.

B. Preservation of auxotrophic mutants

The final stage on the isolation of mutants involves the preparation of a filter stock for preservation and storage. For short-term storage, a Whatman filter No. 1 was placed on CMA. With a sterile toothpick, the purified auxotroph was picked and transferred to the CMA plate with the Whatman filter. Within a week, colony growth covered the filter. To insure even coverage, the filters were not lifted until mycelial

growth was observed passed the filter paper. At that time, with sterile forceps, the filter is lifted and set to dry in a sterile, empty 9 cm petri dish. Once the filter was dried, with sterile scissors the filter was cut and stored in sterile containers in the refrigerator @ 4° C.

Another short-term storage technique involved transferring 100µl of freshly harvested spores to 1ml sterilized PDB in a 2ml microfuge tube at room temperature. These cultures were viable even after eight months of the initial inoculation.

Cryopreservation is the freezing and storage of cells at very low temperatures. This procedure was used for long-term storage of strains and auxotrophs. Freeze-drying involves the removal of water or other solvent from a frozen product by a process called sublimation. Pre-freezing of a suspension to be freeze-dried is required to ensure starting with a solid product. Reconstitution of the freeze-dried product requires the addition of water back to the dried suspension and rehydration time varies depending on the organism dried.

To prevent damage to cell suspensions as they are freeze-dried, cryoprotectants are used. For fungi the recommended cryoprotectants are 20% sterile skim milk (Difco 0032)(1:1 for a final skim milk concentration of 10%, 5% DMSO and for freezing, 50% glycerol. For bacteria use 20% skim milk unless growth is inhibited by milk or on cultures which are grown on chemically defined media, such as chemolithotrophs (like nitrosomonas). In that case use 24% sucrose (1:1 for a final sucrose concentration of 12%), Reagent 20 (100g BSA FV, 200g sucrose in 1L dH2O), filter sterilized, or trehalose. Dispense into sterile cryovials in aliquots of 200 µl. Freeze drying was accomplished using using LabConco FreeZone 4.5 Liter Freeze-dry System model 77510

(LabConco Corp, Kansas City, MO). The freeze-dried material was stored in the –20°C freezer.

For cultures growing on slants or agar plates, pour the desired content of the cryoprotectant on the slant or plate, scrape and collect with a sterile pipette. Dispense into sterile cryovials in aliquots of 200 µl. Store at –80°C.

For mycelia or mutants use freezing instead of freeze-drying. Recommended cell density is a minimum of 10^6 cells/ml.

C. The wild type testers: benomyl resistant mutants

One million UV mutagenized spores/ml were plated on benomyl Complete Medium (CMB) plates at concentrations of 10, 50 and 100 µg/ml and allowed to grow 24 hrs in the dark to prevent photoreactivation. Benomyl-resistant (B+) colonies emerged approximately 1 week after plating and were transferred to fresh CMB plates to verify tolerance. The benomyl resistant auxotrophs were stored as indicated above.

D. Auxanography: The Holliday Test

Auxanography, the diagnostic screening of the nutritional deficiencies, was made using a modified Holliday Test for bacterial auxotrophs (Holliday 1956).

II. Heterokaryon Formation

A. Pairings by Hyphal anastomosis

1. The Double Pick Method

In the double-pick method inoculum from the edge of the colony of one parent growing on PDA was transferred to a MMA plate by picking with a sterile toothpick With a different sterile toothpick the second parent was picked in the same fashion and co-inoculated into the same hole as parent one

Both the controls and the pairings were picked in the same plate. Heterokaryon formation was seen in 72 hours.

Prototrophic colonies growing on the selective medium (MMA) were considered putative heterokaryons and were transferred to fresh MMA to screen for continued growth. Of the putative heterokaryons that continued to grow on MMA five to ten were chosen at random (whenever possible), microconidia were collected and tested on diagnostic plates. As the nuclei in the heterokaryotic cell can segregate into propagules, analysis of microconidia from heterokaryons can be assumed to represent the heterokaryotic cell (Maheshwari 1998). These heterokaryons were removed from selective pressure and stored in potato dextrose broth (PDB) (Difco 0549-17-9) at room temperature.

In addition, 1 mm x 1mm blocks were cut from the edge of growing colonies and were transferred to water agar for hyphal tipping (Section III-Appendix I). Individual hyphal tips were cut and transferred to Potato Dextrose Agar (PDA) plates. Microconidia collected from sporulating colonies were serially diluted and plated on PDA.

2. Liquid Minimal Media

Another method used to force heterokaryon formation was the liquid minimal media (LMM) method (Appendix I). Ten to twenty pairings were forced by coinoculating equal number of microconidia from the auxotrophic mutants in a 13mm x 100 mm test tube or 2ml microcentrifuge tubes. The tubes were left unshaken to promote germination of the microconidia. The still cultures were incubated at room temperature and monitored daily for growth. Putative heterokaryons were transferred to fresh LMM to look for continued growth. Although many heterokaryotic events could be present in one tube, each test tube was considered as one putative heterokaryotic event. A third transfer involved vortexing the tubes and transferring propagules to MMA plates. Five pairings were further tested in diagnostic plates and stored in PDB as in the DP method above.

Control tubes were coinoculated with microconidia from parental strains with themselves.

B. Pairings by Protoplast Fusion

1. Protoplast preparation

Transfer 10^7-10^8 freshly harvested spores to two 500ml erlenmayer flask and germinate in 100 ml PDB overnight @ 125 rpm (Lab-line orbit shaker). Pour the germinated spores into two sterile 50ml centrifuge tubes. Spin germlings @ 3000 rpm in a table top centrifuge (IEC Centra C12, MA) for 5 minutes to concentrate the mycelial mat. Decant supernatant and wash with 15ml protoplast solution (1.2M $MgSO_4$, 10mM Na_3 Phosphate, pH 5.8) without enzyme. Centrifugeat 3000 rpm for five minutes. Decant

supernatant. Resuspend pellet in 15ml protoplast solution at room temperature still for 30 minutes. Centrifuge at 3000 rpm x 5 minutes. Decant and resuspend pellet in 10ml protoplast solution with enzyme (Novozyme 234 (2g/ml) (Sigma., CA). Shake @ 125 rpm overnight. Spin protoplasts @ 3000 rpm for 5 minutes to pellet. Wash protoplast pellet in osmoticum solution (1.2M sorbitol, 10mM $CaCl_2$,10mM Tris, pH 8) Centrifuge@ 3000 rpm for 5 minutess to pellet. Decant and resuspend pellet in osmoticum solution. Count protoplasts using the hemocytometer (Fisher scientific)

2. Protoplast fusion

In a sterile 15 ml centrifuge tube equal number of protoplasts (10^6–10^7) of each parental strain were coinoculated. Protoplasts were centrifuged at 3000 rpm for 5 minutes the supernatant was decanted and resuspend in 1ml 30% PEG (.375g Glycine (50mM), .147g $CaCl_2$-$2H_20$ (10mM), 30g PEG (MW 8000), pH 7.5 bring volume to 100 ml with sterile dH_20). After 15 minutes at room temperture, the protoplast solution was diluted in 5ml osmoticum solution (1.2M sorbitol, 10mM $CaCl_2$,10mM Tris, pH 8). The fused protoplasts were allowed to recover before they were serially diluted and plated on MMA plus osmoticum to prevent shearing of the protoplasts. The plates were incubated inverted at 30° C and monitored daily for prototrophic growth. Emerging colonies were considered putative heterokaryons, were transferred to MMA and were screened for continued growth. Ten heterokaryons (whenever possible) were chosen at random and further screened on diagnostic plates. These heterokaryons were stored in PDB.

