

Adhesion and Suction Functions of the Tip Region of a Nectar-drinking Butterfly Proboscis

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Abstract

In this study, we investigated the dynamic functions of the tip region of the butterfly proboscis through which liquid is sucked during liquid feeding. The microstructures and flow patterns in the tip region of the proboscis were *in vivo* analyzed. The tip region can be divided into two functional sections: namely adhesion and suction sections. The liquid adheres to the adhesion section during liquid suction. Although the tip region has numerous slits connected to food canal of the proboscis, liquid is mainly sucked through the suction section, which section is submerged in the fluid pulled by the adhesion section and then successfully imbibes liquid. To check the dynamic functions of the tip region, we fabricated a suction tip model having adhesion and suction parts. The *in vitro* model experiments show that the hydrophilicity of the adhesion part and the existence of the suction inlet improve the liquid uptake driven by a suction pump. This study may provide insights for the biomimetic design of nectar-feeding butterflies.

Keywords: butterfly, proboscis, nectar-feeding strategy, suction

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1 Introduction

Microfluidics has been applied to biological and chemical analyses, biomedical sensing, and micro devices designing for fluid analysis^[1]. In these applications, the efficient acquisition of test fluids is essential for successful analyses. Recently, the mouthparts of nectar-feeding insects that effectively handle small amounts of liquids have been investigated and inspired microfluidics^[2–9]. Among nectar-feeding insects, Lepidoptera (butterflies and moths) is one of the most diverse species^[10]. In general, nectar-feeding butterflies belong to the category of an active suction insect which drinks nectar using an elongated tube called a proboscis with the suction pressure created by expansion of a cibarial pump^[8–17].

The nectar-feeding strategies of butterflies have been widely studied to understand the relationship between nectars in flowers and butterflies as an important pollinator^[13–19]. Such strategies have been generally explained on the basis of Hagen-Poiseuille relationship

by assuming that the butterfly's proboscis is a simple straw through which the suction pressure is created by a muscular pump^[8,9,13–15,17]. This assumption successfully accounts for drinking a pool of nectar in flowers and provides useful insights into the relationship between the geometrical configuration, including diameter and length, of the proboscis and the concentration of nectar^[8,9,15].

However, a simple straw model of the proboscis for active suction of nectar seems to barely reflect the morphology of the proboscis. The proboscis of a butterfly does not have a distinct hole at the tip. It has a specialized tip region composed of numerous slits which allow the uptake of liquid^[10,11,20–24]. The suction flow through this area composed of small slits is definitely different from that through the opening of the straw tip^[25]. The liquid-intake flow around the proboscis tip was studied and it was shown that the liquid was sucked through the linear sink located apart from the tip instead of through the apical end of the proboscis^[25]. It was estimated that a very high pressure gradient is required to make fluid

flow through the tapered tip of the proboscis, when the butterfly drinks liquid from a pool^[26]. These results imply that a simple straw model introduced for active suction should be modified by reflecting the function of the tip region as a fluid inlet. To understand the features of the tip region during active suction, the interaction between the tip region and flow during suction process needs to be observed. However, as far as we surveyed, there is little information about this interaction.

Therefore, in this study, the liquid-feeding phenomena at the tip region of the proboscis of a nectar-feeding butterfly (*Pieris rapae*) were experimentally investigated. The flow patterns at the tip region of the proboscis were *in vivo* analyzed by using micro-PIV (particle image velocimetry). Based on these experimental results, the dynamic functions of the tip region for active suction were established. To validate the functions of the tip region for liquid suction, a suction tip model was fabricated and compared with a straw model. The present results would be helpful for understanding the dynamic functions of the tip region of butterflies for effective suction. In addition, the functions of the tip region may inspire the design of efficient liquid-sucking devices.

2 Materials and methods

2.1 Observation of the function of butterfly proboscis

To investigate the dynamic functions of the tip region, a nectar-feeding butterfly, cabbage white (*Pieris rapae*) was selected as a sample. The butterflies were purchased from the Little Pet (Seoul, Republic of Korea). Three proboscises were cut and split longitudinally along the dorsal and ventral linkages to examine the microstructures of a proboscis. The section of the split proboscis was then observed using a SEM (XL30 FEG, Philips, Netherlands). Four living butterflies were fixed on a slide glass by attaching an adhesive tape on their folded wings. The proboscis was carefully uncoiled and straightened with the dorsal side upward using a micro needle. The dorsal part of the proboscis tip was observed using an optical microscope (Eclipse 80i, Nikon, Tokyo, Japan) with a digital camera (D700, Nikon, Tokyo, Japan).

Micro-PIV was then conducted to observe the liquid-sucking process. The four butterflies were induced to feed by covering their straightened proboscis

tips with the cover slip that got wet with water seeded with micro fluorescence tracer particles (mean diameter of approximately 1.0 μm , Molecular Probes, Eugene, OR, USA). Nd: YAG laser ($\lambda = 532 \text{ nm}$, SLOC, Shanghai, China) was used to illuminate the flow on the cover slip. The seeding particles were excited by the laser, and they emitted a fluorescent light with a wavelength of 554 nm. Fluorescence flow images were recorded at a rate of 2000 frames per second by using a light microscope (Eclipse 80i, Nikon, Tokyo, Japan) equipped with an optical long-pass filter ($\lambda > 550 \text{ nm}$) and a high-speed camera (Photron Ultima APX, Fujimi, Tokyo, Japan). The resolution of the high-speed camera is 1024×1024 pixels at 2000 fps.

The PIV velocity field measurement technique is based on measuring the displacement vectors of fluorescence tracer particles between two successive images captured with a certain time interval. Each flow image was split into small interrogation windows. Local displacement of fluorescence tracer particles in each interrogation window was obtained by applying a cross-correlation PIV algorithm to the two consecutive images. The local velocity was then evaluated by dividing the local displacement with the time interval^[27].

2.2 Suction tip model test

The suction tip model composed of a glass capillary tube (O.D. = 2.0 mm, I.D. = 1.0 mm, BF200-100-10, Sutter instrument, CA, USA) and a filter paper (pore size = 0.2 μm , MCA cellulose acetate membrane filter, CHMLAB, Barcelona, Spain). Three different types of suction tip models were fabricated: straw, adhesion, and adhesion/suction models. The straw model is a simple glass capillary tube. A filter paper was cut into a square shape and then bonded to the end of a glass capillary tube by using epoxy glue to prepare the adhesion model. The adhesion/suction model was fabricated by punching a hole with certain diameter in the center of the square filter paper attached to the glass capillary tip. The filter paper was punched with commercially available 22 G (0.72 mm in diameter), 25 G (0.51 mm in diameter), 30 G (0.31 mm in diameter) needles. A hydrophobic PTFE filter paper (pore size = 0.2 μm , PTFE membrane filter, CHMLAB, Barcelona, Spain) was used as the control group to investigate the effect of wettability of the adhesion section. The glass capillary tubes were

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