Genomic Organization and Mechanisms of Inheritance in Arbuscular Mycorrhizal Fungi: Contrasting the Evidence and Implications of Current Theories

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1 Introduction

Most plants, including the majority of crop species, associate with a specific group of soil fungi called arbuscular mycorrhizal (AM) fungi. These fungi facilitate the uptake of plant resources and are increasingly acknowledged as being critical to ecosystem function. This high level of ecological success is not expected for species that reproduce asexually, as they are expected to be vulnerable to accumulation of deleterious mutations (Muller 1932; Kondrashov 1988) and pathogenesis (Hamilton 1980; Lively 1987). Rather, AM fungi have flourished since the origin of plants (Pirozynski and Malloch 1975; Redecker et al. 2000), and appear to be the oldest asexual multicellular eukaryotes. While the ecological importance and evolutionary novelty of these fungi have become clear, the basic genetics of these fungi remain enigmatic. In fact, their genetic structure seems unusual in two ways. Firstly, individual cells always contain many nuclei, into the thousands or tens of thousands. And secondly, individual cells also contain very high levels of genetic variation, with as many as 13 variants at what would be expected to be single copy regions of the genome. It is likely that the presence and maintenance of this high level of standing variation is related to the long-term persistence and ecological success of these asexual species. However, basic issues such as the arrangement of this variation between, or within, nuclei remain controversial.

Two basic organizational structures have been advocated. Firstly, it is possible that all intra-cellular variation is present within individual nuclei and all of the nuclei within a cell are identical, i.e., homokaryotic (Pawlowska and Taylor 2004; Pawloska 2005). Alternatively, much of the genetic variation may be distributed between nuclei, with each cell containing multiple genomes, i.e., heterokaryotic (Bever and Morton 1999; Kuhn et al. 2001; Hijri and Sanders 2005). These two scenarios have very different implications for our understanding of inheritance of

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A. Varma (ed.) *Mycorrhiza*,

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genetic variation and maintenance of this genetic variation in AM fungi. In this chapter, we explore the evidence for these two scenarios and their implications.

2 Mendelian versus Non-Mendelian Inheritance

The modern study of transmission genetics began with Mendel's investigation of the inheritance of variable characters in the domesticated pea (Mendel 1865). Mendel identified the central roles of segregation and sexual recombination in the transmission of particulate inheritance, and these discoveries have since been codified into one of the unifying principles of biology (Suzuki et al. 1986). The simple segregation and recombination patterns predicted by this mode of inheritance are well described by simple statistical distributions that then formed the foundation of the fields of population and statistical genetics. With these tools, we are able to project evolutionary dynamics into the future, and make inference into evolutionary events of the past (Weir 1996; Lynch and Walsh 1998).

Mendelian transmission, however, does not apply to all genetic systems. Mendel's rules have been found to describe transmission of nuclear genes during sexual reproduction of eukaryotes. Eukaryotes, however, also have important non-nuclear genetic systems in the form of genes in mitochondria and chloroplasts. The transmission of these genes does not follow the Mendelian paradigm and they are instead generally inherited solely from the maternal parent.

Non-Mendelian genetic systems are common in fungi, in particular. Many fungi have a prolonged and dominant haploid stage. These nuclei, however, can exist alone in a single cell (monokaryon) or multiple nuclei can co-habit a single cell (di- or multikaryon). These multinucleate stages may eventually proceed to sexual fusion (karyogamy) followed quickly by meiosis. However, for some fungi, the multinucleate stages are long-lived and stable. These multinucleate stages represent interesting challenges as genetic systems. In Basidiomycetes and Ascomycetes, dikaryotic cells are formed by fusion of haploid hyphae during mating prior to meiosis (Davis 1966). Often, the hyphae are from similar "compatibility groups", but the rules for hyphal fusion can be complex. These multinucleate hyphae can be heterokaryotic (i.e., including genetically diverse nuclei). The heterokaryons can fuse with additional monokaryons or other heterokaryons and, conversely, a heterokaryon hypha can lose nuclei or bud-off monokaryons. In addition, there can be occasional non-meiotic recombination of genetic material between cohabitating nuclei within heterokaryons (i.e., parasexuality, Leslie 1993; Alexopoulos et al. 1996). Such unusual genetic processes defy easy statistical description of their transmission properties.

