Asian Honey Bee *Apis cerana* Foraging on Mushrooms

Jun-ichi Takahashi[1*], Kentaro Hosaka[2], Stephen J. Martin[3], Akira Kawabe[1]

[1] Faculty of Life sciences, Kyoto Sangyo University, Kyoto, Japan

[2] Department of Botany, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki, Japan

[3] School of Environment & Life Sciences University of Salford, Manchester UK

Corresponding author:

Jun-ichi Takahashi, Faculty of Life sciences, Kyoto Sangyo University, Kyoto, Japan.

E-mail: jit@cc.kyoto-su.ac.jp

Introduction

Honey bees (*Apis* spp.) are well known for obtaining their nutrition from pollen and nectar collected from a wide range of flowers (Winston, 1991). They also collect honeydew secreted by aphids and scale insects as an additional source of carbohydrate. Many species of bees use plant resins, which when mixed with their saliva produces propolis, a sticky substance used to seal the hive and help combat pathogens and infections (Castro, 2001). In some honey bees e.g. *Apis florea* propolis can be used as an ant deterrent (Duangphakdee, Koeniger, Koeniger, Wongsiri, & Deowanish, 2005). However, in Brazil the Africanized honey bee (*Apis mellifera*) workers gather mycelium and spores from *Cladosporium* sp. of fungi (Modro, Silva, & Luz, 2009) but are not known to visit the fruiting body of fungi. Here we report for the first-time honey bee (*A. mellifera*) workers apparently feeding on the fruiting body of fungi.

Material and Methods

The observational survey was conducted at the Kyoto Sangyo University campus in Kyoto City, Japan in autumn 2017. At the study site, 14 individual fruiting bodies of the same species where located within a 1 m^2 area. Continuous observations were made for at least two hours each day between September 26 and October 3, 2017. There were no

35 flowers to attract bees within a 50-m radius of the study site. Observations of fungi were
36 made by four researchers and recorded using a digital camera (Sony HXR-MC1),
37 resulting in total 48 hours of recordings. During the same period, control observations of
38 honey bee visiting goldenrod (*Solidago canadensis)* flowers around 200 m away from the
39 fungi using the same methodology resulted in a total of 21 hours of recordings. After that,
40 we measured the duration and frequency of visits by the honey bee workers to the
41 fruiting body used the video data.
42
43
44 Results and Discussion
45 Although more colonies of *A. mellifera* than *A. cerana* were in the surrounding area, only
46 *A. cerana* workers were found visiting the fruiting body at a rate of 3.2 times per hour
47 spending an average of 28.2 seconds during each visit (Figure 1). *A. cerana* visited the
48 goldenrod flowers at a similar rate of 3.1 times per hour and stayed for an average of 31.6
49 seconds, whereas *A. mellifera* visited the flowers 3.4 times per hour staying an average
50 of 35.1 seconds (Figure 1).
51
52 We observed *A. cerana* workers gnawing with their mandibles and extending their

53 tongues into the tubes (Figure 2, Video 1) of the fruiting body. The *A. cerana* workers
54 were also observed feeding on the decaying mushrooms later in the study. The fungi was
55 initially identified as *Xerocomus* spp. in the order Boletales (Basidiomycota, Fungi). This
56 identification was later confirmed via DNA sequence analysis, using the internal
57 transcribed spacer (ITS) primers, ITS5 and ITS4, with reaction conditions as described in
58 White, Bruns, Lee, & Taylor (1990). The resultant nucleotide sequence was registered
59 with the DDBJ DNA data bank (accession number LC333562) and had a 96% homology
60 with genus *Xerocomus* spp. as determined by BLAST of the resulting ITS sequence.
61 Additional DNA sequences (accession numbers MG650105 and MG650106) were
62 obtained from RPB1 and ATP6 genes, using the primer pairs bRPB2-6F/bRPB2-7.1R
63 (Matheny, 2005) and ATP6-3/APT6-2 (Kretzer & Bruns, 1999), respectively. These
64 sequences also confirmed *Xerocomus* spp.based BLAST searches.
65
66 It remains unclear why this foraging behavior of *A. cerana* at the fruiting bodies was
67 occurring. The orchid *Cymbidium floribundum* is known to trick *A. cerana* bees into
68 pollinating them for no reward of nectar or pollen by mimicking honey bee secretions
69 (Sasaki, Ono, Asada, & Yoshida, 1991). It is possible that the mushroom may release
70 secretions, which are acting as an attractant or psychostimulant. It has been shown that

71 honey bees preferred solutions with low concentrations of drugs such as nicotine and
72 caffeine over a 20% sucrose control solution (Singaravelan, Nee'man, Inbar, & Izhaki,
73 2005) and several insects are known to become addicted to drugs (Boppre, 1999).
74 Furthermore, we found that some of the *Xerocomus* spp. fruiting bodies were parasitized
75 by *Sepedonium* spp., an ascomycetous mold (Figure 2), which was identified as either a
76 *Sepedonium* spp. (98% homology) or *Hypomyces chrysospermus* (97% homology) based
77 on the ITS sequence obtained using the primers ITS5 and ITS4 (White, Bruns, Lee, &
78 Taylor, 1990). These mold's are known to parasitize Boletaceae fruiting bodies, including
79 the genus *Xerocomus* (Arora, 1986). *Hypomyces chrysospermus* produces sepedonin,
80 which shows anti-fungal and -bacterial activities (Nagao, Yoshida, Iwai, Sakai, Tanaka,
81 & Miyahara, 2006) that are highly prized for their culinary value (Arora, 1986). So, it is
82 possible that *A. cerana* is selectively choosing parasitized *Xerocomus* as their food source.
83 However, these ideas remain highly speculative, until more data is obtained. It is well
84 established that some species of beetles and flies feed on the fruiting bodies of mushrooms
85 (van Emden, 2013), but such behavior in bees is hitherto unknown (Winston 1991; Sasaki,
86 2010).
87
88 In this study, we may have observed the mutualistic relationship between the honey bee and

fruiting body of a fungi. We hypothesize that *A. cerana* workers likely obtain some resources from the mushrooms. Honey bees are most likely to obtain proteins like pollen from fruiting body, but some extracts from the mycelium of amadou (*Fomes*) and reishi (*Ganoderma*) fungi have been suggested to have antiviral effect against pathogenic viruses (Stamets, Naeger, Evans, Han, Hopkins, Lopez, Moershel, Nally, Sumerlin, Taylor, Carris, & Sheppard, 2018). It has also been suggested that *A. mellifera* nurse bees prefers honey that has high antimicrobial activity (Gherman, Denner, Bobiş, Dezmirean, Mărghitas, Schlüns, Moritz, & Erler, 2014). Mushrooms often release secretions from the underside of their fruiting bodies, and the honey bees might be collecting that. In this observation, the type of the substance that the honey bees obtained from the mushrooms remains unknown.

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151 **Figures captions**

152

153 Figure 1

154 Comparison of (a) duration and (b) visits (mean ± s.e.) in the honey bees *Apis cerana* and

155 *Apis mellifera* on the fungi *Xerocomus* cf. *chrysenteron* and goldenrod flowers *Solidago*

156 *canadensis*. N = The total number of honey bees observed.. Observations of fungi and

157 flower were made for 42 and 21 hours respectively.

158

159 Figure 2

160 *Apis cerana* worker foraging on the tubes of the mushroom *Xerocomus* cf. *chrysenteron*

161 (left) and biting the fruit body of the mushroom *Xerocomus* cf. *chrysenteron* parasitized

162 by *Sepedonium* sp. (white mycelia on cap surface) (right). Please click the image to play

163 Video 1.