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Mycodiplosis (Diptera) infestation of rust fungi is frequent, wide spread and possibly host specific

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ABSTRACT

Insect mycophagy is considered common but generally lacking host-specificity. Larvae of some *Mycodiplosis* species (Insecta, Diptera) feed primarily on spores of rust fungi (Basidiomycota, Pucciniales). The number of rust-feeding species and their relative frequency, distribution, and degree of host-specificity are not known. A survey of 200 recent rust collections from around the world, and a systematic survey of 333 herbarium specimens from Maryland show that *Mycodiplosis* infestation is very common. Desiccated larvae were found on specimens dating back as far as 1886, the oldest collection in the survey. Greater than 20 % of all rust collections examined were infested with *Mycodiplosis* larvae. In Maryland infestation frequencies were similar at different spatial scales, but different rust species varied in their frequency of infestation. Primers were designed to target *Mycodiplosis* 28S rDNA, and sequence data revealed genetic variation between *Mycodiplosis* isolates from different rust species.

Introduction

Mycophagy is common among Diptera (true flies) larvae and many feed exclusively on mycelium, yeasts, fruit bodies, or complex decomposing fungal-rich substrata (Hammond & Lawrence 1989). Although it is generally accepted that there is little specialization by flies on particular fungi, studies have not comprehensively addressed Dipteran fauna feeding on particular fungi with the notable exceptions focused on large fleshy fungi (Buxton 1960; Bruns 1984). There are only a few mycophagous Diptera with recognized host-specificity feeding on other groups of fungi.

Rust fungi offer an attractive nutritional resource to potential mycovores. Rust fungi produce copious amounts of thick-walled spores that are presumably fairly uniform in nutritive composition while generating little in the way of vegetative growth outside of the host plant. Rust fungi can be patchily distributed in space and time but display strong seasonality, host-specificity, and some species form systemic infections that may produce spores at the same locations and times of year on an annual basis. However, only a few Coleoptera (beetles) and Lepidoptera (moths and butterflies) are known to make use of rusts as a food source (Hammond & Lawrence 1989). The most common mycovore feeding on rusts are flies in the genus *Mycodiplosis* (Cecidomyiidae – Gall Flies).

Mycodiplosis is cosmopolitan in distribution, but species are restricted to feeding on either rust fungi (Pucciniales) or powdery mildews (Erysiphales) (Holz 1970; R. Gagné, pers comm). This genus is within the Cecidomyiidae, a family that

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includes another specialised group of flies that live within plant galls but feed only on fungi growing within the gall, collectively called ambrosia galls. *Mycodiplosis* larvae feed primarily on fungal spores, for which they have specially adapted mouth parts (Holz 1970), with perhaps occasional grazing on mycelium. Few studies have examined the relationship between *Mycodiplosis* and its fungal food sources. Some researchers have suggested that the relationship results in a significant benefit to the infected plants because the fly grazing reduces fungal spore quantity (Golenia 1961; Kaushal *et al*. 2001). Others have suggested that the flies may serve as inter-plant vectors for the fungi (Eskes 1989; Kluth *et al*. 2001). We are not aware of any studies that have examined frequency of infection or host specialization in the *Mycodiplosis*–Rust relationship.

In the only systematic revision of flies in the *Mycodiplosis* group, Holz (1970) recognized only three species from Europe that feed on rust fungi. Gagné (1994) recognized four other species largely endemic to the Neotropics. There are likely to be many undescribed species in entomological collections, but these collections lack value because they do not include ecological information such as host, and are largely ignored by entomologists because *Mycodiplosis* flies are very small (Gagné 1994, R.J. Gagné, pers comm). There are also likely to be many undescribed species of *Mycodiplosis* present in mycological collections as larvae, but species identification in *Mycodiplosis* largely depends on the morphology of adult males. It is easy to rear *Mycodiplosis* larvae collected with a fungal host to maturity, but because mycologists are largely unaware of the existence of these flies many collections go unrecognized and, once dried and pressed, the insects become unidentifiable beyond genus using standard morphological techniques.