Genetic systems of fungi not only present challenges in understanding inheritance, but simultaneously present novel problems regarding the translation of genotype to phenotype. For example, how does the genetic material in different nuclei of a heterokaryon interact to produce a phenotype? There is much evidence of genetic complementation of mutations between nuclei cohabitating a heterokaryon. For example, two mutants of *Neurospora crassa*, one lacking the ability to synthesize

p-maniobenzoic acid and the other nicotinic acid, are able to grow as a heterokaryon on media that would otherwise inhibit either monokaryons (Ingold and Hudson 1993). This provides evidence that the phenotype can reflect the collective genotype of the multinucleate hyphae.

3 AM Fungal Cells Harbor Many Nuclei and Many Genetic Variants

The genomic structure of AM fungi is unusual in at least two respects. First, AM fungi are multinucleate at all stages of their life history. Individual cells may contain as many as a few hundreds to tens of thousands of nuclei depending on the fungal species and the method of analysis employed (Cooke et al. 1987; Becard and Pfeffer 1993; Hosny et al. 1998). As the AM fungal hyphae lack regular septa and the fungi do not appear to go through a uni-nucleate or sexual stage, the vegetative structures can be thought of as free-flowing populations of nuclei.

The second unusual aspect of their genetics is that individual cells can have very large amounts of genetic variation, with repetitive regions such as ribosomal RNA genes (rDNA) having several genetically different copies derived from single spores (Sanders et al. 1995; Lloyd-MacGilp et al. 1996; Hijri et al. 1999; Clapp et al. 1999; Pringle et al. 2000; Pawlowska and Taylor 2004). While a component of this considerable variation has been found to be due to non-mycorrhizal fungi that cohabit with, and contaminate, AM fungi (Hijri et al. 2002), these contaminants do not negate the high diversity of rDNA of AM fungal origin (Pringle et al. 2003). Moreover, a similar level of variation within spores has been observed within single copy regions of the genome (Kuhn et al. 2001; Pawlowska and Taylor 2004), with, for example, 13 different variants of putatively single copy gene, DNA polymerase 1 (PLS1), being found within individual spores of *Glomus etunicatum*. The processes maintaining the high intracellular genetic diversity may be critical to our understanding of the long-term persistence of these asexual lineages.

4 Alternative Hypotheses on Genomic Organization: The Evidence

The distribution of allelic diversity within the cells, as well as the genetic processes maintaining the variation, has been a matter of considerable dispute. The genetic diversity within single cells could be distributed within or among nuclei. At one extreme, all of the variation could be held within any one nucleus and all nuclei within a cell may then be identical (i.e., they are *homokaryotic*). Alternatively, nuclei within a cell could be genetically different (i.e., they are *heterokaryotic*), in which case there could be substantial variation between nuclei.

Over the last ten years, three types of evidence have arisen which inform this question: (1) attempts to directly score nuclei within hyphae, (2) tests of segregation of variants, and (3) tests of recombination between loci.

4.1 Evidence from Direct Scoring of Nuclei within AMF Cells

Hijri and colleagues (1999) attempted to score individual nuclei by serial dilution of an extract of nuclei derived from a single spore of *Scutellospora castanea*. A solution of nuclei was diluted until an estimated 43% of the total samples contained nuclei and 66% of these samples were expected to be harboring a single nucleus. Hijri and colleagues then amplified and scored the size of a region of rDNA that had been found to be polymorphic within a single spore. Individual amplification was found to contain different sized fragments, which is consistent with expectations from the original spore being heterokaryotic. With this design, there is a risk that DNA had broke free from nuclei before the dilution. However, this would have changed the expectations for the proportion of samples that contain nuclei, and the proportion observed was consistent with these expectations. Moreover, the control amplifications from multinuclear extractions consistently showed strong polymorphisms with the rDNA indicating that the results were not PCR artifacts.