Controls for the protoplast fusion included protoplasted parental survival rate, parental protoplast self-fusion and protoplast lysis.

For all the above methods, plugs from emerging colonies from the final transferred MMA plate, were cut and further transferred to PDB tubes to collect spores. After two days, these spores were harvested for diagnostic screening by plating serial dilutions on PDA plates. The colonies were then picked on diagnostic plates (Table 2) to determine the genotype.

B. Cellophane Test

Auxotrophs might be able to complement each other on MMA by cross feeding rather than hyphal anastomosis. To rule out cross feeding in our pairings we performed each method under conditions that would allow cross feeding but prevented anastomosis, modifying the technique used by Correll (1986).

III. Hyphal Tipping

Plugs from heterokaryons growing in MMA plates were transferred to 1.5% water agar plates poured thinly (4 ml/plate) to allow for sparse growth. Using platinum needles under a dissecting microscope, the hyphal tips of growing mycelia were removed and transferred to CMA plates. Spores were collected from viable tips and were screened on diagnostic plates (table 2) to assess their genotype.

IV. Allozymes

A. Methods

I. Tissue preparation:

$2mm^2$ filter stocks of wild type strains were inoculated on Minimum Media Agar plates (MMA) to induce enzymatic production and the cultures were allowed to grow for four days. Mycelia from the edge of the growing culture was removed using a sterile loop and transferred to 1.5 ml epindorff tubes. Depending on the tested enzyme, tissue samples were homogenized with either 100μl of phosphate grinding buffer pH 7 or a 1:1 phosphate grinding buffer pH7:NADP solution. An electric drill set at high speed was used to grind the samples for 30 seconds to 1 minute. Once homogenized, the samples were centrifuged for 1 minute and kept on ice.

II. Electrophoresis

10μl aliquots were transferred to a super z 12-sample well plate (Helena Laboratories cat 4096). The samples are then stamped to a 76 mm x 76 mm cellulose-acetate gel (Titan III, Helena Laboratories cat 3033) that were previously hydrated in 1:5 gel buffer for at least 20 minutes. Depending on the enzyme, Tris-glycine (TG) buffer pH 8.5 or CAAMP buffer pH 7 was used. The gels were run under a continuous buffer system. With TG, to achieve better banding resolution, the gel buffer was at a higher concentration than the running buffer (1:9).

The following table summarizes the tested enzyme and the optimized protocols used to achieve clearer bands.

ENZYME	EC NUMBER	GRINDING BUFFER	GEL BUFFER	RUNNING BUFFER	INCUBATION
IDH	1.1.1.42	B	C	D	32-37^{O}C
LDH	1.1.1.27	A	C	D	32-37^{O}C
MDH-1	1.1.1.37	A	C	D	25^{O}C
MDH-2	1.1.1.37	A	E	E	25^{O}C
ME	1.1.1.40	A	C	D	32-37^{O}C
PGM	5.4.2.2	A	C	D	25^{O}C
G6PDH	1.1.1.49	B	C	D	25^{O}C
GPI	5.3.1.9	A	C	D	25^{O}C
6PGDH	1.1.1.49	A	C	D	25^{O}C
ACON	4.2.1.3	B	E	E	32-37^{O}C
ALP	3.1.3.1	A	E	E	25^{O}C

A=100 μl 0.1M Potassium phosphate buffer pH 7; B=1:1 (0.1M) Potassium phosphate buffer pH 7.0 : NADP [2mg/ml] ; C= 1:5 Tris-Glycine buffer pH 8.5: dH_2O; D= 1:9 Tris-Glycine buffer pH 8.5: dH_2O; E=Citric Acid 4-(3-Aminopropyl) morpholine buffer (CAAMP) pH 7.0

Running time was set for 20-30 minutes at a voltage of 200 amps under constant current.

III. Staining

The enzymatic activity was assayed using the stain recipes outlined in the manual Methodologies for allozyme analysis using cellulose acetate electrophoresis (Herbert and Beaton, 1993) using the tetrazolium system (MTT), and overlaid with 1.6% water agar. Allowing one minute for the gel to set, the plates were incubated in the dark for a time ranging from 5 minutes to overnight. The agar overlay was removed by gently washing the plate and blotted dry. The zymograms were digitized immediately to a file for scoring and labeling using the HP scan-jet 4P or the Fotodyne gel imager.

IV. Gel Preservation

For long term preservation of the bands, the gel plates were de-stained in water for 20 minutes, blotted dry and incubated @ 60°C for 30 minutes to one hour. Rehydration of the gels reconstitute the banding patterns for reexamination.

APPENDIX II: DATA MATRICES

A. Isolates of *Fusarium* used and the Data bases they were used for.

ISOLATE	SPECIES	FSP	VCG	RACE	ORIGIN	Source	MT	mt-dna	allo	rflp	rapd	Mat	EK
CS 85-1	Oxysporum	niveum	0080	?	?	FNM	XI	x	x				
CS 85-4	Oxysporum	niveum	0082	?	Florida	FNM	XI	x	x	x		x	
PHW 777	Oxysporum	conglutina	0101	1	Japan	HCK	X	x	x				
PHW 699	Oxysporum	raphani	0102		Wisconsin	HCK	IX	x	x			x	
15638	Oxysporum	cubense	0120	?	Malasia	RCP				x		x	x
22411	Oxysporum	cubense	0120	4	Australia								x
22424	Oxysporum	cubense	0120	?	Australia					x			x
22425	Oxysporum	Cubense	0120	4	Australia	RCP		x		x			x
22615	Oxysporum	Cubense	0120	4	Byron Bay						x		
3S1	Oxysporum	Cubense	0120	1	Honduras					x			x
A2	Oxysporum	Cubense	0120	4	Australia	RCP		x		x			x
ADJ1	Oxysporum	Cubense	0120	4	Canary	RCP		x		x		x	x
ADJ2	Oxysporum	Cubense	0120	4	Canary					x			x
BUE1	Oxysporum	Cubense	0120	4	Canary					x			x
C1	Oxysporum	Cubense	0120	4	Canary	RCP				x			x
C2	Oxysporum	Cubense	0120	4	Canary	RCP				x			
F9127	Oxysporum	Cubense	0120	4	So. Africa					x			x
FCJ7	Oxysporum	Cubense	0120	?	Jamaica					x	x		x
GAL2	Oxysporum	Cubense	0120	4	Canary					x			x
GALI	Oxysporum	Cubense	0120	4	Canary					x	x		x
IC1	Oxysporum	Cubense	0120	4	Canary								x
IC2	Oxysporum	Cubense	0120	4	Canary Island					x			
MD401	Oxysporum	Cubense	0120	4	Wamuran						x		
MGSA1	Oxysporum	Cubense	0120	?	So. Africa					x			
NB	Oxysporum	Cubense	0120	?	So. Africa					x			
NH	Oxysporum	Cubense	0120	4	So. Africa					x	x		x
NW	Oxysporum	Cubense	0120	?	So. Africa					x			
O-1219	Oxysporum	Cubense	0120	?	Australia	RCP				x			
O-1220	Oxysporum	cubense	0120	?	Australia	RCP	I	x	x	x	x	x	x
O-1222	Oxysporum	cubense	0120	4	Australia	RCP				x			x
ORT1	Oxysporum	cubense	0120	4	Canary					x			x
ORT2	Oxysporum	cubense	0120	4	Canary								x
Pacovan	Oxysporum	cubense	0120	?	Brazil					x			x
PAJ1	Oxysporum	cubense	0120	4	Canary					x			x
Prata	Oxysporum	cubense	0120	?	Brazil								x
SA3	Oxysporum	cubense	0120	?	So. Africa					x		x	
SA4	Oxysporum	cubense	0120	?	So. Africa	RCP	I	x		x			
SA6	Oxysporum	cubense	0120	?	So. Africa					x	x		
SA8	Oxysporum	cubense	0120	4	So. Africa	RCP	I	x	x			x	
STGM1	Oxysporum	cubense	0120	1	Costa Rica	RCP	I	x	x	x	x	x	x
STGM2	Oxysporum	cubense	0120	1	Costa Rica	RCP	I	x	x		x	x	
STH1	Oxysporum	cubense	0120	1	Honduras	RCP	I	x				x	x
INDO15	Oxysporum	cubense	0120/01	?	Indonesia					x			
INDO18	Oxysporum	cubense	0120/01	?	Indonesia					x			
INDO20	Oxysporum	cubense	0120/01	?	Indonesia					x			
F9130	Oxysporum	cubense	0121	4	Taiwan					x	x		x
GM	Oxysporum	cubense	0121	4	Taiwan	RCP	II	x	x	x	x	x	x

