Drinking With an Unsealed Tube: Fluid Uptake Along the Butterfly Proboscis

KARENA J. KWAUK,[1] DANIEL K. HASEGAWA,[1] MATTHEW S. LEHNERT,[1,2] CHARLES E. BEARD,[1] PATRICK D. GERARD,[3] KONSTANTIN G. KORNEV,[4] AND PETER H. ADLER[1,5]

Ann. Entomol. Soc. Am. 107(4): 886–892 (2014); DOI: http://dx.doi.org/10.1603/AN14027

ABSTRACT Most adult Lepidoptera depend on a proboscis for fluid uptake. Although the proboscis has been regarded as a sealed tube with fluid uptake restricted to the distal end, recent evidence indicates that it is permeable along its entire length in at least some species. We, therefore, tested the effectiveness of the seal during feeding in four species of butterflies. Feeding rates in monarchs (*Danaus plexippus* L.), painted ladies (*Vanessa cardui* L.), and tiger swallowtails (*Papilio glaucus* L.) did not differ significantly when the proboscises were straightened and fully, versus partially, submersed in 1 or 15% sucrose solutions. To explore these results, we tested fluid uptake along the nearly transparent proboscises of buckeye butterflies (*Junonia coenia* Hübner) by applying colored droplets of water to the legular seam between the paired galeae. Colored fluid appeared in the food canal of straightened and naturally flexed proboscises within 10 s, regardless of whether the chemosensilla were stimulated with sugar. Statistically significant entry of fluid, however, occurred ≈30 s after droplets were applied and only if the proboscis was naturally flexed and stimulated with sucrose. The results suggest that fluid uptake along the length of the proboscis is influenced by changes in legular spacing when the butterfly naturally bends the proboscis and on activation of the cibarial pump when chemosensilla are stimulated with sugar.

KEY WORDS feeding behavior, fluid uptake, Lepidoptera, proboscis

The efficiency of feeding can be paramount to fitness, particularly for short-lived organisms constrained by vagaries of the environment, including predators and weather. Feeding efficiency of insects, such as butterflies and moths (Lepidoptera), is typically viewed in terms of time budgets (Hirota and Obara 2000), foraging strategies (Rusterholz and Erhardt 2000), and food handling (Bauder et al. 2011). The mechanical or physical efficiency of the devices used to acquire food is less often considered (Kunte 2007, Kim et al. 2011, Bauder et al. 2013, Lehnert et al. 2014).

Adult Lepidoptera feed from a variety of nutrient sources including floral nectar, rotting fruit, dung, and wet soil (Adler 1982). The feeding device, or proboscis, is composed of two elongated maxillary galeae joined by overlapping dorsal and interlinking ventral cuticular structures, the legulae. The concave medial faces of the galeae form a central food canal (Eastham and Eassa 1955). The distal region ("drinking region" sensu Lehnert et al. 2013) of the proboscis is characterized by enlarged dorsal interlegular spaces that allow fluid to enter the food canal from liquid films and pools (Krenn et al. 2001, Molleman et al. 2005). In species that routinely feed from porous substrates, such as rotting fruit, the enlarged legular spacing is associated with lateral rows of densely packed sensilla styloconica (i.e., elongated mechano- and chemosensilla), which create a brush-like tip on the proboscis that presumably aids fluid uptake (Knopp and Krenn 2003, Molleman et al. 2005). In nectar feeders, elongated sensilla are absent or reduced in number (Krenn et al. 2001).

The proboscis has been compared functionally to a drinking straw (Kingsolver and Daniel 1995, Eberhard and Krenn 2005, Bauder et al. 2013), relying solely on a cibarial pump (Borrell and Krenn 2006) and requiring submersion of the drinking region for fluid uptake (Krenn et al. 2001). The drinking-straw model, however, does not adequately represent proboscis function when Lepidoptera drink from porous substrates, such as wet soil (puddling sensu Arms et al. 1974) or rotting fruit, rather than pools of liquid. Under these circumstances, Lepidoptera capitalize on capillarity via interlegular spaces that pull fluid from pores in wet surfaces into the food canal (Monaenkova et al. 2012, Lehnert et al. 2013).