Trouvelot and colleagues (1999) were the first to attempt to visualize different nuclei types within cells of AM fungi by developing markers of variation in rDNA and labeling the 28S rDNA markers using fluorescent tags. Spores were squashed, nuclei fixed to slides and the DNA labeled with fluorescent markers (via fluorescent DNA-DNA in situ hybridization (FISH)). Using this technique on spores of *S. castanea, Glomus intraradices, G. mosseae,* and *Gigaspora rosea*, they observed what appeared to be nuclei that harbored genetically different rDNA markers, indicating heterokaryotic nuclei within spores. This interpretation, however, is weakened by the fact that the annealing rate of the label was not controlled and low rates of labeling could generate the appearance of different nuclei types from nuclei that are in fact homokaryotic. Kuhn and coworkers (2001) again used the FISH approach to score DNA of spores of *S. castanea* labeling with two variants of the rDNA internal transcribed spacer sequences (ITS). They again found visual evidence consistent with genetically distinct nuclei being present within individual spores, as some spores were labeled by one of the two tags and other nuclei by both. The authors then developed a statistical argument in favor of heterokaryosis in which the frequency of observation of differently labeled nuclei exceeded that expected by observed frequency of labeling even if one allowed for substantial interference. Some authors, however, suggest caution in interpreting these results (Pawlowska 2005) because of the potential problems in interference of probes access to target DNA in FISH experiments can lead to discrepancies in signal detection (Yilmaz and Noguera 2004). However, the controls used for efficient probe penetration would appear to negate these suggestions, barring strong non-linearities (Kuhn et al. 2001).

Pawlowska and Taylor (2004) reported attempts to amplify rDNA from nuclei that they had dissected from a single spore of *G. etunicatum* and *G. intraradices*. They found that each of the dissected nuclei contain the same set of three ITS rDNA variants and they identified that this was consistent with a homokaryotic arrangement of molecular variants. However, Bever and Wang (2005) noted that the presence of the three types within a nucleus is not a definitive test for homokaryosis as the nuclei could still vary in the numbers for each of the three ITS types as well as in other regions of the genome. Pawlowska and Taylor (2005) suggested that changes in copy number of rDNA are not relevant, citing evidence that copy number can be dynamic within a cell cycle. While there can be rapid physiological accumulation of extra chromosomal elements of circular rRNA repeats (as cited by Pawlowska and Taylor) and these changes would not be relevant to considerations of inheritance, there is also much evidence of rapid evolution in the number of rRNA repeats within chromosomes of fungi (Zolan 1995), and this variation can be ecologically important.

4.2 Evidence from Patterns of Segregation

The homokaryotic versus heterokaryotic arrangements of genetic variation yield two different expectations for the segregation of this variation across generations. In the case of homokaryotic arrangement, all offspring are expected to have identical genetic composition to their own and to their parental spore. However, in the case of heterokaryotic arrangement of genetic variation, genetically different nuclei are expected to segregate out into different frequencies, leading to divergence of offspring hyphae. Over time, this process is expected to lead to the loss of variation from offspring spores (Bever and Morton 1999; Kuhn et al. 2001; Pawlowska and Taylor 2004; Hijri and Sander 2005).

Bever and Morton (1999) presented the first evidence for segregation of different nuclei types through observations of inheritance of spore shapes of *S. pellucida*. They found that replicate single spore isolates from a single population had different spore shapes and that the average spore shapes were highly heritable. Moreover, the cultures also differed in the variance, skewness and kurtosis of the distributions of spore shape (Fig. 1). These observations are difficult to explain under the homokaryotic hypothesis, as it would require positing that the nuclei in different spores differed in many ways, including maternal effects on the distribution of offspring spore shape. However, the results are very consistent with expectations from the heterokaryotic hypothesis. In the course of making this argument, Bever and Morton (1999) introduced a model of inheritance which we will review separately below. Pawlowska (2005) questioned this interpretation, suggesting that the different isolates may be different species. However, the morphological species concept within *Scutellospora* has been supported by both morphological and molecular evidence (Franke and Morton 1994; Morton 1995; Bentivenga and Morton 1996), and our analysis of rDNA sequences indicates that these isolates are indeed the same species (H. Kang, personal observation).

