

Meiotic genes in the arbuscular mycorrhizal fungi

What for?

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The arbuscular mycorrhizal fungi (AMF) are important symbionts of land plants, which are known for their tremendous positive effects on terrestrial ecosystems, their peculiar cellular features, and their very old evolutionary history. To date, no sexual stage or apparatus have ever been observed in these organisms; a remarkable absence for a eukaryotic lineage. For this reason, AMF have long been considered an evolutionary oddity, having evolved for over 500 millions of years in the absence of sexual reproduction and meiosis. Here, we discuss the recent identification across a number of AMF genomes, of many genes that are known to be involved in the process of meiosis in several eukaryotic model species. The presence of these genes in AMF is a previously unsuspected and highly intriguing finding, which suggests the presence of a “hidden” sexual (or parasexual) reproduction that awaits formal observation in these poorly studied fungi.

AMF have been long recognized to represent a peculiar lineage of “ancient asexuals.”^{7,8} This assumption, however has been mostly based on the absence of a recognizable sexual cycle in these organisms, and by a controversial hypothesis that suggest the presence of perpetual heterokaryosis in some species (AMF cells are multinucleated);^{9–11} so it is still virtually possible that AMF may have a hidden sexual reproduction that has not yet been captured using the microscopy tools currently available.¹² In a recent study, we aimed to detect the presence of potential sexuality in AMF by searching their genomes for evidence of genes involved in the process of meiosis.¹³ Meiosis is considered a hallmark of sexual reproduction in eukaryotes, which is essential to recombine different alleles across homologous chromosomes and protect the nuclear content from deleterious mutations.^{14,15}

AMF Genomes Have the Tools to Undergo a Conventional Meiosis

Even though a battery of sequencing and bioinformatics tools are now available to sequence the genome of many organisms, the acquisition of large-scale sequence data from AMF is still a relatively hard task to accomplish. This is because AMF are obligate symbionts of plants with relatively large genomes,¹⁶ and which require tedious and time consuming cultivating techniques for propagation.¹⁷ For some time, the combination of these features has hampered the acquisition of large amount of pure, contaminant-free, nucleic acids that are necessary to perform genome sequencing using the different next

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Arbuscular mycorrhizal fungi (AMF) represent an ancient fungal lineage, with a fossil record dating back to the Ordovician (i.e., over 500 million years ago), and which evolved a close symbiotic association with the roots of most land plants (e.g., the mycorrhizal symbiosis).^{1–6} Their extreme longevity has intrigued scientists for some time, because members of this group are thought to have evolved for this long in the absence of sexual reproduction (i.e. exclusively clonally). Long-term clonal evolution is extremely rare among eukaryotes, and for this reason

generation platform that are currently available (i.e. pyrosequencing).¹⁸

Fortunately, Halary et al.¹³ have recently circumvented this problem by growing many AMF species under axenic conditions, allowing them to extract DNA in quantities large enough to perform deep genome sequencing procedures. Upon sequencing, the authors assembled relevant genome information, which they readily searched for evidence of meiosis. These searches used a set of genes (a total of 87) that are known to be involved at different stages of meiosis in the model eukaryote *Saccharomyces cerevisiae*, as queries against publicly available AMF transcriptome sequence data and newly acquired genome surveys from four species in the genus *Glomus* (i.e., *Glomus irregulare*, *Glomus diaphanum*, *Glomus clarum* and *Glomus cerebriforme*). These in silico approaches were complemented by degenerate PCR procedures, resulting in the identification of 51 AMF genes with strong homology with meiotic genes from other fungi. This gene set encodes for most of those proteins involved in the “core meiotic machinery”; a proteomic repertoire that is generally conserved across all sexual eukaryotes.^{14,15,19} This gene catalog also included seven members whose products are only known to be involved in meiosis (and no other processes) in other

eukaryotes (e.g. *Dmc1*, *Rec8*, *Spo11*, *Mnd1*, *Hop2*, *Msb4* and *Msb5*); including other fungi. Importantly, the orthology of AMF meiosis-specific was confirmed using a variety of phylogenetic methods and gene homologs from many other organisms. The identification of a conserved and expanded catalog of meiotic genes in AMF, and the confirmation of their orthology, both suggest that a typical meiosis could occur in these supposedly ancient asexual fungi. If true, AMF may not be considered as an evolutionary aberration anymore, but rather as being similar to many other well studied sexual fungi that typically undergo meiosis following exchange of genetic material and nuclear fusion.