For >150 yr, the nondrinking region of the proboscis has been regarded as sealed. English naturalist Gosse (1993) described the proboscis in 1838: ". . . it is

[1] School of Agricultural, Forest & Environmental Sciences, Clemson University, Clemson, SC 29634-0310.
[2] Department of Biological Sciences, Kent State University at Stark, North Canton, OH, 44720.
[3] Department of Mathematical Sciences, Clemson University, Clemson, SC 29634-0310.
[4] Department of Materials Science & Engineering, Clemson University, Clemson, SC, 29634.
[5] Corresponding author, e-mail: padler@clemson.edu.

0013-8746/14/0886–0892$04.00/0

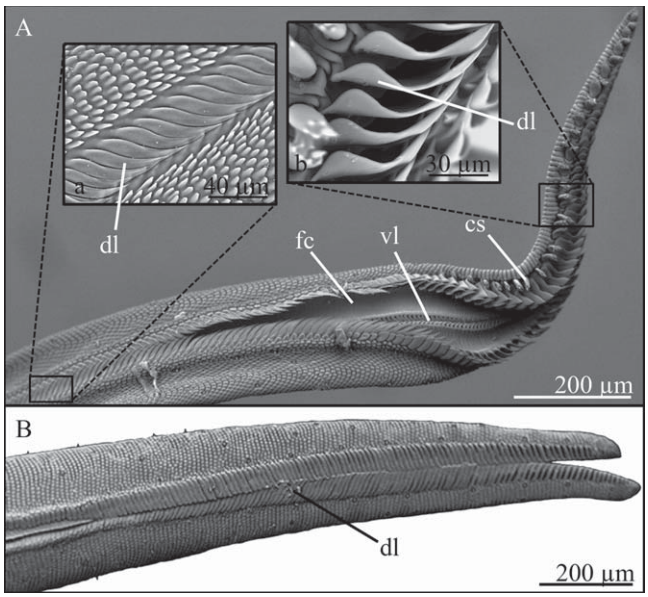

Fig. 1. Scanning electron micrographs of proboscis tips, showing variation in the arrangement of dorsal legulae and architecture of the drinking region. (A) Proboscis of *V. cardui*, showing the two galeae, artificially separated along the dorsal legulae (dl), exposing the food canal (fc) and ventral legulae (vl). The dorsal legulae in the proximal region (inset a) have small interlegular spaces but are widely spaced in the drinking region (inset b). *V. cardui* has enlarged chemosensilla (cs) near the tip that give the proboscis a brush-like appearance; the smooth-tipped proboscis of *D. plexippus* (B) has minute chemosensilla.

composed of two parts perfectly separable, you see, each part being a cylinder, yet when placed side by side, meeting in such a manner as to form a tube quite air-tight between the two lateral ones." The idea of a sealed tube persisted (Eastham and Eassa 1955, Borrell and Krenn 2006, Krenn 2010) until recent experiments showed that water can enter the food canal through interlegular spaces proximal to the drinking region in monarch butterflies (*Danaus plexippus* L.; Monaenkova et al. 2012).

The evidence that water enters the nondrinking region of *D. plexippus* (Monaenkova et al. 2012) suggests that the extent of the proboscis (e.g., drinking region only or the entire tube) exposed to liquid films or pools might influence feeding efficiency by changing the surface area available for uptake. Butterflies and moths drinking from liquid films on substrates, such as soil, typically expose more than the drinking region of their proboscises to fluid (Fig. 3 of Adler 1982). Lepidoptera drinking from floral corollas encounter a range of nectar availability within and among plant species (Davis 2001), and vary in the extent to which they insert the proboscis into a corolla (Krenn 2010). Proboscis length determines the maximum corolla depth that a butterfly can exploit and, therefore, influences choice of floral hosts (Corbet 2000). Additional factors, such as nectar volume relative to corolla volume and excess fluid from rainfall, could influence the extent of the proboscis exposed to fluid, restricting it to the drinking region or presenting an amount sufficient to cover more than the drinking region. Insertion of the proboscis into the corolla also might displace nectar, exposing a greater extent of the proboscis to fluid.