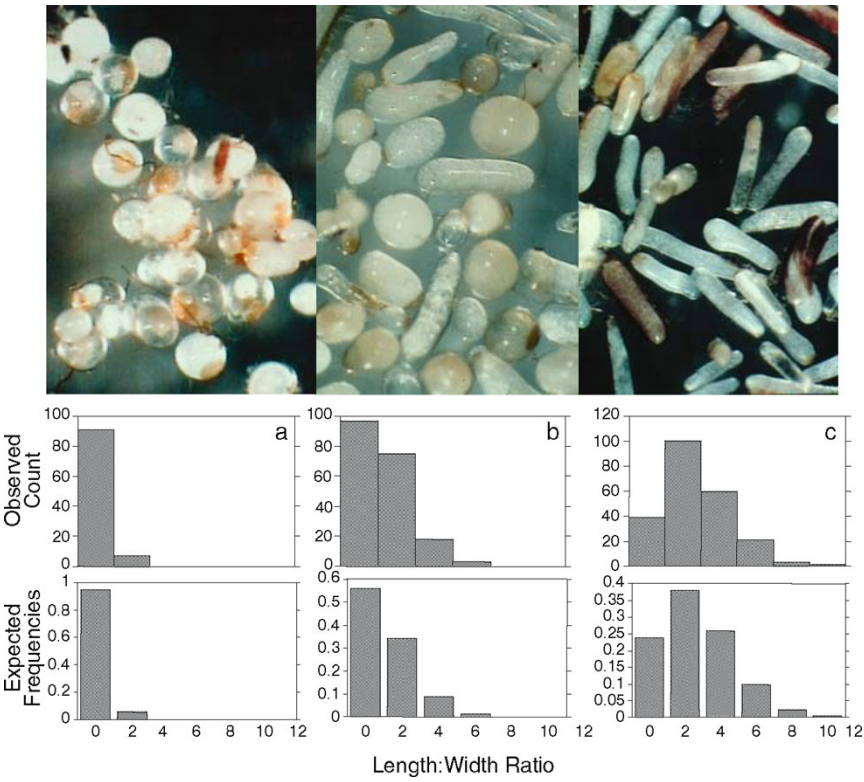

Fig. 1 Spores and spore shape distributions from three of the single spore isolates from Bever and Morton (1999). The *top histograms* represent the observed distribution of spore shape as the ratio of length over width. The *lower histograms* represent the expected distributions from the parameters fit by the model of inheritance described by Bever and Morton (1999)

Pawlowska and Taylor (2004) found that high levels of intra-cellular molecular variation within isolates of *G. etunicatum* were not lost due to segregation. They observed that each of 20 single progeny spores had all 13 variants of PLS1, a putatively single copy gene. They argued that the lack of loss of these variants was inconsistent with heterokaryotic organization of the genome, with their statistical confidence in this conclusion coming from simulations of the segregation process that assumed haploidy, no hyphal fusion and no selection. Instead, they proposed that all 13 variants of the PLS1 gene were present within each nucleus (and all nuclei in the hyphae were identical), with the persistence of the large number of variants within individual spores resulting from very high ploidy in these fungi (i.e., at least 13 ploids) and the suspension of gene conversion. This explanation came into conflict with the subsequent observation that *G. etunicatum* is actually haploid (Hijri and Sanders 2005).

Bever and Wang (2005) presented a resolution to this apparent conflict by using a simulation similar to that of Pawlowska and Taylor (2004) to demonstrate that modest

levels of hyphal fusion would allow remixing of the nuclei and reduce the effective rate of segregation to a level consistent with the Pawlowska and Taylor's laboratory observations. With sufficient rates of hyphal fusion, high levels of variation can be maintained within spores over long periods of time, which is consistent with Pawlowska and Taylor's field observations. Pawlowska and Taylor (2005) argued that there is no evidence of the level of hyphal fusion assumed in Bever and Wang's simulation, citing evidence of barriers to hyphal fusion between geographically isolated populations of *G. mosseae* (Giovannetti et al. 2003). However, this same body of work shows very high rates of hyphal fusion within isolates of many species of *Glomus* (Giovannetti et al. 1999, 2001, 2004). The simulation of Bever and Wang simply assumed that offspring from a single spore could fuse, which is exactly what had been demonstrated by the work of Giovannetti and others. Moreover, several studies of Giovannetti's group are of isolates derived from INVAM, which were derived from multiple spores (Morton et al. 1993), and other, similarly derived, INVAM cultures have been shown to be genetically heterogeneous populations (Bentivenga et al. 1997).

