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Mini-Review

Genetic Status of the *Gibberella fujikuroi* Species Complex

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Fusarium moniliforme Sheldon was the name adopted by Snyder and Hansen (1945) to what is now referred to as the *Gibberella fujikuroi* species complex, or *Liseola* section of the *Fusarium* genus. At present, at least 30 named or phylogenetic species are included in this group (Leslie, 1995; Nelson et al., 1983; Nirenberg, 1989; O'Donnell et al., 1998). Some of these species have known teleomorphs, while others have been described solely on the basis of asexual anamorphic characters. Representatives of this group have been cultured from all continents except Antarctica, and are known to colonize hosts as diverse as maize, mango, sorghum, asparagus, and native North American prairie grasses (Leslie, 1995; O'Donnell et al., 1998; Zeller et al., unpublished). Toxins and secondary metabolites produced by these fungi are chemically and biosynthetically diverse and include compounds such as fumonisins (Gelderblom et al., 1988), moniliformin (Marasas et al., 1986), fusaproliferin (Logrieco et al., 1996; Moretti et al., 1996), fusaric acid (Bacon et al., 1996), fusarins (Wiebe and Bjeldane, 1981), beauvericin (Logrieco et al., 1998; Moretti et al., 1996), and gibberellic acids (Cerda-Olmedo et al., 1994; Phinney and West, 1960), with additional toxins remaining to be identified (Leslie et al., 1996).

All of the species in the group are amenable to physiologic and molecular genetic studies as they can be cultured on a defined salts-sugar medium under laboratory conditions (Correll et al., 1987; Puhalla and Spieth, 1983), and DNA can be extracted using standard protocols (e.g., Murray and Thompson, 1980). Species with sexual stages may be analyzed using classical genetic protocols as well (e.g., Klittich and Leslie, 1988a). Currently, all of the species with known sexual stages are heterothallic (Britz et al., 1999; Hsieh et al., 1977; Klaasen and Nelson, 1996; Klittich et al., 1997; Kuhlman, 1982; Leslie, 1991a), and fertile crosses result in perithecia comprised of asci containing eight unordered ascospores (Raju, 1994). Unordered tetrad analysis (Fincham et al., 1979) is possible, but most researchers select ascospores from the cirrhus of exuded spores and analyze them using standard techniques for the analysis of random ascospores (Fincham et al., 1979). Parasexual analysis (Fincham et al., 1979) may be possible, but parasexuality has not been shown to occur in any of the species in this group under either laboratory or field conditions.

Some of the species within this group have been subjected to relatively intense genetic scrutiny. My objectives in this presentation are: (i) to briefly describe the genetic characters known in these fungi, (ii) to identify potential areas for unique further research on the genetics of these organisms, and (iii) to speculate on the genetic returns that might result from further studies of the genetics of these organisms.

Genome Organization

The physical genome of *G. fujikuroi* mating populations A-F has been estimated at between 45 and 55 Mb. Early reports identified four chromosomes (Howson et al., 1963) based on cytological observations of meiosis. Later reports increased this number to at least 10 (N.B. Raju, personal communication), and contour-clamped homogeneous electric field (CHEF) gel electrophoretic analysis identified 12 bands between 0.7 and 12 Mb in size (Xu et al., 1995). All six examined mating populations have the same number of chromosomes, but the chromosome sizes vary somewhat between species. No significant chromosome rearrangements have been identified in field strains. There is preliminary genetic evidence for a chromosome translocation in *G. fujikuroi* mating population A (Zeller and Leslie, unpublished data), and physical evidence for a deletion as a result of a transformation event in *G. fujikuroi* mating population C (Linnemannstöns et al., 1999). Limited cross-hybridization of restriction length fragment polymorphison (RFLP) sequences shows that if the chromosomes are numbered from largest to smallest, then sequences that hybridize to a particular chromosome in one species usually also hybridize to the correspondingly numbered chromosome in the other mating populations. Although exceptions are known (Xu et al., 1995), this pattern suggests that genome organization in all of these mating populations is similar.

