

## RESEARCH

# Switchable Wettability of the Honeybee's Tongue Surface Regulated by Erectable Glossal Hairs

Ji Chen,\* Jianing Wu,\* and Shaoze Yan<sup>1</sup>

Division of Intelligent and Biomechanical Systems, State Key Laboratory of Tribology, Department of Mechanical Engineering, Tsinghua University, Beijing, People's Republic of China.

<sup>1</sup>Corresponding author, e-mail: yansz@mail.tsinghua.edu.cn

\*These authors contributed equally to this work.

**Subject Editor:** Takumasa Kondo

J. Insect Sci. (2015) 15(1): 164; DOI: 10.1093/jisesa/iev143

**ABSTRACT.** Various nectarivorous animals apply bushy-hair-equipped tongues to lap nectar from nectaries of flowers. A typical example is provided by the Italian honeybee (*Apis mellifera ligustica*), who protracts and retracts its tongue (glossa) through a temporary tube, and actively controls the erectable glossal hairs to load nectar. We first examined the microstructure of the honeybee's glossal surface, recorded the kinematics of its glossal hairs during nectar feeding process and observed the rhythmical hair erection pattern clearly. Then we measured the wettability of the glossal surface under different erection angles (EA) in sugar water of the mass concentration from 25 to 45%, mimicked by elongating the glossa specimens. The results show that the EA in retraction approximately remains stable under different nectar concentrations. In a specific concentration (35, 45, or 55%), the contact angle decreases and glossal surface area increases while the EA of glossal hairs rises, the glossa therefore could dynamically alter the glossal surface and wettability in foraging activities, not only reducing the energy consumption for impelling the nectar during tongue protraction, but also improving the nectar-trapping volume for feeding during glossa retraction. The dynamic glossal surface with switchable wettability regulated by erectable hairs may reveal the effective adaptation of the honeybee to nectar intake activities.

**Key Words:** dynamic surface, hairs erection, honeybee, switchable wettability

Researchers have been discovering various drinking strategies (Richard and Errard 2009) of animals according to the morphological, physiological, and environmental constraints (Crompton and Musinsky 2011, Kim and Bush 2012). The Italian honeybee, *Apis mellifera ligustica*, as a typical nectar-dependent insect, feeds on nectar in the pattern of viscous dipping mainly in accordance with their chewing–lapping proboscides (Kim et al. 2011). The proboscis for nectar feeding comprises a pair of galeae, a pair of labial palpi and a densely-hairy tongue (glossa) (Fig. 1a–f) (Cook 1880). Kim et al. (2011) and Yang et al. (2014) discovered that honeybees imbibe nectar in a typical pattern of forward–backward motion of the glossa and proposed two physical models to explain the mechanism of nectar intake considering the glossa as an invariant object. The models are categorized as viscous dipping because they found that glossa uploads nectar during reciprocating movement owing to the viscosity of the liquid. However, they both neglected facts that the actual erection angles (EA) are  $<90^\circ$ , which is assumed by Yang et al., in nectar intake process, and that the wettability of the glossal surface is various with respect to the changing surface structure. More specifically, glossal surface with different EAs of hairs has different wettability, which means the ability of nectar sticking to the glossal surface is different.

The wettability of a surface is determined by both its chemical composition and micro- or nanostructure (Sun et al. 2005). As for surfaces in changeless chemical composition, their functions in most cases depend on the difference or transform of the structure (Barthlott et al. 2010). Diverse wettability in different regions is proved to be beneficial for animals' physiological activities (O'Donnell 1982, Prakash et al. 2008, Lehnert et al. 2013, Wainwright et al. 2013, Wu et al. 2015), whereas switchable wettability in fluid intake process, which may structurally meet the functional demands of effectively loading liquid, has not been clearly revealed yet. In order to further explore the feeding mechanism of a honeybee, we experimentally captured the actual nectar intake process of live honeybees to observe the tongue profiles in the