Accordingly, we tested the hypothesis that drinking rates are influenced by the extent of the proboscis submersed in fluid. We investigated drinking rates of three nectar-feeding species—*D. plexippus*, the painted lady (*Vanessa cardui* L.), and the eastern tiger swallowtail (*Papilio glaucus* L.), the latter of which has male-specific puddling behavior. To visualize and quantify fluid entry in the nondrinking region, we chose the buckeye (*Junonia coenia* Hübner), which has a nearly transparent proboscis, as our model species.

Materials and Methods

Experimental Subjects. We used four species of butterflies with a range of proboscis architecture (Fig. 1): *D. plexippus* with a smooth proboscis (i.e., with minute sensilla) and short drinking region (9% of the total proboscis length); *P. glaucus* with a smooth proboscis and long drinking region (15% of the total length); and *V. cardui* and *J. coenia*, each with a brush-like (i.e., with elongated sensilla) and short drinking region (6–7% of the total length). Representative specimens are deposited in the Clemson University Arthropod Collection.

Butterflies were reared from commercially obtained larvae and pupae (Shady Oak Butterfly Farm, Brooker, FL; Carolina Biological Supply Co., Burlington, NC; Ward's Natural Science, Rochester, NY), although some eastern tiger swallowtails were reared

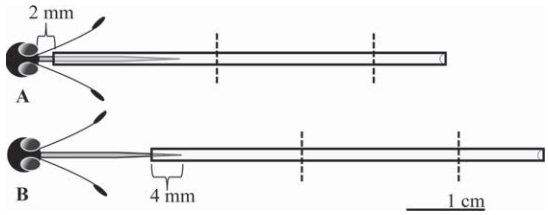

Fig. 2. Setup for butterfly drinking experiments. Dashed vertical lines represent the distance (2 cm) used to estimate fluid uptake rates from fluid-filled 20-μl capillary tubes (inner diameter = 590 μm) shortened to 5 cm. (A) For full submersion treatments, the proboscis was inserted into a capillary tube so that 2 mm of the proboscis remained distal to the head. (B) For partial treatments, the proboscis was inserted 4 mm into a tube, covering the drinking region.

from wild females collected in Clemson, SC. Larvae of *D. plexippus* were fed milkweed (*Asclepias curassavica* L. and *Asclepias syriaca* L.), those of *V. cardui* were fed artificial diet (Carolina Biological Supply Co., Burlington, NC), and those of *P. glaucus* were fed tulip poplar (*Liriodendron tulipifera* L.) All specimens of *J. coenia* were received as pupae. Larvae were reared in an environmental chamber with at 28 ± 3°C, 60–70% relative humidity (RH), and a photoperiod of 16:8 (L:D) h, although pupae of *J. coenia* were allowed to eclose at 25°C, and swallowtail pupae were reared at 32 ± 1°C, 65–70% RH, and a photoperiod of 18:6 (L:D) h .

All laboratory-reared butterflies used in experiments were held unfed in glassine envelopes at 4°C for 24–72 h after emergence to ensure that the proboscis was free of food residues. Butterflies were immobilized temporarily for experiments on a Styrofoam board, with the proboscis straightened either in a capillary tube or with crossed pins, or the proboscis was allowed to flex naturally while cross pinned.

