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Honeybee males use highly concentrated nectar as fuel for mating flights

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ABSTRACT

Honeybees use nectar held in the crop as their main source of energy for flight but the mass of the crop nectar load may be a cost burden. This study investigated whether males of the honeybee *Apis mellifera* adjust their nectar fuel load and concentration to enhance the success of mating flights. When the crop content was compared between males staying in the hive and those departing, the latter group had the larger volume (median, 5.0 μl; range, 0.0–17.8 μl) and higher concentration (median, 71.6%; range, 49.0%–77.6%), indicating that departing males load concentrated nectar as fuel before mating flights. Moreover, the crop nectar concentration was significantly higher in departing males than in departing workers. These results suggest that concentrated nectar is advantageous to males because it provides more sugar for energy at lower mass and secures longer or more effective mating flights for higher chance of reproductive success. No significant effect of age was detected in crop volume, and concentration and amount of dissolved sugars in the crop content. In addition, laboratory experiments showed that males had only about 5 μl of nectar in the crop soon after feeding, irrespective of fed volume (5–15 μl), suggesting they do not hold much nectar in the crop but send it rapidly to the midgut, unlike workers.

1. Introduction

Flight enhances survival and reproduction in insects. It allows them to forage over a wider range and move to better habitats (Visscher and Seeley, 1982; Gathmann and Tscharntke, 2002; Osborne et al., 2008; Kennedy, 1951; Howard and Davis, 2009; Romoser and Stoffolano, 1994). Some insects fly for reproduction-related activities, such as defending territory, locating mates, and searching for oviposition sites (Zmarlicki and Morse, 1963; Sullivan, 1981; Romoser and Stoffolano, 1994). In solitary species, individuals may use both types of flight—for survival and for reproduction—at different life stages. On the other hand, the two flight types are performed by different individuals in some social insects, such as honeybees, in which workers fly primarily for foraging, whereas queens and males fly for mating (Winston, 1987). If there are different requirements for the two flight types, reproductives (queens and males) and non-reproductives (workers) should show different flight adaptations. Flight specialization in social insects might be an important feature promoting colony survival and reproduction. However, few studies have been conducted from this viewpoint. Comparison of behavioral and physiological aspects of flight, including fuel loading, between reproductives and non-reproductives might shed light on unknown differential adaptations.

Insect flight consumes a lot of energy that is supplied from energy reserves such as fat and glycogen stored in the fat body of many insects (Beenakkers et al., 1984). However, honeybee workers, queens, and males have limited glycogen stores (Panzenböck and Crailsheim, 1997) and rely largely on sugars in the digestive tract and hemolymph for energy. To secure sufficient energy for flight, honeybee workers leaving the hive receive nectar as fuel from nest mates (Beutler, 1950; von Frisch, 1967). In workers, nectar held in the crop is sent gradually to the midgut where sugars are absorbed into the hemolymph (Crailsheim, 1988a).

Although nectar provides energy for flight, its mass may be a cost burden for bees. In honeybee workers, flight energy expenditure increases with the mass of taken nectar (Wolf et al., 1989). A large nectar mass might reduce aerial agility and flight speed (Wenner, 1963), resulting in increased predation risk and reduced foraging rate. Due to these costs, perhaps workers adjust the amount of fuel carried from the hive according to several factors, including distance to nectar source and foraging experience (Beutler, 1950; Harano et al., 2013). Males also use nectar taken from hive stores as fuel for the mating flight (Free, 1957). Since workers adjust fuel load to maximize foraging, perhaps males enhance their reproductive success using a similar fueling strategy.

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Honeybee males have no duties in colony maintenance other than mating with queens. They start flying about 1 week after emerging (Howell and Usinger, 1933) and mate with queens at mating sites called 'drone congregation areas' (DCAs) that can be several km from the hive (Zmarlicki and Morse, 1963). Males die at separation after copulation, but return to the hive if they fail to mate. The male flight lasts 27 min on average (Howell and Usinger, 1933) but young males tend to make short (<6 min) orientation flights to learn landmarks around the hive. Since they never forage during the mating flight (Currie, 1987), all fuel for the flight must be carried from the hive. To increase the chance of a successful mating, males must stay as long as possible at the DCA. However, carrying more nectar to sustain a longer flight has a cost of increased mass, possibly reducing aerial agility, which might be another important factor for successful mating at the DCA. One answer might be to load more-concentrated nectar, which reduces mass without reducing sugars for energy.