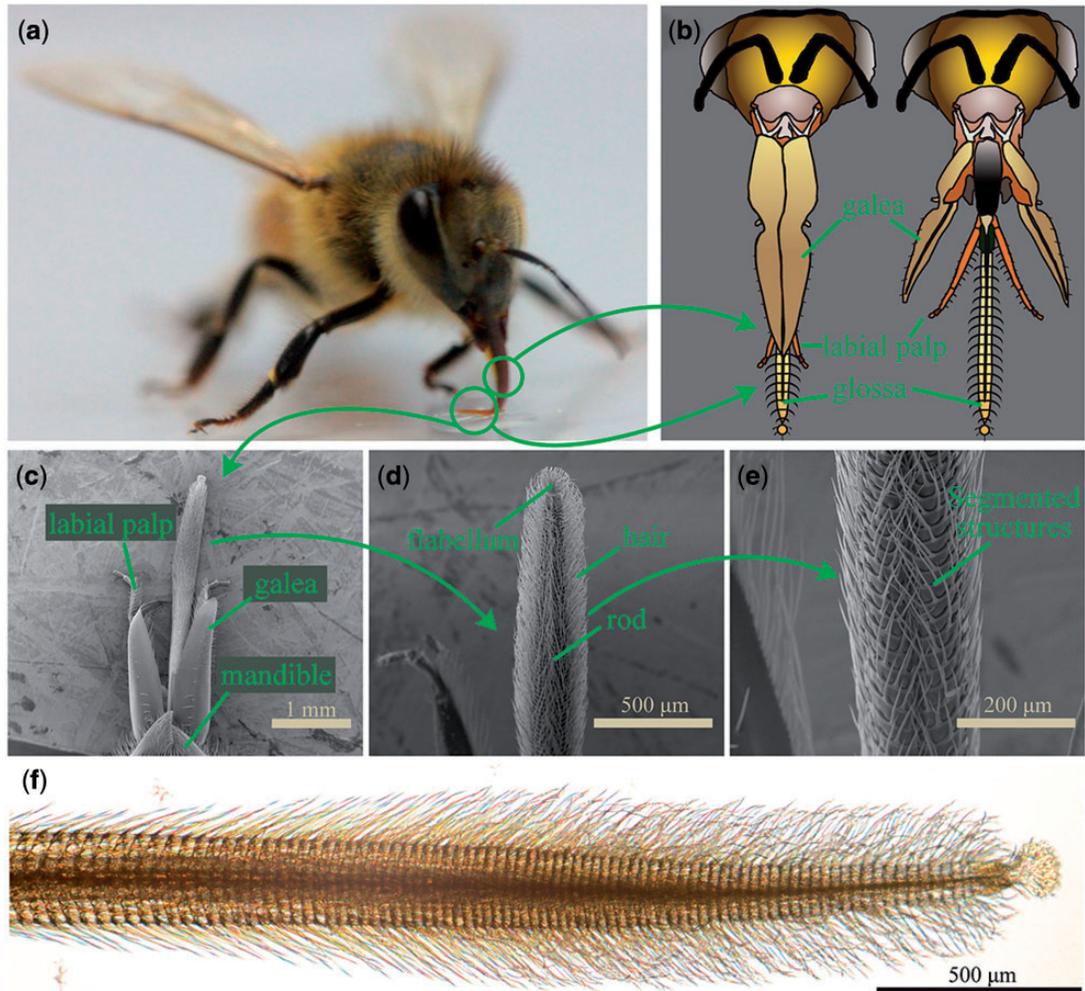
feeding cycles. In addition, we developed a novel testing method to detect the wettability of the functional surface (Fig. 2b). By stretching the glossa, we mimicked different glossal configurations of the in-feeding bees, and measured the contact angle (CA) with sucrose solutions under a series of ascending concentrations.

## Materials and Methods

**Honeybee Specimens.** We collected the honeybee specimens (*A. m. ligustica*) from Beijing, China (40.00°N, 116.33°E) and fed them with 35% (wt/wt) sugar water in a glass-made beehive, keeping the temperature and humidity at 25°C and 50%, respectively. We affirm that these locations required no particular permissions, and that we did not use endangered or protected animals. To get rid of chemical contamination, all of the experiments were conducted in a clean chamber, and latex gloves and masks were adopted as well (Wu et al. 2015).

**SEM Imaging.** Ten live worker bees were captured from the beehive and fixed in 100% ethanol to ensure that tongues are straightened from the mouthparts. These dissected glossa specimens of bees ( $3.32 \pm 0.13$  mm,  $n = 10$  bees) were soaked in a 50% glutaraldehyde solution, dehydrated by an ethanol series (75, 80, 85, 90, 95, 100%, ~30 min each), coated in gold palladium (10 nm in depth), and observed under a scanning electron microscope (SEM, FEI Quanta 200, Czech Republic) (Wu et al. 2015).