Many genome properties are as expected. Telomere sequences from *F. oxysporum* (Powell and Kistler, 1990)

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hybridize to all of the bands separated on the CHEF gels (Xu et al., 1995). rDNA sequences hybridize to chromosome 2. No centromeres have been identified, and regions of relatively decreased recombination, as might be expected near centromeres, are not obvious on the existing genetic maps (Xu and Leslie, 1996). If centromeres are to be localized in this organism, then it will probably require unordered tetrad analysis to locate a genetic region that can then be correlated to the existing maps. Physical evidence suggests that multiple copy dispersed transposable elements are rare; no multi-copy RFLP probes were detected except for those associated with the rDNA coding region (Xu and Leslie, 1996). Genetic evidence suggests that such elements may be present, however, as the frequency with which spontaneous *nit* mutants arise varies widely in natural populations and is heritable as a quantitative trait (Klittich et al., 1988).

Techniques and Technology

The techniques described in this section are primarily those that work in my laboratory. While these techniques work, other research groups may use other techniques and obtain similar, or perhaps even better, results. Nomenclature generally follows that proposed by Yoder et al. (1986) for filamentous fungal plant pathogens. Some groups follow the conventions of Clutterbuck (1973) or Demerec et al. (1966), however, and it is possible that the same gene may be called different names by different research groups, e.g. *nit1* and *niaD* both designate the locus that encodes the holoenzyme for nitrate reductase.

Classical genetics. Crosses are usually made on carrot agar (Klittich and Leslie, 1988a), although some other groups use V-8 agar (Kathariou and Spieth, 1982; Puhalla and Spieth, 1983, 1985), and incubated right-side up in a lighted incubator at 25°C. Normal asci contain eight unordered ascospores. Nonviable ascospores usually abort and disintegrate before maturation. Thus, crosses that result in dead progeny, e.g., $sk^K \times sk^S$ (Raju, 1994), result in tetrads with fewer than eight viable spores. The strain serving as the female parent is inoculated first and allowed to grow for a week, and then is spermatized with a conidial suspension from the male parent. Crosses mature in 2-4 weeks, and we identify fertile perithecia as those that exude a cirrhus of ascospores. We collect random ascospores by taking the ascospore cirrhus from a single perithecium and spreading it along the edge of a 3% water agar slab (Haefner, 1967). Individual ascospores are separated using a fine glass needle in a micromanipulator. To be 95% certain that no two ascospores originate from the same ascus (a fundamental assumption underlying random ascospore analysis), no more than 20 ascospores are used from a single perithecium (Leslie, 1991b). Ascospores are allowed to germinate *in situ* on the water agar for 16-24 hours. Agar blocks with the germlings are cut from the agar slabs and transferred to other media once the germlings are clearly visible. Ascospore viability is highest when crosses are fresh and decreases as the crosses age or as the cross plates dry out. The window of maximum fertility is usually no more than one week once the ascospores have been extruded from the perithecium.

Molecular genetics. Most molecular techniques that work with other filamentous fungi can be used for members of the *G. fujikuroi* species complex with relatively little modification. DNA extracted using a CTAB technique, e.g., Murray and Thompson (1980) as modified by Kerenyi et al. (1999), usually gives high quality DNA, but is certainly not the only available technique, e.g. DuTeau and Leslie (1991). Strains may be transformed using eith calcium-shocked protoplasts, e.g. Brückner et al. (1992) or Linnemannstöns et al. (1999), or with intact cells that have been permeabilized with lithium acetate (Leslie and Dickman, 1991). Resistance to benomyl and hygromycin B is commonly used as selectable markers, but some care using these genes needs to be exercised as field isolates can develop tolerance or resistance to these drugs in the absence of the foreign resistance genes and since the background resistance levels may differ significantly by mating population (Yan et al., 1993). Multiple-copy transformants appear to be MIP but not RIP when passed through classical genetic crosses (Leslie and Dickman, 1991).

Genetic Maps

The first genetic map of *F. moniliforme* was constructed by Puhalla and Spieth (1985). This map contained 12 markers on four linkage groups. Additional three markers could not be tied to any of these four linkage groups.

