A scientific note on a protozoan pathogen of the small hive beetle

Natasha Wright, Donald Steinkraus

► To cite this version:

Natasha Wright, Donald Steinkraus. A scientific note on a protozoan pathogen of the small hive beetle. *Apidologie*, 2013, 44 (2), pp.173-175. 10.1007/s13592-012-0164-7 . hal-01201280

HAL Id: hal-01201280

<https://hal.science/hal-01201280>

Submitted on 17 Sep 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

A scientific note on a protozoan pathogen of the small hive beetle

Natasha WRIGHT, Donald STEINKRAUS

Department of Entomology, University of Arkansas, Fayetteville, AR 72701, USA

Received 23 April 2012 – Revised 29 June 2012 – Accepted 19 July 2012

Aethina tumida / Malpighian tubule / parasite / neogregarine / biological control

The small hive beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae), is an invasive pest of European honey bee colonies. Chemical and cultural controls are typically used to keep SHBs at a tolerable level. Little work has been done on the biological control of SHBs. While some generalist fungal pathogens and commercially available nematodes have been reported to attack SHBs (Ellis et al. 2004; Mürrle et al. 2006; Cabanillas and Elzen 2006; Ellis et al. 2010), and a yeast, *Kodamaea ohmeri*, forms a mutualistic relationship with SHBs (Torto et al. 2007), to date, there have been no published reports of any host-specific predators, parasitoids, or pathogenic protozoa, fungi, nematodes, bacteria, or viruses. We conducted a study to determine if there are any naturally occurring pathogens of SHB adults and larvae in Arkansas and adjacent states. This is the first report of an obligate internal pathogen of SHBs.

Beekeepers were solicited to submit SHB samples at beekeeper meetings. SHBs were sent to us in 70 % ethanol by beekeepers and bee removers. In some cases, live adults were collected from apiaries and maintained on honey before being dissected. In 2011, we dissected 749 adults and 230 larvae from 13 counties in Arkansas and one county each in Oklahoma and Missouri. The midgut, Malpighian tubules, and fat body from each SHB were examined under a phase contrast microscope at $\times 400$ for the presence of microorganisms (Undeen and Vavra 1997). Lacto-fuchsin was used for mounting the tissues of preserved specimens, and Ringer's solution was used for fresh specimens (Becanel 1997).

We observed no microbial pathogens in SHB larvae ($n=230$). However, we did find a protozoan

pathogen in adult SHBs from three Arkansas counties: Crittenden, Pulaski, and St. Francis. Forty adults (5.3 %) were found to be infected in preserved and living specimens ($n=749$). Most of these infected beetles were from a single apiary in St. Francis County, AR ($n=201$, 18.4 % infected). Of the 189 living adult SHBs collected from apiaries across Arkansas, three lightly infected beetles were found (less than 50 cysts counted per SHB).

Protozoan cysts were found only in the Malpighian tubules of infected adult SHBs. The severity of infections varied among SHB specimens. Malpighian tubules of uninfected or lightly infected beetles were normal in appearance (Figure 1a) with zero or relatively few cysts. However, Malpighian tubules of heavily infected beetles were greatly swollen, containing thousands of cysts (Figure 1b). The cysts were lemon-shaped (Figure 1c) and measured $9.9 (\pm 0.13) \mu\text{m}$ in length and $7.1 (\pm 0.05) \mu\text{m}$ in width ($n=65$). It seems likely that heavily infected Malpighian tubules had impaired function in life.

We observed no early developmental protozoan life stages (such as primary or secondary trophozoites in the Amoebozoa or sporozoites and merozoites in the Neogregarinida) in preserved or living SHB specimens. Precise identification of this protozoan morphologically, based only on cysts, was not possible. Identification of the cysts would be very difficult even with molecular techniques. Currently, there is no effective method for isolating the cysts from host tissue and DNA. Obtaining nucleic acids and rRNA gene sequences from cysts is extremely difficult and expensive (J. Silberman, unpublished data). Therefore, we were unable to identify this protozoan further at this time.