H1	Oxysporum	cubense	0121	4	Taiwan	RCP	II	x		x			x
ML	Oxysporum	cubense	0121	4	Taiwan					x	x		x
O-1124	Oxysporum	cubense	0121	?	Taiwan					x			
SKC	Oxysporum	cubense	0121	4	Taiwan						x		
T3	Oxysporum	cubense	0121	4	Taiwan	RCP	II	x	x		x	x	
TBR	Oxysporum	cubense	0121	?	Taiwan					x	x		
A1-1	Oxysporum	cubense	01210	1	Florida						x		
A15	Oxysporum	cubense	01210	?	Florida					x		x	
A2-1	Oxysporum	cubense	01210	1	Florida					x	x		
A3-1	Oxysporum	cubense	01210	?	Florida					x			
A4-1	Oxysporum	cubense	01210	?	Florida					x			
CSB	Oxysporum	cubense	01210	1	Florida					x	x		
F2	Oxysporum	cubense	01210	?	Florida					x			
F3	Oxysporum	cubense	01210	?	Florida					x			
FI	Oxysporum	cubense	01210	?	Cuba	RCP	VI	x	x			x	
GG1	Oxysporum	cubense	01210	1	Florida					x	x		x
JC1	Oxysporum	cubense	01210	1	Florida					x			x
JC14	Oxysporum	cubense	01210	1	Florida	RCP	VI	x	x	x	x	x	x
JC4	Oxysporum	cubense	01210	1	Florida						x		x
JC7	Oxysporum	cubense	01210	1	Florida								x
JC8	Oxysporum	cubense	01210	?	Florida					x			
23631	Oxysporum	cubense	01211	4	Wamuran						x		
1372-1	Oxysporum	cubense	01211	?	?	RCP	I	x	x	x	x	x	
SH3142	Oxysporum	cubense	01211	?	Australia	RCP	I	x	x	x		x	x
STNP1	Oxysporum	cubense	01212	?	Tanzania	RCP	IV	x		x	x	x	x
STNP2	Oxysporum	cubense	01212	?	Tanzania					x			
STNP3	Oxysporum	cubense	01212	?	Tanzania								x
STNP4	Oxysporum	cubense	01212	?	Tanzania	RCP	IV	x	x	x	x	x	x
1-1	Oxysporum	cubense	01213	?	Taiwan					x			
1-2	Oxysporum	cubense	01213	4	Taiwan								x
2-1	Oxysporum	cubense	01213	?	Taiwan					x			
2-2	Oxysporum	cubense	01213	4	Taiwan					x			x
4-1-1	Oxysporum	cubense	01213	4	Taiwan					x			x
4-2-1	Oxysporum	cubense	01213	4	Taiwan					x			x
5-1-1	Oxysporum	cubense	01213	4	Taiwan					x			x
6-2	Oxysporum	cubense	01213	4	Taiwan					x			x
ES2-1	Oxysporum	cubense	01213	?	Taiwan					x			
STSUM2	Oxysporum	cubense	01213	?	Indonesia	RCP	II	x	x			x	
MW 2	Oxysporum	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
MW 40	Oxysporum	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
MW 41	Oxysporum	cubense	01214	Mbufu	Malawi	RCP	VII	x	x	x		x	x
MW 42	Oxysporum	cubense	01214	?	Malawi					x		x	
MW 44	Oxysporum	cubense	01214	?	Malawi	RCP				x		x	x
MW 46	Oxysporum	cubense	01214	?	Malawi	RCP				x			x
MW 48	Oxysporum	cubense	01214	?	Malawi	RCP				x			x
MW 51	Oxysporum	cubense	01214	?	Malawi	RCP				x		x	x
MW 7	Oxysporum	cubense	01214	?	Malawi	RCP						x	x
MW 89	Oxysporum	cubense	01214	Harare	Malawi	RCP	VII	x	x	x		x	x
CR1	Oxysporum	cubense	01215	?	Costa Rica					x			
CR2	Oxysporum	cubense	01215	?	Costa Rica					x			
CR4	Oxysporum	cubense	01215	?	Costa Rica					x			
CR5	Oxysporum	cubense	01215	?	Costa Rica					x			

CB1	Oxysporum	cubense	01215/1		Florida	RCP	I	x	x				
CB2	Oxysporum	cubense	01215/1	?	Florida	RCP	I	x	x			x	
LAP	Oxysporum	cubense	0122	4	Philippines					x	x		
P18	Oxysporum	cubense	0122	4	Philippines						x		x
P79	Oxysporum	cubense	0122	4	Philippines					x	x		x
PH2	Oxysporum	cubense	0122	4	Philippines	RCP	I	x	x		x	x	x
PH3	Oxysporum	cubense	0122	4	Philippines					x			
PH6	Oxysporum	cubense	0122	4	Philippines					x			
PW3	Oxysporum	cubense	0122	4	Philippines					x	x		x
PW4	Oxysporum	cubense	0122	4	Philippines								x
PW5	Oxysporum	cubense	0122	4	Philippines	RCP	I	x	x			x	x
PW6	Oxysporum	cubense	0122	4	Philippines					x			x
PW7	Oxysporum	cubense	0122	4	Philippines					x			x
SABA	Oxysporum	cubense	0122	2	Philippines	RCP	I	x	x	x	x	x	x
DAVAO	Oxysporum	cubense	0123	1?	Philippines	RCP	III	x	x	x	x	x	x
F9129	Oxysporum	cubense	0123	1	Taiwan	RCP	III	x	x	x	x	x	x
JLTH4	Oxysporum	cubense	0123	?	Thailand	RCP				x			x
JLTH5	Oxysporum	cubense	0123	?	Thailand					x			x
PH12	Oxysporum	cubense	0123	?	Philippines					x	x		x
PHL1	Oxysporum	cubense	0123	?	Philippines						x		
PHL2	Oxysporum	cubense	0123	?	Philippines					x	x		x
T1	Oxysporum	cubense	0123	1	Philippines					x	x		x
23485	Oxysporum	cubense	0124	1	Mena Creek						x		
A35	Oxysporum	cubense	0124	?	Brazil						x		
A36	Oxysporum	cubense	0124	?	Brazil					x			
B1	Oxysporum	cubense	0124	?	Florida					x	x		
B2	Oxysporum	cubense	0124	2	Florida	RCP	IV	x		x			x
BLUG	Oxysporum	cubense	0124	2	Honduras	RCP	IV	x		x			x
FCJ2	Oxysporum	cubense	0124	?	Jamaica					x			
FCJ3	Oxysporum	cubense	0124	?	Jamaica					x	x		
FCJ8	Oxysporum	cubense	0124	?	Jamaica					x			
FCJ9	Oxysporum	cubense	0124	?	Jamaica					x			
GMB	Oxysporum	cubense	0124	1	Brazil	RCP	IV			x			x
JCB1	Oxysporum	cubense	0124	?	Florida	RCP	IV	x		x		x	
JLTH15	Oxysporum	cubense	0124	?	Thailand					x			
JLTH2	Oxysporum	cubense	0124	?	Thailand					x			
JLTH3	Oxysporum	cubense	0124	?	Thailand								x
JLTH7	Oxysporum	cubense	0124	?	Thailand					x			x
MACA	Oxysporum	cubense	0124	1	Brazil	RCP	IV	x	x	x		x	x
MW 43	Oxysporum	cubense	0124	?	Malawi					x	x	x	x
MW 45	Oxysporum	cubense	0124	?	Malawi					x		x	x
MW 47	Oxysporum	cubense	0124	?	Malawi					x			x
MW 50	Oxysporum	cubense	0124	?	Malawi					x			x
MW 52	Oxysporum	cubense	0124	Sukali	Malawi	RCP	IV	x	x	x		x	x
MW 55	Oxysporum	cubense	0124	?	Malawi								x
MW 58	Oxysporum	cubense	0124	?	Malawi					x			x
MW 64	Oxysporum	cubense	0124	?	Malawi					x			x
MW 65	Oxysporum	cubense	0124	?	Malawi								x
MW 67	Oxysporum	cubense	0124	?	Malawi					x	x		
MW 69	Oxysporum	cubense	0124	?	Malawi					x		x	x
MW 71	Oxysporum	cubense	0124	?	Malawi					x			x
MW 78	Oxysporum	cubense	0124	?	Malawi					x			

