Fungal symbiont of firebrats (Thysanura) induces arrestment behaviour of firebrats and giant silverfish but not common silverfish

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Abstract—We have recently shown that firebrats, *Thermobia domestica* (Packard) (Thysanura: Lepismatidae), carry, and deposit with their faeces, the symbiotic bacterium *Enterobacter cloacae* (Jordan 1890) Hormaeche and Edwards 1960 (Enterobacteriaceae) and the symbiotic fungus *Mycotypha microspora* Fenner, 1932 (Mycotyphaceae)**,** and that these microbes induce arrestment behaviour and aggregation of firebrats. Here, we tested whether giant silverfish, *Ctenolepisma longicaudata* Escherich (Thysanura: Lepismatidae), and common silverfish, *Lepisma saccharina* (Linnaeus) (Thysanura: Lepismatidae), also arrest in response to these two microbes. In dual-choice bioassays, *E. cloacae* arrested firebrats but not giant silverfish or common silverfish, whereas *M. microspora* arrested firebrats and giant silverfish but not common silverfish. As close relatives, firebrats and giant silverfish have similar microclimate and nutrient requirements and may use *M. microspora* as the same aggregation cue when they aggregate in hot and humid microclimates where *M. microspora* proliferates and breaks down cellulose. As a more distant relative to firebrats and giant silverfish, common silverfish seem to require a different as yet unknown aggregation cue or signal, possibly one that is indicative of the type of microclimate (room temperature; high humidity) they prefer.

Résumé—Nous avons démontré récemment que les thermobies, *Thermobia domestica* (Packard) (Thysanura: Lepismatidae), portent et déposent dans leurs fèces la bactérie symbiotique *Enterobacter cloacae* (Jordan 1890) Hormaeche et Edwards 1960 (Enterobacteriaceae) et le champignon symbiotique *Mycotypha microspora* Fenner 1932 (Mycotyphaceae) et que ces microorganismes provoquent un comportement d'arrêt sur place et d'attroupement chez les thermobies. Nous vérifions maintenant si le poisson d'argent géant, *Ctenolepisma longicaudata* Escherich (Thysanura: Lepismatidae), et le poisson d'argent commun, *Lepisma saccharina* (Linnaeus) (Thysanura: Lepismatidae), s'arrêtent aussi en présence de ces deux microorganismes. Dans des essais à deux choix, *E. cloacae* provoque l'arrêt sur place des thermobies, mais non des poissons d'argent géants ni des poissons d'argent communs, alors que *M. microspora* provoque l'arrêt des thermobies et des poissons d'argent géants, mais non des poissons d'argent communs. Les thermobies et les poissons d'argent géants, qui sont proches parents, possèdent des besoins semblables en microclimat et en nourriture et peuvent ainsi utiliser *M. microspora* comme signal commun lorsqu'ils se rassemblent dans les microclimats chauds et humides dans lesquels *M. microspora* prolifère et décompose de la cellulose. Étant des parents plus éloignés des thermobies et des poissons d'argent géants, les poissons d'argent communs semblent nécessiter un signal ou indicateur de rassemblement différent et encore non identifié, possiblement un qui indique le type de microclimat qu'ils préfèrent (température de la pièce, humidité élevée).

Many microorganisms are symbionts to more than one insect host species, because they are physiologically versatile or pervasive in the environment, can provide various functions or benefits, or because they have simply radiated with their host (Bright and Bulgheresi 2010; Woodbury 2012). For example, the bacterium *Enterobacter cloacae* (Jordan 1890) Hormaeche and Edwards 1960 (Enterobacteriaceae) produces the aggregation semiochemical of the desert locust, *Schistocerca gregaria* (Forkskal) (Orthoptera: Acrididae) (Dillon *et al.* 2000, 2002; Dillon and Charnley 2002), helps

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blowflies and fruit flies locate oviposition sites (Emmens and Murray 1983; Jang and Nishijima 1990), and improves the development and survival of various other fruit fly hosts (Fitt and O'Brien 1985; Brummel *et al.* 2004). Firebrats, *Thermobia domestica* (Packard), giant silverfish, *Ctenolepisma longicaudata* Escherich, and common silverfish, *Lepisma saccharina* Linnaeus (all Thysanura: Lepismatidae), inhabit human dwellings with preference for a particular microclimate (firebrats: 32–44 °C, 50–98% relative humidity (Sweetman 1938); giant silverfish: 24 °C, 55–82% relative humidity (Lindsay 1940); common silverfish: 22–32 °C, 75–97% relative humidity (Lindsay 1940)).