**Kinematic Observation of Glossae (Live Honeybees).** The experimental setup for drinking activity observation consisted of a high-speed camera (Metek Miro M110, USA, up to 2,000 fps), a microscope (ZEISS Axiostar plus, Germany), a nectar container, which we made by keeping a distance between two slides of glasses 2 mm and using the ethylene–vinyl acetate copolymer glue to seal two sides and the bottom, and a positioner with a fixture attached to it (Fig. 2a). To start with, the honeybee specimens were starved in an inspection box for 18 hr. Then a live honeybee was affixed in the fixture via its thorax to ensure the free movement of the mouthpart. It could be moved vertically adjusted by the positioner (motion accuracy: 0.01 mm). In addition, we placed the

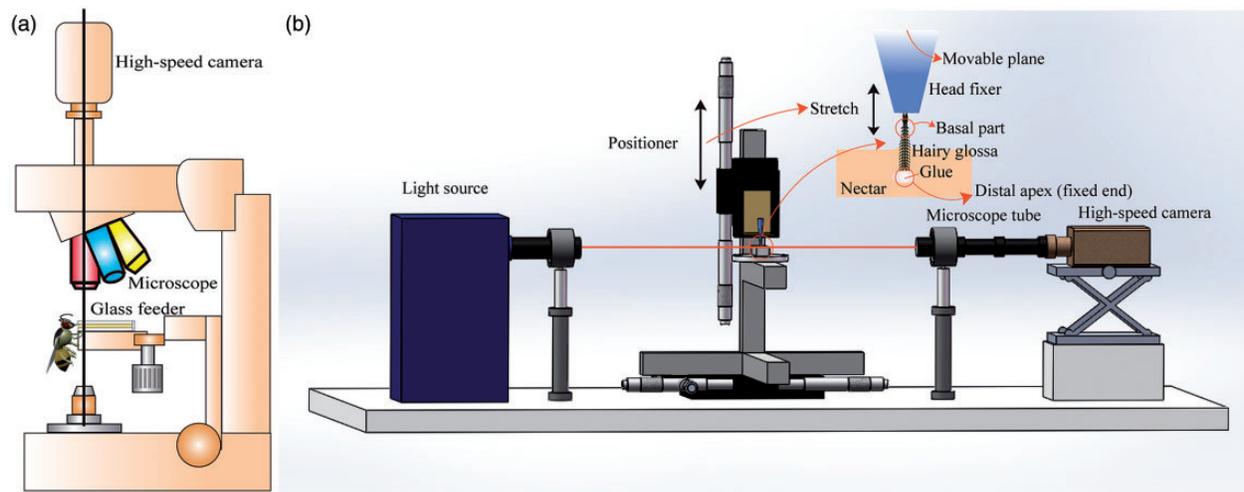


**Fig. 1.** Anatomy of an Italian worker bee's mouthpart and the experimental setups for feeding behavior observation and wettability examination. (a) Lateral view of an in-feeding worker bee. (b) Line tracings of a honeybee's mouthpart. A pair of galeae are distributed on the dorsal side of the glossa and the dual labial palpi are developed on the ventral. In the nectar feeding process, galeae and labial palpi form a sucking tube and the glossa reciprocates through the tube to trap nectar. (c) SEM image of the honeybee's mouthpart which is comprised a pair of mandibles, a pair of galeae, a pair of labial palpi, and a glossa. (d) SEM image of a densely-hairy glossa. The distal apex of the glossa is a spoon-like flabellum covered by setae and sensilla. (e) SEM image of the glossal surface. The glossa appears in a multi-articulate structure and hairs are merely born on the ring-like membranes between segments. (f) Segmented structures of a glossa with bushy hairs under a microscope (magnified by  $5\times$ )

