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Mouthpart separation does not impede butterfly feeding

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ABSTRACT

The functionality of butterfly mouthparts (proboscis) plays an important role in pollination systems, which is driven by the reward of nectar. Proboscis functionality has been assumed to require action of the sucking pump in the butterfly's head coupled with the straw-like structure. Proper proboscis functionality, however, also is dependent on capillarity and wettability dynamics that facilitate acquisition of liquid films from porous substrates. Due to the importance of wettability dynamics in proboscis functionality, we hypothesized that proboscides of eastern black swallowtail (*Papilio polyxenes asterius* Stoll) (Papilionidae) and cabbage butterflies (*Pieris rapae* Linnaeus) (Pieridae) that were experimentally split (i.e., proboscides no longer resembling a sealed straw-like tube) would retain the ability to feed. Proboscides were split either in the drinking region (distal 6–10% of proboscis length) or approximately 50% of the proboscis length 24 h before feeding trials when butterflies were fed a red food-coloring solution. Approximately 67% of the butterflies with proboscides split reassembled prior to the feeding trials and all of these butterflies displayed evidence of proboscis functionality. Butterflies with proboscides that did not reassemble also demonstrated fluid uptake capabilities, thus suggesting that wild butterflies might retain fluid uptake capabilities, even when the proboscis is partially injured.

1. Introduction

Mouthpart functionality of fluid-feeding insects – more than half of all known animal species (Foottit and Adler, 2009) – is an important component of disease transmission and the stability of insect-pollination systems (Kingsolver and Daniel, 1995). Mouthparts of fluid-feeding insects, such as butterflies and moths (Lepidoptera), might be subjected to damage while seeking mates, searching for food, or from predator encounters. Mouthparts rendered nonfunctional, therefore, could affect fitness (Krenn, 1997) and impact insect–flower interactions.

Most Lepidoptera have a coilable, tube-like proboscis that transports fluids, such as nectar, sap, fruit juices, and blood (Adler, 1982) to the insect's gut. The lepidopteran proboscis is composed of two elongated maxillary galeae that are connected by overlapping dorsal and interlinking ventral structures (i.e., legulae) to form a food canal (Eastham and Eassa, 1955; Krenn et al., 2005; Krenn, 2010). The distal 5–20% of the proboscis has dorsal legulae that are elongated and more widely spaced (Krenn et al., 2001) (i.e., the drinking region), which facilitates fluid uptake (Lehnert et al., 2013). The merging of the galeae into a functional proboscis takes place after adult eclosion from the pupa, and consists of coiling and uncoiling actions of the proboscis accompanied by the presence of saliva droplets (Krenn, 1997). Proboscis assembly must occur before sclerotization of the legular cuticle, otherwise the proboscis is putatively nonfunctional and reassembly cannot occur (Krenn, 1997).

A functional proboscis is widely considered a sealed tube that operates similar to a drinking straw (Krenn, 2010; Bauder et al., 2013), solely relying on the sucking pump in the head for fluid uptake (Kingsolver and Daniel, 1995; Eberhard and Krenn, 2003); however, recent experiments have demonstrated that aqueous solutions can enter between dorsal interlegular spaces along the proboscis (i.e., not a sealed tube) (Monaenkova et al., 2012) and that a straw-like structure is not necessary for functionality (Grant et al., 2012). The proboscis employs capillarity via interlegular spaces to build liquid bridges in the food canal for the sucking pump to act on when feeding from liquid films and porous substrates (Monaenkova et al., 2012), such as rotting fruit. Fluid uptake is further regulated by wettability dynamics (i.e., hydrophilicity and hydrophobicity) of proboscis structures (e.g., hydrophilic dorsal legulae, chemosensilla, and the food canal) and surface roughness (e.g. microbumps that create an overall hydrophobic surface, explained using the Cassie-Baxter model, Cassie and Baxter, 1944; Lehnert et al., 2013). Based on our current understanding of the multifaceted fluid uptake system of butterfly proboscides we hypothesized that previously assembled proboscides of two distantly related nectar-feeding butterfly species, the eastern black swallowtail, *Papilio polyxenes asterius* (Papilionidae), and cabbage

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butterfly, *Pieris rapae* (Pieridae), will maintain functionality following experimental splitting of the galeae as long as both galeae are subsequently placed in a feeding solution.