All three Thysanura species aggregate on substrates that have previously been visited by conspecifics and by some heterospecifics (Tremblay and Gries 2003; Woodbury and Gries 2007, 2008). Specifically, substrate previously exposed to firebrats or giant silverfish induces arrestment behaviour of firebrats and giant-silverfish, but not common silverfish (Woodbury and Gries 2007). The arrestment response to *E. cloacae* and *Mycotypha microspora* allows firebrats, which lack any form of long-distance communication, to locate conspecifics and prospective mates in suitable shelters (Woodbury and Gries 2013).

We have recently shown that firebrats carry, and deposit with their faeces, the symbiotic bacterium *E. cloacae* and the fungus *M. microspora* Fenner, 1932 (Mycotyphaceae), and that these microbes induce arrestment behaviour and aggregation of firebrats (Woodbury and Gries 2013; personal observations). Fluorescently labelled *E. cloacae* and *M. microspora* were present within digestive tracts, faeces, and shelters of firebrats, but not within firebrat ovarioles or eggs, indicating that firebrats consume these symbionts and transmit them to new habitats by defecation (Woodbury *et al.* 2013). Like their firebrat host, both symbionts require sustained hot and humid environments (35 °C, >75% relative humidity; personal observation), and proliferate only in those conditions optimal for their host. Firebrats aggregate where their microbial symbionts thrive, responding to and consuming digested cellulose (glucose) produced by *M. microspora* (personal observation). Here, we tested the hypothesis that *E. cloacae* and *M. microspora* induce arrestment in firebrats and giant silverfish but not in common silverfish.

Insect specimens used for bioassays were obtained from three-week-old colonies maintained at the insectary of Simon Fraser University (SFU), Burnaby, British Columbia, Canada under an 8:16 light:dark light-cycle, at 23–35 °C and 70–85% relative humidity, as described for firebrats (Woodbury and Gries 2007). Colonies comprised insects of mixed age and gender that were obtained from autoclave rooms and boilers around SFU.