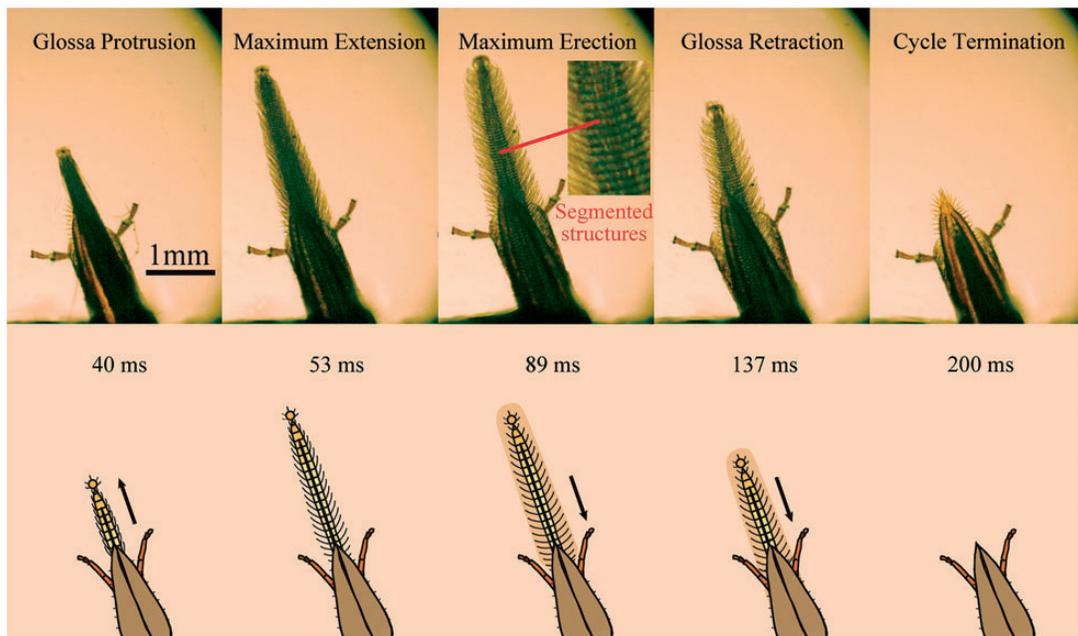
bee near the nectar container to guarantee the glossa horizontally extend into the artificial nectar (35% wt/wt sucrose solution) (Fig. 2a). We recorded a honeybee's drinking sucrose solution from a dorsal shooting angle at a speed of 500 fps. A series of images captured by the high-speed camera were magnified  $5\times$ . Honeybee specimens ( $n = 10$  bees, body length  $15.07 \pm 0.15$  mm) were initially starved in an inspection box for 18 hr. Then, we recorded honeybees' feeding activities by using 35% (wt/wt) sucrose solution from a dorsal shooting angle using a microscope equipped with a high-speed camera (Metek Miro M110, USA) with a recording rate of 500 fps (Fig. 2a).

**CA Measurement of Glossae (Postmortem Honeybees).** The setup for examining wettability contains a white light source (100 W) and a high-speed camera (METEK MIRO M110, USA, up to 2000 fps) accompanied with a horizontally placed microscope tube (Keyence, VH-Z50L, Japan, up to the magnification of  $50\times$ ) (Fig. 2b). For the CA measurement of the tongue under different EA, we first fixed the tongue through a head fixture, used glue to stick the distal apex to the bottom of the nectar container, then injected the sugar water into the container, certifying the glossa was half-submerged in the liquid and finally adjusted a precision positioner

connected to the head fixture to elongate the glossa as well as avoid breaking it. The tongue, nectar, and air, namely the solid, liquid, and gas phases formed a meniscus. We started recording when the tongue was straight and the hairs were attached to the tongue ( $EA \approx 0^\circ$ ). Then, we tuned the positioner handle for  $360^\circ$  every time, developing a quasi-static process, to make the tongue extend step-by-step. After each step, the tongue was elongated for 0.5 mm in length. Between each step, the positioner was kept still for 2 s to reach the static EA and CA. Accordingly, the EA increased from  $0^\circ$  to  $80^\circ$  as the glossa extended for  $1.8 \pm 0.2$  mm ( $n = 12$  glossa specimens). Under each EA, different appearances of meniscus were formed. Through the microscope tube, the magnified contour of the wetted tongue was recorded by the high-speed camera (Fig. 2b). The CA, at a location where the tangent along the liquid-gas interface intersects the liquid-solid interface, is the most common method to evaluate the wettability (Mittal 2006). As a subtle tongue structure, glossal hairs can be erected by stretching the glossa (Simpson and Riedel 1964). We designed an experimental setup to examine the CAs under different EA (where the tangent along the hair on the middle of the glossa intersects the outline of the rod surface) by elongating the glossa (Fig. 2b).



