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Application of flow cytometry for exploring the evolution of *Geosmithia* fungi living in association with bark beetles: the role of conidial DNA content

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ABSTRACT

Geosmithia belongs among fungi living in symbiosis with phloem-feeding bark beetles. Several species have altered their ecology to that of obligatory symbiosis with ambrosia beetles, which has led to a shift in their phenotype and caused formation of large spherical conidia. In this study, we pose the following questions; (1) Is the conidial DNA content of *Geosmithia* correlated with conidial volume?; (2) Is the DNA content of *Geosmithia* related to the degree of mutual dependence between *Geosmithia* and their vector? There was a positive and strong correlation between conidial DNA content and conidial volume in *Geosmithia*. Also species more narrowly associated with the vector tend to have a larger conidial DNA content and volume than less narrowly associated species. Ambrosia fungi achieved the biggest conidial DNA content and volume compared to other species. We suppose that polyploidisation occurred during the evolution of ambrosia species in the genus *Geosmithia*.

Introduction

Genome sizes among eukaryotic organisms differ by almost 80 000-fold (Gregory et al., 2007) and this range is thought to be even larger (Gregory, 2001). It was recognised early that genome size does not correlate with organismal complexity (Mirsky and Ris, 1951). This disproportion was termed the C-value paradox (Thomas, 1971). The C-value paradox was resolved by the discovery that non-coding DNA could create the majority of DNA (Cavalier-Smith and Beaton, 1999; Gregory, 2001). Bennett (1971) pointed out that DNA influences the development not only by a gene but also by its mass, termed the nucleotype. Since then, many publications have explored the correlation between genome size and cell size

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(reviewed in Gregory, 2001). The mechanism that underlines this phenomenon has remained unknown until now. Cavalier-Smith and others declared that a correlation between cell size and genome size is maintained by the need for preservation of karyoplasmic volume ratio for balanced growth (Cavalier-Smith and Beaton, 1999; Cavalier-Smith, 2005). Modulation of genome size is therefore the consequence of organismal selection for optimal cell size. This assumption was supported by manipulations of genome size in yeast (Jorgensen et al., 2007) and fission yeast (Neumann and Nurse, 2007). In fungi, a relationship between spore size and life-history strategies (Kauserud et al., 2008, 2011; Meerts, 1999; Philibert et al., 2011) or climate (Kauserud et al., 2011; Pentecost, 1981) has been found. This indicates the great importance of spore size in exploring fungal ecology and evolution. Explanations of these relationships stem from the physical characteristics of spores (floating and impact ability, drag minimisation, nutrient and water content). Additional explanations due to differences in genome size/DNA content should also be considered. The role of genome size in species adaptations to environmental conditions was found repeatedly in plants and animals (Barow and Meister, 2003; Beaulieu et al., 2008; Smith and Gregory, 2009; te Beest et al., 2012; Vesely et al., 2012), but few studies exist in mycology, and they focus mostly on yeasts (Conant and Wolfe, 2007; Lidzbarsky et al., 2009; Talbot and Wayman, 1989).

