Wood-inhabiting insects can function as targeted vectors for decomposer fungi

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ARTICLE INFO

ABSTRACT

Most wood-inhabiting fungi are assumed to be dispersed primarily by wind, with the exception of a few species involved in mutualistic relationships with insects. In this study we tested whether several species of wood-inhabiting insects can function as dispersal vectors for non-mutualistic fungi, which would indicate that wood-inhabiting fungi can benefit from targeted animal-mediated dispersal. We sampled wood-inhabiting beetles (Coleoptera) from freshly felled wood experimentally added to forests and used DNA metabarcoding to investigate the fungal DNA carried by these insects. Staphylinid beetles rarely contained fungal DNA, while *Endomychus coccineus*, *Glischrochilus hortensis* and *Glischrochilus quadripunctatus* frequently carried fungal DNA with a composition specific to the insect taxon. A large proportion of the obtained fungal sequences (34%) represented decomposer fungi, including well-known wood-decay fungi such as *Fomitopsis pinicola*, *Fomes fomentarius*, *Trichaptum abietinum* and *Trametes versicolor*. Scanning electron microscopy further showed that some of the fungal material was carried as spores or yeast cells on the insect exoskeletons. Our results suggest that insect-vectored dispersal is of broader importance to wood-inhabiting fungi than previously assumed.

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1. Introduction

Dispersal is an integral aspect of community ecology and population dynamics. It is a key component of community assembly (Myers and Harms, 2009; Chase, 2010) and influences the response of species to disturbances such as fragmentation (Johst et al., 2002; Cordeiro and Howe, 2003; Montoya et al., 2008) and climate change (Brooker et al., 2007; Engler et al., 2009). Given the rapid, human-driven habitat changes presently occurring on a global scale (Cardinale et al., 2012; Haddad et al., 2015), it is crucial to understand how species disperse in order to conserve both biodiversity and ecosystem functions.

For sessile terrestrial organisms such as plants or fungi, the propagule vector is highly important for dispersal efficiency. The most important abiotic vector is wind, with water playing a minor role, whereas a multitude of different animals can serve as biotic vectors (Watkinson et al., 2015). While wind dispersal is generally considered a random process, animal-mediated dispersal can be targeted towards suitable habitats. This fundamental difference between wind- and animal-mediated dispersal results in important ecological differences between species employing these different dispersal modes. For instance, animal-dispersed plant species seem to tolerate habitat fragmentation better than wind-dispersed species (Purves and Dushoff, 2005; Montoya et al., 2008; Marini et al., 2012), as long as their dispersal agents are present in habitat fragments (Cordeiro and Howe, 2003; Galetti et al., 2006; Cramer et al., 2007).

Seed dispersal has been extensively studied for both wind-dispersed and animal-dispersed plants (Nathan and Muller-Landau, 2000; Nathan et al., 2002; Wang and Smith, 2002; Schupp et al., 2010), but studies of fungal dispersal are less

exhaustive, partly due to the high diversity of fungal species and their variable ecology (Watkinson et al., 2015). Many fungi are wind-dispersed (Ingold, 1953; Piepenbring et al., 1998; Halbwachs and Bässler, 2015), but there are also several examples of animal-mediated dispersal (Blackwell, 1994; Johnson, 1996; Piepenbring et al., 1998; Halbwachs and Bässler, 2015). Animal-vectored dispersal of fungi is an understudied field where many interactions probably remain to be discovered (Malloch and Blackwell, 1992). Although some interactions between fungi and animal vectors are co-dependent mutualisms (Batra, 1963; Slippers et al., 2011), there is a continuum of insect-fungus interactions of differing specificity and opportunism which may lead to dispersal of fungal propagules (Talbot, 1952; Wilding et al., 1989; Blackwell, 1994; Tuno, 1998; Greif and Currah, 2007).

Fungi living in dead wood perform an essential ecosystem service by decomposing woody material and constitute a major component of forest biodiversity, including many species threatened by extinction (Gärdenfors, 2010; Rassi et al., 2010; Henriksen and Hilmo, 2015). Wood-inhabiting fungi are generally assumed to be dispersed by wind (Junninen and Komonen, 2011; Norros, 2013), with the exception of fungi associated with bark beetles, ambrosia beetles, termites or wood wasps (Batra, 1963; Martin, 1992; Harrington, 2005). However, these mutualistic species only represent a small fraction of the great diversity of arthropods and fungi that inhabit and decompose dead wood (Tikkanen et al., 2006; Stokland et al., 2012), and there are indications that animal-mediated spore dispersal of wood-decay fungi could be more widespread than previously assumed. For instance, several wood-inhabiting beetles visit fruit bodies of wood-inhabiting fungi during sporulation (Hågvar, 1999; Krasutskii, 2007b, 2010; Schigel, 2011), presumably feeding on spores, and these species could disperse spores of wood-inhabiting fungi in much the same way as invertebrates that feed on spores of soil fungi contribute to their dispersal (Rantalainen et al., 2004; Lilleskov and Bruns, 2005; Seres et al., 2007; Halbwachs et al., 2015). The few studies that have tested the effect of wood-inhabiting insects on the establishment of fungi in dead wood did find significant differences between the fungal communities that established with and without insects, but these studies all focused on bark beetles (Müller et al., 2002; Persson et al., 2011; Strid et al., 2014).