**Fig. 2.** Experimental setups for nectar-intake observation and contact angle measurement. (a) The experimental setup for kinematic observation of tongue's nectar intake process. (b) The experimental apparatus for contact angle examination of the glossa



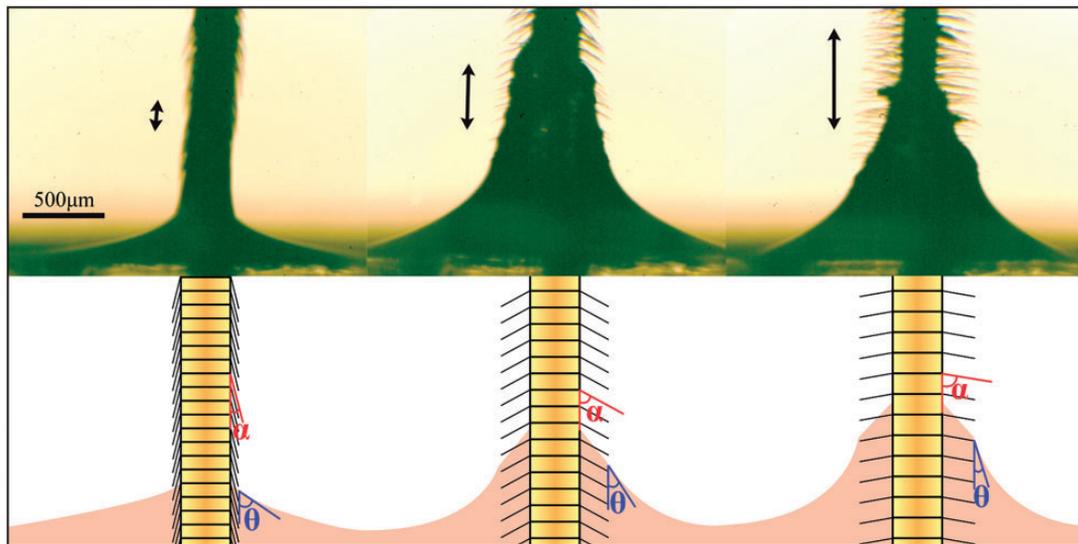
**Fig. 3.** Snapshots (upper) and sketches (lower) of a set of frames of tongue kinematics and hairs erection in a feeding cycle. The inset in the third image shows the appearance of glossal segmented structures. Time elapsed from the beginning of one cycle is given each frame. Black arrows indicate the moving direction of the tongue. Deep yellow area shows the trapping nectar

## Results

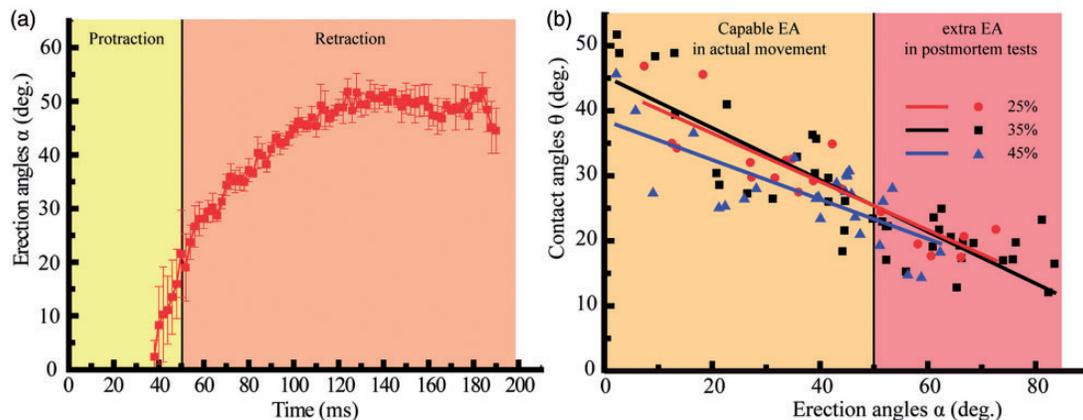
A color high-speed movie of glossa movement in nectar intake process demonstrated that hairs erect rhythmically keeping pace with the glossa's reciprocating movement, conspicuously changing the surface configuration in motion (Fig. 3, and Supplementary movie S1). Unlike the hummingbird which uses its dynamic liquid-trapping device on the tongue tip to transport nectar (Rico-Guevara and Rubega 2011), honeybee immerses its glossa into the nectar and take advantage of reciprocating movement as well as rhythmical hair erection to intake nectar. The duration of an entire feeding cycle is  $200 \pm 10$  ms and protraction lasts  $53 \pm 5$  ms, while the retraction lasts  $150 \pm 5$  ms (three cycles per one bee,  $n = 10$  bees). We designated the moment when the tongue was about to emerge from the tube as the beginning of a feeding cycle, labeled as 0 ms. As the glossa protrudes, hairs are firmly attached to the glossa rod. At 53 ms, the glossa reaches the maximum extension with hairs slightly

