Sexual and Parasexual Variability in Soil Fungi with Special Reference to *Fusarium moniliforme*

Gurmel S. Sidhu

Assistant professor, Department of Plant Pathology, University of Nebraska, Lincoln 68583-0722.
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Soilborne fungal pathogens continuously adapt to a changing soil environment. Successful adaptation depends on the availability of genetic variation maintained through various mechanisms. Among these, sexual and parasexual recombination are by far the most important sources of genetic variability.

The sexual cycle essentially involves a regular alternation of karyogamy and two sequential, highly coordinated meiotic divisions. The parasexual cycle differs in one or more steps from a normal sexual cycle and lacks a fixed sequence and fine control over the entire process (4). For example, plasmogamy and karyogamy are essentially identical in both processes. The main differences, however, exist in reduction division and its coordination. Parasexuality, as compared to sexuality, involves less coordinated reduction division and proceeds through irregular steps. The process of evolution normally proceeds from uncertainty to certainty. Therefore, the view has developed that sexuality may be a highly evolved form of parasexuality (16).

Sexual reproduction in fungi portrays a tremendous range of genetic variation. Three distinctive features, ie, life cycle, pattern of sexuality, and the sexual mechanism, are involved in sexual variation (18). Combination of these three determines the mode of sexual reproduction of a given fungal species. Raper (18) provides some examples that also include certain soilborne fungal parasites.

Parasexuality. The standard parasexual cycle, first established in *Aspergillus nidulans* (15), passes through various sequences of events (9). The first sequence brings together the two haploid nuclear strains into a heterokaryon (plasmogamy or anastomosis). In the second step, two genomes fuse (karyogamy) to give a somatic diploid. The diploid nuclei divide mitotically along with the haploid nuclei in the heterokaryon. The diploid nuclei may form a discrete sector and can be propagated as separate diploid strains in some cases. However, often the diploid nuclei are unstable and give rise to segregants by "mitotic recombination" through intra-chromosomal crossing over and "haploidization" through chromosomal nondisjunction involving the entire chromosome. The haploidization involves a random loss of chromosome(s) by mitotic nondisjunction and generates different levels of aneuploids (eg, 2n-1, 2n-2, 2n-3). However, normally the balanced haploids survive in the population. The parasexual process, therefore, generates a population of haploids, which are recombinants of parental haploid homokaryons. This is essentially what the sexual cycle achieves; however, the whole process of parasexuality is uncoordinated and involves mitosis. The recovery of recombinant haploids is influenced adversely at every step in the parasexual cycle. Various steps in parasexual cycle will be considered with pertinent examples of soilborne pathogens.

Anastomosis. The occurrence of the parasexual cycle depends on the nature of anastomosis, ie, the fusion of two hyphae. Certain factors affect anastomosis adversely. For example, cell wall dissolution and incompatibility factors may operate during different stages of anastomosis between a pair of hyphae: hyphae may repel each other, fusion may fail to take place, fusion may take place with no cytoplasmic mixing, and cells may collapse after anastomosis. All these events have been observed in anastomosis of *Thanatephorus cucumeris* (7).

Anastomosis is a complex phenomena and relatively little is known about its occurrence in pathogenic fungi. It is difficult to observe an apparent fusion and determine compatibility between the two hyphae. Therefore, anastomosis is usually established on the basis of functional criteria. Despite the difficulties, the fusion between germ tubes of macroconidia has been observed in *Fusarium* (2).

Heterokaryosis. Evidence for heterokaryosis has been found in many pathogenic fungal species (4,9). Specific examples of heterokaryosis and parasexuality in soilborne fungi are shown (Table 1). Heterokaryosis has been reported to occur between strains or races, formae speciales, and species of soilborne pathogens (22). It should be emphasized that most studies on heterokaryosis involve pairing between selected laboratory isolates differing in morphology or in certain nutritional requirements. Little attempt has been made to isolate and study the natural occurrence of heterokaryosis in pathogenic fungi. An account of naturally occurring heterokaryons in various fungal species was given by Caten and Jinks (5).