MW 80	Oxysporum	cubense	0124	?	Malawi						x	x	
S?	Oxysporum	cubense	0124	?	Jamaica					x		x	
STD2	Oxysporum	cubense	0124	1	Honduras	RCP	IV	x	x	x	x	x	x
STJ1	Oxysporum	cubense	0124	?	Jamaica								x
STJ2	Oxysporum	cubense	0124	?	Jamaica					x			
STN1	Oxysporum	cubense	0124	2	Nicaragua	RCP	IV	x	x			x	x
STN2	Oxysporum	cubense	0124	?	Nicaragua	RCP	IV	x	x	x	x	x	x
STN5	Oxysporum	cubense	0124	?	Nicaragua					x			
STN6	Oxysporum	cubense	0124	?	Nicaragua					x			
STN7	Oxysporum	cubense	0124	?	Nicaragua					x			
STPA2	Oxysporum	cubense	0124	?	Tanzania					x	x		x
MW54	Oxysporum	cubense	0124	?	Malawi								x
23534	Oxysporum	cubense	0124-5	1	Orneau						x		
CVA	Oxysporum	cubense	0124-5	?	Florida						x		
EA 23	Oxysporum	cubense	0124-5	?	East Africa	RCP	V	x	x			x	
EA 25	Oxysporum	cubense	0124-5	?	East Africa	RCP	IV	x	x			x	
JLTH1	Oxysporum	cubense	0124-5	?	Thailand					x			x
JLTH16	Oxysporum	cubense	0124-5	?	Thailand					x			x
JLTH17	Oxysporum	cubense	0124-5	?	Thailand					x			x
JLTH18	Oxysporum	cubense	0124-5	?	Thailand					x			x
MW 11	Oxysporum	cubense	0124-5	?	Malawi					x		x	x
MW 15	Oxysporum	cubense	0124-5	?	Malawi						x		
MW 39	Oxysporum	cubense	0124-5	?	Malawi					x			x
MW 5	Oxysporum	cubense	0124-5	?	Malawi						x	x	x
MW 53	Oxysporum	cubense	0124-5	?	Malawi					x			x
MW 56	Oxysporum	cubense	0124-5	?	Malawi					x			x
MW 60	Oxysporum	cubense	0124-5	?	Malawi					x			
MW 61	Oxysporum	cubense	0124-5	?	Malawi					x	x		x
MW 63	Oxysporum	cubense	0124-5	?	Malawi					x			
MW 66	Oxysporum	cubense	0124-5	?	Malawi					x			x
MW 68	Oxysporum	cubense	0124-5	?	Malawi						x		
MW 70	Oxysporum	cubense	0124-5	?	Malawi					x			x
MW 86	Oxysporum	cubense	0124-5	?	Malawi					x		x	x
MW 9	Oxysporum	cubense	0124-5	?	Malawi						x		
8605	Oxysporum	cubense	0125	1	Tallebudger						x		
8606	Oxysporum	cubense	0125	?	Australia					x			
22417	Oxysporum	cubense	0125	?	Australia					x			
22468	Oxysporum	cubense	0125	?	Australia					x			
22479	Oxysporum	cubense	0125	1	Australia					x			x
22541	Oxysporum	cubense	0125	?	Australia					x			
22600	Oxysporum	cubense	0125	?	Australia					x			
23480	Oxysporum	cubense	0125	1	Tallebudger						x		
1S	Oxysporum	cubense	0125	?	Jamaica								x
1S?	Oxysporum	cubense	0125	?	Jamaica					x	x		
4S1	Oxysporum	cubense	0125	1	Honduras						x		
5S1	Oxysporum	cubense	0125	1	Honduras						x		
8610	Oxysporum	cubense	0125	1	Australia	RCP	IV	x	x			x	
8611	Oxysporum	cubense	0125	1	Australia	RCP	IV	x	x	x	x	x	x
A1	Oxysporum	cubense	0125	1	Australia								x
A4	Oxysporum	cubense	0125	?	Australia					x			
JLTH20	Oxysporum	cubense	0125	?	Thailand					x			x
JLTH21	Oxysporum	cubense	0125	?	Thailand								x

O-1223	Oxysporum	cubense	0125	?	Australia					x			
STNP5	Oxysporum	cubense	0125	?	Zaire						x		x
STPA3	Oxysporum	cubense	0125	?	Uganda					x	x		x
S1	Oxysporum	cubense	0126	?	Honduras					x	x		
STA2	Oxysporum	cubense	0126	1	Honduras					x	x		x
STB2	Oxysporum	cubense	0126	1	Honduras	RCP	VI	x	x	x	x	x	x
STM3	Oxysporum	cubense	0126	1	Honduras	RCP	VI	x	x	x	x	x	x
22993	Oxysporum	cubense	0128	2	S.Johnstone						x		
22993	Oxysporum	cubense	0128	?	Australia					x			
22994	Oxysporum	cubense	0128	2	Australia					x	x		x
A47	Oxysporum	cubense	0128	2?	Comores	RCP	IV	x	x	x		x	x
8604	Oxysporum	cubense	0129	?	Australia					x			
8623	Oxysporum	cubense	0129	4	Mooloolah						x		
8627	Oxysporum	cubense	0129	4	Australia					x			x
22401	Oxysporum	cubense	0129	4	Australia								x
22402	Oxysporum	cubense	0129	?	Australia					x			
22507	Oxysporum	cubense	0129	?	Australia								x
23549	Oxysporum	cubense	0129	4	Wamuran						x		
0-1221	Oxysporum	cubense	0129	1	Australia	RCP	I	x	x	x		x	x
N5331	Oxysporum	cubense	0129	4	Australia	RCP	I	x		x			
N5443	Oxysporum	cubense	0129	4	Australia					x			x
	Oxysporum	melonis	0130		?	HCK	IX	x	x			x	
PT1	Oxysporum	melonis	0131		?	HCK	IX	x	x			x	
MC265	Oxysporum	melonis	0134		?	HCK	IX	x	x			x	
SC626	Oxysporum	lycopersici	Folr1	1	Italy	HCK	VIII	x	x	x		x	
SC761	Oxysporum	lycopersici	Folr3	3	Florida	HCK	VIII		x	x		x	
247	Oxysporum	pisi		5	Washington	HCK	IX	x	x			x	
77137	Solani Pisi	mpvi				HCK			x			x	
Ca-Ha-3	Acuminatum					LH			x				
Ka-Cv-1	Crookwellen					HN/LH			x				
NoT&S-4	Sambucinum					HN/LH			x				
S-66	Solani	mpv				HCK			x			x	
SC548	Oxysporum	lycopersici		?	Florida	HCK	VIII			x			
So-BB-3	Equiseti	n/a				LH			x				
	Oxysporum	Dianthi			?	HCK	IX	x	x				