Based on the organ specificity (only the Malpighian tubules were infected) and the size and shape of the cysts, this newly discovered protozoan pathogen of

Corresponding author: N. Wright,
nawright@uark.edu
Manuscript editor: David Tarpy

Figure 1. **a** Healthy Malpighian tubule from an adult SHB (prepared in Ringer's solution). **b** Heavily infected, swollen, Malpighian tubule from an adult SHB containing many protozoan cysts (prepared in lacto-fuchsin). Arrows point out some protozoan cysts. **c.** Close up of a single protozoan cyst.

SHB could be a species in the order Amoebida, perhaps in one of the three genera reported as pathogens of insects: *Malameba*, *Malphigamoeba*, or *Malpighiella* (Undeen and Vávra 1997). However, the cysts also resembled those of protozoa in the order Neogregarinida. We consulted two prominent insect pathologists. They stated that the cysts appeared to be neogregarines but they could not be certain without seeing the immature stages (H. Kaya and J. Lord, unpublished data). Even though we lacked early stages, it is evident that this protozoan is an obligate internal pathogen. While there are some commensal or weakly pathogenic protozoa found in insects (such as the eugregarines), neogregarines and entomogenous amoebae found in the Malpighian tubules are considered to be obligate pathogens (Tanada and Kaya 1993). Future research should include bioassays with this protozoan and SHBs to

examine as many life stages of this protozoan as possible, allowing for more precise identification.

ACKNOWLEDGMENTS

We thank the California State Beekeeper Association for funding. We would also like to thank the beekeepers, bee removers, and Jon Zawislak for supplying SHBs. Drs. Jeff Silberman, Harry Kaya, and Jeff Lord examined our specimens and provided advice.

Note scientifique sur un protozoaire pathogène du petit coléoptère des ruches

Eine wissenschaftliche Notiz über ein Einzeller-Pathogen des Kleinen Beutenkäfers

REFERENCES

- Becnel, J.J. (1997) Complementary techniques: preparations of entomopathogens and diseased specimens for more detailed study using microscopy. In: Lacey, L.A. (ed.) Manual of techniques in insect pathology, pp. 337–353. Elsevier, Amsterdam
- Cabanillas, H.E., Elzen, P.J. (2006) Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae). *J. Apic. Res* **45**, 49–50
- Ellis, J.D., Rong, I.H., Hill, M.P., Hepburn, H.R., Elzen, P.J. (2004) The susceptibility of small hive beetle (*Aethina tumida* Murray) pupae to fungal pathogens. *Am. Bee. J* **144**, 486–488
- Ellis, J.D., Spiewok, S., Delaplane, K.S., Buchholz, S., Neumann, P., Tedders, W.L. (2010) Susceptibility of *Aethina tumida* (Coleoptera: Nitidulidae) larvae and pupae to entomopathogenic nematodes. *J. Econ. Entomol* **103**(1), 1–9
- Mürle, T.M., Neumann, P., Dames, J.F., Hepburn, H.R., Hill, M.P. (2006) Susceptibility of adult *Aethina tumida* (Coleoptera: Nitidulidae) to entomopathogenic fungi. *J. Econ. Entomol* **99**, 1–6
- Tanada, Y., Kaya, H.K. (1993) Insect Pathology. Academic, San Diego
- Torto, B., Boucias, D.G., Arbogast, R.T., Tumlinson, J.H., Teal, P.E.A. (2007) Multitrophic interaction facilitates parasite-host relationship between an invasive beetle and the honey bee. *Proc. Nat. Acad. Sci. USA* **104**(20), 8374–8378
- Undeen, A.H., Vávra, J. (1997) Research methods for entomopathogenic Protozoa. In: Lacey, L.A. (ed.) Manual of techniques in insect pathology, pp. 117–151. Elsevier, Amsterdam