A new map was produced by Xu and Leslie (1996). This map contained 150 markers, primarily RFLPs, but also segregating were mating type (*mat*), spore-killer (*sk*), two RAPD polymorphisms, a gene involved in the biosynthesis of fumonisin (*fum1*), and two auxotrophic markers (*nic1* and *arg1*) that were first mapped by Puhalla and Spieth (1985). Twelve linkage groups were identified, and these linkage groups were correlated with bands on a CHEF gel (Xu et al., 1995). The linkage groups and chromosomes identified by Xu et al. (1995) and Xu and Leslie (1996) are numbered based on physical size. Linkage groups I and II of Puhalla and Spieth (1985) correspond, respectively, with chromosomes 5 and 3 of Leslie and Xu (1996). The total map length was estimated at approximately 1500 map units, or centiMorgans (cM). Of the 150 mapped markers, 135 could be placed in linkage groups, 122 were assigned

to linkage groups based on hybridization to Southern blots from the CHEF gels, and 15 could not be assigned unambiguously to any of the chromosomes. One chromosome, number 11, had members of two different linkage groups assigned to it based on Southern hybridization, but the remaining chromosomes all had only a single linkage group. Four of the chromosomes, numbers 3, 10, 11 and 12, averaged less than one crossover per meiosis and were thought to possibly be subject to distributive pairing (Grell, 1976; Hawley et al., 1993a, 1993b). Although the Xu and Leslie (1996) map was a major improvement over that of Puhalla and Spieth (1985), it still had at least 22 regions in which there was no identified marker for at least 20 cM.

The Xu and Leslie (1996) map has now been updated by Jurgenson et al. (1999). These investigators added over 500 amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995) to the map. They used the same mapping population as that used by Xu and Leslie (1996), and confirmed the positions of all of the markers identified and inferred by Xu and Leslie. The addition of more markers expanded the length of the map to nearly 4500 cM, approximately 10 kb/cM, on average. The new map has 27 intervals with no marker for $\geq$ 20 cM. These intervals are found all chromosomes except 7, and are usually distal. The increased size of this map is attributable both to detection of more recombination events and to the inclusion of additional regions that had not been included previously. The biggest disappointment of this map is that the *fum1* locus is still not bracketed closely enough by other markers to make it possible to clone it via chromosome walking.

Dispensable Chromosome (B Chromosome)

The smallest chromosome in the *F. moniliforme* genome is approximately 700 kb in size. Xu and Leslie (1996) found that three progeny of the 121 in the mapping population used to construct their map had lost this chromosome and that one additional progeny had apparently had this chromosome rearranged through a duplication and deletion. Loss of this chromosome resulted in no detectable change in phenotype. Traits tested include morphology, rate of growth, ability to induce stalk rot in maize, and ability to produce fumonisins. When this chromosome is lost, the material it contains also is lost, since hybridizing RFLPs that map to this chromosome now disappear completely. There is evidence from Northern analyses that expressed genes are carried on this chromosome. Eight field strains of *G. fujikuroi* mating population A carried a chromosome of approximately this size, although it is not known if these chromosomes are homologues. Similarly, the representatives of the six mating populations examined by Xu et al. (1995) all contain chromosomes of approximately this size (700-900 kb), but their homology is unknown.

Speciation/Mating Populations

Definitions of species within this group are varied. At a minimum, eight mating populations, or biological species, have been defined. *Fusarium* anamorph names have been proposed for all of these mating populations, but not all of these names have been widely accepted. Based on morphological and/or phylogenetic criteria, an additional 20 species have been described (see O'Donnell et al., 1998 for a recent compilation). Finally, eight additional phylogenetic lineages have been identified, but not formally described as distinct species (O'Donnell et al., 1998). Undoubtedly additional biologically significant entities remain to be identified and described.

