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**Hyphal Fusion Can Maintain the Multigenomic Structure
of Arbuscular Mycorrhizal Fungi***

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Hyphal fusion can maintain the multigenomic structure of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi reproduce asexually, are multinucleate, and have high levels of genetic variation within single cells. Pawlowska and Taylor¹ recently found that genetic variation within AM fungal cells was not lost due to segregation and they interpreted this as evidence that the variation was present within each nucleus and that all nuclei within individual spores were genetically identical (i.e. homokaryotic). Here, we show that the empirical observations of Pawlowska and Taylor are also consistent with a distribution of genetic variation between nuclei within spores (i.e. heterokaryotic), given that there is fusion of fungal hyphae. This analysis, along with complementary studies²⁻⁴, suggest that AM fungi have an unusual genomic structure in which multiple, genetically diverse nuclei are maintained within cells through remixing by hyphal fusion.

Pawlowska and Taylor observed that each of 20 single progeny spores had all thirteen variants of a putatively single copy gene, DNA polymerase 1. They argued that the preservation of these variants was inconsistent with heterokaryotic organization of the genome because under this genomic structure, stochastic loss of variants would be expected. Their statistical confidence for this conclusion comes from simulations of the segregation process that assumed haploidy, no hyphal fusion and no selection. We relaxed the first two assumptions and demonstrated that both diploidy and hyphal fusion

could delay the loss of variation (Fig 1). Hyphal fusion, in particular, would have strong effects because it allows the remixing of previously separated nuclei types, thereby stemming the loss due to drift³. By allowing fusion of hyphae derived from a single spore, as has been observed empirically^{5,6}, high levels of variation can be maintained within spores over long periods of time, assuming either haploidy or diploidy (Fig. 1).

We calculated the likelihood of losing variants from spores with 13 variants within one generation as done by Pawlowska and Taylor¹, but varied the rate of fusion (Electronic Supplement). As demonstrated 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 levels to those 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? While 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 to 85 % of contacts between hyphae derived from spores from the same cultures^{5,6}. Given this observation and the observation that the species of fungi that Pawlowska and Taylor investigated are haploid⁴, 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 (2004) also amplified the ITS region from micro-

dissected 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 ITS types, as well as in other regions of the genome.

1. Pawlowska, T. E. & Taylor, J. W. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* **427**, 733-737 (2004).
2. Kuhn, G., Hijri, M. & Sanders, I. R. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* **414**, 745-748 (2001).
3. Bever, J. D. & Morton, J. B. Heritable variation of spore shape in a population of arbuscular mycorrhizal fungi: Suggestions of a novel mechanisms of inheritance. *American Journal of Botany* **86**, 1209-1216 (1999).
4. Hijri, M. & Sanders, I. R. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* (In press).
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6. Giovannetti, M., Fortuna, P., Citernesi, A., Morini, S. & Nuti, M. The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. *New Phytologist* **151**, 717-724 (2001).

Supplementary Information accompanies the paper on *Nature's* website (<http://www.nature.com>).

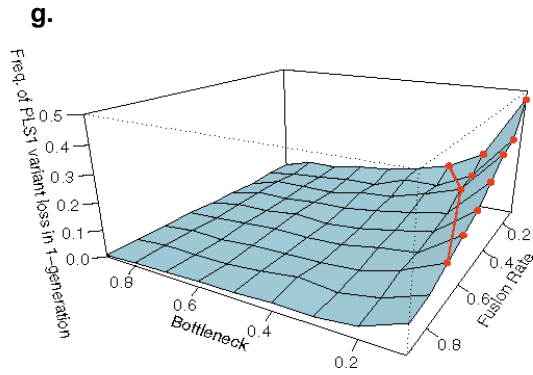
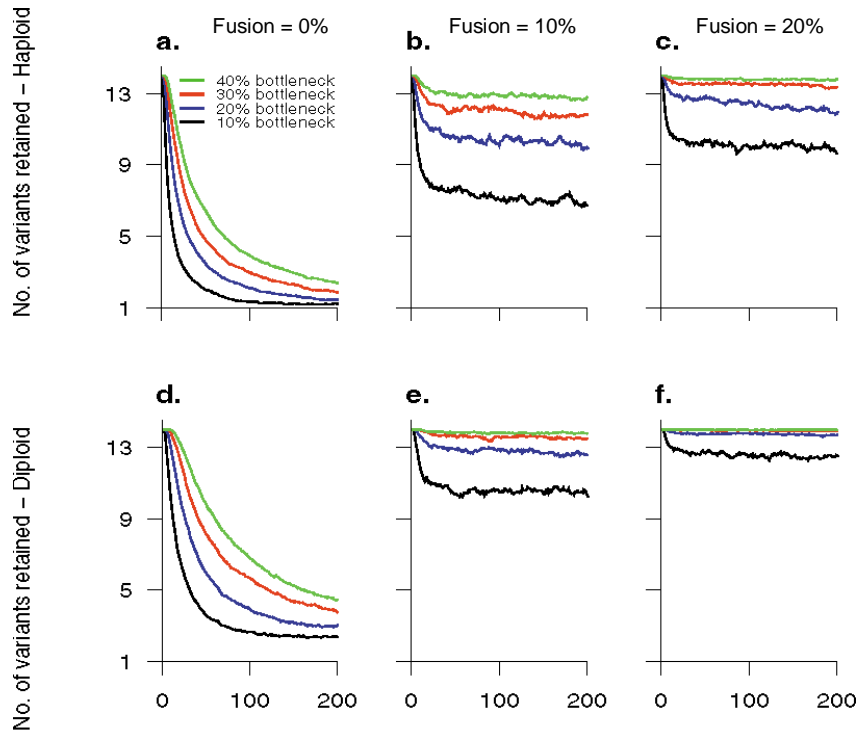
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Figure Legend

Figure 1. Retention of genetic variants in 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). Plots a-c are for haploid nuclei and plots d-f are for diploid nuclei. Rates of hyphal fusion of 0%, 10% and 20% generated plots a, d, plots b, e, and plots c, f, respectively. **g**, The probability of losing at least one variant in one generation given that a haploid parental spore had 13 variants. The red points indicate the parameter

region that can be excluded at the 5% significance level by Pawlowska and Taylor's data. Their data are consistent with most of the parameter space.

Figure 1



Electronic Supplement:

Our simulations generally followed those of Pawlowska and Taylor (2004). 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.

We calculated the frequency of losing at least one variant from spores with 13 variants as described in Pawlowska and Taylor (2004). To make these estimates, we averaged the proportion of 1,000 offspring that lost at least one variant among 2,000 different parental spores that had 13 variants obtained from the simulations that started with spores containing 14 variants. As with Pawlowska and Taylor (2004), we 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.

Pawlowska, T. E. & Taylor, J. W. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* **427**, 733-737 (2004).