

The Influence of Genotype and Environment on the Physiological and Metabolic Diversity of *Fusarium compactum*

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Accepted for publication September 30, 1996

Talbot, N. J., Vincent, P., and Wildman, H. G. 1996. The influence of genotype and environment on the physiological and metabolic diversity of *Fusarium compactum*. *Fungal Genetics and Biology* 20, 254–267. Fungal species produce a large variety of secondary metabolites which are of considerable interest to the pharmaceutical industry. It is clear that the secondary metabolite production of a species varies significantly in strains from different geographic locations and from different habitats. The influence of genotype and environment on metabolite production is, however, poorly understood. In this study we examined the influence of genotypic variability, physiological variability, environmental location, and habitat on metabolite production by *Fusarium compactum*. Isolates of the fungus from two geographic locations and two distinct habitat types were examined for growth on 95 different carbon sources, and genotypic variability was determined using RAPDs and rDNA–RFLP analysis. In a blind test secondary metabolite production was assessed using HPLC profiles of methanolic cell extracts. A number of correlations were observed between genotypic groupings, as determined using parsimony, and specific metabolite production. Similar correlations were also observed with physiological groups although genotypic analysis proved to be a more sensitive predictor

of metabolite variability. The data suggest a complex relationship between environment, genotype, and metabolite production but highlight the use of genetic screening as a means of optimizing the chances of identifying a wide range of metabolites from a given species. © 1996 Academic Press

Index Descriptors: Molecular markers; RAPD; rDNA-RFLPs; fungal systematics; secondary metabolite production; fungal biodiversity; natural products.

Fungi are a rich and diverse source of secondary metabolites which are once again of considerable interest to pharmaceutical companies. Following the decade of rational drug design and chemical synthesis programs which many companies adopted, there has now been a general reversion to screening widely among fungi for novel natural products. This has largely been the result of the development of automated chemical screening processes which have reduced the cost of these programs, making them economically attractive. Screening for new antibiotics, or to a greater extent for novel therapeutic activities, has resulted in screening not only new fungal species (Brill *et al.*, 1996; Fischer *et al.*, 1996; Norman *et al.*, 1996), but also many diverse isolates of well-studied species (Dreyfuss and Chapela, 1994; Munzberg *et al.*, 1996). A clear example of the latter is the discovery of lovastatin from *Aspergillus terreus* by Merck and Co. (Vagelos, 1991).

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With fungal screening programs common in the pharmaceutical industry, one of the most frequent dilemmas faced is how to optimize the chances of finding novel metabolites. The main question here is how many isolates of a particular species should be screened, and from how many sites? By analyzing many different fungi over the years, it is evident that there are variations in secondary metabolite production between different isolates of the same species from different substrates and in different habitats. It is not clear, however, whether these differences mirror the diversity of genotypes within a sampling site or if the diversity is a result of habitat and epigenetic variability. A better knowledge of the relationship between genotypic diversity, habitat variability, and metabolic diversity would therefore be desirable as it might provide guidance for the design and implementation of more effective screening programs.

In this study we have set out to determine the influence of genotype and habitat on the physiological and metabolic diversity of a number of *Fusarium compactum* (Wollenw.) Gordon individuals. These fungi are from communities colonizing wood collected from two distinct habitats within the Jornada Long Term Ecological Research (LTER)² site in New Mexico and the Silverbell site belonging to the University of Arizona (Wildman, 1995). The sites chosen each represent a different desert environment, and two habitats—standing dead wood and dead wood on the soil surface—have been sampled from each.

The physiological diversity of the fungi collected has been measured by determining their ability to utilize 95 distinct carbon sources. In a blind test we have examined the genotypic variability of the same isolates using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) analysis. Finally, diversity in secondary metabolism has been measured by taking extracts from shaken, liquid-grown cultures and analyzing them by high performance liquid chromatography. The results have then been analyzed using cluster analysis and parsimony. We present data suggesting that genotypic diversity, as measured by molecular markers, is correlated with both physiological variation and metabolite production. Interestingly, genetic markers appear to be powerful predictors of metabolite production, although the relationship between genotype, physiology, and habitat variability is complex.

² LTER, Long Term Ecological Research site; RFLP, restriction fragment length polymorphism; RAPD, random amplified polymorphic DNA; CM, complete medium; SSPE, standard saline-phosphate-EDTA.

MATERIAL AND METHODS

Geographic Sites

The Jornada LTER is located in the northern Chihuahuan Desert near Las Cruces, New Mexico, and is considered a warm temperate high (altitude) desertland (Brown, 1982). The Silverbell site is located in the Sonoran Desert near Tucson, Arizona, and is considered a woody tropical-subtropical desertland (Turner and Brown, 1982). Further site details are provided in Wildman (1995).

Samples

The *F. compactum* individuals used in this study were isolated from creosotebush (*Larrea tridentata* Coult.) wood pieces from two habitats in each desert—standing dead (i.e., wood pieces wired to the branches of the bushes) and surface dead (i.e., wood pieces wired to the soil under bushes). The wood pieces were placed in each environment for a period of 9 years (Wildman, 1995). Fifteen different individuals, as determined by somatic incompatibility tests (Leslie, 1993), were used for this study: 2 from the standing dead wood and 6 from the surface dead wood in the Chihuahuan Desert, and 1 from the standing dead wood and 6 from the surface dead wood in the Sonoran Desert. Three isolates of each individual were produced using a hyphal tip isolation procedure.

Fungal Isolates and Culture Conditions

Isolates of *F. compactum* used in this study are given in Table 1. Isolates were routinely stored as agar plugs in sterile distilled water at room temperature (Jones *et al.*, 1991) in the laboratories of N.J.T. (Exeter University) and H.G.W. (Glaxo-Wellcome, Stevenage). *F. compactum* was grown on solid complete medium (CM) (as described by Talbot *et al.*, 1993) for 5 days at 24°C using a 12-h light-dark cycle to promote sporulation. For DNA extractions a small agar block was removed and used to inoculate 20 ml of CM broth. Cultures were incubated in the dark without shaking for 7 days until the fungus had formed a submerged mycelial mat. The mat was then removed, blotted dry between paper towels, and used directly for DNA extractions or frozen in liquid nitrogen for storage at -80°C.