Correct identification of the group to which a strain belongs is essential for many genetic analyses. Classical genetic crosses between strains belonging to different species are only rarely successful (Perkins, 1994). Similarly, the secondary metabolites produced by different species vary (Leslie et al., 1996; Logrieco et al., 1998; Moretti et al., 1996; Thrane and Hansen, 1995), and genetic analysis of secondary metabolism will be successful only if the strain(s) being analyzed should normally produce the secondary metabolite of interest. The recent identification of the *spc1* mutant (Leslie and Zeller, 1997) may provide a handle that can be used to identify genes involved in speciation, as this single gene mutant can form viable heterokaryons with representatives from several different species. Another approach to studying speciation may be to study the progeny of interspecific crosses and to identify regions that appear to be unique to strains of one of the two participating species.

Nutritional Mutants

Two types of nutritional mutants are known in *G. fujikuroi* mating population A, traditional auxotrophs, e.g. mutants that require a nutritional supplement for growth, and those that cannot utilize as wide a spectrum of nitrogen (Klittich and Leslie, 1988a) and sulfur (Correll and Leslie, 1987) sources as can the normal wild type strains. Traditional auxotrophs are known in the "A" and "C" mating populations of *G. fujikuroi* (Avalos et al., 1985; Puhalla and Spieth, 1985; Sidhu, 1983). Genetic analysis of these mutants is limited. None of these genes has been cloned, and only a few that were isolated in the "A" mating population have been mapped (Puhalla and Spieth, 1985; Xu and Leslie,

1996).

The nitrate-nonutilizing (*nit*) mutants and the chlorate-resistant nitrate-nonutilizing (*crn*) mutants are the best studied class of nutritional mutants in *G. fujikuroi*. The *nit* mutants encode proteins that function in the reduction of nitrate to ammonium and in the breakdown of purines (Correll et al., 1987; Klittich and Leslie, 1988a). The *nit1* locus encodes the holoenzyme for nitrate reductase. The NitM loci (*nit2*, *nit4*, *nit5*, *nit6*, and *nit7*) jointly encode a molybdenum co-factor that is essential for action of nitrate reductase and purine dehydrogenase. The *nit3* locus encodes a regulatory locus that senses nitrate and is responsible for the induction of the entire pathway. Based on the pathway as known in *Neurospora crassa* and *Aspergillus nidulans*, at least two additional loci are also involved, but mutants in these loci are not known in *G. fujikuroi* mating population A. The global nitrogen regulator, termed *nit2* in *N. crassa*, *areA* in *A. nidulans* and *nnu* in *G. zeae*, has been identified in *G. fujikuroi* mating population C (Dickman and Leslie, 1992; Exley et al., 1993; Johnstone et al., 1990; Leslie, 1983, 1987; Marzluf, 1997). The nitrite reductase structural locus, termed *nit-6* in *N. crassa* and *niiA* in *A. nidulans*, has not been identified in any of the *G. fujikuroi* mating populations. *nit* mutants in *Fusarium* species usually are recovered spontaneously following growth on media containing chlorate, a toxic analog of nitrate. *nit* mutants become resistant to chlorate when they lose the ability to metabolize chlorate via the nitrate reduction pathway. *crn* mutants differ from *nit* mutants in that the *crn* mutants are both chlorate resistant and capable of utilizing nitrate as a nitrogen source. The *crn* mutants fall into two broad categories. In the first, the mutation occurs in a gene that can also be mutated to a *nit* phenotype, e.g., *nit1* and *nit3*. These mutants result in greatly reduced nitrate reductase levels (Klittich and Leslie, 1989) that are apparently still sufficient for the cell to reduce enough nitrate to survive, but the enzyme level is low enough that the amount of chlorate converted to chlorite is too small to be lethal. Mutants in this category usually are identified by the *nit* locus that they affect. The second category of *crn* mutants have no known similarities to the *nit* mutants but have normal or greater levels of nitrate reductase. These mutants map to at least three additional different loci and may be involved in the transport of nitrate (or chlorate) into the cell, similar to the *crn* mutants of *A. nidulans* (Brownlee and Arst, 1983) or *chl* mutants of *Arabidopsis thaliana* (Braaksma and Feenstra, 1982; Doddema et al., 1978) or tobacco (Marton et al., 1982). Little additional work has been done with these mutants.

