

RESEARCH ARTICLE

A quick tongue: older honey bees dip nectar faster to compensate for mouthpart structure deterioration

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ABSTRACT

The western honey bee, *Apis mellifera* L. (Hymenoptera), is arguably the most important pollinator worldwide. While feeding, *A. mellifera* uses a rapid back-and-forth motion with its brush-like mouthparts to probe pools and films of nectar. Because of the physical forces experienced by the mouthparts during the feeding process, we hypothesized that the mouthparts acquire wear or damage over time, which is paradoxical, because it is the older worker bees that are tasked with foraging for nectar and pollen. Here, we show that the average length of the setae (brush-like structures) on the glossa decreases with honey bee age, particularly when feeding on high-viscosity sucrose solutions. The nectar intake rate, however, remains nearly constant regardless of age or setae length ($0.39 \pm 0.03 \mu\text{g s}^{-1}$ for honey bees fed a 45% sucrose solution and $0.48 \pm 0.05 \mu\text{g s}^{-1}$ for those fed a 35% sucrose solution). Observations of the feeding process with high-speed video recording revealed that the older honey bees with shorter setae dip nectar at a higher frequency. We propose a liquid transport model to calculate the nectar intake rate, energy intake rate and the power to overcome viscous drag. Theoretical analysis indicates that *A. mellifera* with shorter glossal setae can compensate both nectar and energy intake rates by increasing dipping frequency. The altered feeding behavior provides insight into how *A. mellifera*, and perhaps other insects with similar feeding mechanisms, can maintain a consistent fluid uptake rate, despite having damaged mouthparts.

KEY WORDS: Nectar intake rate, Glossal setae, Dipping frequency, Adaptive behavior, Feeding habits

INTRODUCTION

The underlying physical mechanisms by which organisms acquire and transport liquids for feeding is of significance to a wide variety of disciplines (Gillett, 1967; Kim and Bush, 2012; Yang et al., 2014). Several fluid-uptake mechanisms have been described, which often depend on material properties, including morphology, chemistry and physiology (Kim and Bush, 2012; Crompton and Musinsky, 2011; Lehnert et al., 2013; Harper et al., 2013). Fluid-feeding insects are of particular interest because they have mouthparts that are adapted to acquire and transport nanoliter

amounts of liquids (Kim et al., 2011; Lehnert et al., 2017; Hischen et al., 2018). The western honey bee, *Apis mellifera* L. (Hymenoptera), for example, rapidly dips floral nectar using a tongue (glossa) covered with brush-like setae (Snodgrass, 1956; Simpson and Riedel, 1964; Krenn et al., 2005; Wu et al., 2015).

The first drinking model to elucidate the viscous-dipping feeding mechanism of *A. mellifera* simplified the glossa as a bald rod (Kim and Bush, 2012). Subsequently, Yang et al. (2014) proposed a model that considered the effects of the setae (erectable, brush-like structures on the glossa) and used experimental data to validate theoretical predictions on volumetric flow rate and energy intake rate. Considering that the back-and-forth movements of the glossa occur at a frequency of ~ 5 Hz (Li et al., 2015) (similar to a sewing needle), we hypothesize that the high-intensity work and fast dipping frequencies cause wear or damage to the glossal setae, which could result in the gradual deterioration of nectar-loading capabilities. This situation, however, creates a paradox because it is the older worker honey bees (i.e. those likely most prone to setae damage) that forage pollen and nectar (Amdam and Omholt, 2002). We hypothesize that if mouthpart damage does occur, *A. mellifera* employ a method of mechanistic or behavioral compensation to overcome the structural wear of the glossa in order to maintain optimum fluid uptake rates (Abrams et al., 2015).

MATERIALS AND METHODS

Western honey bee rearing and colony maintenance

Approximately 2000 western honey bees, *A. mellifera*, were collected from Guangzhou, China (22°N , 112°E), where no specific collecting permits were required, and were housed in a hive with drones and a queen. The entire system was maintained at 25°C at 50% humidity, and bees were fed a 35% (w/w) sucrose solution and an inorganic salt solution (Kim et al., 2011) (Fig. 1). Pupae were removed from the hive and placed into a container ($28\text{--}30^\circ\text{C}$). Upon adult emergence, individual *A. mellifera* workers were color coded with a unique mark on the tergum (solution composed of acetone and oil painting dye) that was used to identify their age (Huang et al., 1991).

