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Drag reduction effects facilitated by microridges inside the mouthparts of honeybee workers and drones

Q1 Chu-Chu Li[a,b,1,2], Jia-Ning Wu[b,1,3], Yun-Qiang Yang[a,*,4], Ren-Gao Zhu[a,b,5], Shao-Ze Yan[b,*,6]

[a] School of Engineering and Technology, China University of Geosciences, 100083 Beijing, PR China
[b] Division of Intelligent and Biomechanical Systems, State Key Laboratory of Tribology, Department of Mechanical Engineering, Tsinghua University, 100084 Beijing, PR China

HIGHLIGHTS

- We recorded the feeding process of live honeybee workers and drones.
- The effects of drag reduction regulated by especial microridges were compared.
- The mouthparts of workers were more capable of drag reduction than those of drones.
- A link between microridge dimensions and drag reduction capability was established.

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ABSTRACT

The mouthpart of a honeybee is a natural well-designed micropump that uses a reciprocating glossa through a temporary tube comprising a pair of galeae and labial palpi for loading nectar. The shapes and sizes of mouthparts differ among castes of honeybees, but the diversities of the functional microstructures inside the mouthparts of honeybee workers and drones remain poorly understood. Through scanning electron microscopy, we found the dimensional difference of uniformly distributed microridges on the inner galeae walls of *Apis mellifera ligustica* workers and drones. Subsequently, we recorded the feeding process of live honeybees by using a specially designed high-speed camera system. Considering the microridges and kinematics of the glossa, we constructed a hydrodynamic model to calculate the friction coefficient of the mouthpart. In addition, we test the drag reduction through the dimensional variations of the microridges on the inner walls of mouthparts. Theoretical estimations of the friction coefficient with respect to dipping frequency show that inner microridges can reduce friction during the feeding process of honeybees. The effects of drag reduction regulated by specific microridges were then compared. The friction coefficients of the workers and drones were found to be 0.011 ± 0.007 (mean $\pm$ s.d.) and 0.045 ± 0.010, respectively. These results indicate that the mouthparts of workers are more capable of drag reduction compared with those of drones. The difference was analyzed by comparing the foraging behavior of the workers and drones. Workers are equipped with well-developed hypopharyngeal, and their dipping frequency is higher than that of drones. Our research establishes a critical link between microridge dimensions and drag reduction capability during the nectar feeding of honeybees. Our results reveal that microridges inside the mouthparts of honeybee workers and drones reflect the caste-related life cycles of honeybees.

* Corresponding authors.
E-mail addresses: Cugb_lcc@163.com (C.-C. Li), wujn09@mails.tsinghua.edu.cn (J.-N. Wu), meqqy@163.com (Y.-Q. Yang), zhurengaocn@163.com (R.-G. Zhu), yansz@mail.tsinghua.edu.cn (S.-Z. Yan).

[1] These authors contributed equally to this study.
[2] One of the first authors who wrote the manuscript and performed the experiments.
[3] One of the first authors who found the special microstructure on the inner wall of the galeae and propounded the idea of the possible difference among castes.
[4] A corresponding author who conceived the project and designed the experiments.
[5] The general author who fed the honeybees and performed the experiments.
[6] A corresponding author who conceived the project and designed the experiments.

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1. Introduction

The word "biofluiddynamics" is proposed to describe fluid mechanic problems in biology (Lighthill, 1975). Drinking is a principal route for water intake and is critical in the sustenance of most animals. Many studies have explored the morphological properties and drinking strategies of animals (Kim and Bush, 2012; Daniel and Kingsolver, 1983; Andersson et al., 1984; Cundall, 2000). Reis et al. (2010) and Crompton and Musinsky (2011) studied the drinking strategies of cats and dogs, respectively, and demonstrated that these animals use inertial forces generated through the lapping of tongues to overcome gravity during water intake. A number of studies analyzed the physiological mechanisms and behavior toward various sucrose concentrations of nectarivorous animals under different experimental conditions (De Brito Sanchez, 2011; Lee et al., 2014a, 2014b). Some vertebrates, such as bats, hover in front of flowers and use long tongues to collect nectar (Winter and Von Helversen, 2003). Nectarivorous bats possess highly specialized extendable tongues to gather nectar (Harpera et al., 2013). Various insects and birds, such as butterflies (Krenn and Penz, 1998; Lee et al., 2014a, 2014b; Tsai et al., 2014) and hummingbirds, feed on floral nectar using tubes formed from proboscises or tongues (Jensen et al., 2013). Other studies investigated the dynamic models of the nectar drinking of hummingbirds and butterflies (Kingsolver and Daniel, 1983; Pivnick and Mcneil, 1985). To observe the drinking rates of butterflies, Kingsolver and Daniel (1979) became the first to propose nectar drinking through a tube as a constrained optimization problem. Meanwhile, an experimental and theoretical investigation into the feeding dynamics of ruby-throated hummingbirds (Rico-Guevara and Rubega, 2011) found that hummingbirds use a self-assembling tube and special tongues to ingest nectar. In other studies, the nectar drinking patterns of honeybees were extensively investigated, and various mouthpart modes were found to be involved in the drinking mechanisms (Kim et al., 2011; Yang et al., 2014).