Genetics of Secondary Metabolites

Fumonisins. Several of the genes required for fumonisin biosynthesis are located in a cluster on chromosome 1 (Desjardins et al., 1992, 1995, 1996; Plattner et al., 1996; Xu and Leslie, 1996). The enzymatic functions of these genes are not all known, but include hydroxylation at positions C-5 and C-10, the polyketide synthase used for fumonisin biosynthesis. Another gene in this region, *fum4*, may be involved in regulation of fumonisin biosynthetic gene expression. Fumonisins are known to be phytotoxic under some conditions (Gilchrist et al., 1992; Lamprecht et al., 1994), but their active expression is not necessary for a strain to be an effective plant pathogen (Jardine and Leslie, 1999).

Fusaric acid. Fusaric acid is made by all of the members of this group that have been tested (Bacon et al., 1996). The genetic basis of the biosynthetic pathway is unknown, but recently has become a target for genetic analysis (Kuldau et al., 1999).

Gibberellic acid. Gibberellic acids are made by members of *G. fujikuroi* mating population C via a complex pathway of at least a dozen steps (Cerdá-Olmedo et al., 1994). Mutants defective in gibberellic acid production are known from field populations (Bearder, 1983; Bearder et al., 1973; Spector and Phinney, 1968) and as a result of mutagenesis with chemicals or ultraviolet light (Avalos and Cerdá-Olmedo, 1987; Bearder et al., 1974; Candau et al., 1991; Takenaka et al., 1992). The genes encoding farnesyl diphosphate synthase (Homann et al., 1996), two geranylgeranyl diphosphate synthases (Mende et al., 1997; Tudzynski and Hülter, 1998), 3-hydroxy-3-methylglutaryl-CoA reductase (Woitek et al., 1998), copalyl diphosphate/*ent*-kaurene synthase (Tudzynski et al., 1998), and four cytochrome P450 monoxygenases (Tudzynski and Hülter, 1998) have been cloned. Many of these genes appear to map to a cluster on chromosome 4 (Linnemannstöns et al., 1999; Tudzynski and Hülter, 1998). Efforts to clone these genes via REMI-mutagenesis procedures have not been successful (Linnemannstöns et al., 1999).

Mating Type

The fungi in this group all appear to be typical heterothallic ascomycetes. Mating type in *G. fujikuroi* is dimictic, i.e. it is controlled by a single gene with two idiomorphic alleles. Fertile crosses can occur only if the two parents carry opposite mating type alleles. Strains of either mating type can serve as the male or as the female parent. Sequence similarities are known between mating type loci in several heterothallic ascomycetes (Coppin et al., 1997; Turgeon, 1998). These similarities are particularly conserved in the HMG binding region of one of the alleles (*MAT-2*). Arie et al. (1997) designed degenerate primers that could be used

to amplify this region from a number of ascomycete fungi. These primers have been used successfully to amplify this region from strains of *G. fujikuroi* mating population A (Covert et al., 1999; Kerényi et al., 1999; Steenkamp et al., 1999). This region has been sequenced and primers designed to amplify it specifically. These primers also can be used to amplify similar regions from all of the other characterized mating populations and some strains of *F. oxysporum* (Kerényi et al., 1999). Until recently, mating type designations had been arbitrarily assigned as "+" and "–", with the investigator who described the mating population and identified the tester strains making the designations. With the identification of idiomorphic molecular alleles at mating type, the form of the terminology has changed. The allele that carries the HMG box is now termed *matX-2*, where the "X" represents the mating population to which the strain belongs, and is structurally related to the *a* allele of *Neurospora crassa*, the *FPR1* allele of *Podospora anserina*, and the *MAT-2* allele of *Cochliobolus heterostrophus*. The alternate allele, which carries a conserved alpha box sequence and is similar to the *A* allele of *N. crassa*, the *FMR1* allele of *P. anserina*, and the *MAT-1* allele of *C. heterostrophus* is termed *matX-1*. Sequencing of the mating type alleles from several of these fungi is in progress and these sequences and some functional analyses should be available in the near future (Steenkamp et al., 1999; Yun et al., 1999).