Mouthpart morphology

Fifteen-day old adult *A. mellifera* were removed from the hive and randomly placed into beakers ($170\text{ mm} \times 270\text{ mm}$) with either 35% or 45% (w/w) sucrose solution. The sucrose solutions were based on sucrose concentration measurements from nectar (acquired with a polarimeter, Autopol IV) collected from three species of plants (*Sophora japonica*, *Physostegia virginiana* and *Paulownia tomentosa*) located near the bee hive. Each beaker was provided with 10 ml of their respective solution daily. The glossa of each honey bee was measured every 2 days using a light microscope (Eclipse 90i) at $4\times$ magnification. During each measurement period, two individuals were randomly selected and placed into 100% ethanol for dehydration and further studied with scanning electron microscopy (SEM; FEI Quanta 200). Because setae

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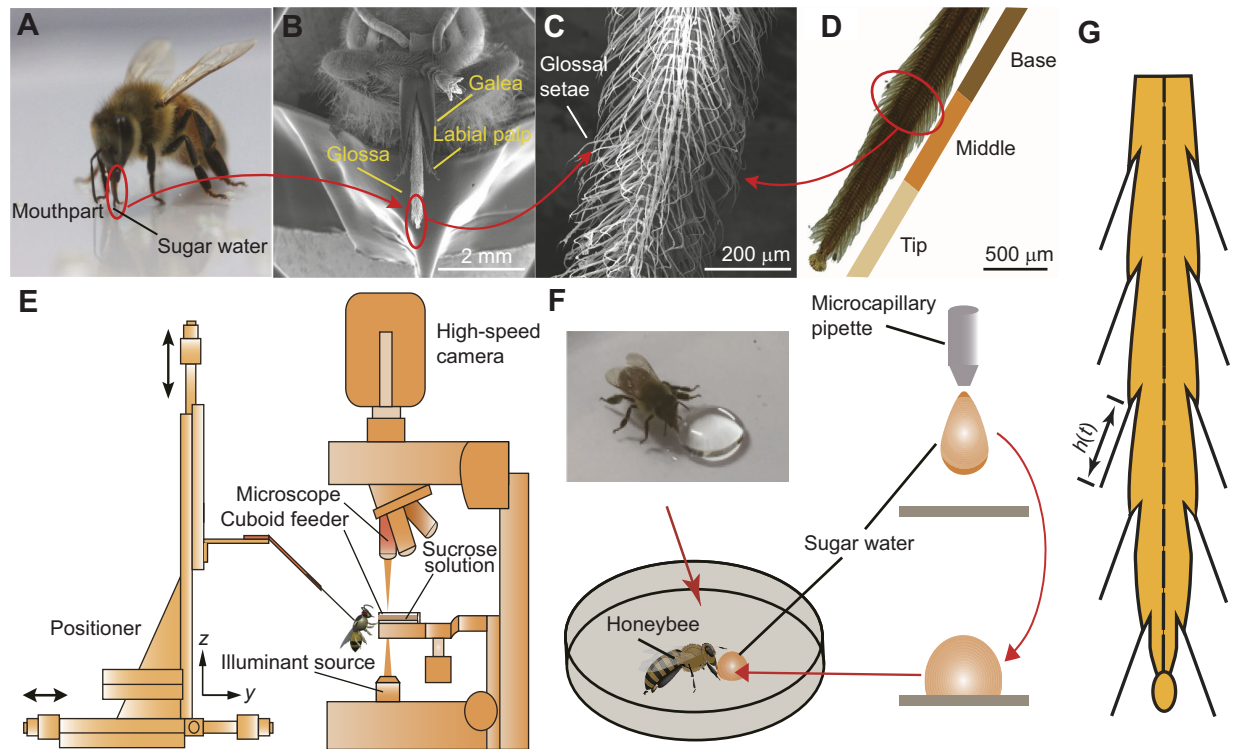


Fig. 1. Mouthpart morphology of *Apis mellifera* and experimental setup. (A) *Apis mellifera* feeding on a droplet of sugar water. (B) Scanning electron microscopy (SEM) image showing the mouthparts of *A. mellifera*, which are composed of a pair of galeae, a pair of labial palpi and a tongue (glossa). (C) The glossa is covered by erectable setae. (D) Light microscopy image of the glossa. The glossa was divided uniformly into three sections (the tip, middle and base) to determine the average setae length per individual. (E) The experimental setup for studying the setae length and dipping frequency consisted of a positioner, a high-speed camera, a microscope, an illuminant source and a cuboid feeder. (F) The nectar intake rate was determined by feeding *A. mellifera* individuals with a droplet of a sucrose solution (10 or 20 μl) in a Petri dish. (G) The glossal setae length at time t , $h(t)$, was measured from the base to the distal tip.

length h differed along the length of a single glossa, we determined an average setae length for each individual by randomly selecting and measuring 10 setae from three uniform sections of the glossa (30 setae total): the tip (distal region), middle and base region (Fig. 1D). The average setae length of individuals within the same age class (15 days and older) was determined for both sucrose solution concentrations.

