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A diverse assemblage of moths feeding on aphid honeydew

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ABSTRACT

Adult butterflies and moths (Lepidoptera) use their proboscises to feed on flower nectar and other types of liquid food. Aphids frequently secrete honeydew from their anuses, attracting various types of insects, such as ants. Adult lepidopterans are also known to feed on aphid honeydew. However, very few studies have clarified the species composition and morphology of moths feeding on aphid honeydew. In late June 2017, we found nocturnal moths sipping honeydew secreted by *Shivaphis celti* and *Sitobion cornifoliae* (Hemiptera: Aphididae) on leaves of *Celtis sinensis* (Cannabaceae) and *Cornus kousa* (Cornaceae), respectively, at the edge of a secondary forest in central Japan. The moths were observed to uncoil their proboscises and feed on honeydew on the leaves. No moths were observed to feed on honeydew directly from aphids. Nocturnal moths of 60 species (11 families) and 16 species (7 families) were collected from honeydew on *Ce. sinensis* and *Co. kousa* leaves, respectively. Eleven moth species were shared between the two types of honeydew. The most abundant species, *Oncocera semirubella* (Lepidoptera: Pyralidae), accounted for 44.7% of all individuals. The sex ratio of *O. semirubella* was female biased. Of the 65 moth species collected on aphid honeydew, 52.3% (34 species) have been previously reported to visit flowers. Moths visiting honeydew had relatively short proboscises and small body size. These results suggest that aphid honeydew is an important, accessible food resource for moths of small size.

Introduction

Adult butterflies and moths (Lepidoptera) use their proboscises to feed on various liquids, such as floral nectar, tree saps, fruit juice, and honeydew (Norris, 1936; Gilbert and Singer, 1975; Nishio, 1986; Sugiura and Yamazaki, 2007). Lepidopteran adults have evolved characteristic proboscises to feed on liquid foods such as flower nectar (Krenn, 2010; Lehnert et al., 2016). For example, some species of the moth family Sphingidae have extremely long proboscises to suck nectar from long and narrow floral tubes (Johnson and Anderson, 2010), while moths of the subfamily Calpinae (Erebidae) have barbed proboscises adapted for piercing fruit skins to feed on fruit juice (Zenker et al., 2011). Therefore, feeding habits are closely related to proboscis morphology in Lepidoptera (Kunte, 2007; Krenn, 2010).

Aphids (Hemiptera: Aphidoidea) frequently secrete honeydew from their anuses, attracting various types of insects, such as ants (Delabie, 2001; Stadler and Dixon, 2005). Adult lepidopterans are also known to feed on aphid honeydew (Norris, 1936). For example, several lycaenidid species use aphid honeydew as food (Banno, 1988; Norris, 1936). Nocturnal moths also reportedly feed on aphid honeydew (Johnson and Stafford, 1985; Nishio, 1986; Norris, 1936; Sansum, 2013). However, very few studies have clarified the species composition and morphology of moths consuming aphid honeydew. Because aphid honeydew is more easily accessible to nectar-seeking insects, it is predicted to attract diverse moths, including small species with short proboscises. Determining the species composition and proboscis length of moths feeding on aphid honeydew would help to clarify relationships between feeding habits and morphology in moths.

In late June 2017, we found nocturnal moths sipping aphid honeydew on the leaves of two woody plant species, *Celtis sinensis* Pers. (Cannabaceae) and *Cornus kousa* Buerger ex Hance (Cornaceae), at the edge of a secondary forest in central Japan. To characterise the community structure and morphology of moths feeding on aphid honeydew, we investigated the species composition, proboscis length, and body size of moths sampled from aphid honeydew.

Materials and methods

Field observations and sampling were conducted at the edge of a secondary forest in Arimafuji Park, Sanda City, Hyogo, central Japan

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(34°54′N, 135°13′E, 216 m above sea level). This forest is mainly composed of *Quercus serrata* Murray (Fagaceae) and *Pinus densiflora* Siebold et Zucc. (Pinaceae). In late June, we found honeydew secreted by *Shivaphis celti* Das on *Ce. sinensis* leaves, and honeydew secreted by *Sitobion cornifoliae* (Shinji) on *Co. kousa*. In Japan, the aphid species *Sh. celti* reproduces on the undersides of *Celtis* leaves from late April to early November, while *Si. cornifoliae* reproduces on the undersides of *Cornus* leaves in June and September (Matsumoto, 2008).

We used an insect net (pole length: 1.2–6.3 m) to collect moths sipping aphid honeydew from the leaves of one *Ce. sinensis* tree (height: 3.5 m) and one *Co. kousa* tree (height: 2.6 m). We did not find any moths on leaves without aphid honeydew. We observed and sampled moths on *Ce. sinensis* at 30-min intervals during 21:00–22:00 on 28 June 2017 (22.2–22.9 °C; temperature data from the Sanda Meteorological Station), 21:00–22:00 on 29 June 2017 (23.6–24.1 °C), and 20:00–23:00 on 1 July 2017 (24.2–26.2 °C); observation and sampling of moths on *Co. kousa* were conducted at 30-min intervals during 22:00–23:00 on 1 July 2017 (24.2–25.0 °C). Sampled moths were killed with ethyl acetate before being brought to the laboratory, where they were preserved in freezers (ca. −15 °C). We identified moths based on external and/or genital morphology (Hirowatari et al., 2013; Kishida, 2011a, 2011b; Nasu et al., 2013). The sex of each moth was also determined. To investigate the morphology of moths, we used a digital calliper to measure the proboscis length and body size (forewing length) of nearly all collected moths to the nearest 0.01 mm. Proboscis length and body size are closely associated with feeding habits in butterflies (Kunte, 2007). We thawed the moths in hot water before measuring proboscis length. To measure proboscis length, we straightened the coiled proboscis by inserting the sharp tip of a forceps into its centre. Extremely short proboscises (< 2.0 mm) were measured with an ocular micrometre under a stereomicroscope. Forewing length was measured as the distance between the centre of the base of a flattened wing and the farthest point of its perimeter (Loudon and Koehl, 2000). The mean value of the right and left forewings of each moth was used as the forewing length. When one side of the forewing was damaged, the value of the other side was used. Forewing lengths have been frequently used as a body size index in lepidopteran adults (Miller, 1977; Haber and Frankie, 1989; Hawkins and Lawton, 1995; Krenn et al., 2001; Calvo and Molina, 2005).

