brief communications arising

in other regions of the genome. James D. Bever*, Mei Wang†

*Department of Biology, Indiana University, Bloomington, Indiana 47405, USA e-mail: jbever@indiana.edu †Department of Statistics, University of Chicago, Chicago, Illinois 60637, USA doi: 10.1038/nature03294

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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 nuclei3. Bever and Wang suggest4 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 forma-

tion 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 mosseae9, indicating that AM fungi have self-recognition mechanisms that are equally sophisticated and operate like those in other fungi. In our simulation model of heterokaryosis³, 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 cell¹⁰. The model of heterokaryosis proposed for AM fungi², 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 results³ 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 fungi¹³. 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 genes¹⁴. 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.

Teresa E. Pawlowska*, John W. Taylor

Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA

*Present address: Department of Plant Pathology, Cornell University, Ithaca, New York 14853, USA e-mail: tep8@cornell.edu

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