Wild type strains of *Fusarium oxysporum* f.sp. *cubense* were obtained from the worldwide collection of Dr. Randy C. Ploetz located at the University of Florida, Tropical Research and Education Center in Homestead, Fla. Formae Speciales (f.sp.) wild types were provided by Dr. H. Corby Kistler (University of Florida) Gainsville. The different species were provided as indicated above. HCK=Dr. H. Corby Kistler, UF-Gainsville, FNM=Dr. Frank N. Martin, LH=Linda Hanson, Cornell University, HN=Dr. Helgard Nirenberg, Federal Biological research Centre for Agriculture and Forestry

B. Nexus files for the original data bases

P A U P *
Version 4.0b3 for 32-bit Microsoft Windows

I. Electrophoretic Karyotype

Boehm, EWA, Ploetz, Rc, and Kistler, HC, 1994 Statistical analysis of electrophoretic karyotype variation among vegetative compatiblity groups of Fusarium oxysporum f.sp. cubense MPMI 7(2): 196-207

[!3 6 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
25 26 26.5 27 28 29 29.5 30 31 32 33 34 35 36 37 38 39 39.5
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52 53 54 55 56 57 58 59 60 61 62 63 63.5 64 65 66 67 68
69 70 71 72 81 96 CN]
[!CN=CHROMOSOME NUMBER]

NEXUS

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1100001000000001000000100000100010000001000000010000000100000000000000E
stpa2
1100000000100001000000000001000010000000011000000001000000000000000000E
b21
1110000001000000000010000001000100000000010000000101000000100000000000G
jlth3
1100000001000001000001000000010000000001000001000001000000100000000000F
jlth7
1100000001000001000000100000010000000001000001000001000000100000000000F
jlth4
1100000000000100000001000001000000100000000100100000000000000000011000E
jlth5
1100000100000100000000001000010000000000000100100000000000000000000001E
mw5
0110100000000100000100000010000100001000000000100000000000000000000000E

mw11
01101000000001000001000000100001000001000000000100000000000
10000000000000000000E
mw39
01001000000000101000000100100001010000000100000100000000000
00000000000000000000F
mw53
01000010000001001000001000100010000100000000000000000000000
00000000000000010000F
mw56
11001000000001001000001010000001000001000000000000000000000
00000000000000000000E
mw61
11010000010000100000000000100010010000000000010000000100000
00000000000000000000D
mw66
11010000010000100000001010000010000000000010000000000100000
00000000000000000000E
mw70
11010000010000100000000000100110000100000001000000000000000
00000000000000000000E
mw86
11010000010000100000000000100001010000000001000000000000000
00000000000000000000D
jlth1
01100000001000000100001000000001000000000000000101000000000
10000000001000000011E
jlth16
01100000001000000100000100000001000001000000000000000100000
00100000000000100000E
jlth17
01000100001000000000000100000101001000000000000000000100000
00100000000000100000D
jlth18
01000100001000000100100000001001001000000000000000000100000
00100000000000100000D
a1
01001000010000001000001000001000001000000001000001000000000
00000000010000000000F
F8611
01001001000000001000001000000000001000000100000000100000000
10000000001000000000G
F22479
01001000000000000001000000000000100000000000000001001000000
10000001000000000000G

F1s
0010010000000000000000010000010010000100010000000100000000010000000F
stpa3
0100000010000000100000100000100001000000000100000000001000000000000E
stnp5
0100000000010000000000100000001000100000000000010000000001000000000F
jlth20
0100000010000000000010000001000010010000000101000100000000010000000G
jlth21
0100000010000000000000100001000010010000000001000100000100001000000G
stm3
0100001000010010000000010000001000000100010000000100000000010000000D
sta2
0100001000010010000000010000001000000000010001000100000000010000000D
stb2
0100000010000100000100010000000010010000010000000100000000010000000D
F22994
0100010000000010010000000000100010000001000000000100000100001000000C
a47
0100010000000001000000000000010100000100000000010000000010001000000C
n5443
0000010000010000001000001000000001000000010000010000000000001000000009
F8627
0000010000010000001000001000000001000000010000010000000000001000000009
F22401
0000010000010000001000000000100001000000010000010000000000001000000009
F1221
0100000010000000100000100000000100010000010000010000000000001000000C
F22507
0100000010000000100000100000000100000010000010000000000000001000000009

gg1
0100001000000100001000100000010000000000000100000100000000000000000F
jc1
0101000000100001000010000010000001000000010000010001000000000000000E
jc4
0101000000100001000010000010000001000000010000010001000000000000000E
jc7
0101000000100001000010000010000001000000010000010001000000000000000E
sh3142
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stnp1
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stpn3
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F12
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F22
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F411
0001000000100000100010000000000010000000000100000000000000000000000C
F421
0001000000100001000010000000000010000000000000100000000000000000000C
F511
0010001000001000000010000000000100000000000000100000000000000000000D
F62
0010001000001000000010000000000100000000000000100000000000000000000D
mw2
0100010000000100000000100000010001000001000000010000010000000000010E
mw7
0100010000000100000000100000010001000001000000010000010000000000010E

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mw40
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
mw41
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
mw44
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
mw46
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
mw48
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
mw51
0100010000000100000000100000001000100000010000010000010000
000000000000010000000E
;
END;
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II. Allozymes

Blanca Cortes (2000) PhD Dissertation, Horizontal Genetic Transfer in Asexual Fungi Dept Biological Sciences Florida International University, Miami, Florida 33199 USA

NEXUS

[!1=idh2,2=idh1,3=ldh,4=mdh3,5=mdh2,6=mdh1,7=me,8=pgm3,9=pgm2,10=pgm1, 11=gpi3, 12=gpi2, 13=gpi1, 14=G6H-1 15=6pgdh2, 16=6pgdh1, 17=acon1]

BEGIN DATA;
DIMENSIONS ntax=52 nchar=17;
format symbols="012345" ;

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stgm1	00111001011010121
stgm2	10121011011010121
t3	10010001012010120
gm	00010001012010120
saba	00110001011010121
ph2	00010001011010121
davao	00310001012010121
std2	10220011021010120
stm3	20210001012010121
stb2	30121011011011121
jc14	10120011011021121
F13721	30120011011010121
stnp1	00210001022010000
CaHa3	10440021013010100
Kacv1	10130021033140120
NotS4	10120011024140200
Sobb3	00010021034050121
S66	43511131015150130
NH77137	02511021043150100
CB1	00010001012011130
CB2	00010001012011121
sa8	00111001011010121
sh3142	20120001011010121
pw5	00210001011010121
F01221	00120001011010020
stsum2	10121011011010121
F9129	00010011012010121
stnp4	00210001022010000
stn1	30220021021010121
maca	10220011021010120
MW52	00010001022011110

```
ea25            00210001022010121
F8611           00210001022010121
F8610           20210001022010130
a47             20211011021010121
ea23            00200001022010000
f1              00110001011021121
mw2             10221211111011121
mw41            10221001111011201
mw40            30010001112011121
mw89            00221211113011121
sc626           00010020022010000
sc761           31220121023010111
FP247           32220021022010111
dianthi         30221121022010111
phw699          32210021022010111
melonis         00011021022010120
pt1             00210021022010120
mc265           30210021022010111
phw777          02010020023030111
cs851           30220021021010111
cs854           00010020022030120
;
END;
```

