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

A response to Bruns et al. (2018) ‘Glomeromycota: 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) ‘Glomeromycota: 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. 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) documented 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 intraspecific 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 (Mirzagharder & 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 Lemoineae), 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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ORCID

Ian R. Sanders http://orcid.org/0000-0002-9591-8214

Ian R. Sanders

References


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