Nectar intake rate measurements

Honey bees of different ages were placed into glass beakers (1.357 cm^3), sealed with a piece of wet gauze, and kept at 25°C and 50% relative humidity. Droplets of 35% and 45% sucrose solutions (10 or 20 μl volume per droplet) were dispensed using a microcapillary pipette (1–200 μl , DragonLab) onto dish feeders placed at the bottom of the glass beakers (Fig. 1F). A timer was used to determine the duration t_F of ingesting a droplet and the average nectar intake rate was calculated as $\dot{Q}_F = V_F/t_F$, in which V_F is the volume ingested (i.e. 10 or 20 μl).

Dipping frequency

The dipping frequency of the glossa was studied using a setup composed of a positioner, a high-speed camera (Phantom M110), a microscope (Axiostabilizer Plus, Zeiss) and an illuminant source (100 W) (Fig. 1E). A cuboid feeder fabricated with glass slides was placed between the LED light source and the high-speed camera. In addition, a 3-degrees of freedom motorized positioner (motion accuracy of 1.0 μm) was used to adjust the cuboid feeder position. During feeding observations, a live honey bee was glued via its

thorax to the precision positioner so that the insect could be moved vertically, thereby allowing the mouthparts to reach the sucrose solution. The honey bees were fed through a feeder filled with either 35% or 45% sucrose solution, and the temperature was maintained at 25°C. We selected 20 bees for each nectar concentration at ages ranging from 17 to 25 days. All feeding cycles were recorded at 500 frames s^{-1} , and the dipping frequency was calculated by first averaging five dipping cycles per individual, then using these values to determine the average dipping cycle per honey bee age. A Pearson's correlation was used to determine whether there was a relationship between dipping frequency and setae length.

RESULTS

Mouthpart morphology and nectar dipping rate

The average glossal setae length decreased with respect to age from 17 to 25 days ($n=120$) (Fig. 2). The absolute values of the slopes of the linear fits for honey bees fed 35% (k_1) and 45% (k_2) sucrose solutions were 3.82 and 4.34, respectively, and represent the rate of reduction of glossal setae length over time (measured as honey bee age). These values indicate that the deterioration rate of the average setae length of bees fed the 45% sucrose solution was greater than that of bees fed the less-viscous 35% sucrose solution ($n=60$ for both treatments). By *in vivo* then postmortem examination, we found that the dipping frequency increased with respect to deterioration of the glossal setae length (Fig. 2). Experimental data indicated a correlation between the average length of glossal setae h and dipping frequency f ($R^2=0.927$), which can be fitted as $h=-15.435f+212.04$.

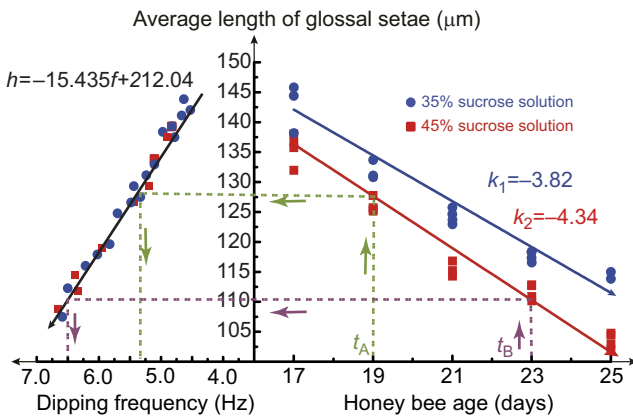


Fig. 2. Relationship between glossal setae length, dipping frequency and honey bee age. The average length of the setae decreased as the age of a honey bee increased. In addition, the wear of glossal setae was more extensive on honey bees fed the 45% sucrose solution (red line) versus those fed the 35% sucrose solution (blue line). The parameters k_1 and k_2 are the slope for the 35% and 45% sucrose solutions, respectively, which represents the rate of reduction in glossal setae length. Green and purple dashed lines provide examples of the relationship between setae length (h), dipping frequency (f) and honey bee age. For example, at 19 days of age (t_A , green dashed line), the mean setae length was 127.5 μm and the dipping frequency was 5.47 Hz. For older bees at 23 days of age (t_B , purple dashed line), the mean setae length was 110 μm with a dipping frequency of 6.61 Hz.