4.3 Evidence of Recombination from Field Samples

Tests of recombination can inform our understanding of mechanisms of inheritance in AM fungi by providing evidence for the frequency of genetic exchange. Genetic recombination could result from an unseen sexual stage (consistent with homokaryotic arrangement within hyphae), but it is more likely that such genetic exchange would result from nuclear exchange following hyphal fusion of genetically different hyphae (as is possible in the heterokaryotic model).

There have been several tests of recombination from multilocus datasets generated from field collected spores. Of these, one study found evidence of genetic exchange in two of the six unmanaged populations tested (Vanderkoornhuyse et al. 2001). This study, however, used loci identified by DNA fingerprinting and therefore the AM fungal origin of the different bands cannot be confirmed, giving rise to the possibility that the recombining bands represented patterns of infection of a contaminant micro-organism (Stuckenbrock and Rosendahl 2005; Pawlowska 2005).

A study by Stukenbrock and Rosendahl (2005) used three codominant genetic markers of confirmed AMF origin to estimate the genetic structure of two AM fungal populations from agricultural fields, and they did not find evidence of significant recombination. The clonal structure of these populations is not surprising given that the populations are continually disturbed due to cropping practice, and are likely to have been recolonized from neighboring populations, and genetically distinct initial founders of the population will generate high indices of association. Left undisturbed and assuming selective neutrality, hyphal fusion and nuclear mixing would be expected to reduce the indices of association overtime. Such decay association indices overtime would provide a much stronger test for remixing and should be the focus of future work in this area.

5 Alternative Hypotheses on Genomic Organization: The Implications

Individual scientists differ in their interpretations of the weight of available evidence on the genomic organization of AM fungi. Pawlowska (2005) is unconvinced by the work suggesting AM fungi are heterokaryotic and argues that the current work favors the homokaryotic hypothesis. While reviewing the same studies, we feel there is good evidence that AM fungi are heterokaryotic. However, at some level, the "homokaryotic" versus "heterokaryotic" dispute on the nature of AM fungal genomic structure could be regarded as simplistic. Given that AM fungi do not go through a life history stage involving a single nucleus per cell, then individual mutations will create differences between nuclei, thereby forcing heterokaryosis. The real issue then is not whether AM fungi are heterokaryotic, but to what extent are they heterokaryotic. This "degree of heterokaryosis" issue has important implications for our understanding of the evolutionary genetics of AM fungi.

5.1 Implications of Homokaryotic Genomic Organization

If AM fungi have large amounts of genetic variation contained within each nucleus, then this suggests that concerted evolution is unusually weak in these species (Pawlowska and Taylor 2004). Concerted evolution is a homogenizing force within multicopy gene families and results from infrequent unequal crossing over events occurring during mitosis and/or meiosis, which are followed by gene conversion. Over time, these processes can reduce variation within multicopy gene families, accounting for the low variation within rDNA gene families in other organisms (Hamby and Zimmer 1992; Avise 2004). It would be surprising if gene conversion were slowed in AM fungi, and would beg an explanation.

Furthermore, explanation for the long-term persistence of the asexual AM fungi in the face of mutation pressure is a challenge under the homokaryotic hypothesis. However, it is possible that the high redundancy due to polyploidy, combined with high hyphal population sizes, would slow the deleterious effects of mutation accumulation (Muller 1932) and thereby contribute to the longevity of these asexual lineages (Pawlowska and Taylor 2004).

5.2 Implications of Heterokaryotic Genomic Organization

If substantial amounts of variation are maintained between nuclei, we would need to identify what processes could contribute to the coexistence of multiple genomes, as nuclear segregation during hyphal growth and division would be expected to reduce nuclear diversity. There are several possibilities, including balancing selection and

constrained coordinated movement of nuclei. But perhaps the most straightforward mechanism is the possibility of occasional fusion of genetically differentiated hyphae.