In the current study we investigate whether insect-vectored dispersal could be of broader importance to the fungal community in dead wood, potentially involving several species of insects and fungi. Studies of fungal dispersal have previously been restricted due to the difficulty of identifying spores, but the rapid development of molecular methods has presented new possibilities in this field of research. In this paper we use metabarcoding of fungal DNA from a broad range of wood-inhabiting beetle species to ask the following questions:

1. Do wood-inhabiting beetles not involved in obligate insect-fungus mutualisms frequently bring fungi to dead wood?
2. If so, what kind of fungi do the beetles carry? Specifically, do the beetles bring wood-decay fungi to newly available dead wood largely uncolonized by fungi?
3. Is the composition of fungal taxa specific to the beetle taxon?

2. Materials and methods

2.1. Sample sites

In March 2014, 17 aspen (*Populus tremula*) trees from the same stand in Ås municipality in Norway (Lat. 59.66, Long. 10.79, 92 m a.s.l.) were felled and cut into 1 m long logs with 20.5–36.4 cm diameter. The trees were felled shortly prior to insect sampling, since our intention was to study fungal dispersal to new, uncolonized habitat.

The logs were transported to two landscapes: Losby forest holdings in Østmarka (Lat. 55.98, Long.10.68, 150–300 m a.s.l.) and Løvenskiold-Vækerø (LV) forest holdings in Nordmarka (Lat. 54.49, Long. 21.24, 200–500 m a.s.l.). Both landscapes are within the southern boreal vegetation zone (Moen, 1998) and consist of forest dominated by spruce (*Picea abies*), with pine (*Pinus sylvestris*), birch (*Betula pubescens*) and aspen as subdominants. The forest holdings were managed as production forests within the regulations of the PEFC (the Programme for the Endorsement of Forest Certification schemes, Norway, http://pefcnorway.org/). Twenty-four logs were divided between four sites in each landscape with a mean distance of 1574 m between sites within a landscape and with an average of six logs per site. All selected sites were in semi-shaded, mature spruce forest.

No fungal fruit bodies were apparent on the logs during the first season (2014). In the second season (2015), fruit bodies of *Chondrostereum purpureum* appeared on all logs. No other macrofungi fruit bodies were apparent on the logs during the two seasons of field work, and thus the logs could be considered largely uncolonized habitat for wood-inhabiting fungi that might be vectored by the insects to the logs.

2.2. Insect sampling

Insects, specifically beetles (Coleoptera), were sampled from the aspen logs at each site during May to August in 2014 and 2015. To avoid contamination among samples the insects were sampled individually with tweezers either from sticky traps or directly from the logs. The tweezers were sterilized with ethanol and a gas burner between handling of each insect.

DeLaval™ fly sheets (60 × 30 cm) were used as sticky traps. At each site, one sheet was divided between three different logs and exposed for one or 2d before insect sampling. During sampling from the sticky traps, all insects found on the logs were also sampled. In total, insects were sampled on 11 occasions from each site.

Each insect was placed in a separate Eppendorf-tube (2 ml) and killed by freezing at − 80 °C, which was also the storage temperature. Insects sampled in 2014 and during the first sampling occasion in 2015 were rinsed in sterilized water to separate fungal DNA from the inside and the outside of the insects. However, it became clear that the insects defecated in the tubes, thereby contaminating their exoskeleton and the water with gut content. We, therefore, omitted rinsing the insects for the remaining sampling occasions.

The insects were identified to species or genus using available literature in a sterile environment and using sterilized equipment. Insects that could not be confidently identified at least to genus by the first author (RMJ) were not analyzed further (<20 individuals). We selected 343 beetle individuals for DNA analysis (Table S1) and put aside an additional nine individuals of some of the most abundant species for scanning electron microscopy (SEM). These were wood-inhabiting genera or species, i.e. insects with larval development either in dead wood or in fungal fruiting bodies on dead wood (Wheeler and Blackwell, 1984; Dahlberg and Stokland, 2004).

2.3. Scanning electron microscopy

We used a scanning electron microscope (Zeiss EVO 50 EP) to investigate whether any of five individuals of *Endomychus coccineus* or four individuals of *Rhizophagus* sp. carried fungal material on