Apidologie (2024) 55:59
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https://doi.org/10.1007/s13592-024-01099-z

Original article

Fuel provisioning for pollen collection by solitary bee, *Andrena taraxaci orienticola*

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Received 25 February 2024 – Revised 26 June 2024 – Accepted 10 July 2024

Abstract – Female solitary bees collect pollen for their brood at the expense of using sugars in the nectar as fuel for flight. When flowers provide both pollen and nectar, solitary bees can refuel from the flowers to sustain pollen foraging. However, not all flowers provide both pollen and nectar. This study investigates how females of the solitary bee *Andrena taraxaci orienticola* secure fuel to sustain pollen foraging when flowers provide scarce nectar. By using DNA barcoding, we identified the Japanese dandelion *Taraxacum platycarpum* as their primary food source. This dandelion produces almost no nectar in the early morning when female bees collect almost no nectar but collect substantial amounts of pollen. We also found that these bees leave the nest with significantly more nectar at the first flight of the day than at later flights. When we restricted their fuel load by preventing nectar feeding, significantly more females failed to collect pollen on the first flight of the following day. These results suggest females hold back some part of the nectar collected at the end of a day's foraging and use it the following morning as fuel to sustain pollen foraging from flowers providing little nectar.

Andrena* / DNA barcoding / Foraging / Nectar / Solitary bees / *Taraxacum

1. INTRODUCTION

Bees require high energy levels to sustain foraging flights. Their primary energy source or "fuel" is sugars in nectar (Beenakkers et al. 1984; Nachtigall et al. 1989; Suarez et al. 2005). During foraging, bees hold collected nectar in the crop and send only a small portion to the midgut where sugars are absorbed into the haemocoel to supply energy for flight (Crailsheim 1988; Gmeinbauer and Crailsheim 1993; Snodgrass 1956). As long as bees can obtain nectar from flowers, they suffer no energy shortage for flight.

However, flowers do not always supply sufficient nectar to bees. Some plants provide only pollen as a reward (Odintsova 2008; Woodcock et al. 2014) and other plants produce nectar at limited times (Edge et al. 2011; Galetto and Bernardello 2004; Pacini et al. 2003). Nectar availability can also be reduced to zero by intra- and inter-specific competition (e.g., Harano and Hrncir 2023). Bees suffering energy shortages cannot continue foraging and even risk death from starvation. Social bees, such as honeybees, stingless bees, and bumblebees, can refuel when returning to the nest (Beutler 1950; Harano and Nakamura 2016; Harano et al. 2020) because their colonies may have large honey stores (Allen et al. 1978; Vit et al. 2013; Winston 1987). In contrast, many solitary bees, including the Andrenidae, cannot take advantage of nectar stores because they do not store nectar in liquid form but instead mix collected nectar with pollen to form pollen masses as nest brood provisions (Danforth et al. 2019; Michener 2000).

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Manuscript editor: Margarita M Lopez-Uribe

One solution to reducing the risk of starvation during foraging is to carry a large fuel load, but this incurs costs, such as increased flight energy expenditure, reduced flight speed and agility, and reduced capacity of crop to hold harvested nectar (Harano 2014; also see Wenner 1963; Wolf et al. 1989). Social bees reduce the cost of carrying fuel by adjusting the amount of fuel loaded into the crop before leaving the nest, depending on foraging conditions (Beutler 1950; Harano et al. 2013, 2020; Harano and Nakamura 2016; Tan et al. 2015). However, this adjustment is impossible for solitary bees with no liquid nectar stores in the nest. Alternatively, they might optimize their fuel load in a different way by adjusting the amount of nectar held back in the crop as fuel for the next trip when unloading (regurgitating) collected nectar in the nest between foraging trips.

Here, we hypothesize that female solitary bees hold back more nectar when unloading to meet the increased requirement of fuel for pollen collection from flowers with insufficient nectar supply. We examined this hypothesis using the solitary bee, *Andrena taraxaci orienticola.* As in other *Andrena* bees, we postulate that this species does not store liquid nectar in the nest although this is yet to be confirmed by examining the nest interior. *Andrena taraxaci orienticola* is suggested to exploit the Japanese dandelion *Taraxacum platycarpum* as its main food source based on frequent visits to this plant and daily foraging activity (mainly 08:00 to 11:00) which overlaps the dandelion flowering time (Harano 2022). Although their specialization to this plant is still far from confirmed, the using of a single floral resource facilitates the study about the impact of nectar availability on the female ability to provision their nests.