Drinking Trials With Fully or Partially Submersed Proboscises. To evaluate the influence that length of the proboscis exposed to fluid has on uptake rates, we manually inserted proboscises of three species (*D. plexippus*, *P. glaucus*, and *V. cardui*) fully or partially into fluid-filled 20-μl capillary tubes (Fig. 2). To ensure that fluid emptied only from the distal end of the capillary tube during drinking, the proximal end was covered with Parafilm with a hole made for the proboscis by a size 0 insect pin. Each proboscis was uncoiled with an insect pin and directed into a capillary tube (shortened to 5 cm) adjacent to a metric ruler (Fig. 2). Full-submersion trials were conducted with the proximal end of the tube 2 mm from the head, covering the drinking region and most of the nondrinking region. In partial submersion trials, the proboscis was inserted 4 mm into the tube, covering the drinking region (up to 2.7 mm of the proboscis length in tested species) and the distal-most end of the nondrinking region. The straightened proboscises were horizontal in each test to minimize the influence of gravity.

After inserting a proboscis in a capillary tube, we used a 1-ml microsyringe (Hamilton Co., Reno, NV) to fill the tube with 1 or 15% sucrose so that the meniscus of the fluid was at the distal end of the tube. A 15% solution was intended to simulate sugar concentrations within the lower range of typical butterfly flowers (Heinrich 1975); a 1% solution was used because butterflies would not drink distilled water in preliminary trials.

We used 3–10 adults per gender per treatment (partial or full submersion) for each sucrose concentration (1 or 15%), for a total of 55–80 butterflies per species. Each butterfly and capillary tube was used only once. To determine if butterfly size influenced uptake rates, we measured forewing and proboscis lengths of each specimen.

Feeding trials were video-recorded (JenOptik ProgRes, JenOptik AG, Jena, Germany) and timed with a stopwatch at 28 ± 3°C and 60–70% RH. Uptake rates were calculated based on the time to drink 2 cm (=5.4 μl) of solution from the middle portion of the capillary tube, beginning when the meniscus of the sucrose solution was 1.0 cm from the distal end of the tube. The capillary tubes, with an inner diameter of 590 μm, accommodated the proboscises of the largest species (*D. plexippus*, $n = 20$), which had a mean diameter of 483.8 ± 8.78 μm (greatest width) in the bend region and 213.6 ± 5.50 μm in the mid-drinking region.

The capillary tubes used in our experiments have inherent capillary pressure. Our setup for evaluating feeding rates measured changes in volume over time, without accounting for the dynamics of meniscus movement (Tsai et al. 2014). As the liquid meniscus in the capillary tube moves toward the tip of the proboscis, the pressure differential per unit length of the liquid column between the cibarial pump in the head and the meniscus changes as a function of wetted capillary area in the tube; thus, the meniscus does not move at a constant velocity. Lacking the resolution to detect rate changes along the distance of the capillary tube, we used an average uptake time to provide estimates of uptake rates.

We used *t*-tests to compare forewing and proboscis lengths between genders within species and proboscis diameters between genders of *P. glaucus.* We used analysis of covariance (Milliken and Johnson 2001) to evaluate the influence of gender and submersion (full or partial) on the feeding rate for each species, with proboscis length and forewing length as potential covariates. Neither proboscis length nor forewing length, however, was useful as a covariate. We, therefore, analyzed the data for each species as a two-way analysis of variance, investigating the influence of gender, submersion (full or partial), and their interaction on feeding rate for each sucrose concentration (1 or 15%). Least-squares means were used to investigate significant interactions and main effects. SAS version 9.2 (SAS Institute, Cary, NC) was used for these and all subsequent analyses, and a significance level of $P < 0.05$ was used for hypothesis tests. Sample statistics are reported as means ± SEs.

