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Inter- and intra-specific genetic variation in *Fusarium*

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Abstract

Genetic variation occurs at all levels across the genus *Fusarium*. In some cases such variation has been used to define species, and in others to describe populations or lineages. When amplified fragment length polymorphisms (AFLPs) are evaluated, strains in different species usually share at least 60% of the fragments and those in different species 40% of the fragments, or less, with isolates sharing between 40 and 60% of the fragments in an indeterminant situation. This gray area also is reflected in morphological characters, usually indistinguishable, and cross-fertility, usually some cross-fertility but often not as fertile as are strains that are more closely related. In terms of DNA sequence, the genes used for species diagnostics often have not been tested on large numbers of strains. For example, the *TRI101* gene of *F. graminearum* contains at least 25 single nucleotide polymorphisms (SNPs) from 36 strains and yielded 17 alleles that have been proposed as a means to subdivide this species into at least nine. However these subdivisions fare poorly as more strains are analyzed, with the number of alleles increasing to >40 when ~500 strains from Korea and South America are sequenced. Some of the newly identified alleles cannot be correctly assigned to one of the nine subdivisions based on the proposed diagnostic SNPs. Before SNPs are proposed as characters to define species, it is important to verify their specificity based on a sufficiently large sample and to evaluate the genetic variation present in terms of an independent measure of genetic relationships. Only in such a manner can names that are meaningful in the context of trade and quarantine regulations be developed.
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Keywords: Amplified fragment length polymorphisms; Biological species; *Fusarium graminearum*; *Gibberella zeae*; Phylogenetic species; *TRI101*

1. Species definitions

Species of *Fusarium* have traditionally been defined with morphological characters. These characters include the shape and size of the macroconidia, the presence or absence of chlamydospores, and the presence/absence, shape and supporting structures of the microconidia (Leslie and Summerell, 2006). Such morphological species definitions have been the basis for much of the taxonomic work that has been done with *Fusarium*, *e.g.*, Wollenweber and Reinking (1935), Snyder and Hansen (1945), Gerlach and Nirenberg (1982) and Nelson et al. (1983). Taxa were most commonly defined at a species level, but varieties were recognized in some species and *forma specialis* that differ in plant pathogenicity recognized in others. The strains within a *forma specialis* often differ significantly based on DNA sequence markers and need not be monophyletic in origin (Baayen et al., 2000; O'Donnell et al., 1998b). Thus, plant pathogenicity traits of significant economic importance need not be evolutionarily conserved characters and may not be part of a reliable species description.

By the mid-1980s, it was apparent that many morphologically described species contained multiple distinct entities that could be differentiated if additional traits were used. The most common of these was cross-fertility, which leads to an application of the biological species concept to *Fusarium*. The various groups were first termed mating populations and some of these were identified within both *Fusarium moniliforme* (Hsieh et al., 1977) and *Fusarium solani* (Matuo and Snyder, 1973). Based on a biological species definition, all of these mating populations were distinct species.

Anonymous DNA markers such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), and Amplified Fragment Length

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Table 1
Relationship between sexual name, and asexual name, and mating population of species in the *Gibberella fujikuroi* species complex

Fusarium species	*Gibberella* species	*G. fujikuroi* mating population	Reference
F. verticillioides	*G. moniliformis*	A	Seifert et al. (2003)
F. sacchari	*G. sacchari*	B	Leslie et al. (2005)
F. fujikuroi	*G. fujikuroi*	C	Samuels et al. (2001)
F. proliferatum	*G. intermedia*	D	Samuels et al. (2001)
F. subglutinans	*G. subglutinans*	E	Samuels et al. (2001)
F. thapsinum	*G. thapsina*	F	Klittich et al. (1997)
F. nygamai	*G. nygamai*	G	Klaasen and Nelson (1996)
F. circinatum	*F. circinata*	H	Britz et al. (2002)
F. xylarioides	*G. xylarioides*	I	(Geiser et al., 2005; Lepoint et al., 2005)
F. pseudonygamai	Not yet named	J	Leslie et al., unpublished

Polymorphisms (AFLPs) often have been used as presence/absence markers in species diagnostics. For RFLPs and RAPDs individual bands may be of critical importance, or a fingerprinting type pattern could be developed based on a sequence that often included a portion of a transposable element. For AFLPs, individual bands usually are not critically important, with the overall similarity, in terms of the proportion of bands present in both isolates, the value assessed. Within the *Liseola* section, strains that share ~60% or more of their bands are usually in the same species, those that share <40% of the bands are in different species and those that share 40–60% of their bands require additional evaluation to determine whether they are in the same or different species (Marasas et al., 2001).

