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Honeybees have Hydrophobic Wings that Enable Them to Fly through Fog and Dew

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Abstract

Honeybees have received public attention for their remarkable performance in low-altitude flying and their outstanding airborne hovering capability. However, minimal attention has been given to their capability to fly through the harshest climatic conditions. In this study, we used a high-speed camera and recorded an interesting phenomenon in which honeybees (*Apis mellifera ligustica*) flew effortlessly through mists or drizzling rain. To identify the mechanism behind honeybees flying through mists, the microstructure of their wings was examined via atomic force microscopy and scanning electron microscopy. Experimental results showed that the surface of a honeybee wing was rough, with bristles distributed on both the dorsal and ventral sides. The measurement results of the contact angle proved that the surface of honeybee wings was hydrophobic. Furthermore, hydrophobic proteins, which contained at least one hydrophobic tetra-peptide (*i.e.*, AAPA/V), were obtained. The rugged surface and hydrophobic proteins caused the hydrophobicity of honeybee wings. These results identify the hydrophobic mechanism of honeybee wings, which will be useful in designing hydrophobic structures.

Keywords: hydrophobicity, microstructure, biological coupling, hydrophobic peptide, honeybee wing

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1 Introduction

For hundreds of millions of years, insects have been evolving to develop superior capabilities that enable them to adapt to rugged environments[1]. Traditional studies have focused on insect flight in a clean, well-controlled environment[2–6]. In nature, however, insects experience various weather conditions, such as mist, rain, fog, and dewfall, and complex environments. The current study focuses on how honeybee wings can withstand disturbances caused by fog and moisture during flight. The wings of honeybees do not get wet even when they are exposed to steam. On this basis of this phenomenon, can an assumption be made that honeybee wings can continue flapping through dew? Do the wings have a nanoporous microstructure that enables them to exhibit a hydrophobic property?

Scientists worldwide have devoted decades to study water-repellent surfaces. Several empirical models have been proposed to illustrate surface wetting properties based on various experimental data; these models include the well-known Wenzel[7] and Cassie–Baxter models[8]. Several scholars have summarized the developments in superhydrophobic surfaces[9–11]. Wagner *et al*.[12] examined the surfaces of the wings of 97 insect species from all major groups via high-resolution Scanning Electron Microscopy (SEM); they demonstrated that the microstructure of a wing played a key role in its hydrophobic property. Wan *et al*.[13] studied the hydrophobic mechanism of the wings of *Apis cerana fabricius*. They found that bristles distributed in a manner similar to that of a parallelogram on both the dorsal and ventral sides of the wings. Contact Angle (CA) is one of the evaluation indexes for hydrophobicity[14–17]. Zhao *et al*.[18] proposed a simplified surface model for an anisotropic patterned surface with dual scale roughness. The free energy, as well as free energy barriers versus instantaneous CAs, was analyzed using a thermodynamic method to calculate the equilibrium CA and the CA hysteresis on a given surface. Surface roughness is another important index for hydrophobicity[19–22]. Michael *et al*.[23] formulated a stability criterion and iden-

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tified the mechanisms of composite interface destabilization. They indicated that these mechanisms were scale-dependent and concluded that surface roughness enhanced the hydrophobicity of a solid surface with high CAs. However, hydrophobicity is not only related to surface topography but also to the chemical constituents of honeybee wings.

The wettability of solid surfaces is an important issue that has drawn the interest of scientists toward the chemical constituents of surfaces[24–28]. Blossey[29] studied self-cleaning surfaces and obtained a complementary DNA microarray on a silanized glass plate. Strachecka *et al.*[30] investigated the proteolytic activity of the body surface of the honeybee *Apis mellifera ligustica*, they determined that the concentration of hydrophobic proteins on the body surface of these bees was higher than that of hydrophilic proteins. Kucharski *et al.*[31] found highly hydrophobic polypeptides that exhibited the sequence motifs characteristic of cuticular proteins and distinctly novel features. Similarly to many cuticular proteins, the apidermin protein family is highly hydrophobic, with alanine being the predominant amino acid, particularly, the hydrophobic tetra-peptide AAPA/V[32]. All the aforementioned works have contributed significantly to the analysis of the chemical constituents of honeybee wings.

In the current study, the microstructure of the wings of the honeybee *Apis mellifera ligustica* was first observed via Atomic Force Microscopy (AFM) and SEM. As a third step to improve the understanding of the hydrophobicity of honeybee wings, CA was measured using a CA measurement device (*i.e.*, OCA 25). In addition, the constituents of honeybee wing were analyzed via mass spectrometry. The result indicated the relationship between hydrophobicity and the special proteomics of honeybee wings. This study is significant because it elucidates the importance of hydrophobicity and the development of biomimetic materials.

2 Materials and methods

2.1 Sample preparation

Honeybee (*Apis mellifera ligustica*) specimens were kept in a hive, where temperature and humidity were maintained at 25 ˚C and 50%, respectively. We confirmed that no specific permission was required for such locations and activities, and that field studies did not involve endangered or protected species.

2.2 Discovery of honeybee traveling through water mist

Images of a honeybee traveling through mists were captured using a high-speed camera (Olympus i-SPEED TR, Tokyo, Japan) operating at 1,000 frames per second with a zoom lens (Navitar 12× Zoom, Rochester, NY, USA). Water was sprayed from the top of the hive using a spraying device. This device was mainly composed of a diaphragm pump (8816, DERTIN, China), a filter head (polyethylene cotton self-suction head with diameter of 6.3 mm, NOVAQINGYUAN, China), a PE pipe (polyethylene with diameter of 6.3 mm, NOVAQINGYUAN, China), and a spray head (MIST01L, NOVAQINGYUAN, China). The working voltage was 24 VDC, the rated current was 2 A and the maximum pressure was 135 PSI. The diameter of the droplet was approximately 45 μm – 75 μm.

2.3 Microstructure observation experiments

The surface topography of honeybee wings was captured using an AFM (Nanoscope IIIa, Veeco, USA). The lateral resolution was 0.1 nm, and the vertical resolution was 0.01 nm. In addition, the microstructure of honeybee wings was observed using a SEM (FEI Quanta 200, FEI Company, Eindhoven, Netherlands). The samples were sputtered with platinum and observed under low vacuum mode (30 kV). The resolution of the images was 3.5 nm.

2.4 Measurement of water CA

In the experiments, the wings were fixed on a glass slide with double-sided tape. Subsequently, CAs were measured using an all-purpose measuring device OCA 25 (DataPhysics, Germany). The measurement accuracy of the system was ± 0.1˚, and the volume of a water droplet was set to 3 μL.

2.5 Identification of chemical constituents

A total of 80 pairs of wings from deceased honeybees were collected and ground in liquid nitrogen solution. The resulting solution was centrifuged using an ultracentrifuge (Optima MAX-XP, Beckman Coulter, USA). Then, the proteins were separated and purified via sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Finally, the chemical constituents of the honeybee wings were determined through mass spectrometry.