Fluid Uptake in the Nondrinking Region of the Proboscis. The weakly pigmented, nearly transparent proboscis of *J. coenia* allowed us to visualize fluid entry

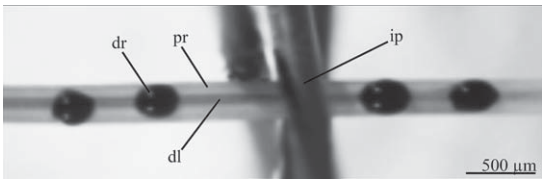

Fig. 3. Two pairs of 10–20-nl droplets of colored water (dr) on the dorsal legulae (dl) of the paired galeae (i.e., legular seam) at 1.0 and 2.0 mm distal and proximal to the bend region of the proboscis (pr) of *J. coenia*; crossed insect pins (ip) are at the bend region.

into the food canal when we used a solution of 20% blue food coloring (Southern Home, Mauldin, SC; vol:vol distilled water). Thus, we were able to examine factors that might influence fluid entry in the nondrinking region. Accordingly, we used the following five treatment groups of butterflies, with four individuals per group: 1) straightened proboscis without sucrose stimulation, 2) straightened proboscis with sucrose stimulation, 3) naturally flexed (bent) proboscis without sucrose stimulation, 4) naturally flexed proboscis with sucrose stimulation, 5) straightened, epoxy-filled proboscis.

For all the five groups, we dispensed two 10–20-nl droplets of colored water from a microsyringe (Hamilton Co., Reno, NV) onto the external surface of the dorsal legulae at 1.0 ± 0.2 and 2.0 ± 0.2 mm, both distal and proximal to the natural bend region (sensu Krenn 1998), for a total of four droplets on each proboscis (Fig. 3). All droplet experiments were conducted at 25.2 ± 0.3°C.

To determine if fluid entry in the nondrinking region was influenced by sucrose stimulation of feeding behavior (groups 2 and 4), butterflies were provided 0.1–0.2-μl droplets of 15% wt:vol sucrose solution with yellow food coloring (Southern Home, Mauldin, SC) from a syringe at the distal 10% of the proboscis 10 s after the blue-colored water droplets were placed on the legulae. All butterflies passed the yellow-colored sucrose solution through their food canals in <30 s, displacing the blue-colored water.

To evaluate the influence of evaporation (group 5), we placed droplets on proboscises that were removed from individuals of *J. coenia* and straightened with crossed pins on a Styrofoam panel. The droplets were prevented from entering the food canal through the interlegular spaces by filling the food canal, via capillarity, with epoxy (Henkel Corporation, Rocky Hill, CT) from the proximal end and allowing it to harden.

To evaluate consistency within individuals, we repeated experiments on each butterfly once a day, for three consecutive days, while maintaining the same proboscis treatment. To clear residual blue-colored water from the food canal between daily droplet applications, an equivalent volume of yellow-colored 15% sucrose was provided in the drinking region of all butterflies. The outside of the proboscis was cleaned by wiping a drop of uncolored distilled water across the dorsal legulae, and the butterfly was returned to 4°C.

Images of droplets were acquired with a Canon EOS Rebel T3i digital camera (Canon U.S.A., Melville, NY) mounted (Martin Microscope MDSLR-RZ adapter, Easley, SC) on a Meiji Techno RZ stereoscope (Meiji Techno, Santa Clara, CA) at initial droplet placement (0 s) and at 10 and 30 s subsequently. Using captured images and the ImageJ software (National Institutes of Health, Bethesda, MD), we calculated the two-dimensional area of each droplet for the three time points.

We used a mixed-model analysis of variance to evaluate differences in the percent area of each droplet after 10 and 30 s. Fixed effects in the model were treatment, location of droplet, and their interaction. Random effects included individual and the replicate test of individuals assigned to a given treatment. If the interaction was not significant ($P > 0.05$), pairwise comparisons of location and treatments were investigated to determine if overall tests for those effects were significant.