Recently, Harano and Nakamura (2016) showed that foragers use nectar at different concentrations as fuel, depending on the type of forage target (nectar or pollen) and distance to food source, suggesting an ability to adjust the concentration of nectar used as fuel. Nectar foragers use nectar at a relatively low concentration (44% average) although more-concentrated fuel seems to facilitate flight. Harano and Nakamura (2016) argue that using less-concentrated nectar as fuel reduces colony-level cost of foraging because nectar is concentrated by using time and energy of in-hive bees and foragers would consume not only sugars in the nectar but also such temporal and energetic investments for concentration when they use concentrated nectar as fuel. If foragers use less-concentrated nectar to maximize foraging at the colony level although more-concentrated nectar is better for flight, males might be expected to use very concentrated nectar for mating flights. To examine this hypothesis, we measured the concentration and volume of nectar used as fuel in males of the European honeybee *Apis mellifera* and compared the fuel load concentrations with those of workers.

In addition, we examined possible fuel adjustment with male age because males fly longer as they get older (Howell and Usinger, 1933; Witherell, 1971). We evaluated the amount of nectar carried by males by measuring crop content but addition of saliva and quick transfer of nectar food to the midgut may cause over- and under estimation errors. To examine if such things occur, crop contents were also measured soon after males were fed with sugar solution of known amount and concentration.

2. Materials and methods

2.1. Study site and bees

A colony of the European honeybee, *Apis mellifera* kept in an apiary of Tamagawa University, Tokyo, Japan, was used unless otherwise stated. The colony consisted of about 16,000 workers and one queen on 8 frames. A frame of male comb was supplied to the colony to obtain adult male. The frame was removed several days before expected emergence and placed in an incubator at 33 °C. Newly emerged males were paint-marked on the thorax within 24 h after emergence to identify age. They were returned to their natal colony soon after marking.

All experiments were carried out during the mating season in Tokyo (April–June) when healthy males were abundant in the colony.

2.2. Measurement of crop content

The volume and concentration of crop content was measured as described elsewhere (Harano and Nakamura, 2016). Bees were fixed with an insect pin on a dissection dish and the crop was removed from the body. The crop content was collected using a 5-μl microsyringe (Hamilton, Reno, NE) and volume was measured using the microsyringe scale. When crop content was over 5 μl, it was collected in several parts. Next, the liquid was applied to a handheld refractometer (low-volume refractometer Nectar 50 and 40–85, Eclipe, Bellingham & Stanley, Hampshire, UK) to determine the concentration of sugars as sucrose equivalent (w/w). The total amount of sugar in the crop content was calculated using the volume, concentration and corresponding liquid density.

2.3. Collection of males

To determine the effect of male age on fuel load, males with ages of 7–8, 13–14, and 20–21 days were captured at take-off from the hive using an insect net between 13:00 and 15:00 (mating time of *A. mellifera* (Yoshida et al., 1994)) on sunny days (11 May 2015 for ages of 7–8 and 13–14 days, and 18 May 2015 for ages of 20–21 days). Captured males were killed immediately by cold spray (134a-QREI, Sunhayato Corp., Tokyo, Japan) and stored in a Styrofoam box ($18 \times 25 \times 16$ cm) with refrigerant. Crop content was, then, measured as described above.

In addition, males of the same ages were also collected from inside the hive on the same days between 09:00 and 11:00 to compare crop contents with captured departing males.

2.4. Comparison between males and workers

The concentration of the crop content was compared between departing males and workers. Males and workers were captured irrespective of age when they left the hive between 12:00 and 15:00 from three queen-right colonies: A, B, and C, each with about 14,000, 25,000, and 24,000 workers, respectively. The bees were caught on fine days: 4 June 2015 for colony A, 20 May and 10 June 2015 for colony B, and 21 May 2015 for colony C. The bees were killed immediately after capture by cold spray to determine the concentration of the crop content.

2.5. Feeding experiments

Experiments were performed to investigate how much food is retained in the crop of males after feeding. Sixty 7-day-old males in the hive were collected between 09:00 and 11:00; they were immobilized in the head-up position by securing their wings with a metal clip (Fig. S1). Soon after immobilization, 30 males were fed 5, 10, or 15 μl of 60% sucrose solution using a 50-μl microsyringe. The males were killed by cold spray approximately 5 min after feeding and the volume and concentration of crop content were measured.

Other 30 7-day-old males were similarly fed 5 μl of 15%, 30%, or 60% sucrose solution to examine whether nectar is diluted by saliva during swallowing. Crop contents were measured using the same procedure as the feeding experiment described above.

2.6. Statistical analysis

The effects of age on the crop volume, concentration and amount of dissolved sugars were tested by Kruskal–Wallis tests. The crop content was compared between males caught upon leaving the hive and those caught in the hive within the same age-group by Mann–Whitney *U*-tests. The same statistical test was used to examine a difference in fuel concentration between males and workers. The relationship between crop volume and concentration in males was described using Spearman's correlation coefficient. In feeding experiments, the effect of volume or concentration of food given to males was first examined by a