III. Mitotypes

D'Alessio, Naomi, (1998) PhD Dissertation, Mitochondrial inheritance during a parasexual cycle in Fusarium oxysporum f.sp. cubense, Florida International University Miami, Florida 33199 USA

#NEXUS
[MacClade 3.01 registered to Naomi, NSUU]

BEGIN DATA;
DIMENSIONS NTAX=11 NCHAR=576;
FORMAT MISSING=? GAP=. INTERLEAVE DATATYPE=DNA SYMBOLS="01" ;
OPTIONS MSTAXA=UNCERTAIN ;

MATRIX
MT-XI
TATTTGTAACTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTGATTA
MT-X
TATCTGTAATTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-IX
TATCTGTAATTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-VIII
TATCTGTAATTAATGCGCCGGCGGCTAGAGGGG.CTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-VII
TATGTGTAACTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-VI
TATCTGTAATTAATGGGCTGGCGACTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-V
TATCTGTAATTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-IV
TATCTGTAGTTAATGCGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-III
TATCTGTAATTAATGTGCTGGCGGCTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA
MT-II
TATCTGTAATTAATGGGCTGGCGACTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA

MT-I
TATCTGTAATTAATGGGCTGGCGACTAGAGGGGGCTATTATGCTTCCCTATATAAAATA
TTTATTA

MT-XI
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-X
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-IX
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-VIII
GCGCTAGCTAAAGCTCTAGCTAATGAAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-VII
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-VI
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-V
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-IV
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-III
GCGCTAGCTAAAGCTCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT
MT-II
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GCTAAGT
MT-I
GCGGTAGCTAAAGGGCTAGCTAATGCAGGAAAAGTTTCCATAGGTAGGGTAAGATATTT
GCTAAGT

MT-XI
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CTTTAGC
MT-X
ATTATGTTTTATACATTTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC

MT-IX
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-VIII
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-VII
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-VI
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-V
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-IV
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-III
ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-II
AWTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC
MT-I

ATTATGTTTTATACATCTAGGTGTTCGAATCACCTCTTTTCCGTAGTCTGCATGTATAG
CTTTAGC

MT-XI
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.....ATTCCGGGACGGAGGCT
GTAGCGT
MT-X
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.....ATTCCGGGACGGAGGCT
GTAGCGT
MT-IX
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GTAGCGT
MT-VIII
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GTAGCGT
MT-VII
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.....ATTCCGGGACGGAGGCT
GTAGCGT

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MT-VI
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.............ATTCCGGGACGGAGGCT
GTAGCGT
MT-V
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.............ATTCCGGGACGGAGGCT
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MT-IV
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.............ATTCCGGGACGGAGGCT
GTAGCGT
MT-III
TTTAGCTTTTAGCTTTAGCTTTTAGCTTT.............ATTCCGGGACGGAGGCT
GTAGCGT
MT-II
TTTAGCTTTTAGCTTTAGCTTTTAGCTTTAGCTTTTAGCTTTATTCCGGGACGGAGGCT
GTAGCGT
MT-I
TTTAGCTTTTAGCTTT.........................ATTCCGGGACGGAGGCT
GTAGCGT

MT-XI
ATTTTATGGGG.AGGGAACTTATAGCTTGCGCGAACTTTGTTCGCGCAAGCTA...GTA
CCCCCTC
MT-X
ATTTTATGGGGGAGGGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTT
MT-IX
ATTTTATGGGGGAGGGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTT
MT-VIII
ATTTTATGGGGGAGGGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTC
MT-VII
ATTTTATGGGG.AGGGA.CTTA.....................................
.......
MT-VI
ATTTTATGGGGGAGCGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTC
MT-V
ATTTTATGGGGGAGGGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTT
MT-IV
ATTTTATGGGGGAGGGA.......GCTTGCGCTTACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTC
MT-III
ATTTTATGGGGGAGGGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTT
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MT-II
ATTTTATGGGGGAGCGA.......GCTTGCGCGAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTC
MT-I
ATTTTATGGGGGAGCGA.......GCTTGCGCTAACTTTGTTCGCGCAAGCTATAAGTA
CCCCCTC

MT-XI
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-X
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-IX
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-VIII
CTATAAAGAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-VII
.TATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-VI
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGGAGC
AGTCTAT
MT-V
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-IV
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-III
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGCAAC
AGTCTAT
MT-II
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGGAGC
AGTCTAT
MT-I
CTATAAAAAATATAGCAAGCAGCATAAATATGCATAGCTTGTTTATAGCCAGCTGGAGC
AGTCTAT

MT-XI
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTTGC.........
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MT-X
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.....A
MT-IX
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MT-VIII
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TAGGGAA
MT-VII
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MT-VI
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTGGC.....
.....G
MT-V
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTTGC.....
.....G
MT-IV
GTATACTTCTTATATCGGAAAATGTAWTAGTGC?AACAAAGTATGTTTGC.....
.....G
MT-III
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTTGC.....
.....G
MT-II
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTTGC.....
.....G
MT-I
GTATACTTCTTATATCGGAAAATGTATTAGTGCTAACAAAGTATGCTTGC.....
.....G

MT-XI
CCGAAATATGGAAGTCA??AT?AAA.TACATTTTT.AGGCGC.....
.....
MT-X
CCGAAATATGGAAGTCAAAAAAAAAATACATTTTT.AGGCGC.....
.....
MT-IX
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGC.....
.....
MT-VIII
TAGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGCAGCTTCGCGCGAATAGC
TTGCGCG
MT-VII
CCGAAATATGGAAGTCAAAAAAAAAATACATTTTTGAGGCGC.....
.....

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MT-VI
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.......
MT-V
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGC................
.......
MT-IV
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGC................
.......
MT-III
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGC................
.......
MT-II
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGC................
.......
MT-I
CCGAAATATGGAAGTCAAAAAAAAA.TACATTTTT.AGGCGCAGGCGC..........
.......