To understand how to secure foraging fuel and the significance of this behavior on foraging success in *A. taraxaci orienticola* females, we addressed the following questions: (1) How much does this bee rely on *T. platycarpum* as a food source? (2) Does the nectar availability of *T. platycarpum* vary within a day? (3) Do bees change the size of fuel load with the nectar availability in the field? (4) Does reduced fuel load lower bee's pollen foraging success?

2. MATERIALS AND METHODS

2.1. Bees and plants

We studied *A. taraxaci orienticola* females at an aggregate nest site on the campus of Tamagawa University, Tokyo, Japan (Harano 2022). This species is ground-nester with a univoltine life cycle. In this population, after emerging and subsequent mating in late March, females dig a nest and forage until early May. The females' active period roughly overlaps with the *T. platycarpum* flowering season. *Andrena taraxaci orienticola* females are the primary visitors to *T. platycarpum* at this study site (Harano, pers. obs.). We conducted all experiments and observations in April 2016 to 2023 at the aggregated nest site or nearby *T. platycarpum* populations.

2.2. Identification for food source plants

We identified *A. taraxaci orienticola* food source plants by DNA barcoding pollen loads collected from 33 female bees on 9 and 18 April 2016 (17 and 16 bees, respectively). We collected pollen loads from females returning to the nest using procedures described elsewhere (Harano 2022). Briefly, we confined individual females returning to the nest with pollen loads in a separate plastic cylinder (ø4.5 cm × 6.0 cm long) in the dark to allow natural pollen unloading. When unloading finished, we collected all pollen from the container and weighed the fresh weight to the nearest 0.1 mg on an electric balance (ML 104 T; Mettler Toledo, Tokyo, Japan).

DNA barcoding analysis was performed by following procedures. A small amount of pollen taken from the pollen loads was homogenized in 50 µl of 0.2 N NaOH containing 1% SDS for 1 min. The homogenate was diluted 100 times with TE (10 mM Tris HCl, 1 mM EDTA, pH = 7.0) to neutralize the solution pH. An aliquot of the diluted homogenate was used as the template for PCR. Internal transcribed spacer 1 (*ITS1*) of rRNA genes was amplified by using

Ex Taq PCR Kit (Takara Bio, Shiga, Japan) and primers ITS1 (5′-tccgtaggtgaacctgcgg-3′) and ITS2 (5′-gctgcgttcttcatcgatgc-3′). The PCR cycle condition was initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 30 min; and final extension at 72 °C for 5 min. The PCR products were purified using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co., Ltd., Tokyo, Japan). Direct sequencing of the PCR products was performed by Fasmac Co., Ltd. (Kanagawa, Japan). The obtained sequences were analyzed by BLAST against the database of the website of the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to estimate species identity.

2.3. Nectar availability in food plants

We investigated the diel change in the nectar availability of *T. platycarpum*, a putative primary food source for *A. taraxaci orienticola*. Hourly observations were made from 08:00 to 12:00 on 7, 11, and 12 April 2021. This time covers the main foraging period (08:00 to 11:00; Harano 2022) of *A. taraxaci orienticola* females. Direct measurement of *T. platycarpum* nectar volume is difficult because flowerheads are composed of many florets each with too little nectar to quantify. Therefore, we used the proportion of florets with nectar as an index of the nectar abundance in each flower. We sampled 10 florets per flowerhead and inspected for the presence of nectar under a dissection microscope (Olympus SZX7, Evident Corp., Tokyo, Japan). Florets were sampled along the flowerhead centerline at an equal interval to reduce spatial bias. Closed florets were excluded from the samples.

2.4. Temporal changes in nectar and pollen harvests

We measured the amount of collected nectar and pollen by catching female bees returning to the nest at different times. The fresh weight of the two pollen loads was measured for each bee as described above on 31 March and 6 and 16 April 2016.