Honeybees have three castes: workers, drones, and the queen. Workers and drones show morphological, physiological, and behavioral differences. Although the anatomy and feeding behavior of honeybees have been reported, the microstructures in the mouthparts of honeybees are not clearly understood. This type of various special structures can be found in nature. McCullough et al. (2014) constructed biomechanical models of the horns of different rhinoceros beetle species to evaluate the functional performance of these horns in response to both species-typical and species-atypical fighting loads. Rico-Guevara and Araya-Salas (2014) suggested the role of sexual selection in the evolution of the overall bill morphology; this suggestion is an alternative hypothesis to the prevailing "ecological causation" explanation for bill sexual dimorphism in hummingbirds. Therefore, revealing the functional diversities of the microstructures of honeybees may improve our understanding of the physiology of honeybees and their adaptability to environmental constraints.

Our study focuses on the specific microridges inside the mouthparts of honeybees. The microridges on the inner wall of the galeae of honeybees can affect drag reduction (Li et al., 2015). However, few studies have examined the effect of microstructures on the drag reduction of honeybee workers and drones, and the mechanism responsible for mouthpart divergence remains largely unexplored. This study examines the differences in and the similarity of the mouthpart microstructures of *Apis mellifera ligustica* workers and drones. The numerical results of the biofluiddynamic analysis based on the microridges of both workers and drones are revealed, and the environmental adaptability of honeybee is investigated.

2. Materials and methods

2.1. Material species and feeding mechanism of honeybees

We used honeybees (*A. m. ligustica*) from Xiangshan, Beijing, China (39.99°N, 116.18°E), where no specific permissions were required. Nearly 2000 workers and a few drones with a queen were housed in an indoor artificial beehive measuring 200 mm × 300 mm × 300 mm. The beehive was connected to an inspection box through which the workers and drones could be observed and captured. The entire system was equipped with artificial ventilation to maintain the temperature at 25 °C and the humidity at 50%. The honeybees were raised almost entirely on honey, pollen, sucrose solution, and inorganic salt solution.

A. m. ligustica is a typical insect whose mouthpart has been investigated extensively with respect to its morphology and function (Snodgrass, 1984). The mouthpart of a honeybee generally comprises a pair of galeae, a pair of labial palpi, and a glossa (Fig. 1). As shown in Fig. 1, the glossa appears as a slightly tapering brush-like organ that is densely covered with glossal setae (Briant, 1884). The elongated galeae and the long flattened first two segments of the labial palpi form a tube around the glossa when the honeybee feeds on nectar (Krenn et al., 2005). In addition, the glossa inside the tube exhibits a reciprocating movement that allows the nectar to be loaded onto the mouth of the honeybee. The erection of the glossal setae to trap nectar during the viscous dipping process of honeybees should be considered (Yang et al., 2014).

2.2. Scanning electron microscope observation

Six specimens (three workers and three drones) were prepared for scanning electron microscopy (SEM) observation. To avoid the chemical contamination of the mouthparts, the laboratory-reared specimens were handled using latex gloves. Respirators were utilized given that the chemical reagent used to prepare the specimens may be harmful to humans. The six specimens were first processed with 2.5% glutaraldehyde solution and washed with 0.1 mol/L phosphate buffer (pH = 7) synthesized from sodium dihydrogen phosphate and disodium hydrogen phosphate. All the specimens were then dehydrated from 70% to 100% using graded ethanol. Afterward, tert-butyl alcohol solution was used for permutation, and the samples were finally desiccated for 10 min in a drying box. The six samples were observed via SEM (FEI Quanta 200, Czech Republic) to obtain detailed information about the structure and morphology of the mouthparts and to identify their differences in workers and drones.

2.3. High-speed video observation

The drinking processes of the honeybees were filmed using a high-speed camera (Fig. 2). The experimental equipment comprised a positioner, a high-speed camera (Phantom M110, USA), a microscope (Axiostar Plus, Zeiss, Germany), a cold light source, and a cuboid feeder. The cold light source and the high-speed camera were located in the same line to provide light. To capture a clear video, the cuboid feeder was placed between the cold light source and the high-speed camera with appropriate focal distance. The glass cuboid feeder (1 mm thickness) contained sucrose solution, which was prepared at a concentration of 35% (wt/wt), and the temperature was set to 25 °C. As a preliminary step, the honeybees used for the experiments were starved for 24 h. To encourage the honeybees to ingest nectar continuously and to ensure that their mouthparts remained fixed, the honeybees were glued to a positioner via their thoraxes. Notably, the position of the honeybees could be adjusted accurately through the positioner

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