MT-XI
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MT-X
.......GAACGAAGTTCCCTCCCGTAACAAAGGAGGAGTACACT000
MT-IX
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAGTACACT000
MT-VIII
ATTCCCGGAGGGAA.TACCCTCCCCTAACAAAGGAGGAGTACACT000
MT-VII
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAATACACT010
MT-VI
.......GAACGAAGTTCCCTCCCCTAA......AGGAGTACACT100
MT-V
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAGTACACT000
MT-IV
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAGTACACT000
MT-III
.......GAACGAAGTTTCCTCCCCTAACAAAGGAGGAGTACACT000
MT-II
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAGTACACT001
MT-I
.......GAACGAAGTTCCCTCCCCTAACAAAGGAGGAGTACACT101
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END;
```

IV. RFLPs

Koenig, R.L., Ploetz, R.C., Kistler, H.C. 1997 *Fusarium oxysporum* f.sp. *cubense* consists of a small number of divergent and globally distributed clonal lineages Phytopathology 87: 915-923

#NEXUS

[!7DRA, 2=7HAE, 3=7EV, 4=30DRA, 5=30EV, 6=30HAE, 7=120HAE, 8=162DRA, 9=162EV, 10=162HAE, 11=204EV, 12=204HAE, 13=260DRA, 14=260EV, 15=187EV, 16=261EV, 17=225EV, 18=177EV, 19=228EV]

BEGIN DATA;
DIMENSIONS ntax=172 nchar=19;
format Missing=? symbols="023456789ABCDEFG";
[!A=10,B=11,C=12, D=13, E=14, F=15, G=16]

matrix

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F01219          1111112111222215111
F01220          111111211222215111
F01222          111111211222215111
F15638          111111211222215111
F22424          111111211222215111
F22425          111111211222215111
A2              111111211222215111
ADJ1            111111211222215111
BUE1            111111211222215111
C1              ?111112111?2215F11
C2              11111211222215?11
F9127           111111211222215211
FCJ7            11111121112?215111
GAL1            111111211222215111
GAL2            111111211222215111
IC2             111111211222215111
MGSA1           1111112111222215D11
NB              11111211222215?11
NH              111111211222215111
NW              111111211222215111
ORT1            11111211222215?11
ORT2            111111211222215111
PAJ1            1111112111222215E11
PAKOVAN         1111112111721215F11
SA3             1111112111222215F11
SA4             1111112?112222?5G11
SA6             ?11111211122?215?11
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STGM1	1111112111222215111
F01124	1111112?11222645114
F9130	111111A211722697l1?
GM	3111112211824625119
H1	111111A211222645114
ML	111111A211222645114
A15	1111112111722646636
A21	1111112111222646111
A31	1111112111222646111
A41	?1111112111222846111
CSB	1111112111222646111
F2	1111112111222646111
F3	1111112111222646111
GG1	1111112111222646111
JC1	1111112111222846F11
JC14	1111112111222646111
JC8	1111112111122?646111
F13721	1?1111211122271511?
SH3142	1111112111222215111
STNP1	21111?1111B11312B21
STNP2	2111111111111312B21
STNP4	211111111111312?21
F11	111111B211222645114
F12	111111B211222645114
F22	111111B211222645114
F411	111111B21122?645114
F421	111111B211222645114
F511	11111?B211222645114
F62	111111B211222685114
ES21	211111B111C41645211
MW2	1111115111321486713
MW40	1111115111?21496213
MW41	1111115111321596213
MW42	1111115111321986715
MW44	1111115111321496215
MW46	1?11115111421486213
MW48	1111115111321986735
MW51	1111115111321A86213
MW89	1111115111421448213
LAP	1111116111222615?11
P79	1111116111722615911
Ph3	1111116111222615?11
Ph6	1111116111222615911
PW3	1111116111222615?11
PW6	1111116111222615?11
PW7	1111116111222615911

SABA	1111116111222615911
DAVAO	2111112111541893211
Ph12	411111B111541883F11
PhL2	211111B111441642211
T1	211111B111441642217
.A36	2211111111111111221
.B1	2211111111111111221
.B21	2211111111111111221
BLUG	2211111111111111221
FCJ2	2211111111111111221
FCJ3	221111111B11111221
FCJ8	2211111111111111221
FCJ9	221111111111?112221
GMB	221111111B11111621
JCB1	2211111111111111221
JLTH15	221111111111?1114621
JLTH2	2211113111111?111221
JLTH3	2?11113?11???111225
JLTH4	2111113111441641211
JLTH5	211111?111141642211
JLTH7	2211111?11111111221
MACA	221111111B11111221
MW43	2211111111111112221
MW45	2211111111111112221
MW47	2211111?11111112621
MW50	221111111111?1112221
MW52	2211111111111114621
MW58	2211111111111114621
MW64	221111111111?13221
MW69	2211111111111113221
MW71	2211111111111113227
MW78	2211111?11111111221
S?	2211111111111111221
STD2	2211111111111111221
STJ2	2211111111111111621
STN2	2211111111111111221
STN5	2211111111111111221
STN6	2211111111111111221
STN7	2211111111111112D21
STPA1	2211111111111111627
STPA2	2211111111111111221
JLTH1	221111111111??11122?
JLTH16	?211111111?1112221
JLTH17	221111111??1115221
JLTH18	221111111?111146?1
JLTH19	2211111111113112221

MW11	2211113111111111221
MW39	2211111111111112221
MW53	221111311111112221
MW56	2211113111111111221
MW61	2211111111111112221
MW63	2211111111111112C21
MW66	2211111111111112221
MW70	2211111111111112221
MW86	2211111111B11114221
F01223	?2111111111111?21
F1S?	2211111111111111221
F22417	2211111111111112221
F22468	2211111111B1111322?
F22479	2211111111111C13221
F22541Z	2211111111111112621
F22600	2211111111111111221
F8606	2211111111B11113221
F8611	22111?1111111112221
A4	2211111111111111221
JLTH20	2211111111113112221
STPA3	2211111111B11111221
STA2	1111112111222216111
STB2	111111B11122?246111
STM3	1111112211122?216211
F22993	2211111111111112221
F22994	2211111111111112221
A47	1211111111B?1?1?B21
F01221	?111112111?22151?1
F22402	1111112111122?215111
F22507	111111211122?215111
F8627	1111112111222215111
N5443	?111112111222215111
F8604	1111112111222215111
F5331	1111112111222215111
F41	2211111111111113221
F63	2211113111111111621
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F124	1111112111?2215111
R1	2111?14411622659A37
R2	2111?14411622659C56
F9129	211111B111441646211
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V. RAPDs

S. Bentley, K. G. Pegg and J.L. Dale, 1995 Genetic variation among a world-wide collection of isolates of Fusarium oxysporum f.sp. cubense analyzed by RAPD-PCR fingerprinting. Mycol. Res. 99(11): 1378-1384

NEXUS

[!RAPD banding pattern generated using primers RCO9 & SSO1
RC09:1=5,2=7,3=11,4=12,5=14,6=18,7=21,8=23,9=25,10=26,11=28,12=29,13=30,14=3
2,15=35,16=40,17=55,18=56,19=58,20=60,21=70,22=77,23=4
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Valid character-state symbols: 01 Missing data identified by '?']

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SA6	11110010010000010110011101001
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S1              00110010001000010110011101101
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F5S1            00110010001000010110011101101
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AII             00110010101001010110011101001
A21             00110010101001010110011101001
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VI. Mating type genes

Hemmings, Clarence (2000) Master's Thesis, Florida International University, Miami, Florida 33199 USA

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#NEXUS

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AGCTGTTCCC CGATACCCAG CAGAAGAATG CCTCCGGTTT CCTGACCCAG
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CTATTCCTTT CTTCGCGATC AACTCGGCAA GGGTCCTGTT AACTTGTCCG
CCTTCCTTGG CATCGCTTGC CCTTTAATGA
AB015641fuji
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CATTCCTTGG TATCGCTTGC CCTTTGATGA
AF100925fuji
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G..TCCTTTT ACCTATGGCA CCTATTGACC AGATCTAGCC TACTATCTGA
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clonedea23
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f16davao

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f74stb2

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n110fsolani
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FORMAT MISSING=? GAP=.  DATATYPE=DNA ;
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davaov3ph
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END;
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Isolates of Fusarium that have been identified as having mating type genes

DNA #	Isolate #	VCG	Name	Subset ?	Mat1 frag	Mat 1 seq	Mat 2 frag	Mat 2 seq	Mito frag	Mito seq
1	120100	0120	ADJ1		X				X	
2	120200		SA8		X		X	X	X	X
3	120300		Stc2				X	X		
4	120400		STGM1	X	X	X				X
5	120500		22425							
6	120600		STH1		X				X	
7	120700		34661				X			
8	120900		STGM2		X					
9	1201000		SA3		X	X				
10	1202000		15638		X	X				
11	121100	0121	T3		X	X				
12	121200		GM	X	X	X	X	X		X
13	122100	0122	PW2		X		X	X		
14	122200		PW5		X					
15	122300		SABA	X	X	X				X
16	123100	0123	DAVAO	X	X	X	X	X	X	X
17	123200		F9129	X	X					
18	123800		RPTH10		X					
19	124100	0124	STN1							
20	124200		MACA	X	X					
21	124300		GMB				X	X		
22	124400		BLUG							
23	124500		JCB1		X					
24	124600		STD1		X				X	
25	124700		B2-1				X	X		
26	124800		S?		X					
27	124900		STD2	X	X		X	X		
28	1241000		MW52	X	X	X	X	X	X	
29	1241100		MW57				X	X		
30	1241200		MW64							
31	1241300		MW69		X		X		X	
32	1241400		MW67							
33	1241500		MW38		X				X	
34	1241600		MW43		X				X	
35	1241800		MW49		X				X	
36	1242100		MW47							
37	1242200		MW45							
38	1242400		MW73							
39	1242500		MW80							
40	12451000	0124/5	EA1		X				X	
41	12452000		EA2				X	X		

42	12454000		EA5		X				X		
43	12455000		EA6		X		X	X	X		
44	12456000		EA4				X	X			
45	12457000		EA7								
46	12458000		EA8								
47	12459000		EA								
48	124510000										
49	124511000		EA14		X						
50	124512000		EA15								
51	124514000		EA17		X						
52	124517000		EA19								
53	124519000		EA23		X	X			X		
54	124521000		EA25		X				X		
55	124522000		EA27				X	X			
56	124524000		EA29		X						
57	124527000		EA32		X		X	X	X		
58	124528000		EA33		X		X	X	X		
59	124529000		EA35		X						
60	124530000		EA36								
61	124531000										
62	124532000		EA38								
63	124533000		EA41								
64	124535000		MW53		X						
65	124536000		MW60								
66	124537000		MW66				X	X			
67	124538000		MW86								
68	124540000		MW5		X				X		
69	124541000		MW9								
70	124542000		MW11		X				X		
71	125100	0125	8611	X	X	X					
72	125200		8610				X	X			
73	126100	0126	STM3	X	X	X			X		
74	126200		STB2	X	X	X	X		X		
75	126400		STM1								
76	128100	0128	A47	X	X	X					
77	129100	0129	O-1221	X	X	X			X		
78	1210100	01210	JC14	X	X	X			X	X	
79	1210200		Cuban F-1		X						
80	1210400		JC4								
81	1210500		A15		X						
82	1210600		LES A		X						
83	1211100	01211	SH3142	X	X	X	X	X	X	X	
84	1211200		1372-1	X	X	X			X	X	
85	1212100	01212	STNP4	X							
86	1212400		STTNZ3				X	X			

87	1213100	01213	ST5M2		X					
88	1214100	01214	MW2	X	X	X				X
89	1214200		MW41	X						
90	1214400		MW89	X	X	X	X	X		
91	1214500		MW 7		X				X	
92	1214600		MW42		X		X	X		
93	1214700		MW44		X					
94	1214800		MW46							
95	1214900	01214	MW51							
96	12141000		MW55		X		X		X	
97	1215200	01215								
98	131100	0131	F.o.mel		X		X		X	
99	134200	0134	F.o.mel		X					
100	200300	F.o.lyco	FOLR	X		X				
101	300100	F.pisi	247Fop		X					
102	300200		F.o.pisi							
103	300300		F.o.pisi		X				X	
104	400100	F.o.con g	F.o.cong		X					
105	500400	F.o.raph	F.o.rap							
106	900100	F.o.niv	F.o.niv	X	X	X				
107	1000100	F.acum	Acum		X				X	
108	2000100									
109	3000100									
110	6000100	F.solani	Solani		X	X			X	
111	7000100	F.S-pisi	S-pisi		X					

C. Nexus file of subset of *Fusarium* isolates during Phase III common to most data bases