TABLE 1
Fusarium compactum Individuals Used in This Study

<i>F. compactum</i> individual	Hyphal tip reisolate no.	Origin
Cd-1b1	44-46	Standing dead wood, Chihuahuan Desert
Cd-1 2	47-49	
Cs-3b 1	50-52	Surface dead wood, Chihuahuan Desert
Cs-5a 2	53-55	
Cs-7b 3	56-58	
Cs-8b 4	59-61	
Cs-1b 7	62-64	
Cs-9b 8	65-67	
Sd-2c	68-70	Standing dead wood, Sonoran Desert
Ss-4b 1	71-73	
Ss-6c 2	74-76	Surface dead wood, Sonoran Desert
Ss-8b 3	77-79	
Ss-8b 4	80-82	
Ss-8b 5	83-85	
Ss-11b 6	86-88	

Note. All individuals were defined by somatic incompatibility tests.

Measurement of Physiological Diversity of *F. compactum*

Physiological differences between isolates of *F. compactum* were determined as patterns of carbon-source utilization using 96-well format BIOLOG GN MicroPlates (BIOLOG, Inc., CA) using a modification of Bochner's (1989) method for testing Gram-negative bacteria (Wildman, 1995). The data were first analyzed using a reversed Jaccard dissimilarity coefficient (SPSS, 1990) and clustered using an average linkage clustering program (SPSS, 1990). Parsimony analysis was then carried out using a heuristic search program in PAUP 3.0s with branch-swapping and nearest neighbor interchanges (Swofford, 1992) with 200 bootstrap replications to measure branch strengths.

Genomic DNA Extraction and Manipulations

DNA was extracted from *F. compactum* using a CTAB extraction procedure (Talbot *et al.*, 1993a). Briefly, mycelium was ground in liquid nitrogen and the powder removed to 4 ml of CTAB buffer [2% hexadecyltrimethyl ammonium bromide (Sigma), 10 mM EDTA, 0.7 M NaCl] at 65°C. Then 40 ml of β -mercaptoethanol was added and the mixture was incubated at 65°C for 20 min and extracted twice with chloroform before nucleic acids were precipitated with isopropanol. DNA was resuspended in

TE-RNase (10 mM Tris, 1 ml EDTA, pH 8.0, 1 unit ml⁻¹ RNaseA) and stored at 4°C. DNA was quantified, digested with restriction endonucleases, fractionated by electrophoresis, and blotted to Hybond N (Amersham) using standard procedures (Sambrook *et al.*, 1989). RFLP analyses were routinely carried out using the restriction endonucleases *Bam*HI, *Eco*RI, or *Hind*III. DNA hybridization probes were labeled by the random priming method (Feinberg and Vogelstein, 1983) using the Stratagene Prime-It kit. The ribosomal RNA gene probe used was pMY60 containing the rDNA repeat unit from *Saccharomyces carlsbergensis*. DNA gel blot hybridizations were conducted by standard methods, washed to high stringency (65°C in 0.1% sodium dodecyl sulfate, 0.1% PP_i, 0.2 × SSPE), and exposed to X-ray film (Amersham).

Random Amplified Polymorphic DNA Analysis

Ten primers were used in RAPD analysis of *F. compactum* isolates using an adaptation of the method of Williams *et al.* (1987). The sequences of the primers were 5' CCATTACGC 3' (1), 5' AGAAGCATG 3' (2), 5' GGAAGCAAC 3' (3), 5' TCCGACGTAT 3' (4), 5' AGGTTCTAGC 3' (5), 5' CGGATAACTG 3' (6), 5' TCCCTTTAGC 3' (7), 5' AGGATACGTG 3' (8), 5' CAATGCGTCT 3' (9), and 5' GTGCAATGAG 3' (10) (Genosys, Cambridge, UK). Briefly, the conditions used were as follows: approximately 10 ng of *F. compactum* genomic DNA was added to a 0.5-ml microfuge tube (Eppendorf) containing 20 pmol of the oligonucleotide primer, 2 mM (each) dATP, dTTP, dGTP, and dCTP (Pharmacia), 1 unit of *Tth* polymerase (Promega), 1 × *Tth* buffer (Promega), and between 1.5 and 3 mM MgCl₂ depending on the primer-template combination. The optimum MgCl₂ concentration and optimum DNA template concentrations were determined prior to each experiment.

Amplifications were carried out in a Perkin-Elmer Cetus 2400 DNA thermal cycler using the following cycling conditions: 2 min at 94°C, 5 cycles of 94°C for 30 s, 36°C for 30 s, and 72°C for 120 s, followed by 30 cycles of 94°C for 20 s, 45°C for 20 s, and 72°C for 120 s. This was followed by an incubation at 72°C for 10 min. Reaction products were separated in 1.5% agarose gels containing 0.5 mg ml⁻¹ ethidium bromide. RAPD bands selected for scoring were those amplified with complete reproducibility and showing an unambiguous presence or absence in individuals.

Measurement of Metabolic Diversity of *F. compactum*

Metabolite production by *F. compactum* was determined by high performance liquid chromatography (HPLC) separation of a methanolic extract of a fermentation medium that has been shown to reliably induce production of secondary metabolites in many fungi (Wildman, 1995). A semiquantitative approach was used to score the metabolite production profiles of the isolates: 1, small peak (<0.2 absorbance units); 2, intermediate peak (>0.2 and <1.0 absorbance units); and 3, peak off scale (>1.0 absorbance units). Profiles were reported in the form $A_1B_2C_3$, where A, B, and C are metabolites and 1, 2, and 3 are their respective peak heights. Metabolic data were also grouped to record common general profiles. For example, the expression $D_{2/1}E_{0/1}F_2$ describes individuals producing metabolite D with a peak height of 1 or 2, metabolite E with a peak height of either 0 or 1, and metabolite F with a peak height of 2.