unfolded. As the protraction ends, hairs continue to deploy, reach the maximum erection at 90 ms, and then stay erect throughout the glossa retraction. The nectar, coating the whole glossa, is then loaded into the mouthpart. After the whole tongue retracts into the tube, it shapes the same as the initial profile with hairs attached. The EA with respect to time is shown in Figure 3 (five cycles,  $n = 5$  bees). In the initial 40 ms, the EA is  $0^\circ$ . From 40 to 130 ms, the average EA increases from  $0^\circ$  to  $50.0 \pm 4.7^\circ$ . From 130 to 200 ms in the retraction phase, the average EA stays at  $48.0 \pm 4.0^\circ$ . Glossal hairs have larger average EAs in retraction phase than in protraction phase.

According to the Wenzel wetting theory (Wenzel 1936), a rough surface is more wettable than a flat surface. We indeed discovered that the surface wettability is strengthened in retraction phase because the rougher glossal surface is created by erected hairs. According to a series of images in CA measurement, as the glossal hairs erected, segmented



**Fig. 4.** Snapshots (upper) and line tracings (lower) of a tongue under different EAs. Black arrows denote the stretching directions. The variation of segmented length, EAs ( $\alpha$ ) and contact angles ( $\theta$ ) are shown in line tracings



**Fig. 5.** The relationship between EA and time as well as contact angle and EA. (a) EAs throughout one nectar feeding cycle. Two colored regions connote the protraction phase and retraction phase, respectively. (b) The scaling of contact angles with EAs under 25, 35, 45% sucrose concentration. Wettability with EAs  $<50^\circ$  is reachable in actual nectar feeding, whereas wettability with EAs  $>50^\circ$  could only be realized in postmortem tests

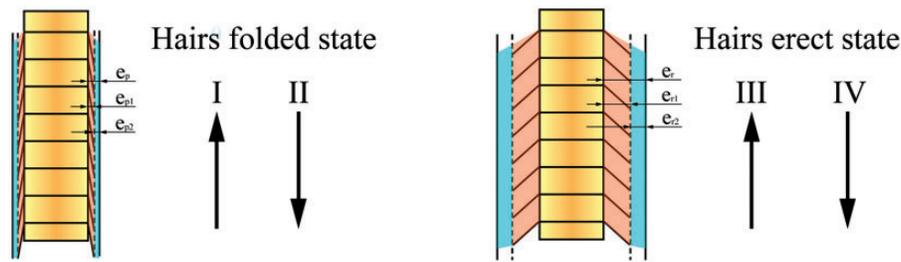
length augmented and peaks of hairs formed a new solid surface with gaps among them, around which the contact line extended but the CA decreased (Fig. 4, Supplementary movie S2). Fitted curves of CA–EA in 25% (fitting function:  $\theta = 43.95 - 0.37\alpha$ ), 35% (fitting function:  $\theta = 45.31 - 0.40\alpha$ ), and 45% (fitting function:  $\theta = 38.51 - 0.30\alpha$ ) are shown in Figure 5. When glossal hairs erect more, the surface area contacting the liquid will increase while the CA will decrease. The results indicate that the hydrophilicity of the glossal surface is strengthened by the erecting hairs. Furthermore, the nectar tends to infiltrate the glossa more easily when the glossal surface becomes more hydrophilic. Thus, higher wettability and larger surface contact area of the glossa imply a stronger absorptive capacity to sugar water when hairs are more erect. As the EA is  $50^\circ$ , the larger viscosity of the liquid leads to more wettability of the glossal surface, especially when the viscosity is very high. However, wettability of glossal surface soaked in 25 and 35% sucrose is close to each other. When EA is  $<50^\circ$ , the glossal surface in 35% sucrose shows the least hydrophilic.

## Discussion

We found that glossal hairs are distributed on the intersegments and can rhythmically erect during nectar feeding to form a dynamic surface

which has the property of switchable wettability. A variety of natural surfaces with special wettability provide possibilities for liquid transportation (Parker and Lawrence 2001, Zheng et al. 2010). And section-wise wettability may be beneficial to the nectar intake rate (Wu et al. 2015). Recently, it is reported that transformable protrusions on the tongue surface of a nectarivorous bat are beneficial for augmenting the nectar intake rate (Harper et al. 2013). And the dynamic surface of a honeybee's tongue with erectable hairs may also enhance the nectar intake rate.