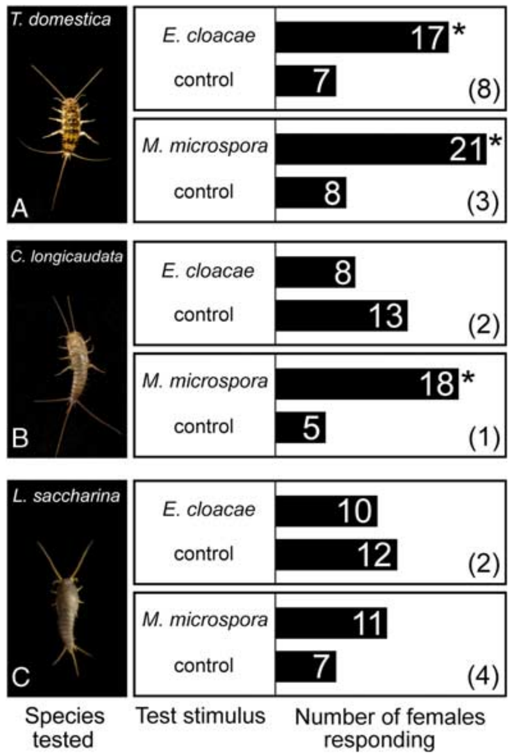
To test the response of single adult female insects to *E. cloacae* and *M. microspora*, bioassays were run in custom-built still-air, three-chamber olfactometers consisting of two lateral Pyrex® glass Petri dishes (Fisher Scientific Company, Whitby, ON, Canada), connected to a central dish (all dishes 3×9 cm) via Pyrex® glass tubing (2.5×2 cm). This olfactometer design mimics the natural still-air shelters of firebrats. Before the start of bioassays, a folded cone of sterile filter paper (Whatman® No. 1, 125 mm diameter; Whatman International Ltd., Maidstone, United Kingdom) was placed (tip facing central chamber) into each lateral dish to serve as an artificial shelter. Treatment and control stimuli were randomly added to each lateral dish that was capped to prevent insect escape (Woodbury and Gries 2007). The randomly assigned treatment chamber received a 0.5-mg (wet-weight) aliquot of *E. cloacae* (1×10^8 colony forming units (CFU) or *M. microspora* (1×10^6 CFU) from GlcNAc-agar cultures (originally isolated from firebrats) that was applied to the centre of a 1/8th piece of filter paper (Whatman® No. 1, 125 mm diameter), smeared into a single 0.5-cm^2 spot, and dried in ambient air before bioassays. Control chambers received an aliquot of water absorbed from uninoculated GlcNAc agar, and applied onto a 0.5-cm^2 spot of a 1/8th piece of clean filter paper that was air-dried before bioassays. As firebrats, giant silverfish, and common silverfish are nocturnal foragers that arrest in shelters during daylight, an individual insect was released into an olfactometer during the dark period, allowing it to explore the chambers during 10 hours of darkness, and to arrest in one of the shelters during 6 hours of light. Any insect not found in contact with either the treatment or control paper was recorded as a non-responder. All experimental replicates ($n = 22$–32 for each experiment) were run at 21 ± 2 °C and 30–60% relative humidity, which are conditions conducive to the response of all three Thysanura (Tremblay and Gries 2003; Woodbury and Gries 2007, 2008). Olfactometers were washed with LiquiNox detergent (Alconox® Inc., White Plains, New York, United States of America), and were oven-dried (Pacific Combustion Engineering Co., Torrance, California, United States of America) between each bioassay. Choice data of responding insects in each experiment were analysed by χ^2 test, using JMP™ software tables (SAS®, Cary, North Carolina, United States of America). Insects not in contact with the treatment or control paper at the termination of experiments were considered non-responders and were not included in statistical analyses. Voucher specimens of all three species were deposited in the museum collection of Simon Fraser University.

Paper inoculated with *E. cloacae* induced arrestment by firebrats but not by giant silverfish or common silverfish (Fig. 1), and paper inoculated with *M. microspora* arrested firebrats and giant silverfish but not common silverfish (Fig. 1). These results confirm reports that firebrats and giant silverfish interchangeably respond to the arrestment cue(s) conspecifics or heterospecifics deposited on substrate (Tremblay and Gries 2003; Woodbury and Gries 2007). The data also suggest that the fungal symbiont *M. microspora*, which we have isolated from field-collected firebrats and giant silverfish (data not shown), may represent or contribute to a common arrestment cue.

Firebrats and giant silverfish as close relatives (Mendes 1991) seek shelters with similar microclimates for reproduction and offspring development (Sweetman 1938; Lindsay 1940). Firebrats aggregate in the sustained hot and humid environments (35 °C; >75% relative humidity) where *M. microspora* proliferates and breaks down cellulose to glucose, which firebrats consume (personal observations). As giant silverfish have similar microclimate and nutrient requirements as firebrats (Sweetman 1938; Lindsay 1940), they may accrue the same benefits when they arrest in response to the presence of *M. microspora* (Fig. 1)

No arrestment response by common silverfish, which are more distant relatives to firebrats and giant-silverfish (Mendes 1991), to paper inoculated with *M. microspora* (Fig. 1), or to paper exposed to firebrats or giant silverfish (Tremblay and Gries 2003; Woodbury and Gries 2007), implies that common silverfish require a different as yet unknown aggregation cue or signal, possibly one that is indicative of the type of microclimate (room temperature; high humidity) they prefer.