The mating populations within the *Liseola* section, approximately *F. moniliforme* as defined by Snyder and Hansen, were initially three and then expanded to five (Leslie, 1991). These five mating populations are now recognized as distinct species (Table 1) and at least five additional mating populations/species have been described. Identification of a sexual stage under laboratory conditions usually requires a cross with a strain that is highly female-fertile. In some cases field strains can be used for this purpose, *e.g. F. verticillioides*, but in other cases one, or more, crosses are required to generate a suitably fertile strain for one or both of the mating types. In such cases one of the mating type tester strains usually serves as the holotype for the species, *e.g. F. thapsinum* (Klittich et al., 1997) and *F. circinatum* (Britz et al., 2002). In practice, all members of a single biological species belong to the same morphological species, but multiple biological species, *i.e.* sibling species, may be found within a single morphological species.

With the advent of DNA sequencing, sequence similarity at one or more diagnostic loci has become an important criterion in distinguishing species. The sequences most commonly used to distinguish *Fusarium* spp. are portions of the genomic sequences encoding translocation elongation factor 1-α (*tef1*), β-tubulin (*tub2*), and the internally transcribed spacer regions in the ribosomal repeat region (*ITS1* and *ITS2*). Not all sequences work equally well for all species, with *tef1* the most widely useable across the genus. The ITS regions do not work well within the *Liseola* section and its near relatives, and *tub2* does not work well within the *F. solani* species complex. Genes encoding other proteins, *e.g.*, histone H3, calmodulin, *TRI101* and mating type amongst others, also have been used to distinguish species in different portions of the genus. A critical question often is, "How different must two DNA sequences be for them to represent two different species?" The answer to this question is "enough" with "enough" best defined by a separate measure indicating a species level distinctness. "Enough" can vary by the species being compared and by the DNA sequence being examined.

The most robust species definitions are those in which the three species concepts — morphological, biological and phylogenetic — all give the same answer. Within the *Liseola* section, biological and phylogenetic species definitions have usually yielded the same answer, although a possible exception has been reported recently within *F. subglutinans*. Whether such exceptions will become more common as more strains are evaluated remains to be seen, but such problems seem more likely to be found in populations recovered from non-economic environmental settings and crops grown by subsistence farmers than from commercial production agriculture. Species concepts are by definition human constructs and strains may not all fall neatly into a particular box with a pre-existing label. Evolution should continually generate new sequences, and intermediates between various species might not yet have been completely eliminated from a population. Such intermediates may frustrate species definitions, while simultaneously validating the evolutionary process that ultimately leads to their differentiation.

2. *F. proliferatum* and *F. fujikuroi*

A problem occurs with *F. proliferatum* and *F. fujikuroi* as these two species are difficult to resolve based solely on morphology. *F. fujikuroi* is found primarily on rice in Asia where it causes the bakanae disease through the overproduction of gibberellic acid. *F. proliferatum* is widespread and occurs on hosts ranging from maize to mangoes, but it usually is not the dominant pathogen. *F. proliferatum* also can be recovered from native grasslands that have not been used agriculturally. In some cases both *F. proliferatum* and *F. fujikuroi* are recovered concurrently from rice. These two species are closely related to one another, being separated by only 15 of 1067 steps in the phylogenetic analysis of O'Donnell et al. (1998a). The AFLP similarity of the testers was ~90% for testers in the same species and ~50% if the strains compared were from different species.

The mating type tester strains for *F. proliferatum* are reliable and produce numerous fertile perithecia when crossed under appropriate conditions (Leslie and Summerell, 2006). The mating type testers for *F. fujikuroi* are neither as reliable nor as productive as those for *F. proliferatum*. In some cases, the tester strains for these two species will cross and produce a few ascospores that have reduced germinability (Leslie et al., 2004b). Amongst the progeny from such a cross approximately 1/3 of the loci segregated in the expected 1:1 Mendelian manner, another third of the loci were marginally different from 1:1, *i.e.* the probability of the observed segregation ratio was

between 1 and 5%, and a final third of the loci had ratios highly skewed (probability of a 1:1 segregation ratio was <1%). This level of cross-fertility is one that Perkins (1994) suggested might be inadequate for membership in the same species and could be indicative of inter-specific cross-fertility.

An unusual strain, KSU X-10626, was recovered from the Konza Prairie Biological Station, a native tallgrass prairie just south of Manhattan, Kansas (Leslie et al., 2004a). This strain appears to be a naturally occurring hybrid between *F. fujikuroi* and *F. proliferatum* that is between 49 and 69% similar to the mating type tester strains representing these species. X-10626 also is cross-fertile with the testers of both species with >80% of the loci in both crosses segregating in a 1:1 manner and <5% of the loci segregating in a highly skewed manner, *i.e.* the probability of a 1:1 segregation ratio was <1%. Thus this strain behaves as an intermediate between *F. fujikuroi* and *F. proliferatum*. *Fusarium* strains collected from rice by Desjardins et al. (1997) also were cross fertile with tester strains from both species. One interpretation of these data is that the apparent hybrids result from the formation of hybrid swarms (Mayr, 1963). These hybrid swarms could provide a mechanism by which new species are generated, or could enable the transfer of traits, *e.g.* mycotoxin production between species. Distinguishing the inter- and intra-specific source of such genetic variants and variation will be difficult, if not impossible.