The volume of the crop content was determined on 11 and 12 April 2016 using a modified version of an established method (Gary and Lorenzen 1976; Stone 1994). After anesthetizing female bees with CO_2, we gently pressed their abdomen between finger and thumb to force them to regurgitate the crop content which was then collected using a glass capillary (Microcaps 10 µl or 20 µl, Drummond Scientific Co., Broomall, USA). We determined the volume of crop content based on the length of regurgitated liquid in the capillary. Preliminary experiments showed that this method collects most of the crop content (mean ± SD, 87.1 ± 34.8%, $N = 7$), and we could measure crop content without sacrificing bees. Although crop dissection provides a more accurate measure, we avoided this method to protect the *A. taraxaci orienticola* population. We also measured the crop content sugar concentration using hand-held refractometers (low-volume refractometer Nectar 50 and 40–85, Eclipse, Bellingham & Stanley, Hampshire, UK) when the volume was sufficient (> ca. 0.4 µl). We calculated the amount of sugars dissolved in the crop content of samples with successfully measured concentration based on the volume, sugar concentration, and specific density of the solution. We assumed sucrose as a solute in the crop content and used a specific density of sucrose (Atago 1987) to calculate the amount of sugar in the crop content.

2.5. Fuel load at nest departure

We measured the volume and sugar concentration of crop content in female bees departing the nest on 15 and 19 April 2016 and on 3, 5, and 10 April 2023. Data were analyzed separately for the first departures of the day and for the second and later departures. First-departing females were caught at the nest entrance in a semi-transparent plastic cylinder (ø4.5 × 6.0 cm long) before starting foraging. Females at the second and later departures were caught in the same way after observing their return to the nest.

Measurements of crop contents were made as in returning females.

2.6. Effect of reduced fuel load on foraging

The above observations revealed (see Section 3) that *A. taraxaci orienticola* females depart the nest with more nectar in the early morning when flowers of their major food plant *T. platycarpum* have limited nectar. As a result, we investigated the significance of the fuel load of female bees at nest departure on pollen collection by restricting their fuel load as follows.

We caught a total of 23 females departing the nest and confined them without food at room temperature (around 20 ℃) until dusk when they were returned to the nest. After returning the bees, we blocked the nest entrance with a plastic cup to prevent nest departure. Next morning, when the females appeared at the entrance, the plastic cup was removed to allow foraging. Since there is no nectar storage in the nest, these unfed females carried little nectar at nest departure (see Section 3). After recording nest departure times, we blocked the entrance again to facilitate recording the return of each female. On return, we removed the block and recorded the return time and pollen-load status.

As a control, 23 other females were treated in the same way but were fed ca. 50% sucrose solution ad libitum rather than starved. A preliminary test showed the fed group taking 9.0 ± 3.36 µl (mean ± SD) of this sucrose solution ($N = 10$). The unfed and fed (control) females were paint-marked with different colors to identify each group.

This experiment was performed in separate 2 trials (16 and 17, and 22 and 23 April 2017). Similar numbers of females were assigned to fed and unfed groups at each trial. Foraging was observed from 06:00 to 09:30 because the first foraging flights start at around 08:30 on warm fine days. However, 23 April was cloudy and foraging started later than usual, so the observation period was extended to 10:45 (Table S6).

2.7. Statistical analyses

To examine whether the nectar availability changes in a course of day, we compared the proportion of florets with nectar between time periods using Steel-Dwass tests. The relationships between nectar or pollen harvest and time of day were examined using Spearman's ranked correlation coefficient. Whether the bees possess larger nectar loads at first than second and later departure was tested by Mann-Whitney's *U*-test. Non-parametric tests were used because the data show unequal variance.

The impact of limiting nectar collection on the next day's pollen foraging was tested using Mann-Whitney's *U*-test and Student's *t*-test for departure time and flight duration (time between nest departure and return), respectively. The difference in the proportion of females returning with and without pollen was tested using the chi-square test.

Bellcurve, an Excel add-in software (SSRI, Tokyo, Japan) was used for all statistical tests at the 5% significance level.

3. RESULTS

3.1. DNA barcoding for pollen loads

PCR targeting *ITS1* amplified DNA fragments of about 300 bp. Direct sequencing of PCR products successfully determined sequences from 235 to 269 bp in 32 of 33 pollen samples (Table S1). Sequence alignment revealed that the 235-bp sequence was identical across all 32 samples. All sequences spanning 235 to 269 bp were a perfect (100%) match for *T. platycarpum*. There was no other sequence in the database that completely matched our pollen sequences. Two of the 32 samples had weak signal overlaps, suggesting the presence of pollen from different plant species. However, sequences determined based on stronger signals were a 100% match for *T. platycarpum*.

