

Letters

Sex, plasticity, and biologically significant variation in one Glomeromycotina species

A response to Bruns *et al.* (2018) 'Glomeromycotina: what is a species and why should we care?'

The account of a recent workshop (at the 9th International Conference on Mycorrhiza (ICOM 9), Prague 2017) 'Glomeromycotina: what is a species and why should we care?' by Bruns *et al.* (2018, in this issue of *New Phytologist*, pp. 963–967) summarizes the current state of knowledge on genetic variation, sexual reproduction, phylogeny and species concepts in these important plant symbionts. In the section 'Do they have sex?' Bruns and colleagues summarize current knowledge on the arrangement of genetic variation and whether current evidence points to sexuality in these fungi. Current evidence supports the existence of a monokaryote–dikaryote state in the species *Rhizophagus irregularis*, and the possible existence of meiosis and recombination. However, several points need clarification concerning: (1) assumptions on the biology of this subphylum based on observations in one species; (2) the authors' interpretation that some cited studies promote heterokaryosis as a substitute for sex; (3) the suggested discrepancy among recent studies of genomic organization; (4) the existence of sex in *R. irregularis*.

Point 1

Bruns and colleagues report that I pointed out at the workshop that some evidence for heterokaryosis comes from Glomeromycotina species that have not yet been studied with genomic approaches. However, the point was made as a more cautionary note about the interpretation of data in an evolutionary context. The first published evidence supporting heterokaryosis was generated from *Scutellospora castanea* (Kuhn *et al.*, 2001), but subsequent studies challenged this hypothesis using evidence generated from other species (Pawlowska & Taylor, 2004; Stukenbrock & Rosendahl, 2005) that probably diverged from *S. castanea* at least 300 million years ago. Indeed, the only good evidence for homokaryosis–dikaryosis and possible sex in Glomeromycotina exclusively comes from one Glomeromycotina species, *R. irregularis*, that almost certainly shared a common ancestor with the other studied Glomeromycotina species many millions of years ago. It is flawed to make assumptions about the biology or genomic organization of one species based on features observed in the genome of another species with which it shared a last common ancestor many millions

of years ago. Would we be prepared to predict features of human biology on the basis of features observed in a reptilian or bird species with which we shared a last common ancestor 300 million years ago? Thus, we should certainly be cautious in such interpretations about sex or heterokaryosis in the subphylum, as a whole, until a lot more data are available. Fortunately, major efforts are being made in the genome sequencing of other isolates of at least one species (Chen *et al.*, 2018, in this issue of *New Phytologist*, pp. 1161–1171) and hopefully more from a broad spectrum of the Glomeromycotina subphylum phylogeny will be available in the near future.

Point 2

Bruns and colleagues state that Wyss *et al.* (2016) and Angelard *et al.* (2014) theorized that heterokaryosis in AMF could be a substitute for conventional sex. This is inaccurate. Wyss *et al.* (2016) looked at genetic variation within, and among, *R. irregularis* isolates and neither presented any hypothesis that the observed sequence variation had arisen in the presence or absence of sex, nor suggested that such variation was a substitute for conventional sex.

In the study by Angelard *et al.* (2014), quantitative genetic variation was observed among sibling single spore cultures from one parental *R. irregularis* isolate. The fungi had been maintained *in vitro* and all material was produced vegetatively without the possibility to exchange DNA with any other individuals. Angelard *et al.* (2014) did not consider, or state, that changes in allele frequency in response to the environment were a substitute for sex, but an additional 'alternative' to provide the vegetatively growing fungus 'plasticity in its own lifetime' to simultaneously colonize a heterogeneous environment. The additional possible existence of sex in *R. irregularis* was discussed, and not discounted, and as well as the explanation that if this fungus is sexual, it would not invalidate their ecologically interesting results (see discussion in Angelard *et al.*, 2014).

The results presented in Wyss *et al.* (2016) and Angelard *et al.* (2014) neither attempted to challenge nor dispel the existence of some form of sexual reproduction in *R. irregularis* but simply interpret the results in the absence of any likely sexual processes occurring during the time period of the experiments and the way the fungal strains were cultured. The findings of those studies are not exclusive of the existence of sexual reproduction in this fungus.

