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Host age determines parasite load of Laboulbeniales fungi infecting ants: Implications for host-parasite relationship and fungal life history

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ABSTRACT

Arthropod-parasitic fungi of the order Laboulbeniales are known to exhibit specialization to individual host taxa in most cases. Some species exhibit ecological specificity to multiple, often unrelated hosts in certain microhabitats; and often position specificity to different host body parts. The myrmecophilous *Rickia wasmannii* (Ascomycota: Laboulbeniales) infects *Myrmica* species (Hymenoptera: Formicidae) (host specificity), and occasionally other arthropod inquilines inside the ant nest (ecological specificity). An effect of the position of infection on the thallus densities has also been reported. Another determinative factor that may also exist in the *Rickia-Myrmica* host-parasite system, the chronological age of ant worker hosts, has also been linked to parasite load. Comprehensive studies on the age-related infection intensity, however, are still lacking. Here we investigated whether the level of infection correlates with the age of the *M. scabrinodis* host consistently. We found that older hosts exhibited higher parasite load, even though the infection level of the different colonies varied widely. The results highlight that the level of *R. wasmannii* infections are strongly influenced by host individual and host colony factors.

1. Introduction

The fungal order Laboulbeniales (Ascomycota) contains more than 2100 species of obligate biotrophic ectoparasites of several arthropod taxa (Espadaler & Santamaria, 2012; Kaur & Mukerji, 2006; Rossi, Máca, & Preisler, 2016). Laboulbeniales are known to lack asexual reproduction and sporadic studies on their life cycle and ontogenesis have concluded that their ascospores are short-lived and upon successful attachment to the host by physical contact, they develop into a mature thallus in 10–21 d (Cottrell & Riddick, 2012; De Kesel, 1993, 1996). Little is known about the effect of Laboulbeniales fungi on their hosts and they are generally considered to be of little harm for the host (Benjamin, 1971; Báthori, Csata, & Tartally, 2015a; Csata, Erős, & Markó, 2014; Konrad, Grasse, Tragust, & Cremer, 2015; Majewski, 1994; Nalepa & Weir, 2007). Members of the order are known to exhibit specialization either to a given host species or genus (host specificity) (Benjamin, 1971), or ecological specificity to microhabitats rather than strict host specialization (De Kesel, 1996; De Kesel & Haelewaters, 2014; Seeman & Nahrung, 2000). Position specificity to different host body parts is also well-known (Goldmann & Weir, 2012). Laboulbeniales are relatively rarely recognized by entomologists and difficulties in obtaining a sufficient number of infected insects hinder physiological or behavioral studies in the majority of host groups. Host insects forming colonies (Markó et al., 2016) or aggregates (Wang, De Kesel, Haelewaters, & Pfister, 2016) are best suited to study the ecology and epidemiology of these fungi (Haelewaters et al., 2017).

Ants are peculiar among Laboulbeniales hosts as they live in eusocial colonies in which larvae, pupae and relatively long-lived adults co-occur for a long time in a high density. Infected colonies can provide a substantial number of infected workers that are not only easily maintained in laboratory conditions, but also genetically related. There are only four Laboulbeniales species recorded from ants in Europe: *Laboulbenia camponoti* S.W.T. Batra (Báthori, Pfliegler, & Tartally, 2014; Espadaler & Santamaria, 2012; Gómez, Espadaler, & Santamaria, 2016); *Laboulbenia formicarum* Thaxt. (Espadaler & Santamaria, 2012; Gómez et al., 2016); *Rickia lenoirii* S. Santamaria (Báthori, Pfliegler, & Tartally, 2015b; Santamaria & Espadaler, 2015) and *Rickia wasmannii* Cavara. The latter species is the most widely distributed ant-

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associated member of this order in Europe, parasitizing nine *Myrmica* Latreille ant species (De Kesel, Haelewaters, & Dekoninck, 2016; Haelewaters, Boer, & Noordijk, 2015a, Haelewaters, Gort, Boer, & Noordijk, 2015b) and occasionally, mite deutonymphs and *Microdon* fly larvae living as inquilines in the *Myrmica* nests (Pfliegler, Báthori, Haelewaters, & Tartally, 2016a).

Intensive research on the latter species has made *Myrmica* hosts and *R. wasmannii* one of the best known Laboulbeniales-host system, with the involvement of biogeographic, physiological, behavioral and ecological aspects, laboratory and field observations on two host species (*M. scabrinodis* Nylander and *M. sabuleti* Meinert) and on populations from several locations in Europe (predominantly from the Netherlands, Hungary, and Romania) (Csata et al., 2014, 2017, 2013; Bezděčková & Bezděčka, 2011; Bezděčka & Bezděčková, 2011; Báthori et al., 2015a; De Kesel et al., 2016; Haelewaters et al., 2015a, b; Markó et al., 2016; Pech & Heneberg, 2015; Pfliegler et al., 2016a, Pfliegler, Tálas, Báthori, Tartally, Pócsi, & Szemán-Nagy, 2016b; Santamaria & Espadaler, 2015; Tartally, Szűcs, & Ebsen, 2007; Tragust, Tartally, Espadaler, & Billen, 2016; Witek, Barbero, & Markó, 2014).