2. Materials and methods

2.1. Butterfly rearing and proboscis measurements

Eggs of *P. p. asterius* were obtained from a female captured in North Canton, OH. The larvae were reared on parsley (*Petroselinum crispum*) and kept in Rubbermaid® Takealong containers. Larvae of *P. rapae* were obtained from Carolina Biological Supply Company (Burlington, NC, USA) and reared on artificial diet. Larvae and pupae of both species of butterflies were maintained at 22 °C, 61% relative humidity (r.h.), and an L18:D6 photoperiod in an environmental chamber (Percival Scientific, Inc., Perry, IN, USA). Adults from an F2 generation of *P. p. asterius* also were used for experiments. All adults were fed a dilute honey:water solution (1:5) daily for at least three days before feeding experiments and kept in glassine envelopes in a refrigerator (4 °C) between feeding times.

Proboscis lengths and drinking region lengths were measured to determine possible effects on functionality between treatments. In order to acquire proboscis measurements, butterflies were stabilized on a piece of Styrofoam and proboscides were uncoiled using insect pins. Images of the total length of proboscides (0.78× magnification) and drinking regions (4.0× magnification) were acquired for each butterfly with a Leica M205 C stereomicroscope and an IC 80HD camera (Heerbrugg, Switzerland) and measured using ImageJ software (http://rsbweb.nih.gov/ij/). The drinking region was measured from the tip of the proboscis to a transition point where the dorsal legulae narrow and remain similar in width for the remainder of the proboscis length (Fig. 1A). Although wettability dynamics of proboscides have been reported for other butterfly species (Monaenkova et al., 2012; Lehnert et al., 2013), we demonstrated these dynamics using the proboscis of an individual *P. p. asterius*. The galeae were split and one galea (unstained) was placed on a slide in dH_2O with a coverslip and imaged using an Olympus Confocal Microscope IX81 with DSU (Center Valley, PA, USA) (999.6 ms exposure, 20× magnification, 30 slices, 1.60 average depth slice, CY3 channel). The other galea was stained with Nile red for 24 h and imaged similarly to distinguish hydrophilic and hydrophobic structures. Proboscides of *P. rapae* were dehydrated in an ethanol series (80%, 90%, 100%, 24 h each), air-dried with hexamethyldisalizane, platinum sputter-coated for approximately 1 min, and imaged with a Hitachi TM3000 scanning electron microscope (Hitachi High Technologies America, Inc., Dallas, TX, USA).

2.2. Experimental feeding trials

All butterflies were fed a 20% sucrose solution and kept at room temperature (24 °C, 61% r.h.) in a netted Bug Dorm (BioQuip Products, Rancho Dominguez, CA, USA) 24 h prior to feeding experiments. Randomly selected butterflies were prepped for the experimental feeding trials by separating the two galeae either in the drinking region using an insect pin or had approximately 50% of their proboscis separated proximally starting at the tip (inset in Fig. 1A) immediately after being fed the 20% sucrose solution. Before proboscides were split, all butterflies had their proboscides