Mass intake rate

We measured the mass intake rate of honey bees of different ages and found that individuals imbibed the 35% sucrose solution at a rate of $\dot{M}_{35\%} = 0.39 \pm 0.03 \mu\text{g s}^{-1}$ ($n=15$) ($R^2=0.004$), and the 45% solution at $\dot{M}_{45\%} = 0.48 \pm 0.05 \mu\text{g s}^{-1}$ ($n=10$) ($R^2=0.019$). The mass nectar intake rate of honey bees for each sucrose solution concentration was approximately constant, independent of the average glossal setae length.

Model of nectar feeding with compensation

Here, we introduce a liquid transport model that considers glossal setae length. The glossa can be regarded as a cylinder that is densely covered by setae, and the surrounding galeae and labial palps can be treated as a tube (Wu et al., 2015). A nectar drinking cycle can be divided into three steps: tongue protraction, tongue retraction and interval, with durations denoted by T_1 , T_2 and T_0 , respectively (Fig. 3). The phase apportionments of old bees and young bees may have a consistent ratio of $T_1:T_2:T_0 = 16:30:9$. During the tongue protraction phase, the glossa extends from the tube wall and dips into the nectar until it reaches its maximum extension. The setae then become erect and trap nectar, and the tongue retracts into the tube wall. In the interval phase, the nectar is transported from the mouthparts to the gut by sucking pressure. We presume the average extension and retraction speeds are u_1 and u_2 , respectively. As shown in Fig. 3, the volume of nectar ingested in a cycle can be estimated by regarding it as the volume of a tubular cylinder, minus that of glossal setae immersed in the nectar (Yang et al., 2014). Therefore, the theoretical volumetric intake rate at age t days, namely $\dot{Q}(t)$, can be written as:

$$\dot{Q}(t) = \frac{\pi a u_2 T_2 (2h(t) \sin \theta + h^2(t) \sin^2 \theta / a) - 2\pi^2 n_h a r^2 h(t) u_2(t) T_2(t) / 3}{T_1(t) + T_2(t) + T_0(t)}, \tag{1}$$

in which a is the radius of the tongue body with no glossal setae ($a=85 \pm 3 \mu\text{m}$, $n=40$), n_h is the distribution density of the setae