RESULTS

Experimental Design

Two sites were chosen for sampling that represent distinct environments. The Silverbell site in the Sonoran Desert is a woody, tropical-subtropical desertland with a bimodal rainfall pattern. This site has a greater structural diversity than the Jornada site in the Chihuahuan Desert which is mainly dominated by uniform shrubs such as the creosotebush. The fungal isolates were collected from four plots at each site from creosotebush wood pieces which were removed after a 9-year period. *F. compactum* isolates were collected from three replicate samples of four pieces of wood from each plot. A surface washing procedure was carried out to ensure that all fungi were growing in, or on, the wood and *F. compactum* isolates were isolated as luteous colored (Rayner, 1970) colonies. These were tested in somatic incompatibility tests to provide 15 genetically different individuals for further study. Three reisolations of each individual were prepared by hyphal tip preparations to provide internal controls for subsequent measurements.

The experimental work was carried out as three independent tests. First, physiological diversity was assessed by

assaying carbon-source utilization using 96-well format BIOLOG GN microplates (BIOLOG, Inc.). Second, in a blind test the genetic relatedness of strains was determined by detecting RFLPs at the ribosomal RNA gene locus and using RAPDs. Third, metabolic diversity was measured using a simple methanol extraction procedure and HPLC. The data were then statistically analyzed independently using cluster analysis and parsimony and the results from all three experiments were compared.

Physiological Diversity of *F. compactum*

Carbon-source utilization patterns were assessed by growth on BIOLOG-GN plates and gave the data set presented in Table 2. The data are the median results from examining the growth of three hyphal tip reisolations of the 15 individuals. Very little variability was observed between hyphal tip reisolates. Distinct patterns of carbon-source utilization were, however, observed for the majority of individuals although clear patterns of similarity were obvious even from the raw data. A phylogram was produced from this data set using parsimony analysis and is shown in Fig. 1. A heuristic search using PAUP 3.0s (Swofford, 1992) gave a single most parsimonious tree of 101 steps (consistency index 0.554, retention index 0.615). Branch strengths were analyzed by 200 bootstrap repetitions and are given in parentheses (Fig. 1). The analysis identified five main groups of individuals, labeled A–E. Cluster analysis based on a reversed Jaccard dissimilarity coefficient gave very similar results which are presented elsewhere (Wildman, 1995).

The data show a complex relationship between the geographic origin of an individual and its relatedness based on carbon-source utilization. The Chihuahuan Desert isolates (all prefixed with C in Fig. 1) appear superficially to be more similar to each other than to those from the Sonoran Desert (prefixed with S), regardless of whether isolated from standing or fallen dead wood (prefix s or d, respectively). Three of the five clades, however, contain isolates from both locations, suggesting that individuals from both geographic sites share common lineages based on this method of analysis. One of the groupings, E, was composed of Cs-3b 1, Cs-1b 7, and Cs-8b 4—which are all Chihuahuan Desert isolates. This grouping showed strong bootstrap support, suggesting that physiological variability could resolve distinct groups of related individuals from within a population.