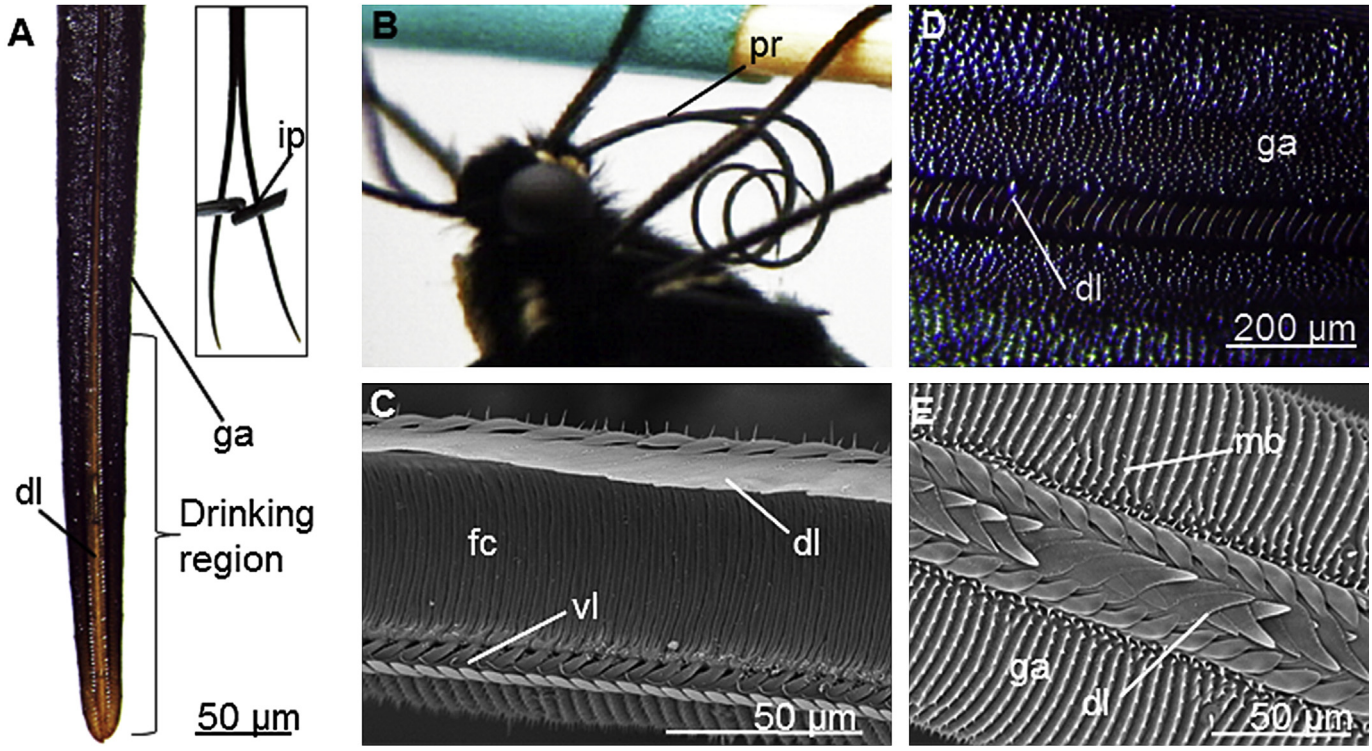

Fig. 1. Proboscis assembly and fluid uptake of split proboscides of butterflies. (A) Stereomicroscope image of an uncoiled proboscis of *P. p. asterius* displaying the galeae (ga) and overlapping dorsal legulae (dl). The dorsal legulae are larger and more widely spaced in the drinking region; the remainder of the proboscis represents the nondrinking region. The inset shows a proboscis of *P. p. asterius* split with insect pins (ip) for the red-50 treatment. (B) Photograph of a *P. p. asterius* obtained shortly after emergence showing the partially assembled proboscis (separated galeae) during the assembly process. (C) SEM image of a single galea of *P. rapae* showing the food canal (fc) and dorsal (dl) and ventral legulae (vl) that interlink during proboscis assembly. (D) Stereomicroscope image of the dorsal legulae of *P. p. asterius* in the nondrinking region; there is little overlap of the dorsal legulae. (E) SEM image of a proboscis of *P. rapae* showing the overlapping dorsal legulae in the nondrinking region and microbumps (mb). The arrangement of the dorsal legulae differs between *P. p. asterius* and *P. rapae*.