A related, but less clear, case involves *Fusarium succisae* and *Fusarium anthophilum*. Morphologically these two species are similar, with their morphological distinctness still a matter of debate (Leslie and Summerell, 2006). No sexual stage is known for either of these species, so tests of cross-fertility are not possible. The two species are separated by 10 steps out of 1067 in the phylogenetic analysis of O'Donnell et al. (1998a). Thus these two species are phylogenetically more closely related than are *F. fujikuroi* and *F. proliferatum*, and the question of whether the genetic differences between these strains are at the intra- or inter-specific level remains to be resolved.

3. *Gibberella zeae*/*Fusarium graminearum*

F. graminearum is the asexual stage of *G. zeae* and has become a major problem in the north central United States (McMullen et al., 1997) where it has caused >$3 billion in losses to US wheat and barley crops in the 1990s alone (Windels, 2000). Initially *F. graminearum* was divided into two groups — Group 1 and Group 2 — with differences in fertility and disease association (Francis and Burgess, 1977). Group 1 strains were heterothallic and associated with crown rot of wheat. Group 2 strains were homothallic and associated with head scab of wheat and barley and with ear rot of maize. In 1999, the Group 1 strains were described as a separate species (Aoki and O'Donnell, 1999a), *Fusarium pseudograminearum*, with a distinct teleomorph, *Gibberella coronicola* (Aoki and O'Donnell, 1999b). The Group 2 strains retained the *F. graminearum*/*G. zeae* names. The two species are difficult to distinguish morphologically, with either sexual fertility or molecular markers usually required for an accurate identification (Leslie and Summerell, 2006).

A proposal has been made to subdivide the *F. graminearum* Group 2 strains into at least nine species (O'Donnell et al., 2004). The nine new species are morphologically indistinguishable (Leslie and Summerell, 2006), with only minor differences in macroconidial morphology reported (O'Donnell et al., 2004). The strains were differentiated on the basis of a series of "species-specific" single nucleotide polymorphisms (SNPs) in eight diagnostic genes. Most of the resolving power comes from SNPs in the *MAT* locus, which is functionally identical in all of these strains since they are homothallic and both the *MAT-1* and *MAT-2* sequences are found in all strains. Within the *Fusarium* community in particular and the mycology and plant pathology communities in general this proposal has received a mixed welcome. Some view the splitting as a clear example of how taxonomy should now be done while others find it to be needless splitting that results in unnecessary problems not only in terms of scientific research but also in terms of trade and quarantine.

3.1. Biological species concept for *F. graminearum*/*G. zeae*

Bowden et al. (2005) evaluated the cross-fertility of the tester strains described by O'Donnell et al. (2000, 2004). In one case, *nit* mutants were made in all of the tester strains and crosses were made between complementary *nit* mutants (Bowden and Leslie, 1992). These crosses were mixed in their success, with some working very well and some producing neither spores nor perithecia. A number of the strains used, however, were unable to produce perithecia homothallicly, suggesting that there were female fertility problems within the tester strain set. Female fertility is a problem in field populations of other *Fusarium* species (Leslie and Klein, 1996); however, many of the strains used by O'Donnell et al. have been maintained in culture collections for an extended period of time and could have lost female fertility during storage. Thus, the results from this diallel cross amongst the identified lineage tester strains were inconclusive.

To bypass this problem, Bowden et al. (2005) used the *MAT*-knockout strains developed by Lee et al. (2003) to test cross-fertility. The three *MAT*-knockout strains are all fertile as a parent in a cross, but are not homothallic, and thus require a partner to supply the missing *MAT* function before they can complete the sexual cycle. If the *MAT*-knockout strains are used as the female parent in a cross, then the perithecia that form are biparental in origin. As the *MAT*-knockout strains are highly fertile as females, the strain used as the male, *i.e.* to fertilize the cross, need not be fertile as a female for the cross to work. Thus, use of the *MAT*-knockout strains removes the problems associated with lack of female fertility that were detected in the diallel cross of the tester strains for the various lineages. All three *MAT*-knockout strains are members of lineage 7.

Bowden et al. (2005) crossed the tester strains with all three *MAT*-knockout strains. All of the tester strains crossed with at least one of the *MAT*-knockout strains at a level that yielded at least 1–10% of the spores produced by the most fertile cross observed. There was no discernable pattern in terms of crosses that was associated with the lineage to which the male parent

belonged. There was, however, evidence of strain-specific interactions. Thus, fertility barriers between strains in different lineages do exist, but these fertility barriers do not appear to be attributable to differences in lineage but instead are due to as yet unidentified sources of genetic variation. To the extent that homothallic reproduction in the population results in inbreeding, the particular interactions could result from making particular recessive meiotic alleles homozygous in a manner similar to that previously reported for *Neurospora crassa* (Leslie and Raju, 1985; Raju and Leslie, 1992). Populations of *F. graminearum* appear to be generally quite outbred, in spite of being homothallic (Schmale et al., 2006; Zeller et al., 2003a, 2004). This outbreeding suggests that most of the progeny are fit enough to persist in the population without being lost immediately to selection.

