

Short communication

Physical properties of fungal rhizomorphs of marasmioid basidiomycetes used as nesting material by birds

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The use of fungal rhizomorphs of marasmioid fungi ('horsehair fungi') as nesting material, especially by tropical bird species, is a widespread but poorly documented phenomenon. I show in this first experimental examination of the physical properties of 'horsehair fungi', that the fungal rhizomorphs utilized by *Icterus pustulatus* have a significantly higher tensile strength and reduced water uptake than alternative fibres used to build nests. These results suggest an adaptive advantage for the use of marasmioid fungi in nest construction.

Most bird species build nests as reproductive structures, which can be defined as 'materials arranged in a particular way' (Hansell 2000). Hansell (2000) distinguished 24 diagnostic categories of naturally occurring materials used in avian constructional behaviour ranging from leaves to pine needles, sticks, bark, grassy fibres, moss, lichens and seaweed. One of the categories listed by Hansell (2000) is the rhizomorphs of the horsehair fungi, characteristic of marasmioid basidiomycetes. Only a very restricted number of publications (Sick 1957, McFarland & Rimmer 1996) focus exclusively on the different natural history aspects of this material, whereas in most studies of avian nesting behaviour (e.g. Collias & Collias 1984, Hansell 2000) fungal rhizomorphs are only superficially treated. To the best of my knowledge this study is the first to report an experimental examination of physical properties of this nest material that is widely used by birds.

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Representatives of the genus *Marasmius* and related genera (Basidiomycetes) are widely distributed throughout the world, including in Africa, Australia, Asia, the Americas and Europe (Singer 1986, Corner 1996). Most species are saprophytic and can be found on fallen dead leaves, needles and small twigs (Sick 1957), but also seem to inhabit the canopy stratum especially of wet tropical forests (Sick 1957, Hedger 1990, Lodge & Cantrell 1995), where their fruiting bodies can even form a component of human diet (Van Dijk *et al.* 2003). Some species are believed to parasitize their host tree; the most notable example, *Crinipellis pernicioso*, is a pathogen causing Witches' Broom Disease of Cacao *Theobroma cacao* in South America (Ploetz *et al.* 2005). Rhizomorphs, between 0.1 and 1.5 mm in diameter, are constructed of a core of many strands of the elongated cells of fungal hyphae that are surrounded by a dark-coloured ring of smaller cells, analogous to a covered cable. The common name 'horsehair fungus' is based on the gross appearance of the rhizomorphs, which is similar to the long tail hairs of horses. A preliminary literature review yielded 41 bird species that make use of fungal rhizomorphs as nesting material (Sick 1957, Foster 1976, 1989, Sherry 1986, Tostain 1988, McFarland & Rimmer 1996, Whitney *et al.* 1996, Botero 2001, Young & Zuchowski 2003, Buehler *et al.* 2004, Greeney 2004), but currently no comprehensive publication on this subject is available (complete list in prep. by B. P. Freymann). It appears that this material is frequently worked into avian nests especially by tropical passerines inhabiting rainforests, as well as temperate ones occurring in subalpine regions. One species using fungal rhizomorphs as nesting material is the Streak-backed Oriole *Icterus pustulatus*. This species ranges from northern Sonora and western Chihuahua (and recently southeastern Arizona, USA) in Mexico through Central America southwards to extreme northwest Costa Rica (Jaramillo & Burke 1999, Gillespie & Walter 2001).

The Streak-backed Oriole builds a nest in the form of a large hanging basket up to 70 cm in length (Jaramillo & Burke 1999). The particular nests examined were two-layered: the outer layer consisted entirely of long fungal rhizomorphs whereas the inner lining of the breeding chamber was made completely out of short grassy fibres. The rhizomorphs appear to be those of *Marasmius* or other marasmioid species, but could not be identified more precisely because fruiting structures were not available (Singer 1976). Moreover, it is unknown if more than one fungal species contributed fibres to the nests. Not only were all the outer layers of the nests composed of rhizomorphs, they were also attached to the tips of *Acacia* tree branches by fungal rhizomorphs, which were woven around them by the nest builder (Fig. 1).

The natural abundance of fungal rhizomorphs is considerably less within a certain habitat – such as the tropical lowland rainforest – than that of grassy fibres which are frequently used as nesting material. Accordingly, the observed use of fungal rhizomorphs requires an increased



Figure 1. Schematic cross-section of a nest of *Icterus pustulatus*. The outer layer consists of fungal rhizomorphs, attaching the nest to an *Acacia* tree. The inner nest cup is made out of grassy fibres.

expenditure of energy for the birds, based on the assumption that an increased time demand is needed to search for appropriate nesting material. If true, the question raised is, what is the adaptive advantage of this behaviour, given

that 'birds build nests to protect themselves, their eggs, and their young from predators and from adverse weather' (Gill 1995)? In this study I tested two hypotheses regarding specific physical properties of these fungal rhizomorphs, which potentially qualify them as very suitable material for bird nests in general and in particular with respect to the protective function a bird nest has to fulfil: (1) Tensile strength: do fungal rhizomorphs have a higher tensile strength than other nesting materials such as grassy fibres? The adaptive advantage of a higher tensile strength of this construction material could lie in improved stability of the hanging nest. (2) Water saturation: due to the hydrophobic characteristics of fungal hyphae in general, bird nests constructed out of fungal rhizomorphs show a lower water uptake than nests made out of grassy fibres. The adaptive advantage of a nest less saturated with water could lie in a less optimal habitat for pathogens potentially harmful to juveniles inhabiting the nest.