These results indicate that these two samples contain pollen from more than two species but most pollen is from *T. platycarpum*. We failed to sequence the one remaining sample due to intense signal overlaps.

3.2. Nectar availability in *T. platycarpum*

The proportion of florets with nectar at 08:00 was quite low (4%) but increased significantly at later times ($P < 0.05$: Figure 1 and Table S2). The highest proportion of florets (40%) had nectar at 12:00 when flowerheads are closing (Harano 2022).

3.3. Nectar and pollen harvests

The volume of crop content at return to the nest (putative nectar harvest) showed a significant positive correlation with time of day ($n = 39$, Spearman's correlation coefficient $r_s = 0.51$, $P < 0.001$: Figure 2A and Table S3). Bees seldom returned with more than 1 µl of nectar in the first half of the foraging period (~ 10:00) but tended to harvest more nectar in the second half. The crop content sugar concentration was 53.5 ± 5.11% (mean ± SD, $n = 30$) and was significantly positively correlated with time of day ($n = 30$, $r_s = 0.44$, $P < 0.05$: Figure 2B and Table S3). Consequently, the amount of sugars in the crop content showed a significant positive correlation with time of day ($n = 36$, $r_s = 0.56$, $P < 0.001$: Figure 2C and Table S3).

The pollen harvest showed the opposite relationship where the fresh weight of pollen loads decreased significantly with time of day ($n = 59$, $r_s = -0.33$, $P < 0.01$: Figure 3 and Table S4).

3.4. Crop loads at nest departure

In general, females departed the nest with a small amount of nectar in their crop (median = 0.16 µl, range = 0.00 to 3.46 µl: Figure 4A and Table S5). The crop content volume at nest departure was significantly larger at the first flight than at later flights ($n = 28$ and 41, $z = 3.78$, $P < 0.001$: Figure 4B). The crop volume was significantly negatively correlated with time of day ($r_s = -0.32$, $P < 0.01$:

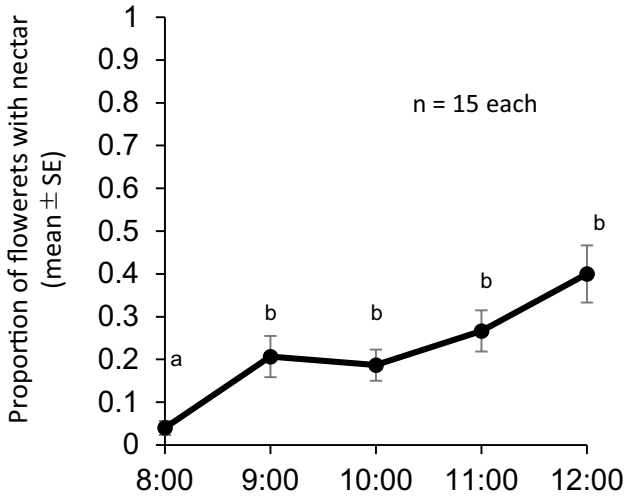


Figure 1. Temporal changes in nectar availability of *Taraxacum platycarpum*. The *y*-axis shows the proportion of florets with nectar, an index of nectar availability calculated from 10 florets per flowerhead. Error bars show standard errors. Different letters indicate significant differences at the 5% significance level by the Steel-Dwass test.