3.2. Phylogenetic species concept

The lineages in *F. graminearum* were first defined by O'Donnell et al. (2000) in which seven lineages were defined and associated with various geographic origins. The lineages were defined in terms of relatedness based on DNA sequences of a series of polymorphic genes and associated with various geographic locations. Ward et al. (2002) added an additional lineage and found that the genealogies of genes in the trichothecene gene cluster were not concordant with those used to define the lineages. O'Donnell et al. (2004) added additional genes and raised the existing lineages and one that had not been previously described to species level based on a phylogenetic species concept. They also identified a series of diagnostic SNPs that could be used to identify these species that were based in sequence differences amongst 36 strains.

We have been evaluating the proposed species by checking them for similarity with AFLP loci (Marasas et al., 2001; Zeller et al., 2003b). Testers in different lineages range between 49% and 67% similar when the bands present are scored and used to determine strain similarity. If the 40% and 60% cutoffs for different species and conspecificity proposed based on studies in the *Liseola* section were used as criteria, then none of the lineage pairs would be clearly different species (<40% similarity), and some pairs would be clearly conspecific (>60% similarity). Many of the lineage pairs have a similarity that falls in the indeterminant region between 40 and 60%. Given the relatively high cross-fertility of these strains, we argue that these strains are all a part of the same species, unlike the *F. fujikuroi*/*F. proliferatum* situation in which the AFLP similarity value is similar but the cross-fertility between the isolates is quite low, if a cross is observed at all.

A second test of the proposed phylogenetic species is to evaluate on a population level the distinctness of the proposed species by sequencing one or more of the diagnostic genes and determining the degree of sequence relatedness. If the data can be adequately represented as a tree, then the hypothesis that the sequences are derived from a series of distinct phylogenetic species is supported. If, however, a genetic network provides a better explanation for the observed data, then a single species, perhaps with a number of semi-discrete subpopulations, is a better model. The data of O'Donnell et al. (2004) for the *TRI101* gene can be represented as both a tree (Fig. 1A) and as a genetic network (Fig. 1B). The 17 identified alleles result in strongly supported branches for lineages 3, 4, 5, 6, 7 and 8 and weakly supported branches for lineage 1 and for subdivisions within lineages 5, 7 and 8. The genetic network contains a single cycle that is needed to accommodate a strain previously identified as a hybrid between lineages 2 and 6 (O'Donnell et al., 2000), although the single species-specific SNP defined by O'Donnell et al. (2004) groups this allele with lineage 2. A second cycle is attributable to the SNP at position 974 being shared by *F. crookwellense* and lineage 3. Note that this cycle means that this SNP might be effective for differentiating lineage 3 from the other *F. graminearum* lineages, but that it would not be effective in distinguishing it from *F. crookwellense*. Lineage 9 has no identified lineage specific SNPs in *TRI101*.

We sequenced a portion of the *TRI101* gene from ~400 strains collected in South America (Vargas et al., 2001) and (~100 strains) collected in Korea and generated both a tree (Fig. 2A) and a genetic network (Fig. 2B) from the data. This data set contains 42 alleles, with some being much more common than others. The data in this set are biased towards lineage 7 as this lineage was the one that was most commonly recovered from these populations. Additional strains of all of the other lineages except for lineages 4 and 5 also were recovered from at least one of the populations examined. In the tree, only the distinctness of lineages 3, 5 and 8 remain strongly supported, while the distinctness of lineage 7 is weakly supported as is the internal branching within lineage 5. The cycles previously observed in the genetic network based solely on the 17 alleles from the lineage testers remain unchanged in the more complicated network. In addition there is another cycle that contains alleles I and II, which cannot be clearly associated with any lineage, three cycles that include alleles closely related to lineage 7 and two cycles that contain only alleles from lineage 7. Several base pair changes may have occurred on multiple occasions or may have been exchanged through gene conversion or recombination (denoted with an * in Fig. 2B). Of these the base change associated with position 287 is perhaps the most interesting as it occurs in lineage 3, lineage 6, lineage 7 and in the alleles closely related to lineage 7.