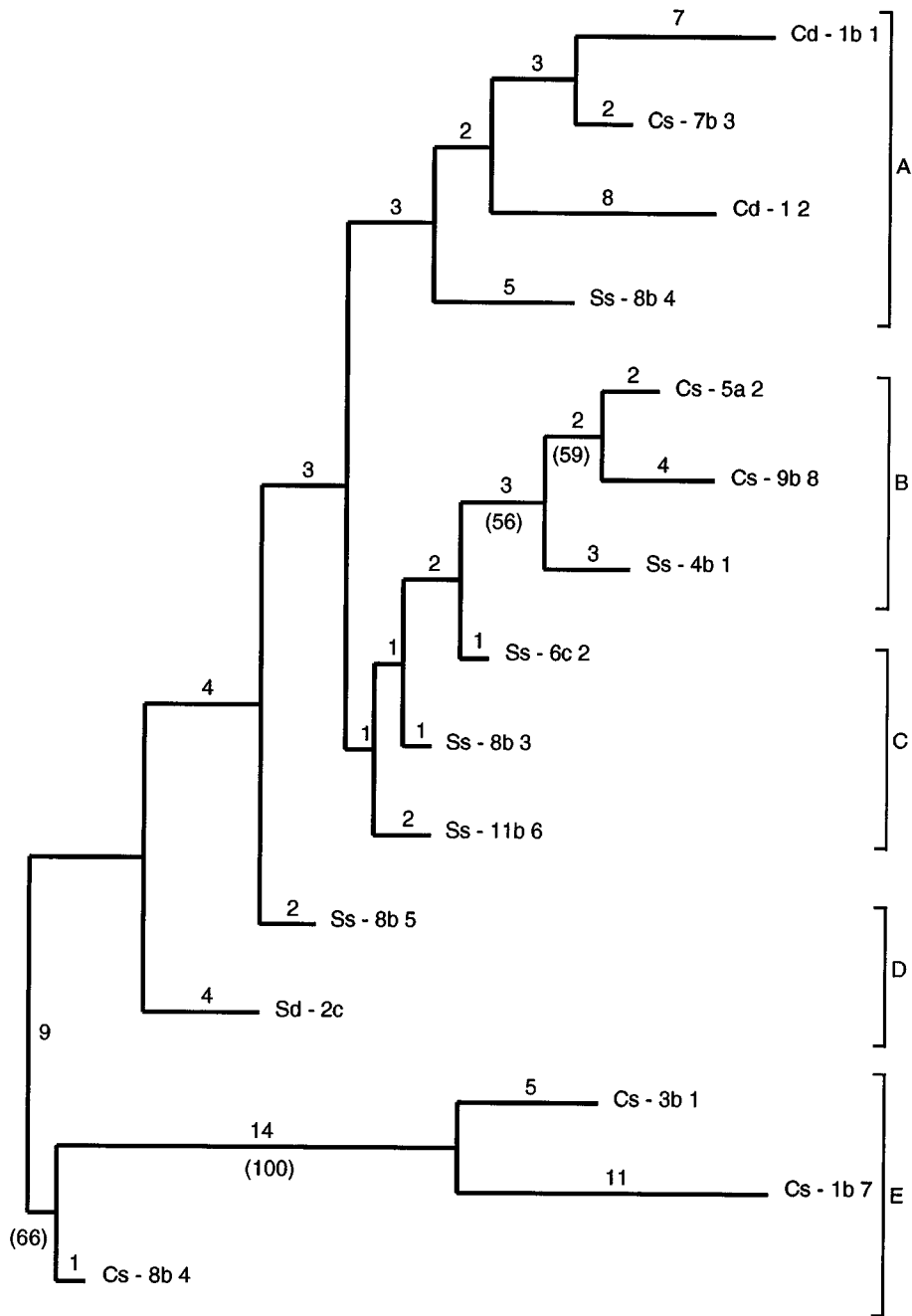


FIG. 1. Phylogram of the most parsimonious tree showing the relatedness of *F. compactum* individuals isolated from the Chihuahuan and Sonoran Deserts based on carbon-source utilization. Relationships were determined using the heuristic search program in PAUP 3.0s. Branch strengths were tested by 200 repetitions of the bootstrap algorithm with branch-swapping (numbers in parentheses). Lengths of branches are shown. The tree was rooted using the midpoint method (Swofford, 1992).

TABLE 3
 Sizes of Major Restriction Fragments Hybridizing to pMY60
 and Inferred rDNA Variant Lengths for *F. compactum* Isolates

Isolate No.	Restriction fragments detected (kb)			rDNA variant length (kb)
	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI	
44 (Cd-1b 1)	5.2, 3.3	>25	>25	8.5
45	5.2, 3.3	>25	>25	8.5
46	5.2, 3.3	>25	>25	8.5
47 (Cd 1 2)	4.7, 3.3	>25	>25	8.0
48	5.2, 3.3	>25	>25	8.5
49	4.7, 3.3	>25	>25	8.0
50 (Cs-3b 1)	4.7, 3.3	8.0	>25	8.0
51	4.7, 3.3	8.0	>25	8.0
52	4.7, 3.3	8.0	>25	8.0
53 (Cs-5a 2)	4.7, 3.3	8.0	>25	8.0
54	4.7, 3.3	8.0	>25	8.0
55	4.7, 3.3	8.0	>25	8.0
56 (Cs-7b 3)	4.7, 3.3	>25	>25	8.0
57	5.2, 3.3	>25	>25	8.5
58	5.2, 3.3	>25	>25	8.5
59 (Cs-8b 4)	4.7, 3.3	>25	>25	8.0
60	4.7, 3.3	>25	>25	8.0
61	4.7, 3.3	8.0	>25	8.0
62 (Cs-1b 7)	4.7, 3.3	8.0	>25	8.0
63	4.7, 3.3	8.0	>25	8.0
64	4.7, 3.3	8.0	>25	8.0
65 (Cs-9b 8)	4.7, 3.3	8.0	>25	8.0
66	4.7, 3.3	8.0	>25	8.0
67	4.7, 3.3	8.0	>25	8.0
68 (Sd 2c)	4.7, 3.3	8.0	>25	8.0
69	4.7, 3.3	8.0	>25	8.0
70	4.7, 3.3	8.0	>25	8.0
71 (Ss-4b 1)	4.7, 3.3	8.0	>25	8.0
72	4.7, 3.3	8.0	>25	8.0
73	4.7, 3.3	8.0	>25	8.0
74 (Ss-6c 2)	4.7, 3.3	>25	>25	8.0
75	4.7, 3.3	>25	>25	8.0
76	4.7, 3.3	>25	>25	8.0
77 (Ss-8b 3)	4.7, 3.3	8.0	>25	8.0
78	4.7, 3.3	8.0	>25	8.0
79	4.7, 3.3	8.0	>25	8.0
80 (Ss-8b 4)	5.0, 3.3	>25	>25	8.3
81	5.0, 3.3	>25	>25	8.3
82	5.0, 3.3	>25	>25	8.3
83 (Ss-8b 5)	4.7, 3.3	8.0	>25	8.0
84	4.7, 3.3	8.0	>25	8.0
85	4.7, 3.3	8.0	>25	8.0
86 (Ss-11b6)	4.7, 3.3	8.0	>25	8.0
87	4.7, 3.3	8.0	>25	8.0
88	4.7, 3.3	8.0	>25	8.0

Chihuahuan isolates while groups IV and V are composed of Sonoran isolates. Interestingly, the three individuals showing 8.5-kb rDNA variants—Cd-1b 1, Cd-1 2, and Cs-7b 3—all appear together as a distinct subclade (group I). Similarly, Ss-8b 4 which has an 8.3-kb rDNA repeat size is an outlying member of group I based on RAPD analysis. An example of the degree of dissimilarity of Ss-8b 4 to other isolates is shown by the RAPD profiles given in Fig. 3. This individual is represented by amplifications from three reisolates in lanes 14, 15, and 16.

The genetic variability of the Chihuahuan Desert isolates was not significantly different from the genetic variability of the Sonoran Desert isolates when compared using Van Valen's modification of Levene's test for variance (Van Valen, 1978) (viz. 3.315 ± 0.815 and 3.168 ± 0.892 , respectively).