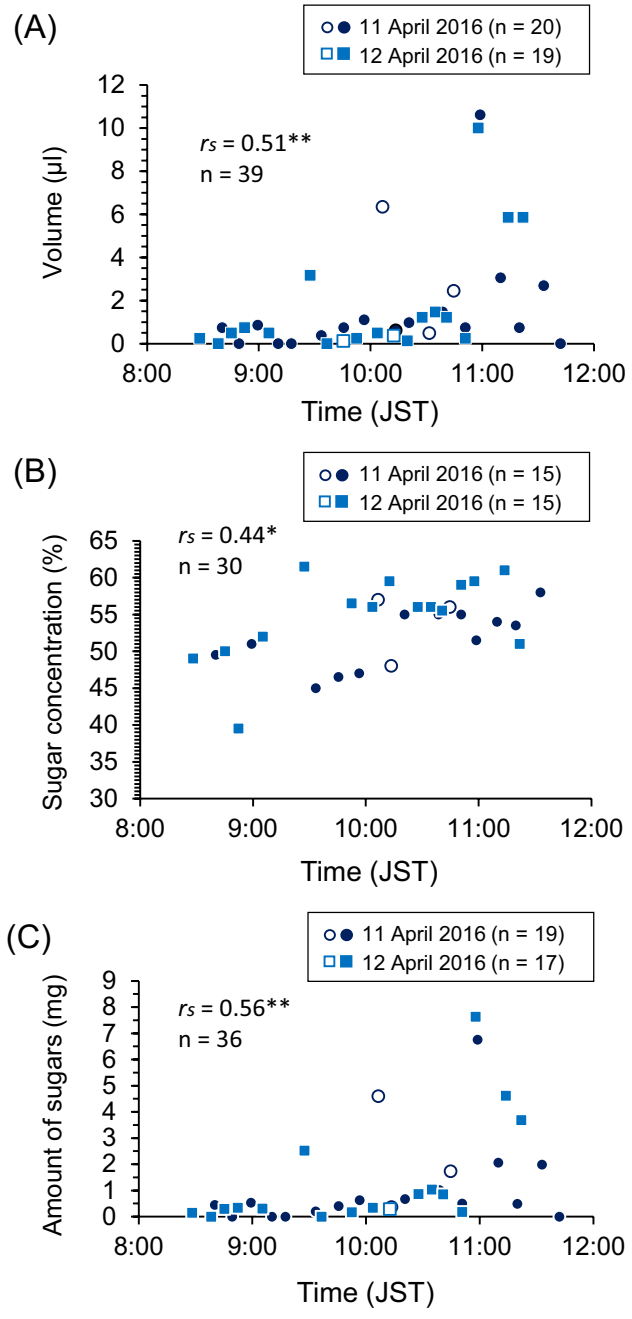


Figure 2. Temporal changes in **A** volume, **B** sugar concentration, and **C** sugar content of crop load of *Andrena taraxaci orienticola* females returning to nest. r_s, Spearman's ranked correlation coefficient. Open symbols, females returning without pollen loads. Solid symbols, females returning with pollen loads.

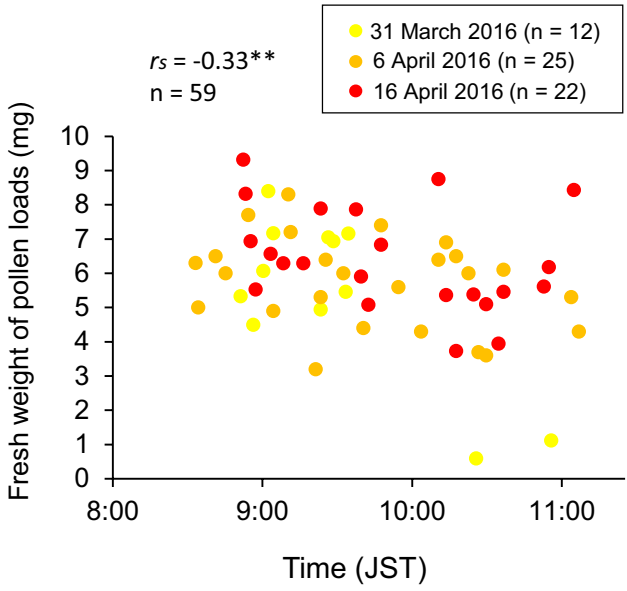


Figure 3. Temporal changes in size of pollen loads for *Andrena taraxaci orienticola* females returning to nest. r_s, Spearman's ranked correlation coefficient.

Figure 4A). The crop content sugar concentration was 58.4% ± 5.73% (mean ± SD, $n = 20$) and decreased significantly with time of day ($n = 20$, $r_s = -0.46$, $P < 0.05$: Figure 4C and Table S5). Although the sugar concentration tended to be higher at the first flight than later flights, there was no statistically significant difference ($z = 1.86$, $P = 0.062$: Figure 4D). The amount of sugars dissolved in the crop content showed a significant negative correlation with time of day ($n = 48$, $r_s = -0.39$, $P < 0.01$: Figure 4E and Table S5) and was significantly larger at the first flight than later flights ($n = 18$ and 30, $z = 4.31$, $P < 0.001$: Figure 4F).