Some of the individual alleles also are interesting, and their origin, in terms of their relationship with holotype allele sequences, not always easy to discern. Allele XVI contains all of the lineage six SNPs and one lineage 3 SNP. The lineage three SNP could have been introduced into the lineage six sequence either by mutation or by a gene conversion or double crossover bounded by base pairs 419 and 566. Allele I appears to be the basal allele in the network. This allele is involved in three of the minimal cycles and is the closest in sequence to the neighboring *F. crookwellense* and *F. culmorum* sequences. This allele contains none of the diagnostic SNPs associated with this locus and would by default, be placed in lineage nine by the classification scheme of O'Donnell et al. (2004), although it differs by two base changes from the holotype sequence for lineage nine. Allele II contains one lineage six SNP (389) and one lineage seven SNP (1176), but no other lineage specific SNPs. Creating

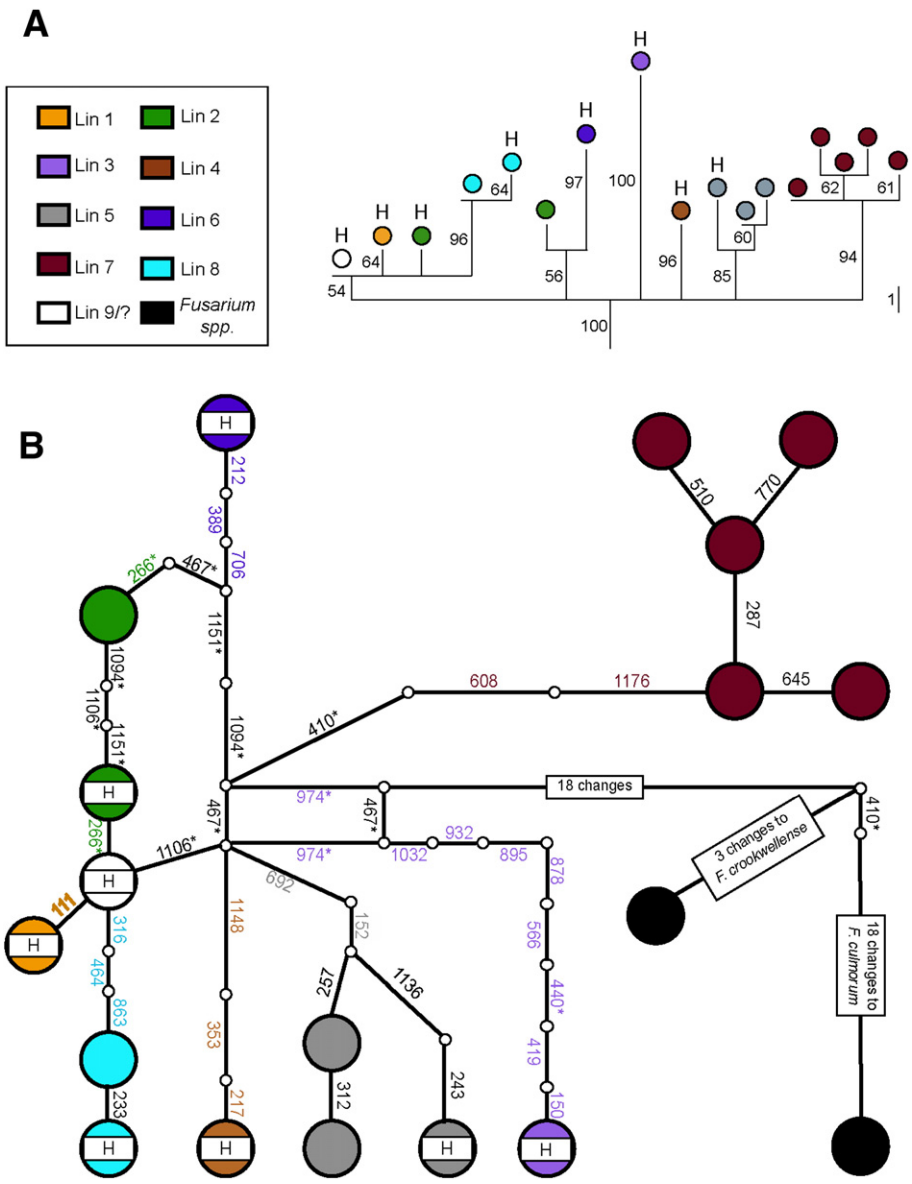

Fig. 1. Phylogenetic tree (A) and genetic network (B) diagramming the relationship of the 17 alleles identified by O'Donnell et al. (2004) in the *TRI101* gene from *F. graminearum*/*G. zeae*. Different colors indicate proposed phylogenetic species/lineages. Each circle represents a putative allele. Large circles correspond to identified alleles and small circles to alleles implied to have occurred in the evolution of the gene sequence. Numbers indicate the position in the coding sequence at which a change occurred. Numbers in color represent SNPs that have been proposed as species/lineage specific. Numbers followed by a * indicate a change that occurs more than once in the genetic network. Alleles denoted with an "H" are those found in the strains designated as the holotypes for the putative species. The tree in A is one of eight most parsimonious trees, has a length of 81, a consistency index of 0.932 and a retention index of 0.977.

this allele requires three crossovers if the holotype sequences for lineages six and seven are used as the parents (between 389 and 410, 608 and 706 and 1151 and 1176); a gene conversion event could be substituted for the two first crossovers.

