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Quantifying functional heterothallism in the pseudohomothallic ascomycete *Neurospora tetrasperma*

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ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form
20 June 2012
Accepted 25 June 2012
Available online 6 July 2012
Corresponding Editor:
Joseph W. Spatafora

Keywords:
Conidia
Heterothallism
Neurospora tetrasperma
Outcrossing
Populations
Pseudohomothallism

ABSTRACT

Neurospora tetrasperma is a pseudohomothallic filamentous ascomycete that has evolved from heterothallic ancestors. Throughout its life cycle, it is predominantly heterokaryotic for mating type, and thereby self-fertile. However, studies of *N. tetrasperma* have revealed the occasional production of self-sterile asexual and sexual spores of a single-mating type, indicating that it can be functionally heterothallic. Here, we report the extensive sampling and isolation of natural, heterokaryotic, strains of *N. tetrasperma* from the United Kingdom (UK): 99 strains were collected from Surrey, England, and four from Edinburgh, Scotland. We verified by phylogenetic analyses that these strains belong to *N. tetrasperma*. We isolated cultures from single germinated asexual spores (conidia) from 17 of these newly sampled UK strains from Surrey, and 16 previously sampled strains of *N. tetrasperma* from New Zealand (NZ). Our results show that the *N. tetrasperma* strains from the UK population produced a significantly greater proportion of self-sterile, homokaryotic conidia than the NZ population: the proportion of homokaryotic conidia was 42.6 % (133/312 spores) and 15.3 % (59/386) from the UK and the NZ populations, respectively. Although homokaryons recovered from several strains show a bias for one of the mating types, the total ratio of *mat A* to *mat a* mating type in homokaryons (UK: 72/61, NZ 28/31) did not deviate significantly from the expected 1:1 ratio for either of these populations. These results indicate that different populations exhibit differences in their life cycle characteristics, and that a higher degree of outcrossing might be expected from the UK population. This study points to the importance of studying multiple strains and populations when investigating life history traits of an organism with a complex life cycle, as previously undetected differences between populations may be revealed.

Introduction

Neurospora tetrasperma is a self-fertile species of the genus *Neurospora* that was first described by Shear & Dodge (1927). The species description of *N. tetrasperma* was based on phenotypic characters that distinguish it from heterothallic species of the genus. In contrast to the heterothallic, eight-spored *Neurospora* species, *N. tetrasperma* produces asci with four

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1878-6146/$ – see front matter
http://dx.doi.org/10.1016/j.funbio.2012.06.006

relatively large ascospores. These ascospores and the germinating mycelia of *N. tetrasperma* are normally heterokaryotic for mating type (*mat A* and *mat a*) and thereby it can complete the sexual cycle without needing to find a compatible mate (Dodge 1927). This mating system is referred to as secondary homothallism, or pseudohomothallism. Pseudohomothallism has evolved independently multiple times throughout the fungal kingdom (Langton & Elliott 1980; Raju & Perkins 1994), and *N. tetrasperma* is one of the best-studied examples of such a mating system in fungi. In association with the evolution of pseudohomothallism in *N. tetrasperma*, the meiotic pathway was reprogrammed so that each ascospore receives *mat A* and *mat a* haploid nuclei produced from a single diploid nucleus. This process is dependent on the segregation of *mat A* and *mat a* idiomorphs [allelic-like sequences at the mating-type locus (Metzenberg & Glass 1990)] at the first division of meiosis, which is assured by the suppression of recombination between the mating-type locus and the centromere (Howe 1963; Howe & Haysman 1966; Merino *et al.* 1996; Jacobson 2005). Recent analysis of high quality genomic data from two strains of opposite mating type found three inversions to be associated with the region of suppressed recombination on the mating-type chromosomes (Ellison *et al.* 2011b). Its novel genetic system and suppression of recombination over much of the mating-type chromosome has meant that *N. tetrasperma* has attracted considerable research attention, primarily for the study of evolutionary genetics.