Fig. 1. Number of adult female firebrats, *Thermobia domestica* (A), giant silverfish, *Ctenolepisma longicaudata* (B), and common silverfish, *Lepisma saccharina* (C) in single-insect bioassays arresting on a piece of filter paper inoculated with the bacterium *Enterobacter cloacae* or the fungus *Mycotypha microspora*. Numbers within bars indicate the number of insects responding to the treatment stimulus (upper bar) or control stimulus (lower bar). An asterisk (*) indicates a significant preference for a particular test stimulus (χ^2 test; $^*P \leq 0.05$). Numbers in brackets indicate numbers of non-responding insects.



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References

Bright, M., and Bulgheresi, S.A. 2010. Complex journey: transmission of microbial symbionts. Nature Reviews Microbiology, **8**: 218–230.

Brummel, T., Ching, A., Seroude, L., Simon, A.F., and Benzer, S. 2004. *Drosophila* lifespan enhancement by exogenous bacteria. Proceedings of the National Academy of Sciences of the USA, **101**: 12974–12979.

Dillon, R.J., and Charnley, A.K. 2002. Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. Research in Microbiology, **153**: 503–509.

Dillon, R.J., Vennard, C.T., and Charnley, A.K. 2000. Exploitation of gut bacteria in the locust. Nature, **403**: 851.

Dillon, R.J., Vennard, C.T., and Charnley, A.K. 2002. A note: gut bacteria produce components of a locust cohesion pheromone. Journal of Applied Microbiology, **92**: 759–763.

Emmens, R., and Murray, M. 1983. Bacterial odors as oviposition stimulants for *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae), the Australian sheep blowfly. Bulletin of Entomological Research, **73**: 411–416.

Fitt, G.P., and O'Brien, R.W. 1985. Bacteria associated with four species of *Dacus* (Diptera: Tephritidae) and their role in the nutrition of the larvae. Oecologia, **67**: 447–454.

Jang, E.B., and Nishijima, K.A. 1990. Identification and attractancy of bacteria associated with *Dacus dorsalis* (Diptera: Tephritidae). Environmental Entomology, **19**: 1726–1751.

Lindsay, E. 1940. The biology of the silverfish, *Ctenolepisma longicaudata* Esch. with particular reference to its feeding habits. Proceedings of the Royal Society of Victoria, **52**: 35–83.

Mendes, L.F. 1991. On the phylogeny of the genera of Lepismatidae (Insecta: Zygentoma). *In* Advances in management and conservation of soil fauna. *Edited by* G.K. Veeresh, D. Rajagopal and C.A. Viraktamath. Oxford & IBH Publishing, New Delhi, India. Pp. 3–13.

Sweetman, H.L. 1938. Physical ecology of the firebrat, *Thermobia domestica* (Packard). Ecological Monographs, **8**: 285–311.

Tremblay, M.N., and Gries, G. 2003. Pheromone-based aggregation behaviour of the firebrat, *Thermobia domestica* (Packard) (Thysanura: Lepismatidae). Chemoecology, **13**: 21–26.

Woodbury, N. 2012. Microorganisms that benefit insects: A guide to their transmission and experimentally-demonstrated benefit. *Edited by* K. Woodman. LAP Lambert Academic Publishing, Saarbrücken, Saarland, Germany. Pp. 1–168.

Woodbury, N., and Gries, G. 2007. Pheromone-based arrestment behavior in the common silverfish, *Lepisma saccharina*, and giant silverfish, *Ctenolepisma lognicaudata*. Journal of Chemical Ecology, **33**: 1351–1358.

Woodbury, N., and Gries, G. 2008. Amber-colored excreta: a source of arrestment pheromone in firebrats, *Thermobia domestica*. Entomologia Experimentalis et Applicata, **127**: 100–107.

Woodbury, N., and Gries, G. 2013. Firebrats, *Thermobia domestica*, aggregate in response to the microbes *Enterobacter cloacae* and *Mycotypha microspora*. Entomologia Experimentalis et Applicata, **147**: 154–159.

Woodbury, N., Moore, M., and Gries, G. 2013. Horizontal transmission of the microbial symbionts *Enterobacter cloacae* and *Mycotypha microspora* to their firebrat host. Entomologia Experimentalis et Applicata, **147**: 160–166.