Natural occurrence of heterokaryosis. The study of natural occurrence of heterokaryosis would give a relatively more realistic estimate of natural genetic variability, particularly in asexual fungal species. However, the experimental demonstration of heterokaryosis in nature is laborious. It requires isolation of wild fungal samples, estimation of proportion of heterokaryons, determination of genetic control of component homokaryons of various heterokaryons, or at least their stability, as judged from successive transfers on media for a number of generations and estimation of nuclear ratios in various heterokaryons in order to observe relative stability.

Early attempts to study natural occurrence of heterokaryons

TABLE 1. Plant-pathogenic soil fungi for which there is evidence of heterokaryosis (H), parasexuality (P), or both (HP)

Ascomycetes	
Aspergillus niger	P
Ceratocystis radicicola	P
Cochliobolus sativus	HP
Penicillium sp.	P
Basidiomycetes	
Rhizoctonia solani	H
Deuteromycetes	
Ascochyta imperfecta	HP
Cephalosporium mycophilum	H
Fusarium solani	H
Fusarium solani f. sp. *cucurbitae*	H
Fusarium fructigenum	H
Fusarium oxysporum f. sp. *pisi*	HP
Fusarium oxypsorum f. sp. *gladioli*	HP
Fusarium oxysporum f. sp. *callistephi*	H
Fusarium oxysporum f. sp. *cubense*	P
Fusarium oxysporum f. sp. *lycopersici*	H
Fusarium oxysporum f. sp. *niveum*	H
Fusarium oxysporum f. sp. *conglutinans*	H
Fusarium moniliforme	H
Gibberella saubinetii	H
Verticillium dahliae	HP
Verticillium dahliae var. *longisporum*	P

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TABLE 2. Characteristics of representative homokaryons recovered from individual hyphal tip colonies of major wild-type heterokaryons of *Fusarium moniliforme* from sorghum

Wild-type isolate		Most prevalent at	Characteristics of major homokaryon colonies		
Designation	Homokaryon		Texture	Growth rate (mm/day)	Sporulation ($\times 10^5$ spores/cm^3)
SORG 1-12:	FmS 2	40 days	Compact, powdery	3.3	4.5
	FmS 36		Velvety	3.0	6.5
SORG 1-19:	FmS 11	60 days	Velvety	5.8	6.0
	FmS 12		Fluffy	5.7	4.5
SORG 1-26:	FmS 15	80 days	Powdery	2.6	5.0
	FmS 37		Rough, scattered	3.8	8.5
SORG 12:	FmS-a	Maturity	Velvety	2.5	2.5
	FmS-b		Fluffy	5.6	6.2

TABLE 3. Percent recovery of *Fusarium moniliforme* heterokaryons (het) when near-equal concentrations of spores of respective homokaryons were plated on various stalk residue media (SRM)

Heterokaryon plated	Percent het recovered on SRM from plant at:[a]			
	40 days	60 days	80 days	Maturity
FmS 2 + FmS 36	*16.2*	6.4	5.8	3.8
FmS 11 + FmS 12	8.1	*20.8*	6.7	4.9
FmS 15 + FmS 37	4.6	7.9	*18.6*	2.8
FmS-a + FmS-b	2.6	5.8	4.2	*4.1*

[a] Italicized values (one in each column) are high percentages of recovery of a given heterokaryon on its related SRM.

were made by Hansen and Smith (8). They examined 47 isolates of *Botrytis cinerea* collected from the field. Some of the cultures produced a variety of different morphologically stable homokaryons, which indicated that both the hyphal cells and conidia were multinucleate. Frequent occurrence of heterokaryosis was observed on potato-dextrose agar, and morphological conidial differences were shown to be chromosomal rather than cytoplasmic in nature. However, Jinks (12) reexamined some of the results of Hansen and Smith (8) and pointed out that the evidence, as it stands, does not rule out a cytoplasmic basis for many presumed examples of heterokaryosis. In *Penicillium*, he demonstrated that two of four presumed heterokaryons were actually heteroplasmons. Jinks (11) showed that *Penicillium* commonly exists in the form of heterokaryons in nature and enjoys an advantage in growth over the homokaryon components. This advantage, however, is conditional and depends on the nutrient balance in the soil. His results demonstrated that heterokaryosis in wild *Penicillium* provides for somatic variation and natural adaptation. The only heterokaryons that survived better were those which were well suited to the nutritional needs of a fungus living on various and often changing substrates. *Penicillium* spp. are mainly saprophytic fungi, and their needs are fulfilled by the dead plant material.