Kim et al. (2011) deduced the following equation from a viscous dipping model only considering the effect of nectar adhesion:  $Q = 2\pi e a u T$ , where  $Q$  is the nectar intake volume in one feeding cycle,  $e$  is the thickness of the adhesion nectar layer,  $a$  is the average radius of the glossa,  $u$  is the retraction speed, and  $T$  is the retraction time. The length of the immersed glossa  $L$  is equal to  $uT$ . In addition, Yang et al. (2014) gave an equation that  $Q = \pi a u T (2h + h^2/a) - 2\pi^2 n a r^2 h u T / 3$ , supposing the length of each hair has the same value  $h$ , the EA is  $90^\circ$  and the amount of hairs is  $n$ . Whereas, in fact, the EA is disparate in different phases, and cannot reach  $90^\circ$  as well. Based on these works, we propose that the actual nectar intake volume  $Q = \pi a u T [2(e_1 + e_2) + (e_1 + e_2)^2/a] - Q_h$ , where  $Q_h$  is the total volume of hairs which remains constant. The value  $e_1 + e_2$  represents the thickness (Fig. 6) of coating nectar around the glossal rod, which can



**Fig. 6.** Two states of glossal surface and four possible kinematic pattern. Hairs folded state means the situation that hairs attach tight to the glossa, namely hairs with minimum EA, which happens at the beginning of protraction phase. Hairs' erect state indicates the situation of hairs erecting with maximum EA in the retraction phase. Two arrows for each state show two possible moving directions. The values  $e_{p1}$  and  $e_{p2}$  denote the thickness of nectar layer among hairs and the thickness of the peripheral nectar dragged by glossa in the protraction phase, respectively. Values of  $e_{r1}$  and  $e_{r2}$  denote those in the retraction phase. Values of  $e_p$  and  $e_r$  are total thickness of the nectar in each phase. Orange and blue strips designate nectar layer among hairs and adhesive nectar layer, respectively

be deliberately divided into two parts:  $e_1$  means the thickness of nectar layer among hairs, determined by the perpendicular distance from the tip of the hair to the glossal surface, while  $e_2$  denotes the thickness of the peripheral nectar, mainly influenced by the wettability of the hairy surface. The increase of either  $e_1$  or  $e_2$  will lead to the extension of value  $Q$  according to the equation. So, large EA and strong adhesive capacity can promote the nectar intake volume. From the other perspective, we notice that with more coated nectar in protraction phase, the tongue consumes more energy because it has to impel the trapped nectar to move together. Less  $e_1$  and  $e_2$  will reduce the energy consumption for actuating the tongue in protraction. We define the situation of hairs with minimum EA as the hairs folded state and that of hairs with maximum EA as the hairs erect state. Figure 6 shows four possible movement patterns, namely the retraction in hairs folded state (I), protraction in hairs folded state (II), retraction in hairs erect state (III), and protraction in hairs erect state (IV). In hairs folded state,  $e_1$  reaches the minimum value  $e_{p1}$ . What is interesting is that  $e_2$  also achieves the smallest value  $e_{p2}$ , since the less the EA is, the weaker wettability the surface holds, which means the lower absorptive capacity it has. Hence,  $e_p = e_{p1} + e_{p2}$  is the smallest total thickness of the coating nectar (Fig. 6). Similarly, in hairs erect state,  $e_{r1}$  and  $e_{r2}$  are both the maximum value of  $e_1$  and  $e_2$ , and  $e_r$  is the largest nectar layer thickness. In pattern II, the glossa with hairs tightly attached to it impels less nectar when dipping into the liquid, which helps the honeybee decrease the energy consumption in one nectar intake cycle. In pattern III, the glossa with erect hairs has a larger surface contact area to the nectar and higher wettability, leading to more nectar intake volume of the honeybee. According to the observation, the glossa movement and hair erection fit pattern II and pattern III, which is beneficial to increasing the energy intake. It is worthwhile to mention that the EA and the glossal surface wettability have a synergistic effect on the volume of carried liquid. The wettability changes by the EA, and maximizes the net intake energy together with EA itself. Such switchable wettability resembles the positive feedback (Tanner et al. 2011) in nature, which amplifies the increment of energy intake in the nectar feeding process. Therefore, the functionally deformable surface of the honeybee's glossa matches the optimized model and reveals the drinking adaptations of honeybees from a perspective of surface wettability.