3.5. Foraging effect of limited nectar feeding

Although some of the observed females did not make a foraging trip during the next morning's observation period (Table S6), this proportion was not significantly different between the fed and unfed groups (0.70 vs 0.83, chi-square test: $n = 20$, 23, $\chi^2 = 0.95$, df = 1, $P = 0.33$). The feeding treatment (fed or unfed) did not significantly affect the departure time of foraging females (Mann-Whitney's U-test: $n = 14$, 17, $z = 0.66$, $P = 0.51$: Figure S1) and flight duration (Student's t-test: $n = 12$, 15, $t = 0.417$, df = 25, $P = 0.68$: Figure S2), but there was a significant difference in foraging outcomes; more females in the unfed group returned to the nest without pollen loads than in the fed group ($\chi^2 = 3.54$, df = 1, $n = 12$, 18, $P < 0.05$: Figure 5). This trend was found in both trials (Table S7).

4. DISCUSSION

Female *A. taraxaci orienticola* departed the nest with larger nectar loads in the early morning when their primary pollen source *T. platycarpum* provides little nectar. Furthermore, females in the unfed group tended not to collect pollen on the first foraging flight of the following day. These results agree well with our hypothesis and suggest that foraging females hold back some collected nectar in the crop as fuel for foraging pollen from flowers providing little nectar the next morning.