The natural occurrence of heterokaryosis has rarely been examined in soilborne fungi. In early study, Buxton (2) claimed to have found heterokaryosis in wild *Fusarium oxysporum* f. sp. *gladioli*, but it is unclear whether the heterokaryons were isolated from soil or infected plant tissue. Ming et al (13) reported natural occurrence of heterokaryosis in *F. fujikuroi* (*Gibberella fujikuroi*). They isolated white-, red-, and purple-pigmented conidia from the hyphal tips. The three types of uninucleate conidia reproduced true to type on successive transfers and differed in amount of gibberellin production. The natural heterokaryons were composed of either any two or all three types of conidia (homokaryons). They resynthesized heterokaryons on artificial media without using selective techniques and recovered all the heterokaryons. Ming et al (13) showed that heterokaryons presumably enjoy a selective advantage over their homokaryons both on rice and on synthetic media. However, Ming et al (13), like Buxton (2), did not indicate whether the natural heterokaryons were actually isolated from the living host plant or from the soil. It would be of interest to know how natural heterokaryons survive and adapt to the living host, which goes through various physiological phases during the growing period. It is conceivable that fungal genotypes may adapt to saprophytic and parasitic phases by virtue of transient or permanent adjustment in their nuclear types in a heterokaryon. In fact, it is well documented that nuclear ratios in a heterokaryon change in response to the components of artificial media (11). The question arises whether a fungal species living on a growing plant would also adapt to the changing environment by forming heterokaryons. This was examined in the case of *Fusarium moniliforme* (Sheld.) on growing sorghum and corn plants (*unpublished*). This experiment will be discussed here in some detail.

Samples of *F. moniliforme* were recovered from different growth stages (40, 60, and 80 days after planting and at maturity) of naturally infected sorghum plants grown in the greenhouse and field. Various heterokaryons were isolated in different proportions from plants of each growth stage. However, only the prevalent heterokaryons were selected for analysis from each stage. The characteristics of the selected heterokaryons are given (Table 2).

Experiments were conducted to investigate the possibility of a correlation between a given heterokaryon and the nutrients in stalks of corresponding maturity. Stalk residues from different growth stages of plants were mixed with the basic ingredients (minus sucrose) of Czapek minimal medium (MM). Each heterokaryon was plated on all the stalk residue media (SRM) prepared from stalks of plants at the different growth stages (Table 3). The percentage recovery and nuclear ratios of a given heterokaryon were higher on the corresponding SRM (Table 3). This was found with all the heterokaryons except FmS-a + FmS-b (Table 3). The heterokaryon FmS-a + FmS-b was recovered from stalks at maturity when the plants were under highest metabolic stress and as a result showed tremendous physiological fluctuations. The nuclear components of most heterokaryons also survived on unrelated SRM, but the survival percentage of the heterokaryons was higher on their related SRM. There seems to be some kind of adaptive adjustment between the heterokaryons and the related growth stages of the plant.

The nuclear ratios of the adapted heterokaryons were also balanced. A heterokaryon usually showed an unbalanced nuclear ratio on unrelated SRM, but gave a balanced nuclear ratio when plated on its related SRM (Table 4). This was true for all but one heterokaryon, FmS-a + FmS-b, which gave an unbalanced nuclear ratio. Nuclear ratios on unrelated SRM were more or less unbalanced. Again, this suggests some kind of preferential adjustment between the heterokaryons and their respective SRM.