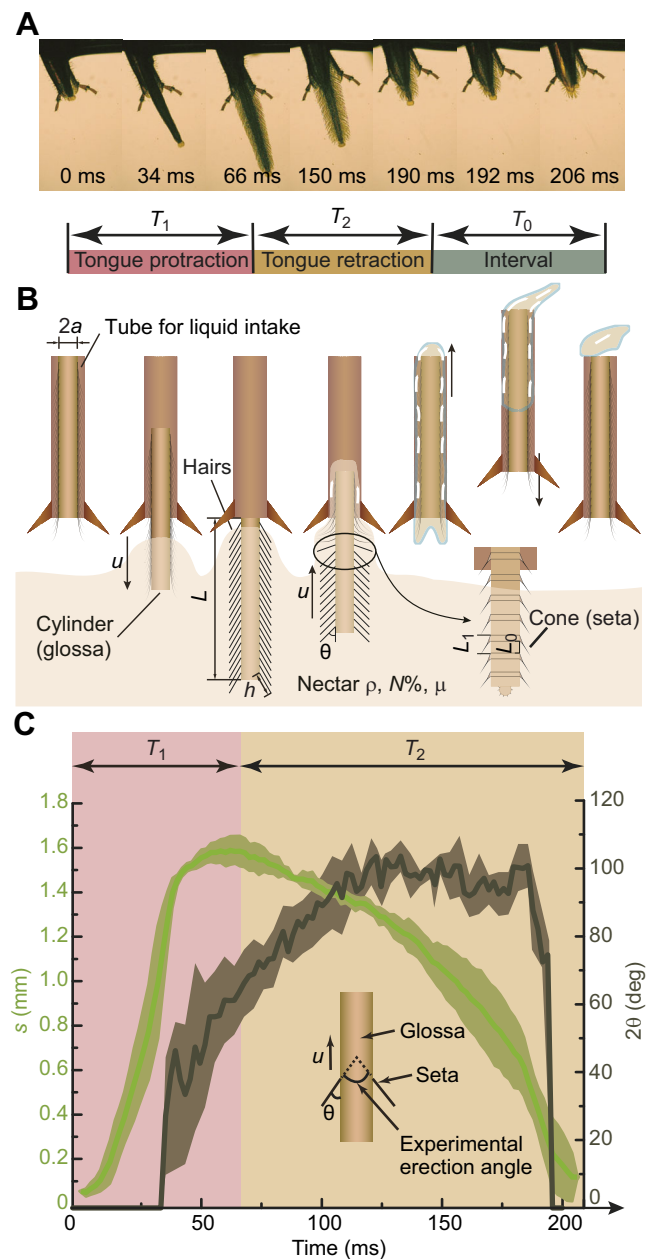


Fig. 3. Feeding cycle and dipping kinematics of a 17 day old *A. mellifera* feeding on 35% sucrose solution. (A) Key events of the feeding process. In the tongue protraction phase (T_1), the brushy glossa extends out of the tube, which is composed of the galeae and labial palpi. At 42 ± 3 ms, the glossal setae start to become erect and the gaps fill with nectar. At 62 ± 5 ms, the glossa protracts to the limit. In the tongue retraction phase (T_2), the glossa withdraws from the liquid, and the erectable glossal setae form a brush-like shape. The glossal setae are fully erect at 135 ± 3 ms. The tongue retracts from the nectar with the setae still erect. After the nectar is loaded into the tube, in the interval phase (T_0), the honey bee transports the liquid to the gut. The setae flatten and return to the same configuration as at time 0 ms. (B) A schematic illustration of movements of the mouthparts and the unfolding pattern of the glossal setae in a feeding cycle. Liquid loading onto the mouthparts consists of the tongue protraction and tongue retraction phases, when the glossa assumes either a spear-like or a brush-like shape. (C) Tongue displacement (s , green) and unfolding angle (θ , black) of one dipping cycle for 19-day-old honey bees ($n=3$) feeding on 35% sucrose solution. The shading indicates the error band of the experimental results. a , radius of the tongue body with no glossal setae; u , velocity; L , glossal length; h , setae length; θ , erection angle of glossal setae; ρ , sucrose solution density; $s\%$, mass concentration of sucrose solution; and μ , sucrose solution viscosity.