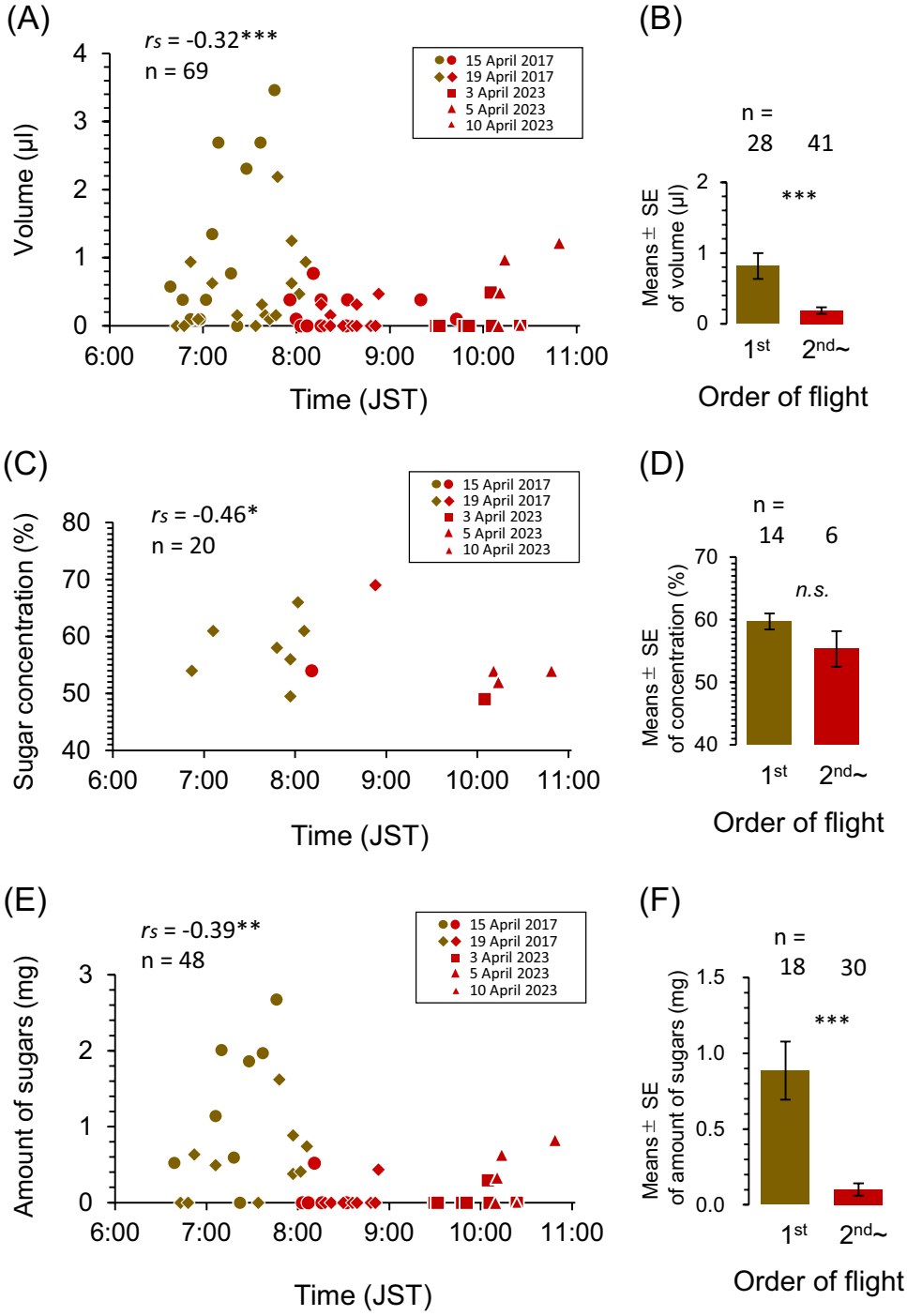


Figure 4. Temporal changes in **A**, **B** volume, **C**, **D** sugar concentration, and **E**, **F** sugar content of crop load of *Andrena taraxaci orienticola* females on departing nest. Comparisons between the first flight of the day and later flights are shown by **B**, **D**, and **F**. Mann-Whitney's *U*-tests were performed for comparisons. r_s, Spearman's ranked correlation coefficient. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

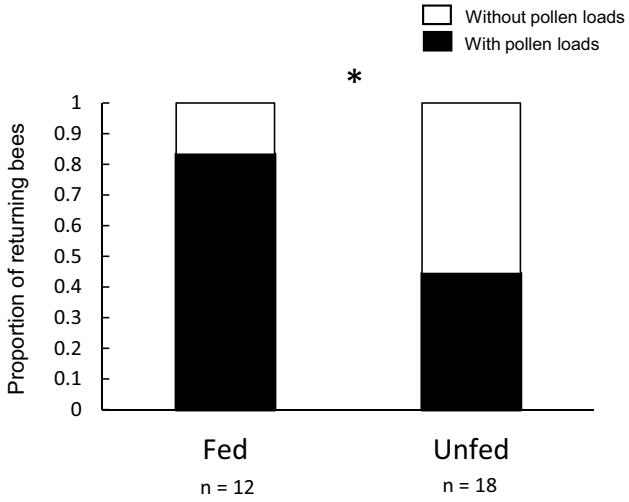


Figure 5. Effect of feeding sugar solution on pollen collection at the following morning by *Andrena taraxaci orienticola* females. Fed females were given sugar solution ad libitum. Unfed females were kept without feeding for 1 day before foraging. $^*P < 0.05$ by the chi-square test.

DNA barcoding showed *T. platycarpum* as the source plant in 32 of 33 pollen-load samples. Most samples (30 of 32) did not show obvious signal overlaps, suggesting one plant species as the source of pollen loads. These results are consistent with previous field observations that *A. taraxaci orienticola* preferentially targets *T. platycarpum* flowers (Harano 2022).

Taraxacum platycarpum florets provide little nectar in the early morning with only 4% of florets having nectar at 08:00. Reflecting this situation, nectar collection by *A. taraxaci orienticola* females was limited at this time. In the early foraging period (~09:00), female bees returned to the nest with only 0.44 ± 0.359 µl of nectar (mean ± SD, $N = 7$: Figure 2), which is comparable to nectar loads on departing the nest (0.64 ± 0.864 µl for females leaving nest before 09:00, $N = 40$; Figure 4) but far from the maximum possible load (> 10 µl: Figure 3). These data indicate that females barely collect any nectar in this early period.

In contrast to nectar, females collected more pollen per trip in the early morning than later. While the reason is unknown, freshly opened flowers in the early morning might provide more pollen because they have not been exploited (Harder 1990). A previous study showed that *A. taraxaci orienticola* females preferentially visit freshly opened *T. platycarpum* flowers than flowers that have been open for several hours (Harano 2022). Solitary female bees are known to assess pollen availability of flowers and alter foraging decisions accordingly (Buchmann and Cane 1989; Burkart et al. 2014); our results suggest that the standing crop of pollen decreases with elapsed time after *T. platycarpum* flowers first open.