The media influence the stability of nuclear ratios (11). Changes in proportions of a given natural medium are reflected in nuclear ratios. If the proportions of a medium that supports balanced ratios is changed, the ratios are also modified accordingly. This aspect was investigated by changing proportions of various SRM and MM mixtures. The media used ranged at 20% intervals from 100% SRM to 100% MM. The results are given in Table 5. The nuclear ratios of all heterokaryons were nearly balanced on their related 100% SRM. With the decrease in proportions of SRM, the related nuclear ratios became unbalanced (Table 5). It is obvious that

relatively more balanced ratios of the heterokaryons were obtained on 100% related SRM. This again confirms that heterokaryons show specific adaptation on related stalk residues. Therefore, the occurrence of a particular heterokaryon at a higher frequency in the stalk of a particular growth stage is not accidental.

Three interesting points emerge from this study: heterokaryons are found in different growth stages of the plant; homokaryons change in morphological and metrical traits with the succession in stalk rot disease syndrome; and an adaptive specificity occurs between heterokaryons and related stages of plant growth. The components of heterokaryons recovered from the diseased tissue were different from those recovered from early stages of plant growth. These experiments indicate that the process of heterokaryosis is not merely a means of bringing together the genes that can result in vigorous and successful individuals, but also a mechanism by which the proportions of various sets of genes can vary with different host genotypes and, particularly, with different growth stages of the plant. This suggests that the variation in relative proportions of the different genes or nuclei contributes towards the adaptive process. Regardless of the initial conidial population in the seedling stages of the plant, the nuclear proportions in the heterokaryons are guided by the corresponding physiological and biochemical changes that occur during the growth of sorghum plants. Thus, the variation in percentages of different heterokaryons is adaptive because the most prevalent heterokaryons are selected according to the characteristics of the substrate.

The process of heterokaryosis, in fact, provides an opportunistic and continuous adaptation. *F. moniliforme*, whose cells are enclosed within a changing host environment, seems to use heterokaryosis for survival. Heterokaryosis protects the constancy of conidial isolates that might otherwise be eliminated during the unfavorable growth stages of the plant. Maturation and environmental stress impose certain changes in the host physiology, which, in turn, serve as selective forces for the promotion of pathogenic isolates. These pathogenic isolates, which are otherwise present throughout the growth stages of the plant, are shielded by heterokaryons and are released at different stages of plant growth in response to an appropriate host environment. Therefore, the survival of different isolates of *F. moniliforme* during different growth stages of sorghum plants seems to be mediated by the mechanism of heterokaryosis. On the other hand, a sexually reproducing fungus would have to go through mitotic-meiotic cycle, before the recombinants capable of adjusting to the continuously changing host environment could evolve. Heterokaryosis enables *F. moniliforme* to dispense with meiosis in

TABLE 4. Shift in nuclear ratios of the heterokaryons of *Fusarium moniliforme* after transferring a single heterokaryotic hypha from a given sorghum stalk residue media (SRM) to other SRM

Heterokaryon	Transferred from SRM medium from plant at age:	Nuclear ratio on SRM from plant at age:			
		40 day	60 day	80 day	Maturity
FmS 2 + FmS 36	40 day	*1.2:1.0*[a]	1.0:5.2	1.0:2.1	1.0:2.6
	60 day	*1.0:1.2*	1.0:1.5	1.0:1.8	1.0:2.8
	80 day	*1.4:1.0*	1.0:1.6	1.0:1.9	1.0:2.0
	Maturity	*1.4:1.0*	1.3:1.0	1.8:1.0	1.0:2.0
FmS 11 + FmS 12	40 day	1.0:2.7	*1.0:1.0*[a]	1.7:1.0	3.0:1.0
	60 day	1.0:1.1	*1.0:1.1*	1.9:1.0	2.8:1.0
	80 day	1.0:2.4	*1.0:1.2*	1.8:1.0	3.0:1.0
	Maturity	2.6:1.0	*1.0:1.4*	2.6:1.0	2.6:1.0
FmS 15 + FmS 37	40 day	1.0:2.1	1.0:2.9	*1.0:1.2*[a]	1.0:2.7
	60 day	1.0:2.6	1.0:2.6	*1.0:1.1*	1.0:3.0
	80 day	1.0:1.8	1.0:1.8	*1.1:1.0*	1.0:2.8
	Maturity	1.0:2.9	1.0:1.6	*1.0:1.4*	1.0:4.0
FmS-a + FmS-b	40 day	1.0:2.0	1.0:2.8	1.0:2.1	*1.0:1.8*[a]
	60 day	1.0:2.6	1.0:3.1	1.0:3.1	*1.0:1.2*
	80 day	1.0:2.6	1.0:2.8	1.0:3.1	*1.0:1.4*
	Maturity	1.0:2.4	1.0:2.4	1.0:1.4	*1.0:1.8*

[a] Italicized values (four in each column) are highly adapted ratios of a given heterokaryon on the relevant SRM.