MATERIALS AND METHODS

A total of 15 nests of Streak-backed Orioles – only nests that contained grassy fibres as well as fungal rhizomorphs, which accounted for about 50% of the total number of nests detected – was collected in the tropical, seasonally dry forest of Palo Verde National Park (PVNP) located in Guanacaste Province, Costa Rica (10°21'N, 85°21'W). Nests were collected in July 2002, but it could not be determined from which breeding season they stemmed. It was recorded whether the fungal rhizomorphs were simply woven around the nest bearing branches of the *Acacia* trees, or whether they were actively growing around them. Experiments were carried out in the same month in the laboratories of Barro Colorado Island (BCI), Panama. The surroundings of both field stations (habitat on BCI: tropical lowland rain forest) were searched for the natural occurrence of marasmioid rhizomorphs. At each site I searched for one week, four to six hours per day, by walking through the direct and wider surroundings of the field stations, thereby visually inspecting the vegetation up to a height of 2–3 m.

Tensile strength experiment

Of each of the 15 nests, five fungal rhizomorph fibres and five grassy fibres were extracted randomly. Each fibre was shortened to a length of 10 cm for standardization. One end of the fibres was fixed to a tripod by means of an alligator clamp. The other end was fixed with another alligator clamp to a free hanging empty plastic bag. Into this water was gradually poured, using a 100 mL measuring cylinder, until the fibres broke. The amount of water added to the mass of the empty plastic bag equalled the maximum load (in grams) that a fibre was able to carry. This parameter was used to characterize the tensile strength of the fibres examined.

Water saturation experiment

One gram of fungal rhizomorphs, as well as 1 g of grassy fibres, was extracted randomly from each of 12 nests. Not all 15 nests could be sampled, because only 12 contained sufficient amounts of fibres of both types. Each sample was placed in a separate open ceramic jar, the empty mass of which was measured at the beginning of the experiment using a digital balance to the closest 0.001 g. The jars were filled completely with water and the samples were allowed to soak for 60 min. Each jar was then placed upside down on a grid for 5 min to let any additional water drip off. The samples were then weighed and placed in a drying oven at 50 °C. After 30, 60, 90 and 120 min the samples were weighed again. The water saturation of the fibres was expressed as the percentage increase of the samples' mass from the initial dry mass.

Culture experiment

From each of the 15 nests, 10 fungal rhizomorph fibres were randomly extracted. These fibres were placed on a standard YM agar medium and incubated for several days in a controlled environment of 24–25 °C to check for hyphal growth. This method (M. Blackwell pers. comm.) was used to try to determine whether the fibres were dead or alive at the time of collection of the nests from the field.

Statistics

Data were analysed using STATISTICA (StatSoft, Inc., version 6.1, Tulsa, OK, USA). For the tensile strength dataset, the mean of the five replicates of the 15 nests for both fibre types were computed and a General Linear Model (GLM) used to test whether there were significant differences between the mean of the maximum load of the two different fibre types. For the water saturation experiment the percentage increase of the samples' mass for the two fibre types was compared at times 5, 30, 60, 90 and 120 min using GLMs to test whether the means of the percentage increase of the two fibre groups were significantly different from each other at each point of time.

RESULTS

The nests collected were attached to *Acacia* branches by fungal rhizomorphs that were simply woven around the nest-bearing branches. The fibres were not growing on the branches laterally to the fixation points, indicating that they had died, probably after being harvested and manipulated by the Orioles. The examined nests consisted of approximately three-quarters fungal rhizomorphs and one-quarter grassy fibres. Naturally occurring fungal rhizomorphs were not detected outside of bird nests despite visually surveying the forest understorey up to a

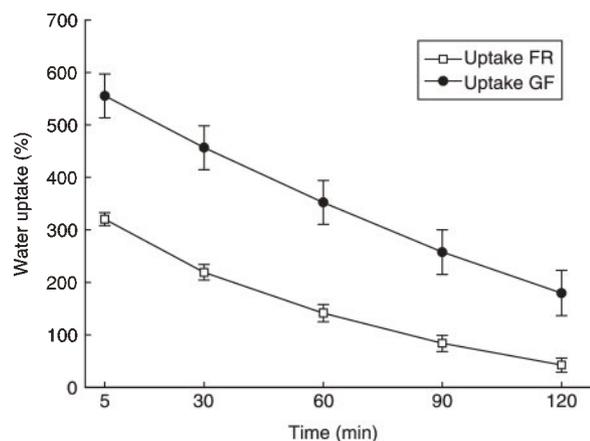


Figure 2. Percentage water uptake of fibre types used as construction material in bird nests (mean \pm SD). FR = fungal rhizomorphs, GF = grassy fibres, $n = 12$.

height of 2–3 m alongside the trails in the surroundings of PVNP and BCI Research Station.