Hyphal fusion has been observed in many different species of AM fungi. While hyphal fusion is inhibited in geographically divergent isolates, hyphal fusion rates can be very high for fungal isolates from the same proximity (Giovannatti et al. 1999, 2001; de la Providencia et al. 2005). Using a stochastic simulation of nuclear segregation, Bever and Wang (2005) demonstrated that modest rates of hyphal fusion can maintain high levels of nuclear variation within spores at equilibrium. In Fig. 2, we further demonstrate that, while very low levels of hyphal fusion (2%) are

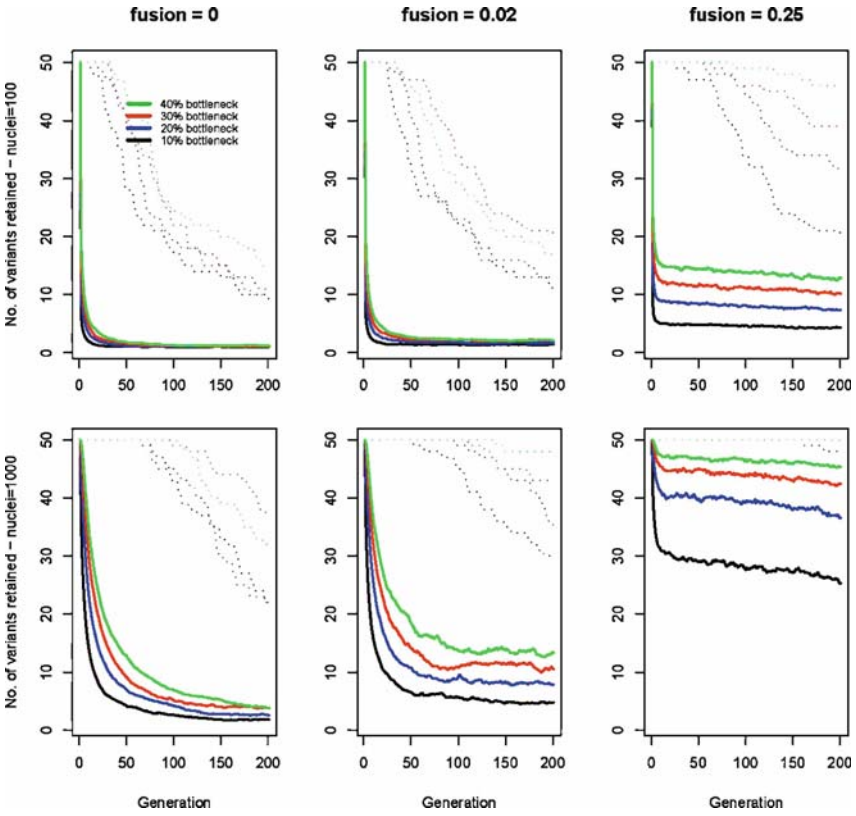

Fig. 2 We explored the implications of heterokaryotic genomic organization for the rate of decay of genetic diversity by simulating the progeny of a single spore that begins with 50 distinct nuclei types. Each generation, the nuclei are chosen from the parental population by chance at a sampling rate determined by the bottleneck rate (40, 30, 20 and 10%) with various combinations of the number of nuclei within the spores, the size of total spore population and the rate of hyphal fusion. The rate of decay in 200 generations is shown for population size of 1,000, numbers of nuclei within the spores of 100 and 1,000, and fusion rates of 0%, 2% and 25%. We monitored the decay of nuclear diversity within spores (*solid curves*) as well as the total nuclear diversity across the entire population (*dotted curves*). The results show that a little fusion goes a long way in maintaining the variants in fungi that have high numbers of nuclei

not sufficient to maintain genetic variation in hyphae that contain a hundred nuclei, these very low rates of hyphal fusion can have large effects in hyphae that contain a thousand nuclei. This result is significant as it matches with patterns of hyphal fusion within AM fungi. While smaller spored species within *Glomus* may have relatively small numbers of nuclei, which makes them very vulnerable to drift, *Glomus* generally have high rates of hyphal fusion which will reduce the rate of genetic drift (de la Providencia et al. 2005; Voets et al. 2006). In contrast, *Scutellospora* and *Gigaspora* generally appear to have lower rates of hyphal fusion (de la Providencia et al. 2005; Voets et al. 2006), but these fungi consistently have