A binomial test was used to compare the observed sex ratio with an equal ratio. The analysis was conducted using R v. 3.3.2 (R Development Core Team, 2016).

The data were deposited in the Figshare Digital Repository (Sakagami and Sugiura, 2018).

Results

In total, we collected 245 moths of 60 species (11 families) from *Ce. sinensis* leaves, and 28 individuals of 16 species (7 families) from *Co. kousa* leaves (Table 1). The moths were observed to uncoil their proboscises and feed on honeydew on the leaves (Fig. 1). No moths were observed to feed on honeydew directly from aphid abdomens. Communities feeding on honeydew on *Ce. sinensis* and *Co. kousa* had 11 species in common. The pyralid moth species *Oncocera semirubella* (Scopoli) was the most abundant, accounting for 44.7% of all individuals (Table 1; Fig. 1B). The sex ratio of *O. semirubella* was female biased (Table 1); the proportion of males (0.27) was significantly different from 0.5 (Table 1; binomial tests, $P < 0.0001$).

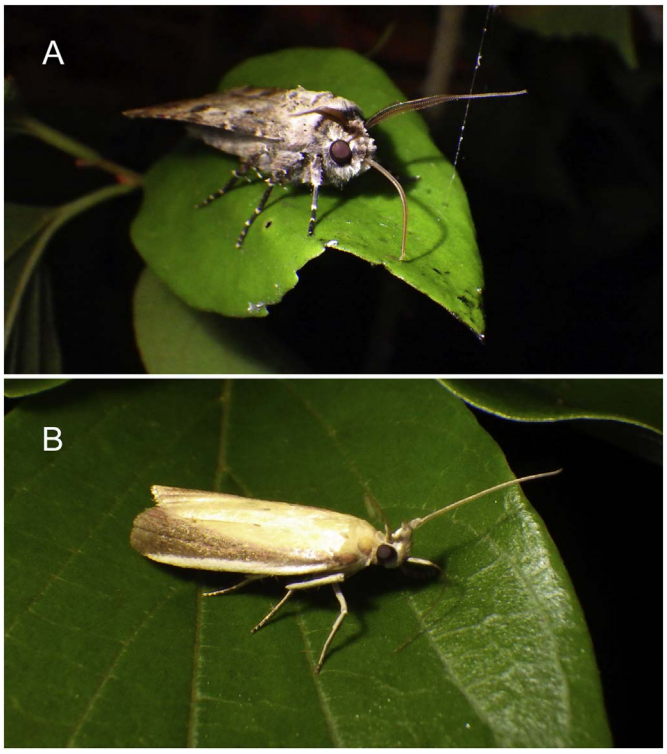

Fig. 1. Nocturnal moths feeding on aphid honeydew on *Celtis sinensis* leaves: (A) *Agrotis segetum* (Denis & Schiffermüller); (B) *Oncocera semirubella*. Mean proboscis length and forewing length were 8.4 and 17.8 in *A. segetum* and 6.2 and 10.9 in *O. semirubella*, respectively (Table 1).

The mean ± SE proboscis length and forewing length of moths feeding on aphid honeydew were 5.6 ± 0.1 mm (range: 0.5–13.2 mm) and 10.7 ± 0.2 (3.0–28.9 mm), respectively (Table 1). No specific characters (e.g., hairs, barbs) were found on moths' proboscises.

Discussion

No studies have investigated nocturnal moths visiting honeydew secreted by *Sh. celti* and *Si. cornifoliae*, although previous studies have described moths feeding on honeydew secreted by other aphid species (Johnson and Stafford, 1985; Nishio, 1986; Sansum, 2013). Our observations showed a diverse assemblage of moths feeding on honeydew secreted by *Sh. celti* and *Si. cornifoliae* (Table 1). The most abundant moth species, *O. semirubella*, accounted for ca. 45% of all individuals (Table 1). The dominance of *O. semirubella* can be explained by the abundance of the larval host plant *Lespedeza cuneata* (Dum.-Cours.) G. Don (Fabaceae) at the study site. The large amount of aphid honeydew excreted by *Sh. celti* and *Si. cornifoliae* may have attracted moths that were already abundant at the study site. In addition, the abundance and species composition of moths visiting aphid honeydew may vary with time and location, as honeydew availability changes spatiotemporally with aphid abundance, so aphid honeydew is not a stable nectar resource for moths.

We also found that the sex ratio of *O. semirubella* was female biased (Table 1). Because aphid honeydew reportedly contains amino acids