To validate our laboratory experiments, we tested straightened and naturally flexed proboscises of field-collected *J. coenia* ($n = 2$) with blue-colored water droplets, males ($n = 3$) and females ($n = 5$) of *P. glaucus* with blue-colored water and blue-colored 15% sucrose, and the following species with blue-colored 15% sucrose: hackberry (*Asterocampa celtis* (Boisduval & Leconte), $n = 1$), comma (*Polygonia c-album* (L.), $n = 1$), and *V. cardui* ($n = 1$). The latter three species had weakly pigmented proboscises, allowing the blue fluid to be viewed in the food canal.

Results

Drinking With Fully or Partially Submersed Proboscises. Drinking rates did not differ significantly ($P \geq 0.05$) for gender, submersion level, or gender × submersion interactions for any species, with one exception (Table 1). For *P. glaucus,* males had significantly ($F_{1,28} = 9.37$; $P = 0.005$) faster uptake rates, with fully submersed proboscises, than did females when drinking a 1% sucrose solution.

Forewing and proboscis lengths did not differ significantly (*t*-test, $P \geq 0.05$) between genders, except for *V. cardui*, in which females had longer forewings than did the males ($t = 2.36$, df = 78, $P = 0.02$). Proboscis diameters of *P. glaucus* did not differ significantly between males and females in either the bend or the mid-drinking region (Welch's *t*-test, df = 11; $P > 0.05$).

Fluid Uptake in the Nondrinking Region. Blue-colored fluid appeared in the food canal of *J. coenia* within 10 s in all treatments, except the epoxy-sealed proboscis, demonstrating that water entered through interlegular spaces in the nondrinking region. The percent of the droplet area remaining on the dorsal legulae, however, did not differ significantly ($P > 0.05$) among the five groups at 10 s (Table 2), suggesting that entry of fluid into the food canal was slow and that the slight reduction (≈7%) in area from the initial placement of droplets was due largely to evaporation. The change in area across the four droplet positions along the proboscis did not differ signifi-

Table 1. Means ± SE (*n*) for feeding rates (μl/s) of three butterfly species, according to the extent of proboscis submersion (full, partial) in capillary tubes with different sucrose solutions (1, 15%)

Species	Gender	1%		15%	
		Full	Partial	Full	Partial
V. cardui	Male	0.25 ± 0.03 (10)	0.28 ± 0.01 (10)	0.21 ± 0.03 (10)	0.27 ± 0.01 (10)
	Female	0.21 ± 0.03 (10)	0.31 ± 0.03 (10)	0.27 ± 0.04 (10)	0.24 ± 0.03 (10)
P. glaucus	Male	1.30 ± 0.07 (8)*	1.27 ± 0.08 (8)	0.99 ± 0.28 (8)	0.99 ± 0.14 (8)
	Female	0.92 ± 0.14 (8)*	1.11 ± 0.13 (8)	0.90 ± 0.25 (4)	0.76 ± 0.18 (3)
D. plexippus	Male	0.43 ± 0.06 (9)	0.36 ± 0.03 (8)	0.50 ± 0.07 (8)	0.64 ± 0.03 (8)
	Female	0.32 ± 0.07 (5)	0.34 ± 0.06 (7)	0.50 ± 0.06 (8)	0.57 ± 0.06 (8)

* Feeding rates differed significantly ($P < 0.05$, least-squares means) only between males and females of *P. glaucus* with proboscises fully submersed in 1% sucrose.

cantly ($P > 0.05$) at 10 s, nor did the interaction between group treatment and droplet position ($P > 0.05$). At 30 s, however, droplet area decreased significantly ($P < 0.0001$) for naturally flexed proboscises when sucrose was applied distally (Table 2), but the effect of droplet position along the proboscis and the interaction between treatment and droplet position were not significant ($P > 0.05$).

We visually confirmed that fluid entered the nondrinking region of wild-caught *J. coenia* when the proboscis was naturally flexed and stimulated with sucrose. We also demonstrated uptake of 15% sucrose droplets in the nondrinking region of field-collected singletons of *A. celtis*, *P. c-album*, and *V. cardui*. In contrast, neither water nor the 15% sucrose measurably entered the nondrinking region of males or females of *P. glaucus* with straightened or flexed proboscises, within 30 s, although the heavily pigmented proboscis precluded visual determination of whether fluid slowly seeped into the proboscis.