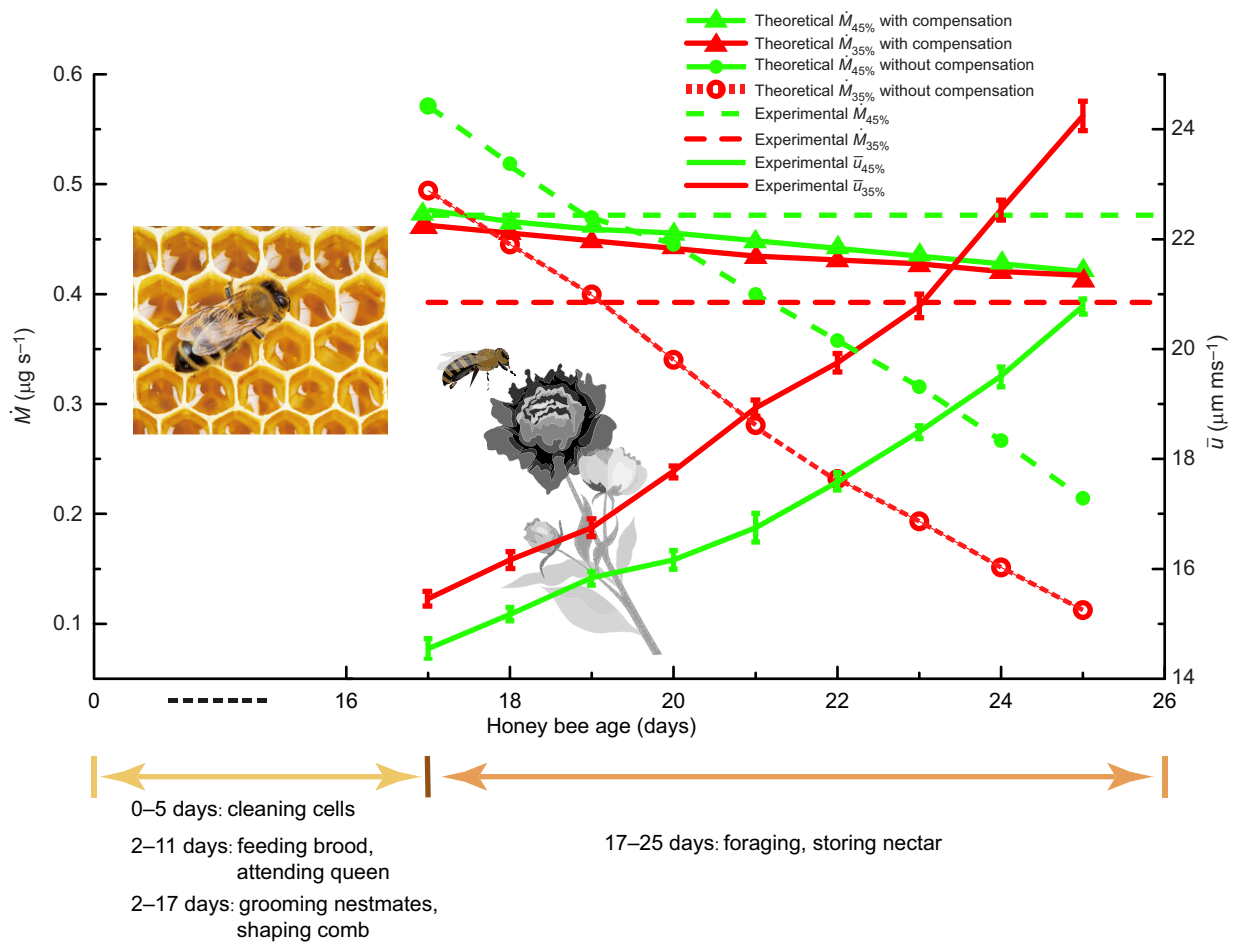


Fig. 4. Model validation of nectar intake rate with respect to honey bee age and sucrose concentration. The relationship between theoretical nectar intake rate (\dot{M}) and setae wear agrees with the experimental data for both the 35% and 45% sucrose solution. \bar{u} , glossal retraction velocity.

($n_h=2500$ strands mm^{-2}), $h(t)$ is the average length of the glossal setae at time t , r is the radius of the glossal setae at the base ($r=3.0\pm 0.2$ μm , $n=40$) and θ is the average erection angle of the glossal setae ($n=40$) (Fig. 3). It should be noted that the deformable tongue structure in nectar feeding plays an important role in increasing the nectar volume intake rate (Zhu et al., 2016). The rhythmical erection pattern of the setae, and protraction and retraction of the mouthpart structures facilitate efficient nectar intake. The glossa is segmented, which allows extension during the feeding process, and one segment will extend the long axis of the glossa with a total length from L_0 to L_1 during the dipping process (Fig. 3); thus, the elongation of the segmented glossa increases the nectar volume intake rate to 3.5 times that of the rigid model (Zhu et al., 2016). The total volumetric intake rate $\dot{Q}_T(t)$, which considers the elongation of the segmented glossa (T), can therefore be written as $\dot{Q}_T(t) = \chi\dot{Q}(t)$ in which χ is a proportionality factor that considers the contribution of the tongue's longitudinal extension to nectar intake augmentation ($\chi=3.5$) (Zhu et al., 2016). In addition, the retraction speed was calculated as $\bar{u}(t) = L/T_2(t)$ in which L is the maximum displacement of the tongue tip ($L=1.60\pm 0.03$ mm, $n=40$ bees). Considering the effect of nectar concentration (Pivnick and McNeil, 1985; Zhao et al., 2017), the nectar density function ρ can be written as:

$$\rho = \frac{N}{N/1.592 + (100 - N)/0.997}, \quad (2)$$

in which $N\%$ is a particular nectar solution concentration. According to Eqns 1 and 2, the nectar mass intake rate \dot{M} denotes:

$$\dot{M} = \dot{Q}_T(t) \cdot \rho. \quad (3)$$

Model validation and energy intake and consumption

By combining the setae length measurements (Fig. 2) with Eqn 2, we calculated the theoretical volume intake rates with the dipping frequency compensation (Fig. 4). The theoretical intake rate without compensation for dipping velocity rapidly decreased from days 17 to 25 for both sucrose concentrations, during a time period when honey bees typically shift from cleaning cells and shaping combs to foraging outside the hive, when feeding efficiency is arguably most important (Seeley, 1982) (Fig. 4). The observations from the feeding trials, however, indicated that the velocity of glossa protraction and retraction increased during this time period. When considering the higher velocity of the glossa, we found that the theoretical nectar intake rate (0.39 ± 0.03 $\mu\text{g s}^{-1}$) when feeding on the 35% sucrose concentration stayed relatively consistent with the actual nectar intake rate recorded from the experiments (0.37 ± 0.02 $\mu\text{g s}^{-1}$). A similar pattern was observed when honey bees fed on the more viscous 45% sucrose concentration, where the theoretical volume intake rate, considering velocity compensation, matched the experimental value of 0.48 ± 0.05 $\mu\text{g s}^{-1}$. The calculations indicate that older honey bees can compensate for the lower nectar intake of shorter glossal setae by increasing dipping frequency.

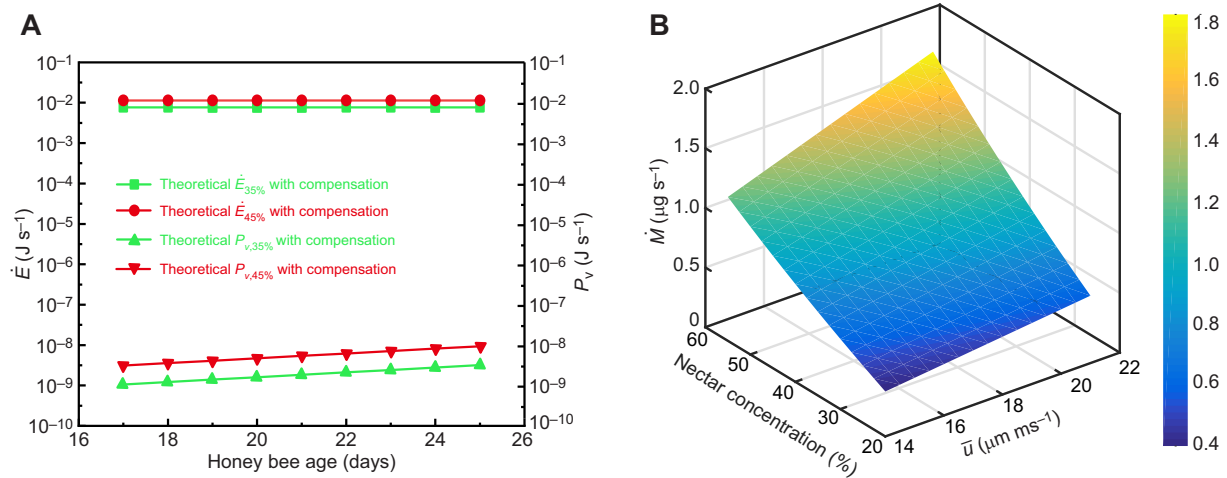


Fig. 5. Energy acquisition and feeding habits among honey bees. (A) Energy intake rate \dot{E} and power to resist viscous drag P_v plotted against honey bee age for bees fed 35% and 45% sucrose solutions. (B) Higher nectar concentrations and higher glossa retraction velocity \bar{u} increase nectar intake rate.

When considering the wear of the setae and dipping compensation, the energy intake rate can be given by $\dot{E}(t) = \dot{Q}(t)\rho cs\%$, where c is the energy content per unit mass of sucrose ($c=16.27 \text{ J g}^{-1}$) (Yang et al., 2014). The sucrose solution has a density ρ and a mass concentration $s\%$. The densities of 35% and 45% nectar are $\rho_{35\%}=1.15 \text{ g ml}^{-1}$ and $\rho_{45\%}=1.20 \text{ g ml}^{-1}$, respectively (Yang et al., 2014). The resistance force of the fluid is similar to that exerted on the surface of a stick (i.e. glossa) that is moving through the fluid at a constant speed u . If the glossa has a diameter d and length L ($L=1.60 \pm 0.03 \text{ mm}$), the drag can be written as $F=S\tau$, where S is the total surface area of the glossa ($S=\pi dL$) and τ is the shear stress ($\tau=C_d\rho u^2/2$, where C_d is the drag coefficient). Using S , τ , $C_d=24/Re$, $Re=\rho du/\mu$ and $\mu=[10^{0.8752N/(100-N)+N^2/9901}]/1009.7$ (where Re is the Reynolds number and μ is the viscosity), the viscosities of 35% and 45% nectar can be calculated as $\mu_{35\%}=0.39 \text{ mPa s}$ and $\mu_{45\%}=0.83 \text{ mPa s}$, respectively. The maximum Reynolds number ranges from 14 to 60 for dipping sugar water of 35% and 45% (Yang et al., 2014; Wu et al., 2018). The drag as a function of time can be calculated from $F(t)=\frac{1}{2}\pi\mu u(t)L(t)$. Therefore, the power required to resist viscous drag P_v with respect to time t is:

$$P_v = \int_0^{T_1+T_2} u(t)F(t)dt. \quad (4)$$

The theoretical analysis indicates that the energy intake rate is approximately 10^6 times as much as the power necessary to overcome viscous drag (Fig. 5). The energy dissipation caused by viscous drag, therefore, can be ignored. Notably, the energy intake rate of 45% sucrose solution is 1.31 times that for the 35% concentration; in other words, feeding on higher nectar concentrations provides a higher net energy intake (Fig. 5). We measured the sucrose concentration in the nectar of wild collected plants and found that the average concentration was approximately 35%, which is similar to the nectar concentration found in other bee-pollinated plants (Yang et al., 2014; Kim et al., 2011), but not as high as the theoretically preferred concentration, for which the energy intake rate would be higher.

DISCUSSION

Adult honey bees that emerge during spring in temperate regions have a mean lifespan of approximately 25–35 days, depending on

complex dynamics involving biotic and abiotic factors (Seeley, 1982). Workers of *A. mellifera* nurse the brood and perform other tasks in the hive during the first 2 weeks; after that, they shift to foraging for nectar and pollen (Amdam and Omholt, 2002) (Fig. 4). As indicated in this study, the glossal setae, which are responsible for trapping nectar during the feeding process (Wu et al., 2015), are shorter and less effective at capturing nectar in older honey bees that are tasked with foraging for nectar. This situation is puzzling – why would individual workers with shorter setae be responsible for foraging for nectar? This study indicates that they have evolved a feeding mechanism that compensates for the damage to glossal setae by increasing dipping frequency.

We found a discrepancy between the theoretical results and natural nectar concentrations on which bees feed, which can be interpreted in at least two ways. First, nectar of higher viscosity causes a faster wear rate of the glossal setae, which likely occurs to a greater extent in the wild compared with the experimental results because of the high intensity of daily work. If glossal setae wear down at a higher rate $|k|$, the glossa would eventually degrade to a bald stick. For a bald stick, the Landau–Levich–Derjaguin theory predicts the volumetric intake rate will be $\dot{Q}_T \approx \mu^{-1/6}$ (Kim et al., 2011), which is approximately 100 times less than for the hairy stick model (Yang et al., 2014); therefore, feeding on thicker nectar might have catastrophic consequences for honey bees. In addition, a reduced volumetric intake rate would impact the pollination rate because the bees would have to spend more time feeding and less time actively pollinating plants. A second reason for the discrepancy pertains to the hypothesis that natural selection favors flowers that maintain a lower sucrose concentration in order to keep their pollinators hungry, thus requiring a higher rate of flower visitation, which would increase pollination rates (Kim et al., 2011). These two aspects are not mutually exclusive, and ultimately result in natural selection favoring a nectar concentration that optimizes pollination.

Natural selection would favor honey bees that feed quickly and efficiently because of the threat of predators and other economic necessities (Roubik and Buchmann, 1984). Honey bees, therefore, have to meet the contradictory demands of keeping visiting time short and maintaining an optimal nectar intake rate. By increasing the dipping frequency, both demands could be satisfied. We are unsure whether foragers possess an adaptive neural mechanism to adjust the feeding frequency, as this requires further study. Considering that the dipping-regulation strategy might be important to the co-evolution of

flowers and honey bees and other nectar-feeding insects that have mouthparts prone to wear, we anticipate this strategy could inspire maintenance plans for performance compensation in human-engineered devices that have easily worn appendages.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Methodology: Y.C., Y.Y., M.S.L.; Software: C.L.; Validation: Y.C.; Formal analysis: Y.C.; Investigation: C.L., M.S.L.; Resources: S.Y.; Data curation: M.S.L.; Writing - original draft: J.W., M.S.L.; Writing - review & editing: M.S.L.; Supervision: S.Y.; Project administration: J.W., C.L., Y.Y., S.Y.

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