Vegetative Compatibility

Genes involved in vegetative compatibility are generally thought to be similar to those that mediate this phenomenon in other filamentous fungi such as *N. crassa*, *A. nidulans*, *P. anserina*, and *Cryphonectria parasitica* (Leslie, 1993). These genes, termed *vic* genes in these fungi, are dispersed throughout the genome. At least nine *vic* loci mapping to chromosomes 2, 3, 4, 5, 8, 10 and 11 have been tentatively identified in *G. fujikuroi* mating population A and are segregating in the mapping population used to construct the genetic map described above (Zeller and Leslie, unpublished data). At least some of these *vic* loci should be sufficiently close to a heterozygous AFLP marker to permit the cloning of the *vic* gene via chromosome walking. An additional locus (*vic1*) that appears to be different from any of these loci was identified by Puhalla and Spieth (1983, 1985), but is not clearly heterozygous in the mapping cross.

In addition to the *vic* loci, at least three other classes of loci (Fig. 1) are involved in the vegetative compatibility process and in the formation of a stable heterokaryon (Leslie and Zeller, 1996, 1997). The first class is the *hsi* mutant class. Strains carrying these mutations are said to be heterokaryon self-incompatible. Strains with this phenotype are unable to form visible prototrophic heterokaryons between complementary mutants that were derived from the same parent (Correll et al., 1989), but have just recently been described in *Neurospora* (Wilson and Dempsey, 1999). These mutants are usually also female-sterile, which means that it is impossible to do classical genetic complementation tests with them as at least one parent of such a cross must be female fertile. Two other classes of genes are also likely to be involved in this process. These genes affect the recognition and reaction pathways and are presumably responding to signals resulting from the interaction between the *vic* alleles, e.g. Loubradou et al. (1999) and Vellani et al. (1994). We have searched for such mutants using classical mutagenesis, by selecting for mutants that could overcome the vegetative incompatibility reaction attributable to differences at multiple *vic* loci. The number of loci that can be so mutated is large, at least 15-20, and the molecular basis for the altered phenotype is not known (Leslie and Zeller, 1997). At least one of these mutants (*spc1*) can also form heterokaryons across species barriers. These mutants suggest that the *vic* loci themselves are probably not directly responsible for the killing that results from an incompatible interaction, but are instead a first step in an intricate and complex set of biochemical reactions.

Spore Killer

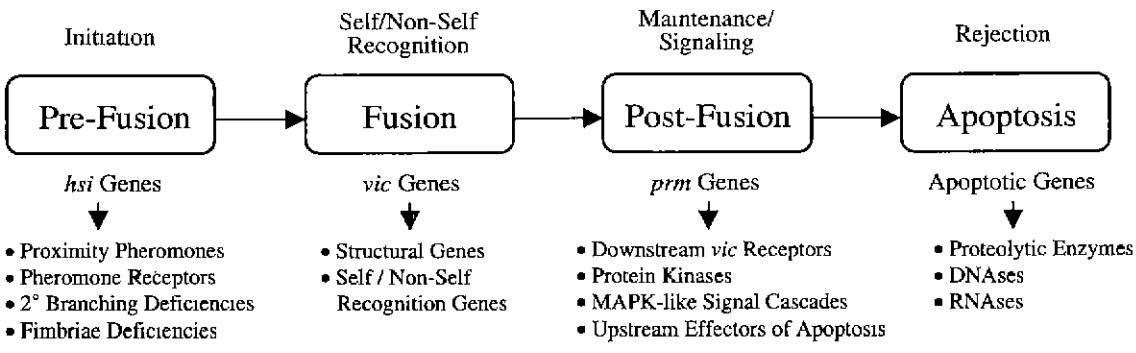

Fig. 1. A model for steps in the vegetative compatibility/incompatibility process (after Leslie and Zeller, 1996, 1997).