The fungal rhizomorphs showed a higher tensile strength than the grassy fibres as indicated by the fact that the mean of the maximum load that the fungal rhizomorphs were able to carry, was significantly higher than for the grassy fibres (GLM: $F_{14,14} = 4.87$, $P = 0.036$). The fungal rhizomorphs could carry between 112 and 233 g of weight before snapping (mean = 145.5 g \pm sd 32.3 g, $n = 15$, 5 replicates), whereas the grassy fibres were capable of carrying only between 68 and 189 g of weight (mean = 118 g \pm sd 35.8 g, $n = 15$, 5 replicates).

At each point in time of the experiment (5, 30, 60, 90, 120 min) the water uptake of the fungal rhizomorphs was significantly smaller than for the grassy fibres (Fig. 2). After 5 min: GLM: $F_{11,11} = 29.15$, $P < 0.001$; after 30 min: GLM: $F_{11,11} = 28.44$, $P < 0.001$; after 60 min: GLM: $F_{11,11} = 22.05$, $P < 0.001$; after 90 min: GLM: $F_{11,11} = 14.75$, $P < 0.001$; after 120 min: GLM: $F_{11,11} = 9.3$, $P < 0.05$.

None of the fungal rhizomorphs produced hyphal growth after incubation on YM agar for several days under standard conditions.

DISCUSSION

This study suggests that fungal rhizomorphs utilized by Streak-backed Orioles as outer nesting material had a significantly higher tensile strength than the grassy fibres making up the interior parts of the same nests. Furthermore, the water uptake of the fungal rhizomorph fibres was significantly reduced relative to that of the grassy fibres. Based on the results of the culture experiment and field observations, the nests probably were constructed out of dead hyphae. This is not certain, because the nests were not in use when they were collected, and there is no way

of knowing the condition of the rhizomorphs when the nests were first built. Results presented here suggest that the nest-bearing fungal rhizomorphs were actively woven around the *Acacia* branches carrying the nests by the birds themselves and did not grow after the initial placement at the nesting site. The repeated searches for naturally occurring rhizomorphs of the horsehair fungi in the surroundings of these two tropical field stations yielded no sightings (in total 30–40 h spent searching at each site). This documents a much lower natural abundance of fungal rhizomorphs of the genus *Marasmius* – at least in this stratum of the forest since the canopy region was not searched – than grassy fibres which were abundant. Accordingly, it can be assumed that those birds using rhizomorphs of horsehair fungi are facing an increased metabolic cost for constructing their nest, based upon a likely longer searching time required to locate the fungal material and transport it to their nesting localities. This is to be seen in comparison to the alternative use of the abundant grassy fibres as the exclusive nest construction material. Given this hypothesized energetic constraint, the results of this study suggest an adaptive advantage for the use of marasmioid rhizomorphs for the outer nest by birds in general because this material is stronger and stays drier than the other more readily available nesting materials.

The two specific physical properties – tensile strength and water uptake – potentially hold advantages with respect to the life history of a particular bird species. A higher tensile strength would enable higher load bearing. This putatively may allow for larger clutch size, as well as an increased body size of adult birds. This could be mediated via a decreased risk of the nest falling due to physical elements such as strong winds but at present the mechanism involved is not well understood. Secondly, a wet or cold nest provides a substrate for various kinds of pathogens (e.g. Singleton & Harper 1998). Accordingly, a drier nest such as achieved by the usage of fungal rhizomorphs to create a water repellent outer protection layer for the actual nesting cup, could result in a lower pathogen load, which ultimately could result in an increased lifetime reproductive success. Melin *et al.* (1947) reported that certain species of the genus *Marasmius* had a marked inhibitory effect upon growth of *Staphylococcus*, although this benefit usually requires active growth of the fungal hyphae. This characteristic could give an adaptive advantage, although the production of antibiotics by the rhizomorphs under the nest conditions would need to be proven. An additional adaptive advantage of horsehair fungi might be that they are simply longer than grassy fibres, allowing the birds to construct large, elaborate nests. Without data on the length of naturally occurring fungal rhizomorphs though, this point has to remain speculative.

This study provides evidence for the hypothesis that the use of fungal rhizomorphs holds adaptive advantages over the usage of other materials: a relatively high tensile strength as well as a low water uptake. Additional studies

to document the potential fitness gain, as well as to answer the question whether fungal rhizomorphs are more abundant at different tropical localities and in the canopy stratum, need to be conducted. It can be assumed that the findings of this study gained by examining nests of Streak-backed Orioles may hold for other bird species using the same nest construction material.

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