Metabolic Diversity of *F. compactum*

The metabolite profiles for each of the 15 individuals are shown in Table 5. Metabolite profiles for all 45 reisolates were determined with broadly similar results within the reisolates of each individual. Ten different secondary metabolite profiles were found among the 15 individuals, with three profiles predominating: $D_{2/1}E_{0/1}F_2$, $D_2F_2I_1$, and $G_3H_3I_2J_1$. The $D_{2/1}E_{0/1}F_2$ profile was found exclusively in Sonoran Desert isolates among half of the surface dead wood isolates. The $D_2F_2I_1$ profile was found in two of the surface dead wood Chihuahuan Desert isolates (Cs-8b 4 and Cs-9b 8) and the single standing dead wood Sonoran isolate (Sd-2c). The $G_3H_3I_2J_1$ profile was found in standing dead wood and surface dead wood isolates from the Chihuahuan Desert and a surface dead wood Sonoran Desert isolate (Ss-8b 4). More secondary metabolite profiles were found among isolates from the Sonoran Desert (7) than among isolates from the Chihuahuan Desert (5). However, the differences in profile of the Chihuahuan Desert isolates were greater qualitatively, and 10 major metabolites were produced by the Chihuahuan isolates in comparison with 7 major metabolites produced by the Sonoran Desert isolates.

Comparative Analysis

A comparison of physiological diversity, genetic diversity, and metabolite production revealed a number of correlations. These are presented with the metabolite profiles in Table 5. The phylograms of physiological and

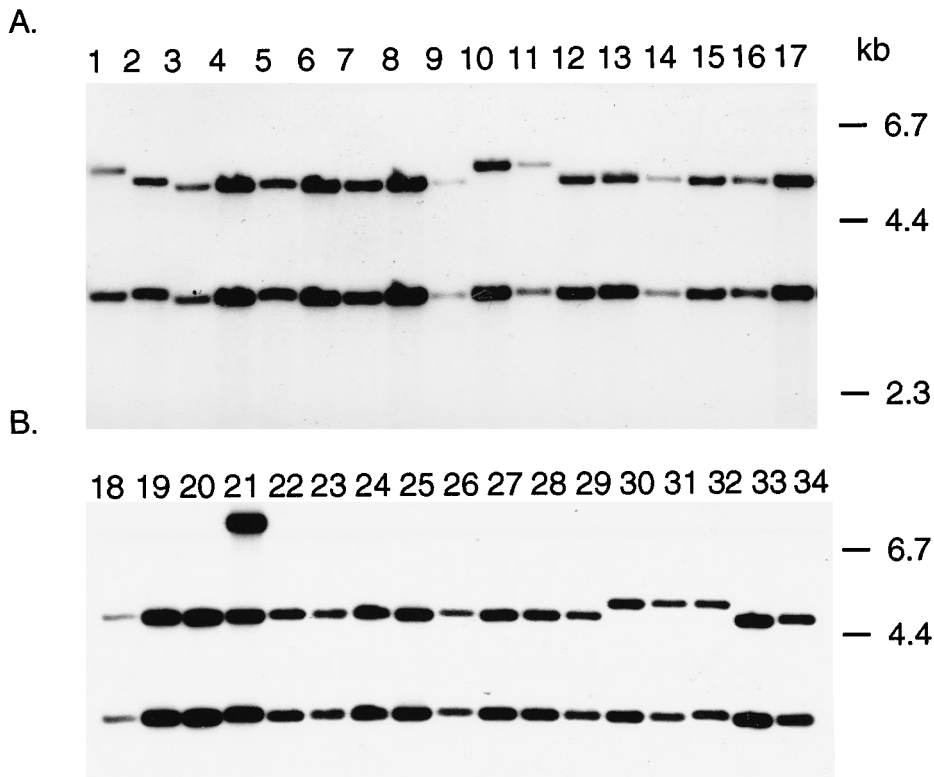


FIG. 2. Southern blot analysis of *F. compactum* individuals from the Chihuahuan and Sonoran Deserts showing variation in nuclear ribosomal DNA (rDNA). DNA was extracted, digested with *Eco*RI, and fractionated in 0.8% agarose gels by electrophoresis before blotting to Hybond-N. Blots were probed with pMY60 which contains the yeast rDNA repeat unit. Lanes contain DNA from the following isolates (reisolate number is given in parentheses): 1, Cd-1 2 (48); 2, Cd-1 2 (49); 3, Cs-3b 1 (50); 4, Cs-3b 1 (51); 5, Cs-3b 1 (52); 6, Cs-5a 2 (53); 7, Cs-5a 2 (54); 8, Cs-5a 2 (55); 9, Cs-7b 3 (56); 10, Cs-7b 3 (57); 11, Cs-7b 3 (58); 12, Cs-8b 4 (59); 13, Cs-8b 4 (60); 14, Cs-8b 4 (61); 15, Cs-1b 7 (62); 16, Cs-1b 7 (63); 17, Cs-1b 7 (64); 18, Sd-2c (68); 19, Sd-2c (69); 20, Sd-2c (70); 21, Ss-4b 1 (71); 22, Ss-4b 1 (72); 23, Ss-4b 1 (73); 24, Ss-6c 2 (74); 25, Ss-6c 2 (75); 26, Ss-6c 2 (76); 27, Ss-8b 3 (77); 28, Ss-8b 3 (78); 29, Ss-8b 3 (79); 30, Ss-8b 4 (80); 31, Ss-8b 4 (81); 32, Ss-8b 4 (82); 33, Ss-8b 5 (83); 34, Ss-8b 5 (84). Molecular mass markers are given on the right.

genetic similarity were broadly similar in revealing a complex pattern of relatedness to geographic origin and habitat. The phylograms also revealed similar outliers to the main group of isolates, based on physiological or genetic relatedness.