Meiosis Has Yet to be Observed in AMF

Obviously, many questions remain as to whether these newly identified genes are truly used for meiosis by AMF; especially because this process has so far never been observed in members of this fungal lineage. AMF nuclei are currently thought to be haploid, so one mechanism that could trigger meiosis in this group is nuclear fusion. In particular, similar cellular events are known to create diploid nuclei in other fungi, which readily

undergo meiosis to produce a haploid nuclear progeny with novel allelic combinations (Fig. 1).²⁰⁻²² This mechanism may also be followed by certain AMF individuals to reduce the load of deleterious mutations that are carried by their nuclei (e.g., Muller's ratchet). Unfortunately, however, although nuclear exchange has been reported to occur between members of one AMF species following anastomoses (cytoplasmic fusion and exchange between hyphae),^{9,23,24} nuclear fusions have yet to be observed in these fungi. Certainly, future studies aimed at detecting the presence of fusion events using, for instance, a variety of microscopy tools are certainly warranted. Specifically, these may reveal the mechanisms that are used by AMF to buffer deleterious mutations, and would shed much needed light on our understanding of their highly successful ecology and evolution by complementing previous work reporting the presence of recombination within some of their populations.²⁵⁻²⁷

AMF May Not be that Shy After All

Several organisms long thought have evolved clonally have ultimately been found to undergo frequent sex,^{8,12,19,28-33} and recent discoveries suggest that AMF may be no different.¹³ Indeed, these

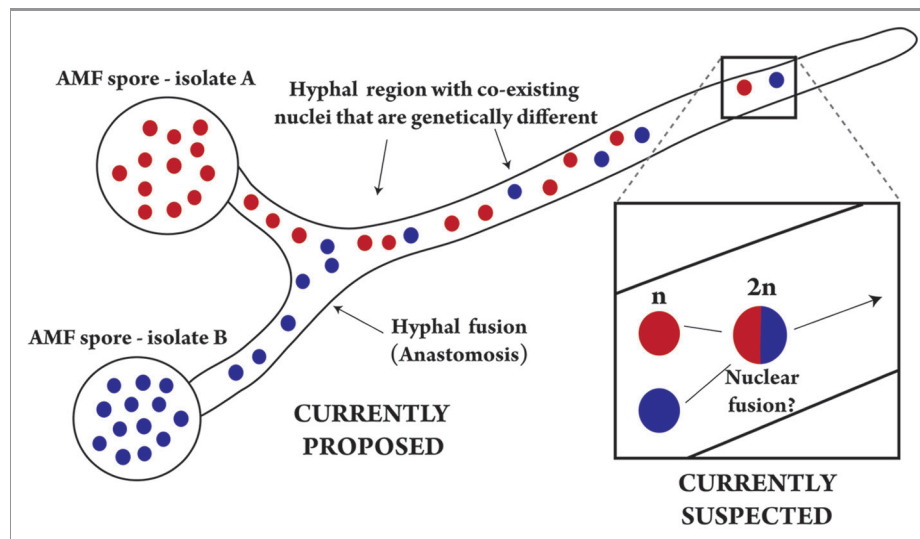


Figure 1. Causes and “potential” consequences of nuclear exchange in the arbuscular mycorrhizal fungi (AMF). Schematic representation of cellular events that may occur between genetically different AMF individuals following anastomosis; based on reference 7. Genetically different nuclei are shown in different colors (red and blue). Meiosis is unknown to occur in AMF and nuclear has only been reported to occur between AMF individuals of one species (*Glomus intraradices*).

supposedly ancient asexual fungi are now known to possess all the tools necessary to undergo a typical meiosis, and this specific phenomenon is likely to have caused the footprints of recombination that have been reported in a number of AMF populations.²⁵⁻²⁷ Hopefully, future studies will provide new insights into the presence, or absence, of sexual reproduction in these organisms; as these features may be used for the application of these obligate plant

symbionts in organic agriculture and environmental practices. In parallel, AMF genomes should also be searched for other genes known to be involved in sexual reproduction and recognition in other fungi; including those that may compose a potential AMF mating type locus.²²

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