Arbuscular mycorrhizal fungi

Hyphal fusion and multigenomic structure

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Arbuscular mycorrhizal (AM) fungi (Glomeromycota) reproduce asexually, are multinucleate, and have high genetic variation within single cells. Pawlowska and Taylor[1] find that genetic variation within AM fungal cells is not lost as a result of segregation, and they interpret this as evidence that the variation is present within each nucleus and that all nuclei within individual spores are genetically identical (that is, homokaryotic). Here we show that their empirical observations are also consistent with a distribution of genetic variation between nuclei within spores (that is, heterokaryotic), given that there is fusion of fungal hyphae. This analysis, together with complementary findings[2–4], suggests that AM fungi have an unusual genomic structure in which multiple, genetically diverse nuclei are maintained within cells through remixing by hyphal fusion.

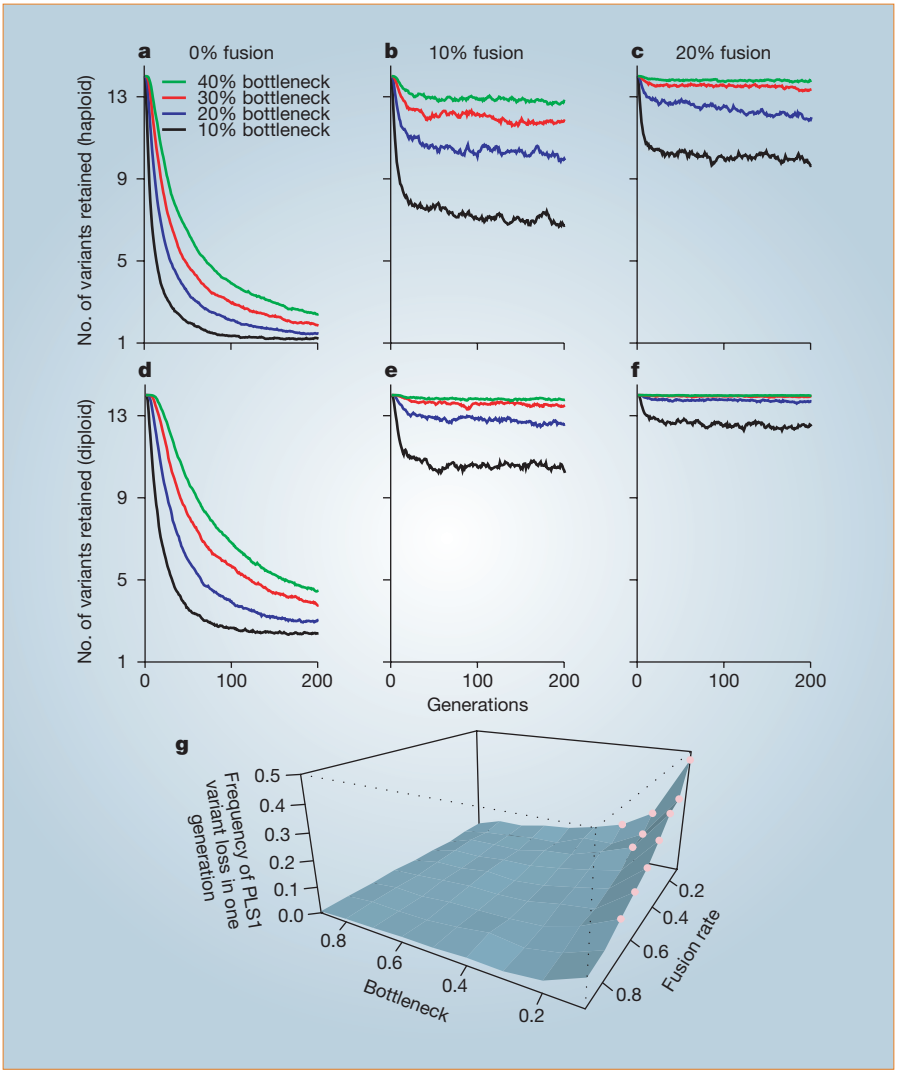

Figure 1 Retention of genetic variants in arbuscular mycorrhizal (AM) fungi. **a–f**, Summary of simulated loss of genetic variants from AM fungi under 10, 20, 30 and 40% rates of bottleneck (indicated by black, blue, red and green lines, respectively); **a–c**, haploid nuclei; **d–f**, diploid nuclei; rates of hyphal fusion, 0% (**a, d**), 10% (**b, e**) and 20% (**c, f**). **g,** The probability of losing at least one variant in one generation, given that a haploid parental spore had 13 variants. Pink points indicate the parameter region that can be excluded at the 5% significance level by the data of Pawlowska and Taylor; their data are consistent with most of the parameter space.

Methods. Our simulations of segregation of nuclei and hyphal fusion in mycorrhizal fungi generally followed those of Pawlowska and Taylor[1]. We simulated the effects of hyphal fusion by mixing a set proportion of all spores following the bottleneck. In these simulations, the total number of spores in the population was assumed to be 1,000. We simulated the rate of variant loss under the assumption of diploid nuclei by assuming that each nucleus contained two variants chosen at random from the total of 14. The frequency of losing at least one variant from spores with 13 variants was calculated as described[1]. To make these estimates, we averaged the proportion of 1,000 offspring that lost at least one variant among 2,000 different parental spores. The parental spores had 13 variants, obtained from the simulations that started with spores containing 14 variants. We also calculated the probability of observing 20 progeny spores that did not lose a variant by taking the 20th power of the probability of not losing a variant[1].