Alleles related to those in lineage seven also have a complex relationship. The changes from allele I to allele IX may have occurred in the order 608, then 1176 (going through allele VII) or in the reverse order (going through allele III). The cycle involving alleles III, IV, V and VI suggests that the observed alleles that have one but not both of the lineage seven SNPs have been in the population for a considerable period of time. Note that the alleles in this cycle are found in both the South American and the Korean populations. The multiple paths from allele I to allele IX also suggests that the coalescence required by the phylogenetic species definition has not yet occurred for this gene. The two internal cycles involving alleles VIII, IX, X and XI and alleles IX, XII, XIII, and XIV, respectively, are expected in the context of a well-established population. We expect that looking at larger numbers of alleles from other lineages would further complicate this network through the introduction of other alleles not clearly identifiable to lineage and the identification of alternative pathways through which an allele sequence might have been derived.

The comparison of the two trees is informative. The Lineage tester only tree (Fig. 1A) is one of eight most parsimonious trees, has a total length of 81, a consistency index of 0.932, and

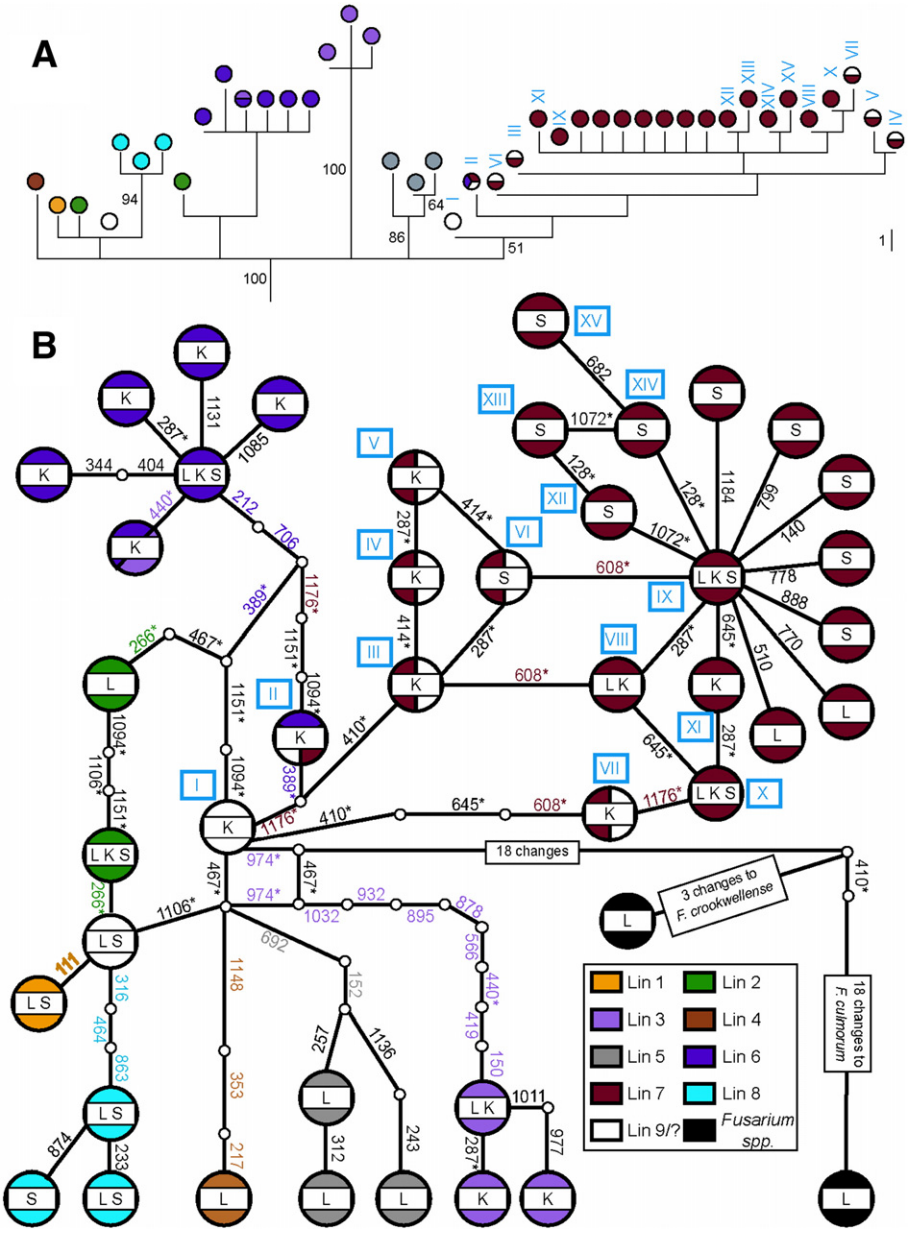

Fig. 2. Phylogenetic tree (A) and genetic network (B) diagramming the relationship of the 42 alleles identified by O'Donnell et al. (2004) and from strains from South America and Korea in the *TRI101* gene from *F. graminearum*/*G. zeae*. Different colors indicate proposed phylogenetic species/lineages. Each circle represents a putative allele. Large circles correspond to identified alleles and small circles to alleles implied to have occurred in the evolution of the gene sequence. Numbers indicate the position in the coding sequence at which a change occurred. Numbers in color represent SNPs that have been proposed as species/lineage specific. Numbers followed by a * indicate a change that occurs more than once in the genetic network. Alleles denoted with an "L" are those found in the strains of O'Donnell et al. (2004), with an "S" are strains from South America and with a "K" are from Korea. Critical alleles in A and B are identified by a Roman numeral to facilitate discussion. The tree in A is one of 8820 most parsimonious trees, has a length of 102, a consistency index of 0.778 and a retention index of 0.918.