However, the study by Angelard *et al.* (2014) and two other studies (Angelard *et al.*, 2010; Ehinger *et al.*, 2012) document very large variation in fungal quantitative traits as well as the enormous variation they cause in plant growth, among single spore siblings produced from the same parent in the absence of sex. The cause of the generation of highly biologically significant variation in *R. irregularis* is highly unusual and clearly needs to be understood.

Bruns *et al.* (2018) pose the question ‘Glomeromycotina: what is a species and why should we care?’ Obviously, the question ‘What is a Glomeromycotina species?’ is important to taxonomists and for understanding the evolution of this fungal subphylum. However, the ‘why should we care?’ part of this question was not addressed at the workshop. Studies like Angelard *et al.* (2010) show that intra-specific variation within Glomeromycotina species, in terms of their effects on plant growth, is very large. This can actually be larger than the differences among Glomeromycotina species (for a review see Sanders & Rodriguez, 2016). Thus, from an ecological perspective, perhaps we should not care too much about defining what a Glomeromycota species is, as it may be of little relevance, and instead focus on understanding the components of genetic or epigenetic variation in these fungi that lead to such differences in plant growth.

Point 3

Bruns and colleagues also suggest that *R. irregularis* isolates, purported to be heterokaryotic by Boon *et al.* (2015) and Wyss *et al.* (2016), were later shown by Ropars *et al.* (2016) to be monokaryotic or dikaryotic. The study by Boon *et al.* (2015) suggested very high levels of heterokaryosis in *R. irregularis* that was clearly not supported by the study of Wyss *et al.* (2016). In fact, the studies of Wyss *et al.* (2016) and Ropars *et al.* (2016) are very similar. One possible scenario, that some isolates are homokaryotic and some isolates are predominantly dikaryotic was, indeed, presented in Wyss *et al.* (2016). At ICOM 9, Tania Wyss presented analyses showing the very close similarity in the independently generated datasets of Ropars *et al.* (2016) and Wyss *et al.* (2016). She showed that many of the same variable, or bi-allelic sites, in the *R. irregularis* genome recorded in several independent replicates by Wyss *et al.* (2016) were also found in the sequence data generated from multiple replicate libraries by Ropars *et al.* (2016). This shows that many of the exact same sequence variants were detected in both studies independently. Obviously, this is highly unlikely to have occurred independently by chance. Bruns and colleagues give the impression of a strong dichotomy between the results of Wyss *et al.* (2016) and Boon *et al.* (2015) on the one hand, and Ropars *et al.* (2016) on the other hand, whereas in reality very few discrepancies appear to exist between Wyss’ and Ropars’ studies. This analysis, presented in a plenary session at the same conference, appears to have been ignored by Bruns and colleagues.

Point 4

The possibility that sexual processes occur in *R. irregularis* is compelling. Indeed, a collection of Swiss *R. irregularis* isolates originating from the same field exhibit footprints of recombination events (Croll & Sanders, 2009), thus ‘dispelling the notion that all AMF are completely clonal’ (Sanders, 2011). In this case, the recombination events must have occurred before the fungi were isolated. The existence of conserved meiosis genes in *R. irregularis*, is compelling yet remains circumstantial evidence for sex. It should be noted that in some plants the presence of meiosis genes and the

process of meiosis is decoupled from sex. The conservation of meiosis genes appears to be selectively important and common in obligate apomictic species that reproduce asexually but undergo meiosis (Mirzaghaderi & Hörandl, 2016). Around 10% of fern species are obligate apomictics that undergo meiosis but then produce clonal gametophytes as the main form of reproduction. There is no obvious selective disadvantage compared to sexual ferns (Liu *et al.*, 2012). Thus, the existence of meiosis genes is not proof of a sexual lifestyle.

Further information on population genetics from a larger number of isolates of *R. irregularis* could greatly help to resolve whether this fungus is indeed sexual (Sanders & Croll, 2010). One expected characteristic of primarily clonal species with very infrequent recombination is a low number of highly divergent genotypes in the environment and widespread occurrence of single genotypes or clones (Sanders & Croll, 2010). A recent genotyping of *R. irregularis* isolates using over 2400 genetic markers revealed an intercontinental distribution of almost identical genotypes, as well as the existence of cryptic species (Savary *et al.*, 2018). While this is not proof of a lack of sex it does suggest strongly that common genotypes of this fungus are unlikely to be recombining very often. Indeed, population genetics models indicate that recombination does not have to be very frequent to purge deleterious mutations. Some plant species are known to grow primarily vegetatively (e.g. some Lemnoideae), and bamboos (Tang *et al.*, 2014; Ge *et al.*, 2016). Indeed, flowering in some bamboo species is synchronous but only every 120 years. Thus, even if *R. irregularis* is a sexual fungus the wait may be so long that I, as well as Bruns and colleagues, may have to accept the possibility of going to the grave before obtaining direct evidence of sexual reproduction in these fascinating fungal symbionts.