Female *A. taraxaci orienticola* left the nest loaded with increased fuel nectar loads in the early morning, especially on the first flight of the day. Based on our hypothesis, this nectar was collected on the previous day because liquid nectar is not stored in the nest. Since more nectar is collected in the latter half of the foraging period, perhaps some of this nectar is used to provision larvae while the remainder is held back in the crop overnight as fuel for the first morning flight. We examined the significance of this held-back nectar on the outcome of foraging the next morning by manipulating nectar fuel loads. Unfed, fuel-restricted females had a significantly

increased likelihood of returning without pollen loads compared to fed non-fuel-restricted females. Unfed females were captured on departing the nest when they were carrying only a trace amount of nectar in the crop (Figure 4) and held overnight without feeding to minimize the nectar fuel load the next morning. On the other hand, fed females are expected to have similar or bigger fuel loads as untreated females departing the nest because they had access to sugar solution approximately equivalent to the maximum nectar harvest. Hence, the difference in foraging outcome between fed and unfed females is likely to be caused by the difference in their energy state. This experiment demonstrated the importance of carrying fuel to accomplish pollen collection when natural nectar flow is low.

Even under natural conditions, *A. taraxaci orienticola* females sometimes return without pollen loads on the first flight of the day but almost always collect pollen on later flights (Harano 2022). As shown in Figure 4, there is a large variation in the size of fuel load at the first flight, and females with little fuel might fail to collect pollen. However, this is unconfirmed because it is difficult to determine the size of fuel load without disturbing subsequent foraging activities.

Why did unfed females in our experiment not collect pollen? One possible but discounted explanation is that they cannot sustain a sufficiently long foraging trip to collect pollen due to the shortage of fuel. However, this is apparently not the case because trip duration was not significantly reduced in unfed females compared to fed females. Perhaps unfed females perceive the risk of starvation or the need for fuel and try to collect nectar on the first flight. Since carrying pollen may increase the energy expenditure for flight (Naumchik and Youngsteadt 2023), they might give up pollen collection due to the expected energy shortage. Females of some solitary bee species, including the genus *Andrena*, are known to make adult feeding trips, which are characterized by a large nectar load but no pollen load on returning to the nest (Danforth 1990; Neff and Simpson 1997). Although the feeding-trip trigger is unknown, perhaps low fuel levels relate to this behavior.

Without liquid nectar stores in the nest, solitary female bees should hold back some nectar in the crop overnight because they cannot forage for nectar at night and bad weather could prevent foraging for several days. This might be one reason why some solitary bees forage for nectar during last flights of the day (Danforth 1990). Females of *A. taraxaci orienticola* also collect nectar late in the foraging period and hold back at least some of it overnight. This behavior undoubtedly contributes to their survival when foraging is not possible. Having an available fuel load at the first flight of the day might also prevent wasting time on adult feeding trips before collecting pollen. The benefit of having an overnight fuel load may be large because bees waste a long time and a lot of energy collecting fuel when the natural nectar flow is low.

The present study demonstrated that *A. taraxaci orienticola* females hold back collected nectar in the crop as fuel for pollen collection from flowers with insufficient nectar. This strategy works only when the availability of nectar and pollen follows a specific pattern. We should confirm whether this strategy is common in solitary bees.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1007/s13592-024-01099-z.

ACKNOWLEDGEMENTS

We thank Dr. Shin-ichi Asada for help with field observations. We also thank Dr. Ryuki Murao for his kind cooperation in bee identification.

AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Ken-ichi Harano. Procedures of DNA barcoding were developed by Tetsuhiko Sasaki. The first draft of the manuscript was written by Ken-ichi Harano, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. KH and TS conceived this research and

designed experiments; KH performed experiments and analysis; KH and TS wrote the paper and participated in the revisions of it.

FUNDING

This study was partially supported by a Japan Society for the Promotion of Science, Kakenhi Grant no. 20K06078.

DATA AVAILABILITY

All data supporting the findings of this study are available within the paper and its Supplementary Information.

DECLARATIONS

Ethics approval This study used an observational approach, where bees were studied with standard professional monitoring methods under standard management procedures.

Consent to participate All people involved in this study gave their consent to participate.

Consent for publication All people involved in this study gave their consent to the publication of this article.

Competing interests The authors declare no competing interests.

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