The most striking correlation observed was that the group of isolates producing the $G_3H_3I_2J_1$ profile of secondary metabolites was also a distinctive group when classified by carbon-source utilization, RAPDs, or rDNA-RFLPs. All had an 8.5- or 8.3-kb rDNA repeat unit lacking a *Hind*III site and were outliers to the main body of isolates based on RAPDs or carbon source usage. Isolates Cd-1b 1, Cd-1 2, and Cs-7b 3 were more closely related to each other genetically than physiologically, although similarity was also apparent in carbon-source usage. Isolate Ss-8b 4, meanwhile, grouped more closely with these isolates

physiologically, although molecular markers also defined it as being in the same class (I). Other correlations between genetic relatedness and metabolite profile were also evident, for example Ss-8b 5 and Ss-11b 6 were very similar genetically (group V) and produced comparable $D_2F_2G_1H_1I_{0/1}$ metabolite profiles. In other cases physiological relatedness was clearly correlated with production of a particular metabolite profile. Production of the $D_{2/1}E_{0/1}F_2$ profile, for example, was only found in isolates from carbon-source group C. The correlation of both genetic relationship and physiological relatedness with metabolite production was, however, at a relatively coarse level. Similar genetic grouping did not always translate into similar metabolite production as illustrated by Cs-3b 1 and Cs-5a 2. The same was also true of physiological relatedness, as with Cs-8b 4, Cs-9b 8, and Sd-2c, which were all

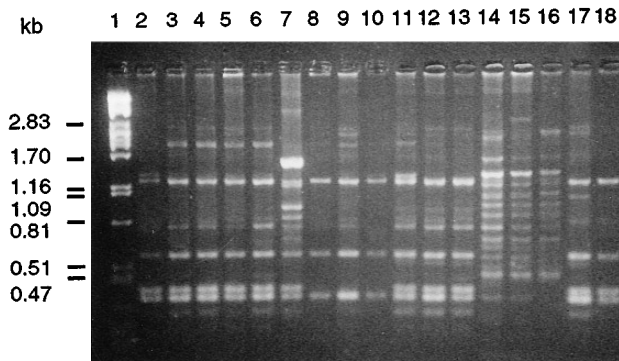


FIG. 3. Gel electrophoresis analysis of RAPD profiles of individuals of *F. compactum* from the Chihuahuan and Sonoran Deserts. Profiles shown were generated using primer 5. RAPD-PCR was performed as described under Material and Methods, amplification products were fractionated in a 1.5% agarose gel, stained with ethidium bromide, and photographed. Lanes contain amplification products from the following individuals (reisolate numbers are given in parentheses): 1, DNA molecular mass marker; 2, Sd-2c (68); 3, Sd-2c (69); 4, Sd-2c (70); 5, Ss-4b 1 (71); 6, Ss-4b 1 (72); 7, Ss-4b 1 (73); 8, Ss-6c 2 (74); 9, Ss-6c 2 (75); 10, Ss-6c 2 (76); 11, Ss-8b 3 (77); 12, Ss-8b 3 (78); 13, Ss-8b 3 (79); 14, Ss-8b 4 (80); 15, Ss-8b 4 (81); 16, Ss-8b 4 (82); 17, Ss-8b 5 (83); 18, Ss-8b 5 (84).

distinct based on carbon source usage but identical in metabolite production (Table 5).

DISCUSSION

This study set out to examine the relationship between the geographic location of a fungal isolate, its habitat, genetic relatedness, physiological relatedness, and its ability to produce secondary metabolites. In order to investigate this complex set of variables we studied isolates of a fungus from two distinct habitats in two geographic areas. These were investigated for each parameter in blind tests using internal controls in the form of hyphal tip replicates to ensure accurate determination of characteristics. Although only a small number of individuals from a single species were examined, the data revealed a number of interesting correlations between each measure of variability and metabolite production. It also highlighted the complex relationship that exists between genotype, environment, and phenotype as expressed in secondary metabolic products.

Physiological diversity was extensive among *F. compactum* isolates, and considerable differences were apparent

in isolates from the Chihuahuan and Sonoran Deserts. The Chihuahuan Desert isolates utilized fewer carbon sources than the Sonoran Desert isolates and the scale of this difference was not the same for isolates from each habitat, the Chihuahuan standing dead wood isolates utilizing 5% fewer and the surface dead wood isolates 16% fewer, than the respective Sonoran Desert isolates. The variation in carbon-source utilization within the Chihuahuan Desert isolates was also significantly greater (see Wildman, 1995). No significant differences were seen in the physiological variability of isolates from different habitats and this was also reflected by the fact that where distinct groupings were significantly supported by bootstrap analysis the isolates grouped more closely by geographic area than by habitat (Fig. 1). Only a small number of isolates, however, was examined from the standing dead wood habitat and a more extensive study would be needed to determine the relative contribution of each habitat to physiological diversity.

In a comparison with metabolite production a clear link could be seen between primary carbon metabolism and secondary metabolism. Similar carbon substrate utilization often translated into similar metabolite profiles. This suggests that prescreening on the basis of carbon-source utilization using an automated approach such as the BIOLOG plate system might be worthwhile in optimization of fungal screening programs. The correlation was, however, not as clear as that between genetic relatedness as measured by RAPDs and it may be that this is where the greater potential lies.

We examined genetic relatedness using two different techniques with the aim of identifying coarse-level and fine-level variation between isolates. The rDNA analysis proved useful in identifying outliers to the main body of isolates and this reflected their secondary metabolic profiles as well. The same outlier isolates were also easily detected using RAPDs which moreover allowed detection of more subtle variability due to the increased number of loci examined. Data analysis for RAPDs is a controversial area (Backeljau *et al.*, 1995) and was chosen to reflect the comparison which would be needed with the physiological data. We analyzed RAPDs using Wagner parsimony which assumes that all characters change from one state to another with equal probability. This is probably not an entirely valid assumption with RAPDs, as a priming site, which leads to an amplification product, probably has a greater chance of being lost than of being gained. Thus the chances of a 1 being converted to a 0 are probably greater than the reverse. To allow for this the results were checked

TABLE 4

Binary Presence–Absence Data Matrix for RAPD Fragments of *F. compactum* from Standing and Surface Dead Wood Habitats in the Chihuahuan and Sonoran Deserts