Spore killer is a meiotic drive phenomenon (Raju, 1994) that is known in *G. fujikuroi* (Kathariou and Spieth, 1982; Sidhu, 1984) and several other fungi, e.g., *Neurospora* spp. (Turner and Perkins, 1979), *Podospora* (Padieu and Bernet, 1967), and *C. heterostrophus* (Bronson et al., 1990), and has homologues in higher eukaryotes such as *Drosophila*, mice, and wheat (Lyttle, 1991a, 1991b, 1993). Spore-killer (*sk*) is a single gene (Kathariou and Spieth, 1982) that maps on linkage group 5 (Xu and Leslie, 1996). Two alleles are known *skK* and *skS*. In crosses that are homozygous for either allele, all asci are normal and have eight viable ascospores. In *skK skS*, however asci contain only four ascospores all *skK*. The efficiency of the killing process can vary somewhat with the alleles involved. The level of killing must be relatively high, usually anything less than a 3:1 *skK*:*skS* ratio cannot be reliably detected, and often approaches near 100% efficiency. The mapping population developed by Xu and Leslie (1996) and also used by Jurgenson et al. (1999) segregates 120:1 *skK*:*skS*. Skewing of segregation ratios around this marker continues to the end of the chromosome in one direction and for at least 62 cM in the other (Xu and Leslie, 1996). Unlike two other well-studied fungi, *C. heterostrophus* (Tzeng et al., 1992.) and *N. crassa* (Campbell and Turner, 1987), there is no evidence for a chromosome rearrangement's association with the chromosomal region that contains *sk*. The molecular basis for segregation distortion has been elucidated in *Drosophila* (Powers and Ganetzky, 1991; Temin et al., 1991), but it is not known if the *sk*-phenomenon in fungi has the same or a different molecular basis.

Population Analyses

The fungi that form this group are not uniform under field conditions. Among traits that are known to vary are: vegetative compatibility (Leslie, 1993), female fertility (Leslie and Klein, 1996), perithecial development (Chaisrisook and Leslie, 1990), mating type (Leslie and Klein, 1996), spore-killer (Kathariou and Spieth, 1982; Sidhu, 1984), and secondary metabolite production (Leslie et al., 1992a, b, 1996; Plattner et al., 1996). No evidence has been found for chromosome rearrangements, suggesting that sexual reproduction is a significant portion of the life cycle.

Researchers studying the genetics of this group of fungi often have focused on population analyses. In many cases, the different biological species all were lumped into the same morphological species, significantly complicating the analysis of the data. The lack of a multiple-copy RFLP probe, such as those used in *C. parasitica* (Milgroom et al., 1992) and *Magnaporthe grisea* (Levy et al., 1991), that permit the assessment of multiple genetic loci at one time, also has hampered genetic studies of populations of *G. fujikuroi*. RAPDs have been used in some cases, but these results are often difficult to repeat from lab to lab and the markers produced do not always segregate in the expected Mendelian fashion.

Some population surveys have been done in which observations were limited to biologically cohesive groups, e.g. Campbell et al., 1992; Desjardins et al., 1994; Kedera et al., 1994; Leslie et al., 1992a; Mansuetus et al., 1997. These surveys have found that *G. fujikuroi* mating population A populations usually have relatively high levels of genotype variation, moderate levels of female fertility, and relatively few isolates from different plants appear to be clones. Mating population D usually resembles mating population A in most of these features, but is usually recovered in smaller quantities making such analyses more difficult. Mating population F appears to be relatively clonal in nature (Mansuetus et al., 1997), with 10 VCGs encompassing 75% of the strains recovered from Kansas sorghum fields (Klittich and Leslie, 1988b).

One objective method for comparing populations is the effective population number *Ne* (Caballero, 1994). Equations adapting this concept to the haploid nature and unusual features of ascomycetes' life cycle were developed by Leslie and Klein (1996) and have been used to analyze a global collection of strains of *G. fujikuroi*. Local populations have also been analyzed in some cases (Britz et al., 1998; *Mansuetus* et al., 1997). Both the absolute frequency of female-fertile strains and the relative frequency of strains of different mating type play a role in determining *Ne*. These equations also can be used to estimate the number of sexual generations that occur per asexual generation. *Ne* is usually limited much more by the number of available female fertile strains than it is by the relative frequency of the two mating type alleles. In some cases the frequency of female fertile strains can be little more than 10%, and sexual reproduction is predicted to occur no more than once every several hundred asexual generations. Even in populations in which sexual reproduction appears to be an important part of the natural history of the organism, the level of female fertile strains appears only rarely to exceed 50%.