### Acknowledgments

We thank the Centre of Biomedical Analysis of Tsinghua University for their assistance with the specimen processing and SEM image capturing. This study was supported by the National Natural Science Founding of China (Grant 51475258) and a Research Project of the State Key Laboratory of Tribology under Contract SKLT11B03.

### Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

### References Cited

- Barthlott, W., T. Schimmel, S. Wiersch, K. Koch, M. Brede, M. Barczewski, S. Walheim, A. Weis, A. Kaltenmaier, A. Leder, et al. 2010. The *Salvinia* paradox superhydrophobic surfaces with hydrophilic pins for air retention under water. *Adv. Mater.* 22:2325–2328.
- Cook, A. J. 1880. The tongue of the honey bee. *Am. Nat.* 14:271–280.
- Crompton, A. W., and C. Musinsky. 2011. How dogs lap: ingestion and intraoral transport in *Canis familiaris*. *Biol. Lett.* 7:8824.
- Harper, C. J., S. M. Swartz, and E. L. Brainerd. 2013. Specialized bat tongue is a hemodynamic nectar mop. *Proc. Natl Acad. Sci. USA.* 110:8852–8857.
- Kim, W., and J. W. Bush. 2012. Natural drinking strategies. *J. Fluid. Mech.* 705:7–25.
- Kim, W., T. Gilet, and J. W. Bush. 2011. Optimal concentrations in nectar feeding. *Proc. Natl Acad. Sci. USA.* 108:16618–16621.
- Lehnert, M. S., D. Monaenkova, T. Andruk, C. E. Beard, P. H. Adler, and K. G. Kornev. 2013. Hydrophobic–hydrophilic dichotomy of the butterfly proboscis. *J. R. Soc. Interface* 10:20130336.
- Mittal, K. L. 2006. Contact angle, wettability and adhesion. *Am. Chem. Soc.* 4:3–28.
- O'Donnell, M. J. 1982. Hydrophilic cuticle—the basis for water vapour absorption by the desert burrowing cockroach, *Arenivaga investigata*. *J. Exp. Biol.* 99:43–60.
- Parker, A. R., and C. R. Lawrence. 2001. Water capture by a desert beetle. *Nature* 414:33–34.
- Prakash, M., D. Quéré, and J. W. Bush. 2008. Surface tension transport of prey by feeding shorebirds: the capillary ratchet. *Science* 320:931–934.
- Richard, F. J., and C. Errard. 2009. Hygienic behavior, liquid-foraging, and trophallaxis in the leaf-cutting ants, *Acromyrmex subterraneus* and *Acromyrmex octospinosus*. *J. Insect. Sci.* 9:1–9.
- Rico-Guevara, A., and M. A. Rubega. 2011. The hummingbird tongue is a fluid trap, not a capillary tube. *Proc. Natl Acad. Sci. USA* 108:9356–9360.
- Simpson, J., and I. Riedel. 1964. Discharge and manipulation of labial gland secretion by workers of *Apis mellifera* (L.) (Hymenoptera: Apidae). *Proc. R. Entomol. Soc. Lond. Ser. A. Gen. Entomol.* 39:76–82.
- Sun, T., L. Feng, X. Gao, and L. Jiang. 2005. Bioinspired surfaces with special wettability. *Acc. Chem. Res.* 38:644–652.
- Tanner, C. J., G. D. Salali, and A. L. Jackson. 2011. The ghost of social environments past: dominance relationships include current interactions and experience carried over from previous groups. *Biol. Lett.* 7:818–821.
- Wainwright, D. K., T. Kleinteich, A. Kleinteich, S. N. Gorb, and A. P. Summers. 2013. Stick tight: suction adhesion on irregular surfaces in the northern clingfish. *Biol. Lett.* 9:20130234.
- Wenzel, R. N. 1936. Resistance of solid surfaces to wetting by water. *Ind. Eng. Chem.* 28:988–994.
- Wu, J., R. Zhu, S. Yan, and Y. Yang. 2015. Erection pattern and section-wise wettability of honeybee glossal hairs in nectar feeding. *J. Exp. Biol.* 218:664–667.
- Yang, H., J. Wu, and S. Yan. 2014. Effects of erectable glossal hairs on a honeybee's nectar-drinking strategy. *Appl. Phys. Lett.* 104:263701.
- Zheng, Y., H. Bai, Z. Huang, X. Tian, F. Q. Nie, Y. Zhao, J. Zhai, and L. Jiang. 2010. Directional water collection on wetted spider silk. *Nature* 463:640–643.