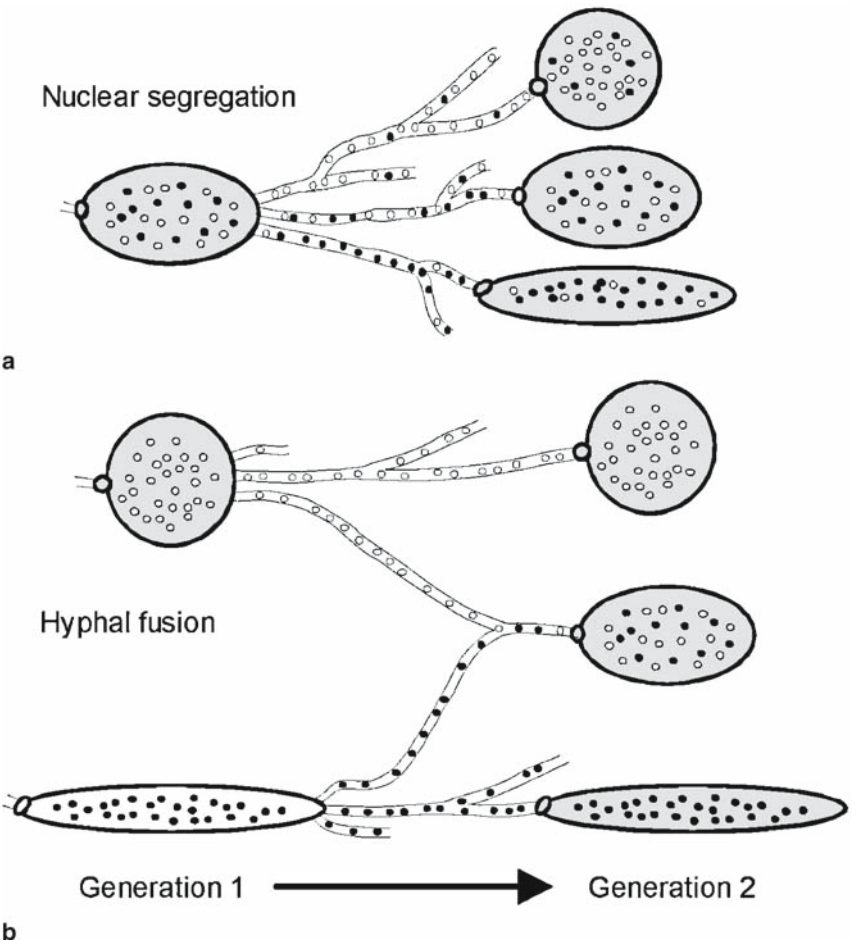

Fig. 3 Under heterokaryotic nuclear organization, the processes of nuclear segregation and hyphal fusion, (**a**) and (**b**), respectively, could segregate and remix variation in an analogous manner as meiosis and sexual gametic fusion. Spore shape is assumed to be a function of the proportion of filled nuclei (following Bever and Morton 1999)

larger numbers of nuclei with estimates ranging from a thousand to tens of thousands (Hosny et al. 1998). As our results show, with these large number of nuclei, the low rates of hyphal fusion can go a long way toward reducing the rate of drift (Fig. 2). These results suggest that hyphal fusion rates are sufficient to offset the force of drift in AM fungi, potentially providing an explanation for persistence of high levels of variation in AM fungal nuclei.

In this heterokaryotic scenario, alternative genetic processes could mimic the benefits of sexual recombination (Bever and Morton 1999). Variation that exists between nuclei would segregate as hyphae grow and divide (Fig. 3a), a process analogous to assortment during meiosis. Fusion of genetically different hyphae could remix and recombine variation (Fig. 3b) in an analogous manner as fusion of gametes in sexual organisms. Assuming that the phenotype is a function of the nuclear composition of the hyphae, this process could mimic the creative process of sexual reproduction by bringing together novel genetic variants into the same functional organism. Because the linkage groups are very large (i.e., contents of a nucleus), this process alone would not prevent the accumulation of deleterious mutations (Muller 1932). However, these processes combined with irregular parasexual recombination may minimize the accumulation of deleterious mutations.