Areas for Future Research

Many areas of research are possible with strains in the *G. fujikuroi* species complex. The availability of standard strains and established techniques for classical, molecular and population genetic analyses endow these organisms with promise that cannot be attached to most other plant pathogenic filamentous fungi. A few areas that I think are particularly promising are outlined below, but this list is meant to be suggestive and is certainly not inclusive.

AFLP vs. sequence. Both AFLPs and the sequences of

individual genes or sequences can be used to build phylogenetic trees and to estimate relatedness between strains. The *G. fujikuroi* group should be of particular interest as relatively large numbers of strains from many of the biological species are available and it should be relatively easy to distinguish intra-specific and inter-specific variation. The global distribution of many of these species also offers the opportunity to examine geographic differentiation of populations and, perhaps, incipient speciation and the process that accompanies it. AFLPs, because they can be selected to be genetically independent of one another, probably will be of more use than the sequences for the analysis of populations.

Genome organization. The relatedness of the different species may also provide a point of reference from which differences in genome organization can be systematically explored. Overall differences can be studied using CHEF gels and cross-hybridization of common markers. However, specific differences may be more interesting. For example, how similar are the small, presumably dispensable chromosomes? The level of variation both within and between species will be of interest. Another question of interest is the similarity between regions that encode for secondary metabolites such as fumonisins and gibberellic acids. Comparing strains from species that do and do not synthesize these compounds could be particularly informative. The clustered nature of the genes governing the synthesis of these metabolites has prompted some to speculate that these genes are acquired or lost as a set. If so, then species that do not synthesize a compound might reasonably be expected to lack these gene sets in their entirety, and not to be lacking a single gene or carrying only inactivated copies.

***vic* genes and hierarchy.** The *G. fujikuroi* species complex is uniquely positioned for studies of the genes that govern vegetative compatibility. Mutants in the *his*, *vic*, and *spc* classes are all available for studies and have been localized, with some accuracy, on a map that should enable their cloning via genetic walking. Comparison of cloned genes with similar function between different species should allow new insights into the molecular basis of the vegetative incompatibility interaction. The *spc1* mutant could give insights into the nature of the mechanism that generally acts to keep different species from forming heterokaryons.

Common controls on secondary metabolism. This area might be the one in which genetic studies could contribute the most to basic understanding of fungal physiology and gene regulation. Most of the species in the *G. fujikuroi* species complex can synthesize several different secondary metabolites, both characterized and otherwise, e.g., Leslie et al. (1996). Yet these metabolites are not all synthesized at the same time, implying that they are sensitive to different physiological signals. Since synthesis is delayed, be definition, until stationary phase, the identification of genes that regulate secondary metabolite biosynthesis in these fungi may uncover genes that affect not only the specific pathways, but also may act in a more global fashion. Identifying a global regulator of secondary metabolism could be of particular interest since it could then be the target of studies designed to inactivate it and limit the degree to which these fungi can contaminate food- and feedstuffs with noxious secondary metabolites.

Native vs. agricultural hosts and communities. Members of the *G. fujikuroi* species complex can be found in both agriculturally important monoculture crops and in native plants that are not of specific economic importance. Comparing strains from these different sources could provide new insights into traits that have been acquired to facilitate the transition from a prairie-grass endophyte to a major plant pathogen. The *Ne* of native populations and the spectrum of secondary metabolites produced also could provide new insights into how these characters have changed in the transition from native flora to agricultural fields.

Conclusions

The *Gibberella fujikuroi* species complex is relatively well-characterized genetically for a fungal plant pathogen. A number of interesting traits are known to be segregating in field populations including vegetative compatibility, spore-killer, and secondary metabolite production. Techniques and standard strains are available for classical, molecular and population genetic analyses, and most procedures can be completed in a timely manner under laboratory conditions. The spectrum of problems available for study is large and diverse and there certainly is room for many more investigators to study this organism than do at present.

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