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References

- Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR. 2010. Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. *Current Biology* 20: 1216–1221.
- Angelard C, Tanner CJ, Fontanillas P, Niculita-Hirzel H, Masclaux F, Sanders IR. 2014. Rapid genotypic change and plasticity in arbuscular mycorrhizal fungi is caused by a host shift and enhanced by segregation. *ISME Journal* 8: 284–294.

- Boon E, Halary S, Baptiste E, Hijri M. 2015. Studying genome heterogeneity within the arbuscular mycorrhizal fungal cytoplasm. *Genome Biology and Evolution* 7: 505–521.
- Bruns TD, Corradi N, Redecker D, Taylor JW, Öpik M. 2018. Glomeromycotina: what is a species and why should we care? *New Phytologist* 220: 963–967.
- Chen ECH, Morin E, Beaudet D, Noel J, Yildirim G, Ndikumana S, Charron P, St-Onge C, Giorgi J, Krüger M *et al.* 2018. High intraspecific genome diversity in the model arbuscular mycorrhizal symbiont *Rhizophagus irregularis*. *New Phytologist* 220: 1161–1171.
- Croll D, Sanders IR. 2009. Recombination in *Glomus intraradices*, a supposed ancient asexual arbuscular mycorrhizal fungus. *BMC Evolutionary Biology* 9: 1–11.
- Ehinger MO, Croll D, Koch AM, Sanders IR. 2012. Significant genetic and phenotypic changes arising from clonal growth of a single spore of an arbuscular mycorrhizal fungus over multiple generations. *New Phytologist* 196: 853–861.
- Ge W, Zhang Y, Cheng Z, Huo D, Li X, Gao J. 2016. Main regulatory pathways, key genes and microRNAs involved in flower formation and development of moso bamboo (*Phyllostachys edulis*). *Plant Biotechnology Journal* 15: 82–96.
- Kuhn G, Hijri M, Sanders IR. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* 414: 745–748.
- Liu H-M, Dyer R, Guo Z-Y, Meng Z, Li J-H, Schneider H. 2012. The evolutionary dynamics of apomixis in ferns: a case study from polystichoid ferns. *Journal of Botany* 11: 510478.
- Mirzaghaderi G, Hörandl E. 2016. The evolution of meiotic sex and its alternatives. *Proceedings of the Royal Society B* 283: 20161221.
- Pawłowska TE, Taylor JW. 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* 427: 733–737.
- Ropars J, Toro K, Noel J, Pelin A, Charron P, Farinelli L, Marton T, Krüger M, Fuchs J, Brachmann A *et al.* 2016. Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. *Nature Microbiology* 1: 1–9.
- Sanders IR. 2011. Fungal sex: meiosis machinery in ancient symbiotic fungi. *Current Biology* 21: R896–R897.
- Sanders IR, Croll D. 2010. Arbuscular mycorrhiza: the challenge to understand the genetics of the fungal partner. *Annual Review of Genetics* 44: 271–292.
- Sanders IR, Rodriguez A. 2016. Aligning molecular studies of mycorrhizal fungal diversity with ecologically important levels of diversity in ecosystems. *ISME Journal* 10: 2780–2786.
- Savary R, Masclaux F, Wyss T, Droh G, Joaquim CC, Machado AP, Morton JB, Sanders IR. 2018. A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus *Rhizophagus irregularis*. *ISME Journal* 12: 17–30.
- Stukenbrock EH, Rosendahl S. 2005. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. *Fungal Genetics and Biology* 42: 73–80.
- Tang J, Zhang F, Cui W, Ma J. 2014. Genetic structure of duckweed population of *Spirodela*, *Landoltia* and *Lemna* from Lake Tai, China. *Planta* 239: 1299–1307.
- Wyss T, Masclaux FG, Rosikiewicz P, Pagni M, Sanders IR. 2016. Population genomics reveals that within-fungus polymorphism is common and maintained in populations of the mycorrhizal fungus *Rhizophagus irregularis*. *ISME Journal* 10: 2514–2526.

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