<i>F. compactum</i> individual	RAPD profile ^a
Cd-1b1	0110000000000111001000000100100110110001010000????????????
Cd-1 2	111000000000001111010101001000001101100010100000010010000000
Cs-3b 1	111011100000000001101001011101001000001110001001000000000010
Cs-5a 2	111011100000000001101001011101001000001110001001000000000010
Cs-7b 3	001010000000001111111101001000001111100010100000000000000100
Cs-8b 4	001010000000000001100000011110000010001111000001001010000010
Cs-1b 7	010101000000000101101100011110001001001110000001001010000111
Cs-9b 8	01010100000000000110100001111000????????????????10010000000011
Sd-2c	00000001110000010110100001110100110111110001001101010000011
Ss-4b 1	010000011110000111100100000001001001101110000001101010000011
Ss-6c 2	111100000000000111101100000100001111111010000001001010000011
Ss-8b 3	111000000000000111100100111110001001111110000001101010000010
Ss-8b 4	11100000000000101100011100100101110000001101010101111011000
Ss-8b 5	111000000000100111100100110101001000001110001001101000000010
Ss-11b 6	1110000000001101111011001???????1000001110000001101000000010

^a The presence or absence of a RAPD marker is specified by a 1 or a 0. Data shown are those reproducible in at least two repetitions from three hyphal tip isolates of each individual. ?, missing data or data not reproducible using these criteria. Ten primers were used.

using Dollo parsimony which allows characters to change only to the positive state (1), but allows unlimited reversals of this process. The data were also analyzed using Jaccard's coefficient which eliminates common negative data (Smith and Stanosz, 1995) and cluster analysis using the UPGMA method. These analyses all gave similar results and so although parsimony analysis has the limitations described, it was chosen because it allowed the same analysis to be performed when comparing physiological and genetic data.

Genetic relatedness as determined by RAPDs was shown to group isolates more closely based on geographic area than by habitat. This was even more pronounced than grouping based on physiological data as only one group defined by genetic analysis contained isolates from both the Chihuahuan and the Sonoran Deserts. The correlation with habitat was less strong, although the Chihuahuan Desert isolates from standing dead wood were very similar. Genetic variability within the Chihuahuan Desert tended to be greater than within the Sonoran Desert though this was not significant. Genetic relatedness also proved to be broadly correlated with metabolite profile. As expected coarse-level variation was most useful. For example, a different RAPD profile observed with many primers was a much more reliable indicator of secondary metabolite

differences than small differences in a RAPD profile generated with a single primer. The most obvious example of this being the group showing the G₃H₃I₂J₁ profile which were easily distinguished by RAPD profiles with numerous primers.

In comparing physiological and genetic relatedness with metabolite production, genetic diversity appeared to be the more reliable indicator of metabolite profile, although both were only broadly predictive. There are a number of possible reasons for this. It may be that the use of RAPD to analyze diversity in a population is simply a higher resolution technique. In this case, for example, the 60 markers scored should correspond to allelic variation at 60 separate loci. This is arguably more likely to identify a greater number of polymorphisms than growth on 95 carbon sources, allowing for the likely redundancy in carbon substrate utilization pathways. Consequently genotype variation may be more closely correlated with secondary metabolite production than carbon-source utilization. Alternatively, it may be that metabolite production and a given carbon-source utilization pattern are not commonly selected for, thus leading to weak correlation. It is difficult at this time, with the limited number of isolates examined, to prove either case, and the situation may well be due to a combination of many reasons. RAPDs have been shown to

