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Homokaryons are more combative than heterokaryons of *Hericium coralloides*

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ABSTRACT

The homokaryotic stage of the basidiomycete lifecycle is generally considered to be short lived, although there is little experimental evidence relating to their longevity in the field. The vast majority of studies on basidiomycete ecology have used only heterokaryons. The few investigations comparing related homokaryons and heterokaryons have revealed no overall trend in differences of extension rate, wood decay or competitive ability. For a rare species the homokaryotic phase may be of greater importance than in common species as it is likely to last longer. *Hericium coralloides*, a rare wood decay basidiomycete, was used to investigate differences between homokaryons and heterokaryons in terms of extension rate and combative ability. Fifteen homokaryons from three fruit bodies and five heterokaryons (obtained by fruit body tissue isolation) were compared at 5–35 °C on malt agar for extension rate, and paired against heterokaryons of 13 wood decay species to assess combative ability. Homokaryons were paired to create ten artificial heterokaryons whose extension rate at 10 and 20 °C was compared to parental rates. There were some significant differences in extension rates between homokaryons and natural heterokaryons, between homokaryons and heterokaryons created artificially from homokaryons, and between homokaryons from different fruit bodies, but no consistent trends. Homokaryons proved more combative than heterokaryons, which was assessed quantitatively as well as qualitatively using a scoring system for outcome of each pairing. Results are discussed in relation to previous findings and in an ecological context.

Introduction

Typically, the lifecycle of heterothallic Agaricomycetes comprises spore germination producing a homokaryotic mycelium, followed at some point by fusion and nucleus exchange with a mating-type compatible homokaryon to yield a heterokaryotic mycelium. Subsequently homokaryotic sexual spores are produced following karyogamy and meiosis, and/or asexual homokaryotic spores, oidia, are derived usually incorporating single nuclei. Homokaryons are generally considered short-lived (e.g. Kauserud *et al.* 2006), and the heterokaryon assumed to be the dominant phase, although there is little evidence for this. Indeed, some homokaryons, for example those of the wood-rotting species *Trametes versicolor* and *Heterobasidion annosum*, can persist in the field for several years, and possibly much longer (Coates & Rayner 1985; Garbelotto *et al.* 1997; Redfern *et al.* 2001). There is also evidence of an inverse relationship between number of

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colonies of a species in the field and the number that are homokaryons (Stenlid 1994). Thus, for a rare species heterokaryotisation may be delayed due to low numbers of potential mates.

The vast majority of studies on fungal ecophysiology and the development and functioning of communities in dead organic resources have employed isolates obtained from colonized resources or from fruit body tissues, i.e. heterokaryotic cultures, and largely ignored the homokaryotic phase. However, there is evidence that heterokaryons and homokaryons exhibit differences in performance (Table 1), for example in terms of extension rate (e.g. Simchen 1966; Hansen 1979; Fryar *et al.* 2002), wood decay rate (e.g. Platt *et al.* 1965; Amburgey 1970; Elliott *et al.* 1979), and interspecific competition (Fryar *et al.* 2002), but there does not yet appear to be a trend for homokaryons or heterokaryons to out-perform the other in any particular activity.

For saprotrophic species that rarely produce basidiocarps there will be relatively fewer airborne propagules compared to frequently fruiting species. In these cases the homokaryotic phase may be of greater significance than for a common species, as this phase may be relatively long, and it will be the homokaryon that establishes the individual in dead organic resources. Thus, homokaryons of rare species must be particularly 'fit' to survive and become heterokaryons, or poor survival of homokaryons may contribute to the rarity of the species.