TABLE 5. The effect of various mixtures of minimal medium (MM) and sorghum stalk residue media (SRM) made from plants at various growth stages on nuclear ratio of heterokaryons of *Fusarium moniliforme*

Heterokaryon	SRM from plants at age:	Nuclear ratios on different mixtures of MM and SRM medium:					
		MM: 100 SRM: 0	80 20	60 40	40 60	20 80	0 100
FmS 2 + FmS 36	40 day	1.0:1.8	1.0:1.6	1.6:1.0	1.0:1.4	1.0:1.4	*1.0:1.0*[a]
	60 day	1.0:1.2	1.0:1.8	1.0:2.1	1.0:2.2	1.0:3.1	1.0:3.1
	80 day	1.3:1.0	1.0:2.1	1.0:2.6	1.0:2.1	1.0:2.1	1.0:4.1
	Maturity	2.2:1.0	1.0:2.1	1.0:1.8	1.0:2.0	1.0:1.8	1.0:3.1
FmS 11 + FmS 12	40 day	1.0:1.2	1.0:1.6	1.0:2.9	1.0:2.0	1.0:2.1	1.0:1.4
	60 day	1.1:1.0	1.0:1.0	1.3:1.0	1.4:1.0	1.2:1.0	*1.0:1.2*[a]
	80 day	1.2:1.0	1.3:1.0	1.0:1.6	1.0:1.6	1.8:1.0	1.8:1.0
	Maturity	1.6:1.0	1.8:1.0	2.0:1.0	1.0:3.1	1.0:2.1	1.0:3.0
FmS 15 + FmS 37	40 day	1.0:1.8	1.6:1.0	2.4:1.0	1.0:1.6	1.0:1.6	1.0:3.0
	60 day	1.0:1.8	1.8:1.0	1.4:1.0	1.0:2.6	1.0:2.6	1.0:2.0
	80 day	1.0:1.1	1.4:1.0	1.0:1.8	1.0:1.2	1.0:1.2	*1.0:1.2*[a]
	Maturity	1.0:1.3	1.0:2.8	1.0:2.9	1.0:2.8	1.0:2.8	1.0:4.9
FmS-a + FmS-b	40 day	1.0:1.3	1.6:1.0	1.8:1.0	1.0:1.9	1.0:1.9	1.0:2.0
	60 day	1.0:1.2	1.0:1.8	1.0:1.6	1.9:1.0	1.9:1.0	2.0:1.0
	80 day	1.0:1.4	1.8:1.0	1.8:1.0	2.0:1.0	2.0:1.0	2.1:1.0
	Maturity	1.0:1.2	1.4:1.0	1.8:1.0	1.6:1.0	1.6:1.0	*1.0:1.4*[a]

[a] Italicized values (four in the last column) are relatively balanced nuclear ratios of a given heterokaryon on 100% SRM.

nature (sexuality is seldom found in nature) and thus helps to utilize mitotic variability more efficiently as an adaptive genetic process.

Somatic diploid. Occasionally, fusion of haploid nuclei in the heterokaryon gives rise to somatic diploids. Somatic diploids are rare; therefore, selective techniques are required for their isolation. Roper (19) has given an account of a selective technique as applied to *A. nidulans*. Similar, or slightly modified, techniques are used to isolate diploids in pathogenic fungi (6). With respect to soilborne pathogens, the somatic diploids have been successfully isolated from *F. oxysporum* f. sp. *cubense* (3) and *Verticillium albo-atrum* (10).