Predominant heterokaryosis for mating type indicates a high degree of selfing in *N. tetrasperma*, and accordingly, observations of homoallellism in recombining regions of the genome support a view of a predominant selfing history for *N. tetrasperma* (Merino *et al.* 1996; Menkis *et al.* 2008). However, the occasional production of homokaryotic progeny would result in functional heterothallism in this species. Indeed, self-sterile homokaryotic (single-mating type) isolates have been collected from nature (Perkins *et al.* 1976; Perkins & Turner 1988) and can be isolated from cultures grown in the laboratory, both from ascospores and conidia. Dodge (1927) was the first to report that *N. tetrasperma* can produce single-mating-type propagules. Raju (1992) later reported on the fraction of spores that harbor only one nuclear type, with estimates as high as 10 % and 16 % for the proportion of homokaryotic ascospores and conidia, respectively, obtained from a single heterokaryotic strain of *N. tetrasperma*. The *N. tetrasperma* cultures derived from such spores are self-sterile and must mate with a strain of opposite mating type to re-establish the pseudohomothallic life cycle. Furthermore, analyses of the distribution of alleles of a heterokaryon incompatibility (*het*) locus indicate that outcrossing occurs in natural populations of *N. tetrasperma* (Powell *et al.* 2001; Menkis *et al.* 2009), but its frequency is yet to be determined.

Neurospora tetrasperma, like the other conidiating species of *Neurospora*, is adapted to growth and sporulation on the surface of fire-scorched vegetation. Strains of *Neurospora* have been extensively sampled at multiple locations around the globe (Perkins *et al.* 1976; Perkins & Turner 1988; Turner *et al.* 2001) with the aim of advancing knowledge of their evolutionary history and ecology. Currently, the largest available collections of *N. tetrasperma* are from the USA and New Zealand (NZ), while smaller samples have been obtained in other locations (Turner *et al.* 2001). More recently, sampling of *Neurospora* in Europe identified 13 % of the total sample (247 strains) to be composed of *N. tetrasperma* (Jacobson *et al.* 2006). These extensive collections of *N. tetrasperma* and other *Neurospora* species have been used in a number of phylogenetic studies.

In the phylogeny of *Neurospora*, *N. tetrasperma* is found within the terminal clade of conidiating species of *Neurospora* (Nygren *et al.* 2011), indicating that it has evolved from a heterothallic ancestor (Natvig *et al.* 1987; Skupski *et al.* 1997; Dettman *et al.* 2003). It has been suggested that *N. tetrasperma* is an ephemeral species that evolved from other heterothallic species at multiple times in the past (Metzenberg & Randall 1995); however, a recent multilocus phylogenetic analysis supports a monophyletic origin for *N. tetrasperma* (Menkis *et al.* 2009). Moreover, using multilocus molecular data and biological characters, Menkis *et al.* (2009) showed that *N. tetrasperma* constitutes a species complex with several reproductively isolated lineages.

While much research has addressed the evolutionary history of *N. tetrasperma* and of the mating-type chromosomes (Merino *et al.* 1996; Gallegos *et al.* 2000; Menkis *et al.* 2008; Ellison *et al.* 2011b), much remains yet to be discovered about the mating system of this organism. For instance, the connection between the proportion of homokaryotic spores produced and outcrossing rates in different populations of *N. tetrasperma* is lacking, with available estimates existing for only two strains (Raju 1992). In this study, we addressed the question of homokaryotic spore production using the largest collections available for individual *N. tetrasperma* populations, newly sampled isolates from the United Kingdom (UK) and an existing sample of strains from NZ. We did this by first confirming that all the investigated isolates of *Neurospora* are indeed a collection *N. tetrasperma* isolates. We isolated homokaryotic strains from the heterokaryotic strains and recorded the proportion of homokaryotic conidia produced in the UK and NZ populations. We found that there are significant differences in the numbers of homokarytotic conidia produced by these two populations of *N. tetrasperma*, and this difference indicates a potential for differences in outcrossing rate between them.

Materials and methods

Field isolation of Neurospora tetrasperma *in the UK*

One hundred strains of *Neurospora*, spotted by the occurrence of orange conidia on the surface of scorched plants (Fig 1), were sampled from Thursley Common National Nature Reserve in Surrey, England (Fig 2), in Jul. 2006 2 weeks after a wildfire. The strains were isolated from four plants, three *Ulex europaeus* and one *Betula pendula*. For each plant, samples were collected from five branches, with five samples per branch (Table 1).

Conidia were collected from visible *Neurospora* colonies by touching the colony with a sterilized strip of filter paper, which was then placed in a sterilized glassine envelope. Collections were transferred to the laboratory and frozen to kill mites. The samples from Scotland were collected from