Hericium coralloides is a rare wood decay basidiomycete included in the provisional Red Data List of British Fungi and red lists of other European countries (Ing 1992; Boddy & Wald 2002). In Japan it is less rare (Y. Obatake pers. comm. November 2007), but information about its occurrence in other countries is not readily available. Little is known of its ecology. It is mostly found (fruiting) on beech (*Fagus sylvatica*) and to a lesser extent on ash (*Fraxinus excelsior*), particularly trunks and large branches, and is long-lived (Marren & Dickson 2000; Boddy & Wald 2002). Heterokaryotic isolates are average to good combatants, not losing any territory to most members of the early stage decay community against which they were tested (Wald *et al.* 2004b). Rarity is not, therefore, likely to result from poor combative ability of established heterokaryons. Here we examine homokaryon 'fitness' and test, in agar culture, the hypotheses that: (1) homokaryons grow more slowly than heterokaryons; and (2) homokaryons are less combative than heterokaryons.

Methods

Isolates

Homokaryotic and heterokaryotic isolates of *Hericium coralloides* and other wood decay Ascomycota and Basidiomycota (Table 2) were maintained on 2 % or 0.5 % (w/v) malt extract agar (MEA; 15 g l^{-1} Lab M agar no. 1 (LabM, Bury, Lancashire, UK) with either 20 or 5 g l^{-1} Munton & Fison Spray Malt Light (Munton Plc, Stowmarket, Suffolk, UK), respectively). Homokaryotic isolates were obtained by collecting spores from fruit bodies in the field on glass slides. Spores were then suspended in sterile distilled water and diluted until a concentration was reached such that when 35 μl of suspension were spread on a 2 % MEA plate there was approximately one spore per field of view at ×100 mag. Up to 20 replicate plates were made, incubated at 20 °C in the dark and checked regularly until germination was seen. Single well-spaced germinating spores were transferred to fresh 2 % MEA plates. Spore prints originated from three fruit bodies: MA126 and MA129, which were produced on the same ash (*Fraxinus excelsior*) tree in Windsor Great Park, Berkshire, UK (Nat. grid ref. SU956740), and MA127 and on a beech (*Fagus sylvatica*) tree in Epping Forest, U.K. (Nat. grid ref. TQ42329853). Heterokaryotic isolates, referred to subsequently as 'natural' isolates, were obtained by fruit body tissue isolations. In addition, ten artificial heterokaryons were created, by pairing selected homokaryons on agar plates to produce stable secondary mycelium (Table 2).

Extension rates

Extension rates were determined on 0.5 % MEA for all homokaryons and the five natural heterokaryons (Table 2) at 5, 10, 15, 20, 25, 30 and 35 °C and for artificial heterokaryons at 10 and 20 °C, with four replicates of each isolate at each temperature. Plugs (6 mm diam), from the growing margin of the colony, were inoculated centrally on 0.5 % MEA in 9 cm non-vented dishes (Greiner Bio-One, Austria) and colony diameter in two dimensions perpendicular to each other was measured regularly during the log phase of extension. Measurements were made to 0.1 mm using Dialmax vernier callipers (Swiss Precision Instruments Inc., Garden Grove, CA, USA). Extension rates were determined by linear regression and compared with one-way ANOVA, or Kruskal-Wallis when data were not normally distributed, using Minitab.

Interspecific interactions

Pairings between the 20 natural *H. coralloides* isolates and the 13 other wood decay species were made on 0.5 % MEA in 9 cm non-vented dishes. Fungi were inoculated (6 mm diam plugs) 3 cm apart at different times according to their extension rates so that they met in the centre of the dish. Plates were incubated in darkness at 20 °C (four replicates per combination). Once colonies had met interactions were observed weekly, and final outcomes recorded after 12–14 weeks as either deadlock (where neither isolate captured territory from the other), replacement (where one fungus had grown over and through the other so that it was no longer recoverable by isolation) or partial replacement (where one fungus was recoverable from some but not all of the territory originally held). Outcomes were confirmed by subculturing from the base of the agar.

As an aid to comparison of overall combative ability, outcome of each replicate of each pairing was given a score: replacement of the antagonist by *H. coralloides* was assigned +2; partial replacement of the antagonist, +1; deadlock, 0; partial replacement of *H. coralloides*, −1; complete replacement of *H. coralloides*, −2. Cumulative values were determined for each isolate.