The detection of somatic diploids requires definitive proof. Generally diploids are detected by their wild-type phenotypes, but this criterion is not sufficient because the heterokaryon, haploid revertants, and nearly balanced aneuploids also produce a normal phenotype. Therefore, some additional criteria, such as DNA content, cell volume, chromosome count, nuclear volume, mating type, segregations of haploid markers, mutation rate, and solopathogenicity (*Ustilago maydis*), are required to ascertain somatic diploidy.

Parasexual cycle. Although there is evidence of parasexuality in certain *Fusarium* species, a clear demonstration of its occurrence is still needed. Among the soilborne pathogens, a clear demonstration of a complete process of parasexuality is available only in *Verticillium* species. The parasexual cycle in *Verticillium* was first described by Hastie (9). It is almost identical to the one found in *Aspergillus*, but two differences are obvious. First, heterokaryons of *Verticillium* are relatively unstable; secondly, the heterozygous diploids of *Verticillium* generate new genotypes more frequently than the diploids of *Aspergillus* (10).

Incompatibility and parasexuality. Incompatibility genes are known to operate at almost all stages of the parasexual cycle. They are known to arrest anastomosis, karyogamy, and heterokaryosis. Mostly they have been studied during heterokaryosis. Heterokaryon incompatibility involves two different, but related, phenomena, eg, true incompatibility and heterokaryotic disadvantage (5). True incompatibility, in which heterokaryons are seldom formed and die soon after formation, is analogous to sexual systems of incompatibility. Heterokaryotic disadvantage involves the persistence of a heterokaryon for only a short time because of its inferiority to its homokaryon components and is analogous to heterozygous disadvantage. Incompatibility factors have been found in *Verticillium* species (17). The information on incompatiblity factors in fungal species was based on the actual formation of heterokaryosis, and no evidence of lack of anastomosis or cytoplasmic mixing was obtained.

Parasexuality and pathogenicity. Changes in pathogenicity occur, and sometimes have been ascribed to heterokaryotic and somatic diploid stages of the parasexual cycle. In some cases, overall pathogenicity of heterokaryons is increased over that of component homokaryons, while in others it is actually reduced (14,22). There is no consistency in the expression of avirulence or virulence by heterokaryons or somatic diploids. The virulence of certain heterokaryons of *Rhizoctonia solani* was enhanced compared to the parental homokaryons, but others experienced a reduced or complete loss of virulence (1). In *F. oxysporum* f. sp. *lycopersici* (20) and *G. fujikuroi* (13), enhanced virulence of certain natural heterokaryons was found.

A few cases of pathogenicity tests involving somatic diploids are also available. It is important to understand the life cycle of a fungus before we interpret relationships between a somatic diploid and its virulence. There is a fundamental difference between pathogens in which infective stage is haploid (ascomycetes and deuteromycetes) and those in which it is dikaryotic (basidiomycetes). Interpretation of virulence tests with somatic diploids of conidial fungi or deuteromycetes needs prior introduction of auxotrophic mutants into the parental homokaryons (5). In fact, only known mutants would allow one to select the somatic diploids. Since genes for virulence are normally recessive, the somatic diploids of deuteromycetes will normally be avirulent because the mutants for virulence seldom occur at the same locus in homokaryons. This implies that the enhanced pathogenicity is a function of an induced mutant rather than somatic diploidy. Therefore, it is more realistic to compare somatic diploids with the wild-type, prototrophic haploids. Such comparisons have been attempted in two soilborne fungi, ie, *F. oxysporum* (2) and *Cochliobolus sativus* (21). In *F. oxysporum* f. sp. *pisi*, heterokaryons and diploids, which originated from race 1 (virulent to pea cultivar Onward), and race 2 (virulent to Onward and Alaska), proved to be virulent to two additional cultivars, Delwiche and Commando, which are otherwise resistant to the two races.

Heterokaryosis in pathogenic fungi is a process of gradual adaptation without immediate dramatic effects on the respective hosts. Microevolutionary changes through adjustments of nuclear ratios in the heterokaryons eventually make them better adapted. Segregants through mitotic crossing over or haploidization may occasionally become better adapted and give rise to virulent variants or races. However, such events are rare in soilborne fungal parasites. This may account for occurrence of fewer races in soilborne pathogenic fungi.

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