6 A Model of Inheritance under Heterokaryosis

In the course of making an argument for the consistency of empirical observations of inheritance of spore shape in *S. pellucida* (Fig. 1), Bever and Morton (1999) proposed a simple model of inheritance under heterokaryosis. We highlight this model here as it provides insight into elements of inheritance when heterokaryotic nuclei are the source of phenotypic variation.

The segregation of heterokaryotic nuclei may be expected to generate an approximately Binomial or Multinomial distribution in offspring spores. If we imagine that two nuclei types control spore shape, one type coding for round spores and a second coding for oblong spores, the initial frequency of the oblong nuclei type would be identified as (p) in the parental spore. The rate of segregation of these nuclear types into offspring spores would be characterized by the effective number of these parental nuclei (n_e). In this model of inheritance, the key genetic parameter is n_e, as this value circumscribes the level of heterogeneity in the offspring distribution. The value of n_e will be a function of the rates of hyphal division (greater rates of division, lower n_e), rates of nuclear replication (higher rates of nuclear replication corresponds to higher average population sizes and higher n_e), and rates of hyphal fusion (higher rates of hyphal fusion will remix the nuclei, thereby raising n_e). These processes will determine how many parental nuclei will be represented in a given length of active fungal hyphae as the spore is produced.

A second set of assumptions is needed to describe the translation of hyphal genotype to phenotype. Work with ascomycetes show that genes from different nuclei can contribute to the phenotype of a hyphae (Ingold and Hudson 1993). We

assumed that there was a linear relationship between the nuclear content of the developing spore and the final shape of that spore (Bever and Morton 1999). With this model, we were able to estimate the genetic parameters, with which we could predict the expected offspring distributions. In Fig. 1, we illustrate both the observed distribution of spore shapes and the distribution of spore shapes predicted by a fit of the Binomial distribution to the data. The fit distributions are very close to the observed distributions and this close matching of the predicted and observed distributions provides support for the genetic model. From this, we suggest that the parental spores differed in their initial proportion of nuclei, with the round spore having a high abundance of nuclei coding for round shape and the oblong spores having a high abundance of nuclei coding for oblong shape. As the fungus grew (Fig. 3a), the nuclei multiplied and segregated into separate hyphae. This segregation resulted in lower variability in offspring spore shape in the lineage that was initially round (and very low p) and higher variability in the shape of offspring spores in the lineage with higher initial proportion of nuclei coding for oblong spores. In this way, the heritable differences in the mean, variance, skewness and kurtosis of spore shape may be the result of a single genetic mechanism: the segregation of heterokaryotic nuclei.

In this scenario, the segregation of nuclei alone would result in the fixation of nuclei types within spores. In fact, given our estimate of $n_e = 7$, we would expect to see noticeable fixation of lineages within our cultures. Instead, the distributions of spore shapes have been consistent over multiple generations, providing indirect evidence of an important role for hyphal fusion within these isolates.

Clearly, the utility of this model of inheritance needs to be tested for other traits and in other AM fungi. Should this model be supported, it may provide a means for gaining inference into patterns of evolution in a similar manner as Mendelian inheritance has allowed for many plants and animals (Weir 1996; Lynch and Walsh 1998).

7 Conclusions

Individual cells of AM fungi have high amounts of genetic variability and large numbers of nuclei. The arrangement of that genetic variation within or among these nuclei has been in dispute. While AM fungal hyphae are certainly heterokaryotic at some level due to the inevitability of mutation, the extent of inter-nuclear variation maintained within hyphae is difficult to assay. In reviewing the available evidence, we feel that there is strong support for substantial amounts of their genetic variation being maintained between nuclei. We then explored the theoretical implications of this arrangement, demonstrating that observed levels of hyphal fusion are sufficient to maintain observed levels of intra cellular variation in a heterokaryotic arrangement. We also present a simple model of inheritance with genetically divergent hyphae.

Acknowledgements We acknowledge the support of an IU Faculty Research Grant and NSF grants to J.D.B.

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