In this study, we assessed the occurrence of *Mycodiplosis* spp. on rust fungi. We used a survey strategy to determine the frequency of infestation on rusts in Maryland and the distribution of *Mycodiplosis* infestation among rust species within Maryland. We surveyed available literature and a sample of recent collections to assess the occurrence of *Mycodiplosis* worldwide and on disparate host fungi. We also developed some basic molecular tools to link mycological and entomological collections while confirming the phylogenetic placement of *Mycodiplosis*.

Methods

Survey of Mycodiplosis *infestation*

Collections of rusts were scanned for the presence of *Mycodiplosis* using a Zeiss Stemi SV11 stereomicroscope. Typically each specimen was examined until two fly larvae were found or for a maximum of 5 min if no insects were found. Some uninfested specimens where material was limited were not scanned for 5 min, but until the available material was thoroughly checked. An initial survey of *Mycodiplosis* infestation was a sample of 200 specimens recently collected from nine countries. A sampling scheme for the survey of the Maryland rusts was constructed using the databases of the U.S. National Fungus Collections (BPI). All rust collections from the state of Maryland housed at BPI total 2077 specimens. Using the random number generator function in Microsoft Excel we randomly chose 208 specimens (10 %) from the Maryland collections to scan. Additionally, we chose all 152 specimens from a single highly collected area, Beltsville, Maryland, to scan. Partially based on results from these surveys we also scanned every collection of *Uromyces ari-triphylli* in the herbarium. We tested individual host species for deviation from the overall infestation rate using a chi squared test of the observed numbers of infested and uninfested samples within a species compared to the expected numbers based on the overall ratio of infested to uninfested samples.

Molecular techniques and phylogenetic analysis

We designed degenerate PCR primers Fly28f (5′-AGAGTCGNG TTGCTTGANAGTGC-3′) and Fly28r (AGACCNGCTGCGGATA TNGGTA) for a region spanning the nuclear large subunit rRNA gene including D1–D7 expansion units based on *Clinodiplosis* sequences in GenBank. We extracted DNA from larvae taken from seven rust specimens using the UltraClean plant DNA extraction kit (Mo Bio Laboratories Inc. Solana Beach, CA, USA). We used up to 15 larvae per extraction, but several extractions contained only a single insect. One μl of each extraction was placed in a 24 μl cocktail containing 12.5 μl of PCR Master Mix (Promega Corp., Madison, WI, USA), 1 μl of each 10 μM primer, and 9 μl sterile water. Amplification reactions were carried out on an Eppendorf Mastercycler thermal cycler for 35 cycles following this profile: 95 °C for 45 s, 52 °C for 90 s, 72 °C for 90 s. Products were visualized *via* electrophoresis in a 1 % agarose gel and target bands were purified using the RECOCHIP kit (Takara Bio Inc., Otsu, Shiga, Japan). Three μl of the purified products were used in sequencing reactions with BigDye terminator cycle sequencing kit 3.1 (Applied Biosystems, Foster City, CA, USA) and the same primers as those used for amplification. Sequencing reactions were precipitated with ethanol and 125 mM ammonium acetate (12:1) and suspended in 10 μl HiDi formamide (Applied Biosystems, Foster City, CA, USA) and sequenced on an ABI3100 Automated Sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were edited using Sequencher 4.1 (GeneCodes Corporation, Ann Arbor, MI, USA).

Sequences were aligned manually with sequences from other flies in the Cecidomyiidae using MacClade 4.08 (Sinauer Associates, Sunderland, MA, USA). Ambiguous and non-overlapping regions were excluded from the analyses. Parsimony and neighbour-joining with maximum likelihood parameters were used to reconstruct a phylogeny of the sequences using PAUP* v.4.0b10 (Sinauer Associates, Sunderland, MA, USA). Because of the small number of taxa we used a branch and bound search algorithm to determine all most parsimonious trees. For bootstrap replicates we also used the branch and bound algorithm with maxtrees set to 100.

Results

Survey of rust collections and general observations

Mycodiplosis larvae were easily detected on fresh collections, and their feeding on rust spores was observed (Fig 1). *Mycodiplosis* larvae were recognized by their mouth morphology