a retention index of 0.977. The population based tree is one of 8820 most parsimonious trees, has a length of 102, a consistency index of 0.778, and a retention index of 0.918. The numerous cycles in the genetic network of the population tree clearly contribute to the much higher homoplasy. The data indicate that sample size is very important in determining whether the observed genetic variation is of an intra- or inter-specific nature. Variants intermediate between alleles previously predicted to be allele-specific are consistent with population-based (intraspecific) genetic variation, but are problematic for species-based (interspecific) genetic variation. We interpret the genetic network (Fig. 2B) based on the population level sample to mean that there is only a single species within *F. graminearum*/*G. zeae* and not nine species as proposed by O'Donnell et al. (2004).

4. Conclusions

Genetic variation is important in distinguishing isolates, populations and species of *Fusarium*, and has become increasingly important with the relative ease of obtaining DNA sequences of diagnostic genes or the banding patterns associated

with a series of anonymous bands on a gel. The utility of these data in determining relationships requires that they be "calibrated" by an independent method and that the data set be large enough to encompass the bulk of the alleles likely to be present. In such cases the distinction between inter- and intra-specific genetic variation is much easier to discern and conclusions regarding the potential evolutionary trajectory of the group easier to discern and evaluate.

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References

Aoki, T., O'Donnell, K., 1999a. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91, 597–609.

Aoki, T., O'Donnell, K., 1999b. Morphological chacterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*. Mycoscience 40, 443–453.

Baayen, R.P., O'Donnell, K., Bonants, P.J., Cigelnik, E., Kroon, L.P.N.M., Roebroeck, E.J.A., Waalwijk, C., 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic *formae speciales* causing wilt and rot disease. Phytopathology 90, 891–900.

Bowden, R.L., Leslie, J.F., 1992. Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Exp. Mycol. 16, 307–315.

Bowden, R.L., Leslie, J.F., Lee, J., Lee, Y.-W., 2005. Cross fertility of lineages of *Gibberella zeae*. Fungal Genet. Newsl. 52, 60 (Supplement).

Britz, H., Coutinho, T.A., Wingfield, M.J., Marasas, W.F.O., 2002. Validation of the description of *Gibberella circinata* and morphological differentiation of the anamorph *Fusarium circinatum*. Sydowia 54, 9–22.

Desjardins, A.E., Plattner, R.D., Nelson, P.E., 1997. Production of fumonisin B_1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. Appl. Environ. Microbiol. 63, 1838–1842.

Francis, R.G., Burgess, L.W., 1977. Characteristics of two populations of *Fusarium roseum* 'Graminearum' in eastern Australia. Trans. Br. Mycol. Soc. 68, 421–427.

Geiser, D.G., Lewis-Ivey, M.L., Hazika, G., Juba, J.H., Miller, S.A., 2005. *Gibberella xylarioides* (anamorph: *Fusarium xylarioides*), a causative agent of coffee wilt disease in Africa, is a previously unidentified member of the *Gibberella fujikuroi* species complex. Mycologia 97, 191–201.

Gerlach, W., Nirenberg H., 1982. The genus *Fusarium* — a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft (Berlin-Dahlem) 209: 1–405.

Hsieh, W.H., Smith, S.N., Snyder, W.C., 1977. Mating groups in *Fusarium moniliforme*. Phytopathology 67, 1041–1043.

Klaasen, J.A., Nelson, P.E., 1996. Identification of a mating population, *Gibberella nygamai* sp. nov., within the *Fusarium nygamai* anamorph. Mycologia 88, 965–969.

Klittich, C.J.R., Leslie, J.F., Nelson, P.E., Marasas, W.F.O., 1997. *Fusarium thapsinum* (*Gibberella thapsina*): a new species in section *Liseola* from sorghum. Mycologia 89, 643–652.

Lee, J., Lee, T., Lee, Y.-W., Yun, S.-H., Turgeon, B.G., 2003. Shifting fungal reproductive mode by manipulation of *mating type* genes: obligatory heterothallism of *Gibberella zeae*. Mol. Microbiol. 50, 145–152.

Lepoint, P.C.E., Munaut, F.T.J., Maraite, H.M.M., 2005. *Gibberella xylarioides sensu lato* from *Coffea canephora*: a new mating population in the *Gibberella fujikuroi* species complex. Appl. Environ. Microbiol. 71, 8466–8471.