The genus *Geosmithia* (Ascomycota: Hypocreales) includes fungal species living in association with bark beetles (Coleoptera: Curculionidae, Scolytinae) or, rarely, occurring as endophytes or on alternative substrata (McPherson et al., 2013; Kolařík et al., 2004, 2005, 2007, 2008, 2011; Kolařík and Kirkendall, 2010; Kolařík and Jankowiak, 2013; Kubátová et al., 2004; Pitt and Hocking, 1997). Some of them are considered to be generalists, living in association with insects on a variety of plant hosts, the others are specialists restricted to vectors living on the single plant family Pinaceae. *Geosmithia morbida* is the only known pathogenic species from the genus and causes necrosis in *Juglans nigra* (Kolařík et al., 2011; Tisserat et al., 2009). Several species, including *Geosmithia microcorthyli*, *Geosmithia eupagioceri*, and likely *Geosmithia* sp. CCF4292, changed their ecology to that of obligatory symbiosis with ambrosia beetles (Kolařík and Kirkendall, 2010). The ambrosia fungi, which are nutritional symbionts of ambrosia beetles, share similar phenotypes (e.g., production of large globose conidia or pleomorphism) (Batra, 1967) and in addition to *Geosmithia* and other Hypocreales (Kasson et al., 2013), these species mostly belong to the orders Ophiostomatales and Microascales (Jones and Blackwell, 1998). Interestingly, *G. microcorthyli*, a large-mitospored ambrosia fungus, is closely related to two non-ambrosial species, *Geosmithia* sp. 8 (Kolařík and Kirkendall, 2010) and *G.* sp. CCF4200 (unpublished), which all have small conidia (Fig 1) and live in association with phloem-feeding bark beetles. This suggests the rapid evolution of large-mitospored ambrosia fungi from small-mitospored species. Sudden genome enlargement is a possible evolutionary step in the evolution of these large-mitospored ambrosia fungi. *Geosmithia rufescens*, that accompanies ambrosial *G. eupagioceri* and *G.* sp. CCF4292 in beetle galleries, is an auxiliary ambrosia fungus (Kolařík and Kirkendall, 2010). This species possesses two distinct conidial types and its phenotype is in transition between ambrosial and non-ambrosial species.

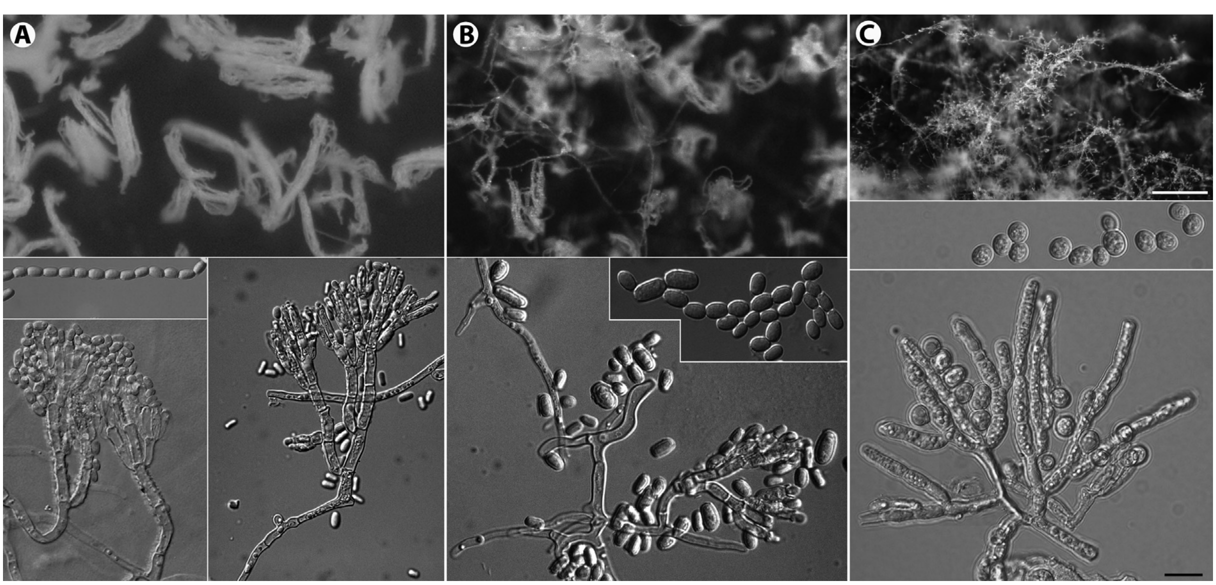

Fig 1 – Morphological variability of studied *Geosmithia* species. The bar is 10 μm (microphotographs) and 100 μm (macrophotographs). (A) *Geosmithia* sp. 8 CCF4528, a generalist producing masses of small cylindrical and catenate conidia, phialide reached 8–11 × 2.0–2.5 μm. (B) *Geosmithia* sp. 24 CCF4294, a specialist restricted to vectors feeding on the trees from the Pinaceae family. Sporulation is moderate and it produces catenate conidia which are very variable in shape. (C) *G. microcorthyli* CCF3861, primary ambrosia fungus producing big and solitary conidia, phialide reached 9–15 × 5–7 μm. All cultures were on MEA medium.