```
#NEXUS

BEGIN DATA;
DIMENSIONS NTAX=26 NCHAR=1187;
FORMAT MISSING=? GAP=. DATATYPE=DNA
SYMBOLS="0123456789ABCDEFGHIJKLMNOPQRSTUVWXYZ";
OPTIONS MSTAXA=UNCERTAIN;

[!combined analysis mat2(1-137)-mitotypes(138-713)-
mat1(714-1043)-ek(1044-1122)-RFLP(1123-1141)-allo(1142-
1158)-RAPDS(1159-1187)]

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stgm1v0CR

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gmv1TW

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jc14v10FL
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sh314211AU

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stnp1v12TZ

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mw40v14

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davaov3PH
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macav4BR

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mw52v4MW

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      1     0     0     0     0     0     0     1     0     0     0
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VITA

Blanca R. Cortes

Born, Rio Piedras, Puerto Rico

1978	A.A., Chemistry Miami Dade Community College Miami, Florida USA
1994	BS, Biology Florida International University Miami, Florida USA
1994-1996	Research Assistant Laboratory of Dr. David N. Kuhn Florida International University Miami, Florida USA
1996	MS, Biology Florida International University Miami, Florida USA
1996	Teaching Assistant, General Biology Florida International University Miami, Florida USA
1996-1998	Research Assistant, Lab Manager Laboratory of Dr. David N. Kuhn Florida International University Miami, Florida USA
1998-2000	Teaching Assistant, Biochemistry Florida International University Miami, Florida USA

AWARDS: Dean's List: 1992, 1993, 1994; Graduate Fee Grant: 1995, 1996, 1998, 1999; Presidential Fellowship award: 1998; Tropical Biology Research Award: 1998, 1999; Tropical Biology Travel Award: 1998, 1999; First Prize: First Annual Biology Research Symposium, 1999; MBRS-RISE Award, 2000.

Cortes, B. R. (July, 1996). Hybrid formation in *Fusarium oxysporum* f.sp. *cubense*. Paper presented at the joint annual meeting of the American Phytopathological Society/Mycological Society of America, Indianapolis, Indiana.

Cortes, B. R. (October, 1996). Parasexuality in *Fusarium oxysporum* f.sp. *cubense*. Paper presented at the First International Biocontrol Workshop, Beltsville, Maryland.

Cortes, B. R. (November, 1996a). Parasexuality in asexual fungi. Paper presented at the Seminar Series of Florida International University, Miami, Florida.

Cortes, B. R. (November, 1996b). Nuclear Fusion in *Fusarium oxysporum* f.sp. *cubense* detected by nuclear staining procedures. Poster presented at the National Minority Research Symposium, Miami, Florida.

Cortes, B. R. (April, 1997). Genetic and molecular analysis of heterokaryon formation in *Fusarium oxysporum* f.sp. *cubense*. Paper presented at the meeting of the Plant Biologists of South Florida, Miami, Florida.

Cortes, B. R. (August, 1997). Nuclear and mitochondrial genomes are not inherited clonally in the asexual phytopathogen, *Fusarium oxysporum*. Paper presented at the joint annual meeting of the Mycological Society of America and the American Institute of Biological Sciences, Montreal, Canada.

Cortes, B. R. (June, 1998). A comparison of heterokaryon formation by hyphal anastomosis and protoplast fusion in *Fusarium oxysporum*. Paper presented at the joint annual meeting of the Mycological Society of America and the ABLS, San Juan, Puerto Rico.

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