Pawlowska and Taylor observe that each of 20 single progeny spores had all 13 variants of a putative single-copy gene — that encoding DNA polymerase I. They argue that the preservation of these variants is inconsistent with heterokaryotic organization of the genome because, under this genomic structure, stochastic loss of variants would be expected. Their statistical confidence in this conclusion comes from simulations of the segregation process that assume haploidy, no hyphal fusion and no selection.

We relaxed the first two assumptions and showed that both diploidy and hyphal fusion could delay the loss of variation (Fig. 1). Hyphal fusion, in particular, has strong effects because it allows the remixing of previously separated nucleus types, thereby stemming the loss due to drift[3]. By allowing fusion of hyphae derived from a single spore, as has been empirically observed[5,6], high levels of variation can be maintained within spores over long periods, assuming either haploidy or diploidy (Fig. 1).

We calculated the likelihood of losing variants from spores with 13 variants within one generation, as did Pawlowska and Taylor[1], but we varied the rate of fusion (see Methods). As shown by Pawlowska and Taylor, we can reject the possibility that AM fungi are both haploid and have no hyphal fusion. However, we cannot reject the possibility that AM fungi are haploid and have low-to-moderate rates of hyphal fusion (Fig. 1g). For example, with a bottleneck of 20%, rates of hyphal fusion greater than 30% will reduce variant loss to that consistent with the observations of Pawlowska and Taylor. In fact, there are many combinations of bottleneck rates and hyphal fusion that can reproduce their results.

What then are reasonable rates of hyphal fusion in AM fungi? Although fusion of hyphae among geographically divergent isolates may be inhibited, rates of hyphal fusion have been found to be very high for fungal isolates from the same proximity, with fusion occurring in 60–85% of contacts between hyphae derived from spores from the same cultures[5,6]. Given this observation and those of haploid genomes in related species of AM fungi[4], we suggest that Pawlowska and Taylor's empirical observation of low rates of loss of variants may be due to heterokaryotic arrangement of the variation within spores that is maintained by hyphal fusion.

Pawlowska and Taylor also amplified the internal transcribed spacer region from microdissected nuclei and found that three variants were present in each nucleus. We note that this is not a definitive test for homokaryosis because the nuclei could still vary in the numbers of the three types of internal transcribed spacer, as well as

in other regions of the genome.

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Pawlowska and Taylor reply — To challenge the hypothesis of multigenomic structure of arbuscular mycorrhizal (AM) fungi[1,2], we presented three lines of evidence consistent with the homokaryotic organization of within-individual genetic variation, including distribution of polymorphic genetic markers among and within field isolates of an AM fungus, and distribution of ribosomal DNA variants among individually microdissected nuclei[3]. Bever and Wang suggest[4] that our data can be explained equally well by heterokaryosis, proposing a model that relies on the assumption that fusions of hyphae of genetically non-identical individuals contribute to the creation and maintenance of a multigenomic status of AM fungal cells. However, we do not believe that this assumption is supported by existing biological evidence.

To support their idea of hyphal fusion in AM fungi, Bever and Wang cite studies[5,6] that present data on successful fusions among hyphae only within an individual mycelium and among mycelia derived from spores representing the same isolate — the studies contain no results that support fusions of genetically different individuals. But Bever and Wang's formula for heterokaryon formation and maintenance requires fusions of hyphae among genetically distinct mycelia. Several studies[7,8] of self versus non-self recognition in fungi have revealed sophisticated mechanisms that prevent fusion of genetically differentiated individuals unless the partners are in the sexual mode, which has never been observed in Glomerales.

In the vegetative mode, genetic compatibility at several loci is required for a successful fusion, which effectively limits fusions of hyphae to those within an individual mycelium or among genetically identical mycelia derived from the same isolate[7,8]. Encounters among non-identical vegetative mycelia initiate a battery of antagonistic responses. Such vegetative incompatibility responses have also been reported in AM fungi during encounters between genetically differentiated isolates of *Glomus mosseae*[9], indicating that AM fungi have self-recognition mechanisms that are equally sophisticated and operate like those in other fungi. In our simulation model of heterokaryosis[3], we therefore explicitly excluded the possibility that vegetative hyphal fusions among genetically differentiated individuals could contribute to the creation and maintenance of multigenomic individuals of AM fungi.

Bever and Wang contest our evidence of the containment of the entire intrasporal rDNA variation in each individually microdissected nucleus, which they claim is not definitive as the nuclei could still vary in the number of copies of each of the rDNA types. However, the quantitative issue of copy number is not relevant to a qualitative distinction between heterokaryosis and homokaryosis. The nucleolar organizer regions, which harbour tandemly repeated rRNA gene copies, are dynamic, and the number of rRNA genes may change even during the lifespan of a single cell[10]. The model of heterokaryosis proposed for AM fungi[2], which we tested by using data from microdissection, made no claims about the number of copies of different rDNA types, but stipulated that different rDNA sequences should be distributed among different nuclei within an individual; we found no evidence to support this idea.

On the basis of our results[3] and of reports of exceptionally large genome sizes in AM fungi[11,12], we speculated that these fungi may have duplicated or polyploid genomes. A recent, considerably smaller genome-size estimate in *G. intraradices* indicates that the sizes of glomeromycotan genomes may not differ markedly from those in other fungi[13]. Bever and Wang cite this estimate as support for heterokaryosis in AM fungi. However, even very small fungal genomes contain arrays of duplicated genes, including rRNA-coding and protein-coding genes[14]. Thus, the evidence of small haploid genomes in AM fungi does not invalidate our conclusion that the intracellular genetic variation observed in these fungi is contained in each of the hundreds of nuclei that populate their cells and spores.

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