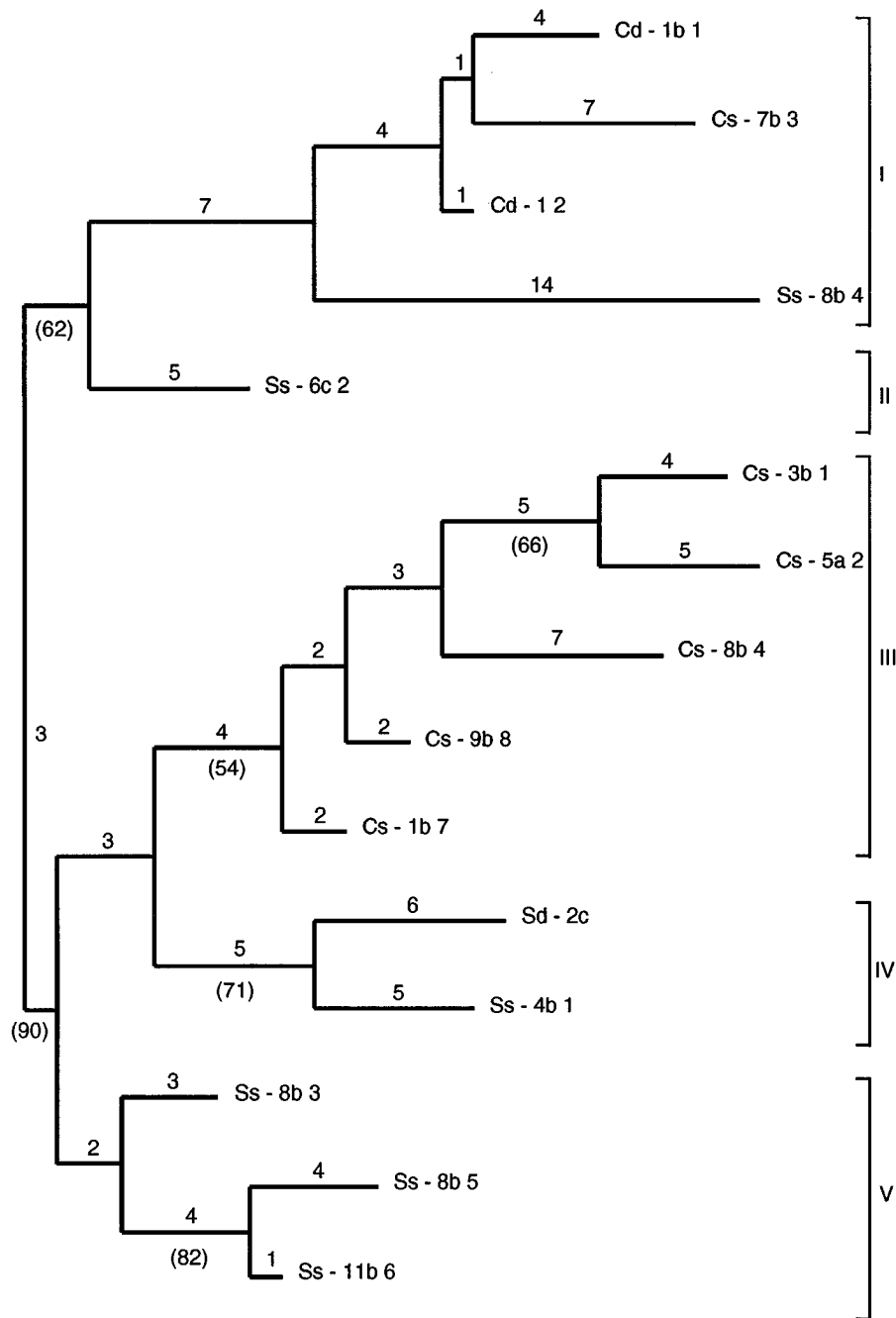


FIG. 4. Phylogram of the most parsimonious tree showing the relatedness of *F. compactum* individuals isolated from the Chihuahuan and Sonoran Deserts based on RAPDs. Relationships were determined using the heuristic search program in PAUP 3.0s. Branch strengths were tested by 200 repetitions of the bootstrap algorithm with branch-swapping (numbers in parentheses). Lengths of branches are shown. The tree was rooted using the midpoint method (Swofford, 1992).

TABLE 5

Metabolite Production by *F. compactum* Isolates from the Chihuahuan and Sonoran Deserts and Comparison with Physiological and Genetic Similarity Measurements

<i>F. compactum</i> individual ^a	Reisolate No.	Metabolite ^b										Physiology ^c	Genetic relatedness ^d		
		A	B	C	D	E	F	G	H	I	J	c-source utilization group	rDNA repeat size (kb)	<i>Hind</i> III site presence	RAPD group
Cd-1b 1	44-46							3	3	2	1	A	8.5		I
Cd-1 2	47-49							3	3	2	1	A	8.0 and 8.5		I
Cs-3b 1	50-52				1	2	2					E	8.0	+	III
Cs-5a 2	53-55	1	1		2	2						B	8.0	+	III
Cs-7b 3	56-58							3	3	2	1	A	8.0 and 8.5		I
Cs-8b 4	59-61				2		2			1		E	8.0		III
Cs-1b 7	62-64		2	1	2	2	1					E	8.0	+	III
Cs-9b 8	65-67				2		2			1		B	8.0	+	III
Sd-2c	68-70				2		2			1		D	8.0	+	IV
Ss-4b 1	71-73				2		2					B	8.0	+	IV
Ss-6c 2	74-76				2	1	2					C	8.0		II
Ss-8b 3	77-79				1		2					C	8.0	+	V
Ss-8b 4	80-82							3	3	2	1	A	8.3		I
Ss-8b 5	83-85				2		2	1	1	1		D	8.0	+	V
Ss-11b 6	86-88				2		2	1	1			C	8.0	+	V

^a C, Chihuahuan Desert; S, Sonoran Desert; d, standing dead wood; s, surface dead wood.^b 1, small peak; 2, intermediate peak; 3, off-scale peak.^c Groups based on parsimony analysis of carbon-source utilization patterns.^d rDNA repeat unit size variation (+, presence of *Hind*III site). RAPD groups based on parsimony analysis.

be diagnostic for pathogenic variants of many plant-pathogenic species and have been extensively used in taxonomic studies of fungi (Samuels and Seifert, 1995). In the few detailed studies linking genetic variability with physiology or morphology, however, correlations have often been very limited (Tedford *et al.*, 1994). The existence of different rDNA repeat unit sizes in different hyphal tip reisolates of the same individual is thought to indicate limited heterokaryosis and the existence of different nuclear types within some of the individuals studied. The relative frequency of heterokaryon formation under natural conditions and the frequency of subsequent genetic exchange is the subject of much speculation but it may represent a potentially adaptive tool for fungi (Anderson *et al.*, 1992).

The greater physiological and genetic variability seen in *F. compactum* isolates from the Chihuahuan Desert may be due to macro- and microclimactic factors and to physical differences between the desert environments. Differences in substrate distribution (patchiness), environmental disturbance, and limitations on the flow of fungal propagules within a habitat might allow more diverse populations of fungi to evolve. This is in accordance with

Levin's (1971) metapopulation concept which recognizes the reality of the uneven distribution of individuals of a species across a landscape. Fungal succession in creosote-bush wood samples in deserts has been studied and suggests that *F. compactum* is very long-lived (J. Zak, personal communication). The possible longevity of fungal isolates in a desert environment as largely quiescent mycelia may therefore also be a factor in the development of distinct genotypes.

The isolates showing the G₃H₃I₂J₁ metabolite profile, for example, all showed a distinct colonial morphology in plate culture and had significantly slower growth rates in liquid culture (data not shown). This morphology is often interpreted as being a degenerate growth form (confirmed by examination by D. Brayford, International Mycological Institute), and is commonly found in fungi following repeated subculture. In this case, however, strains showing the G₃H₃I₂J₁ profile were not the result of subculture, as they were collected and isolated with these features. The scale of the differences observed physiologically and genetically with this group may suggest they are a separate "variety" of *F. compactum* in the sense of Rogers (1992) who suggests that "variety" status should be given to taxa

showing different biosynthetic pathways. The production of a distinct metabolic profile also suggests that strains interpreted as degenerate or senescent may be genetically distinct isolates perhaps due to genetic changes. The occurrence of a unique metabolite (J) in these strains suggests that isolates of this kind might be a potential source of distinct secondary metabolic products.

ACKNOWLEDGMENTS

We are grateful to Mark Macnair (University of Exeter), Paul Nicholson, and James Brown (John Innes Centre) for advice on data processing. Technical help was provided by Ruth Aubrey and Nick Tongue. This work was supported by grants to N.J.T. from the University of Exeter Research Fund and Glaxo-Wellcome.

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