Despite frequent physical contact, workers seem to transmit the infection to larvae extremely rarely (Baumgartner, 1934, pp. 45–47; Haelewaters, Gort, Boer, & Noordijk, 2015b). Additionally, older workers (those with hardened, gradually darker cuticle) have been found to carry a higher parasite load than young ones in the case of *M. sabuleti* and *M. scabrinodis* (Csata, Bernadou, Rákosy-Tican, Heinze, & Markó, 2017; Haelewaters et al., 2015b). In the case of the former species, *M. sabuleti* individuals with low infection rate were mostly infected on the frontal part of the head (Haelewaters et al., 2015b).

In this study, our aim was to determine whether host ant age groups show consistent differences in infection intensity across colonies and populations. For this, we studied the parasite load of workers of nine different colonies, after sorting them to three well-defined age groups (sensu Cammaerts-Tricot, 1974). To see on whether age-related differences in the level of infection simply result from gradual accumulation of thalli, we also compared the proportion of presumably dead thalli in different host age groups.

2. Materials and methods

2.1. Collection of ant colonies and infected ant samples

Colonies of *M. scabrinodis* were collected at the following sites in eastern and northern Hungary: Újléta (May 2013; 47°26′ N, 21°51′ E; 120 m a.s.l.; colonies numbered UF2, UF3 and UF6), Rakaca: Meszes (May 2013; 48°27′ N, 20°47′ E; 165 m a.s.l.; colonies RF1, RF3, and RF5), Gyöngyös: Sár-hegy: Gyilkos-rét (May 2015; 47°48′ N, 19°58′ E; 352 m a.s.l.; colonies GF1, GF4, and GF5). We collected 3 colonies infected by *R. wasmannii* from each site. All colonies contained a few hundred workers of *M. scabrinodis*.

2.2. Determination of thalli load, fungal morphology

Altogether nine infected colonies from three Hungarian sites were used to survey age-related differences in parasite load. From each colony, 20 *M. scabrinodis* workers were killed and stored in 96% ethanol from three of the five known age groups, described by Cammaerts-Tricot (1974) according to the coloration of the cuticle (viz. the older the ants are the darker they become). We selected ants belonging unambiguously to age categories “1”, “3” and “5” (“AGE1, AGE3, AGE5” hereafter; see: Fig. 1) but not to “2” and “4” to avoid the problematic characterization of individuals with intermediate patterns. The infection rate upon the color degree of each individual was investigated. The altogether 540 (viz. 20 workers × 3 age categories × 3 colonies × 3 sites) workers were screened (by FB) for fungal thalli using a Leica MZ125 microscope at 10–160× magnification. We counted all fungal thalli on the whole ant body. Thereafter, thalli were removed from randomly chosen workers of each ant group and slide mounted into Amman's lactophenol as described by Haelewaters et al. (2015c). Thalli were examined with transmitted light and 20–40× magnification using a microscope (Standard 25, Carl Zeiss, Jena, Germany). For identification, thalli were compared to the original description (Cavara, 1899) of *R. wasmannii* and to recent descriptions (De Kesel et al., 2016; Haelewaters, 2012). Thalli with abnormal morphology (teratological forms, De Kesel & Van den Neucker, 2005) but well-developed perithecia, and those with extensive melanization and spore-less perithecia (presumably dead thalli) were observed (Fig. 2) and their proportion in each sample was determined. Thalli in the latter group were presumed lifeless. Altogether 372 thalli were microscopically observed from age group 1 ants, 477 from age group 3, and 604 from age group 5.

2.3. Statistical analyses

Analyses were performed using the R statistical software (version 3.0.2; R Core Team, 2013). Data was analyzed using generalized linear models (GLM) from the R-packages “stats” and generalized linear mixed effects models (GLMM) “lme4” (Bates, Maechler, Bolker, & Walker, 2015). Because in case of factor variables with more than two levels, all factor levels are compared to an arbitrarily selected level (therefore some comparisons are not presented in the model output), in all of the models described we used the general linear hypotheses testing and multiple comparisons method from the R package “multcomp” in order to compare the effects of all factor levels on the tested variables (Hothorn, Bretz, & Westfall, 2008).

2.3.1. Thalli number and age-groups

To test whether the number of thalli increases with the progression of age, generalized mixed-effects linear model (GLMM) was fitted, with negative binomial error distribution, because our data showed considerable overdispersion (higher variance than expected in the data). In the negative binomial GLMM thalli-number was the response variable, while age-group was specified as predictor factor of three levels (AGE1, AGE3, and AGE5). We have specified colony, nested in habitat, as random factors to control to habitat and colony-in-habitat specific variances in the mean number of thalli on ants (random intercepts). Random slopes were also estimated, in order to control for colony-specific differences in the change of thalli number with the progression of age.

2.3.2. Proportion of teratological and presumably dead thalli

When testing whether the proportion of thalli with teratological morphology, or the proportion of presumably dead thalli (characterized by excessive melanization) changes with host age, binomial GLMs were fitted. The proportion of teratological thalli among all living thalli was used as response variable in one model, while in a separate GLM, the proportion of melanized thalli in all (normal and teratological, living and dead) thalli was the response variable. Age-group was specified as predictor factor. Because of the low samples size ($N = 24$ observations) we did not include the habitat and colony of the ants in the models.

3. Results

3.1. Thalli number and age-groups

Workers of older age-groups carried significantly more thalli on