Leslie, J.F., 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 81, 1058–1060.

Leslie, J.F., Klein, K.K., 1996. Female fertility and mating-type effects on effective population size and evolution in filamentous fungi. Genetics 144, 557–567.

Leslie, J.F., Raju, N.B., 1985. Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. Genetics 111, 759–777.

Leslie, J.F., Summerell, B.A., 2006. The *Fusarium* Laboratory Manual. Blackwell Professional, Ames, Iowa.

Leslie, J.F., Zeller, K.A., Logrieco, A., Mulè, G., Morretti, A., Ritieni, A., 2004a. Species diversity and toxin production by strains in the *Gibberella fujikuroi* species complex isolated from native grasses in Kansas. Appl. Environ. Microbiol. 70, 2254–2262.

Leslie, J.F., Zeller, K.A., Wohler, M., Summerell, B.A., 2004b. Interfertility of two mating populations in the *Gibberella fujikuroi* species complex. Eur. J. Plant Pathol. 110, 611–618.

Leslie, J.F., Summerell, B.A., Bullock, S., Doe, F.J., 2005. *Gibberella sacchari*: the teleomorph of *Fusarium sacchari*. Mycologia 97, 718–724.

Marasas, W.F.O., Rheeder, J.P., Lamprecht, S.C., Zeller, K.A., Leslie, J.F., 2001. *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia 93, 1203–1210.

Matuo, T., Snyder, W.C., 1973. Use of morphology and mating populations in the identification of special forms in *Fusarium solani*. Phytopathology 63, 562–565.

Mayr, E., 1963. Animal Species and Evolution. Belknap Press, Cambridge, Massachusetts.

McMullen, M.P., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81, 1340–1348.

Nelson, P.E., Toussoun, T.A., Marasas, W.F.O., 1983. *Fusarium* Species: an Illustrated Manual for Identification. Pennsylvania State University Press, University Park, Pennsylvania.

O'Donnell, K., Cigelnik, E., Nirenberg, H.I., 1998a. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90, 465–493.

O'Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C., 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. U. S. A. 95, 2044–2049.

O'Donnell, K., Kistler, H.C., Tacke, B.K., Casper, H.H., 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U. S. A. 97, 7905–7910.

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., Aoki, T., 2004. Genealogical concordance between the mating-type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. 41, 600–623.

Perkins, D.D., 1994. How should the interfertility of interspecies crosses be designated? Mycologia 86, 758–761.

Raju, N.B., Leslie, J.F., 1992. Cytology of recessive sexual-phase mutants from wild strains of *Neurospora crassa*. Genome 35, 816–825.

Samuels, G.J., Nirenberg, H.I., Seifert, K.A., 2001. Perithecial species of *Fusarium*. In: Summerell, B.A., Leslie, J.F., Backhouse, D., Bryden, W.L., Burgess, L.W. (Eds.), Fusarium: Paul E. Nelson Memorial Symposium. APS Press, St. Paul, Minnesota, pp. 1–14.

Schmale, D.G., Leslie, J.F., Zeller, K.A., Saleh, A.A., Shields, E.J., Bergstrom, G.C., 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96, 1021–1026.

Seifert, K.A., Aoki, T., Baayen, R.P., Brayford, D., Burgess, L.W., Chulze, S., Gams, W., Geiser, D., de Gruyter, D.J., Leslie, J.F., Logrieco, A., Marasas, W.F.O., Nirenberg, H.I., O'Donnell, K., Rheeder, J.P., Samuels, G.J., Summerell, B.A., Thrane, U., Waalwijk, C., 2003. The name *Fusarium moniliforme* should no longer be used. Mycol. Res. 107, 643–644.

Snyder, W.C., Hansen, H.N., 1945. The species concept in *Fusarium* with reference to *Discolor* and other sections. Am. J. Bot. 32, 657–666.

Vargas, J.I., Bowden, R.L., Zeller, K.A., Leslie, J.F., 2001. Comparisons of North and South American populations of *Gibberella zeae*. Phytopathology 91, s91.

Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E., O'Donnell, K., 2002. Ancestral polymorphisms and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc. Natl. Acad. Sci. U. S. A. 99, 9278–9283.

Windels, C.E., 2000. Economic and social impact of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology 90, 17–21.

Wollenweber, H.W., Reinking, O.A., 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekampfung. Paul Parey, Berlin, Germany.

Zeller, K.A., Bowden, R.L., Leslie, J.F., 2003a. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93, 874–880.

Zeller, K.A., Summerell, B.A., Bullock, S., Leslie, J.F., 2003b. *Gibberella konza* (*Fusarium konzum*) sp. nov., a new biological species within the *Gibberella fujikuroi* species complex from prairie grass. Mycologia 95, 943–954.

Zeller, K.A., Bowden, R.L., Leslie, J.F., 2004. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* in the United States. Mol. Ecol. 13, 563–571.