Discussion

We expected that the ability of *D. plexippus* to acquire fluid in its nondrinking region (Monaenkova et al. 2012) would influence feeding rates, depending on the extent of the proboscis exposed to fluid. The lack of significant differences in uptake rates between proboscises fully and partially submersed in fluid, however, led us to explore this paradox further. We discovered that the slow movement of fluid into straightened proboscises through the interlegular spaces yields undetectable differences in feeding rates for fully versus partially submersed proboscises.

Table 2. Water uptake in the nondrinking region of *J. coenia*, with respect to proboscis conformation and sucrose stimulation

Treatment	10 s	30 s
Evaporation control	95.8 ± 0.7	81.9 ± 2.1
Straight	94.3 ± 0.8	80.3 ± 2.4
Straight with sucrose	95.8 ± 0.7	75.4 ± 2.7
Flexed	91.7 ± 2.6	75.0 ± 2.8
Flexed with sucrose	88.7 ± 2.0	38.4 ± 3.0[a]

Mean ± SE for percent droplet area remaining over time (10 and 30 s) for five treatments of butterflies ($n = 4$ per treatment); T, grouping for treatment least squares means ($\alpha = 0.05$).

[a] Least squares means within columns are significant.

Our experiments demonstrate that proboscis conformation, in combination with sucrose stimulation, influences fluid acquisition. We suggest that fluid entry into the food canal, at least in the nondrinking region, is facilitated by expansion of the interlegular spaces as the proboscis transitions from straight to flexed and when chemosensilla are stimulated by sugar. Sugar activates the cibarial pump of Lepidoptera, enhancing the uptake of fluid (Miles and Booker 1998). Active or passive fluid uptake, thus, could occur in the nondrinking region of a lepidopteran with much of its proboscis applied to a fluid film, such as when feeding from a sap flow. Passive entry of fluid via interlegular spaces might occur as dew droplets form on the exposed portion of a coiled proboscis of a resting butterfly, helping to hydrate the insect.

While feeding from flowers, Lepidoptera often flex their proboscises at the bend region and insert only the distal end into the corolla, leaving the nondrinking region largely unexposed to fluid (Fig. 5a of Krenn 2010). However, greater lengths of the proboscis also are inserted into some flowers (Krenn 2010), constraining the extent of flexing, particularly when feeding from narrow corollas. Butterflies, such as *V. cardui* and *J. coenia*, have proboscises longer than the corollas of some flowers from which they routinely feed (e.g., *Lantana camara* L. and *Buddleia davidii* Franchet); yet, they insert a greater length of the proboscis, including the bend region, than the corolla depth can accommodate, suggesting that the extreme distal portion of the proboscis doubles back on itself (K.J.K., unpublished data). Our experiments suggest that the proboscis effectively would be sealed in the straightened portion of the nondrinking region while feeding from flowers with narrow corollas. Feeding periods of Lepidoptera visiting flowers are typically brief, for example, <10 s per flower (Kunte 2007)—insufficient time for significant fluid to enter the food canal in the nondrinking region of a straightened proboscis.

A sealed proboscis might influence not only fluid uptake, but also the efficiency of saliva delivery. Lepidoptera use saliva for multiple purposes, such as solubilizing dried foods (Krenn 2010). The ability to express saliva directly onto a potential food source, such as dried nectar, rather than along the length of the proboscis, should minimize fluid loss.

Our study suggests that fluid entry in the nondrinking region is species-specific, possibly reflecting differences in interlegular spacing. Fluid entry into the food canal, for example, occurs more readily in *J. coenia* than in *P. glaucus.* In *D. plexippus*, interlegular spacing in the nondrinking region ranges from 96 nm near the head to 162 nm more distally (Monaenkova et al. 2012). Legular spacing might reflect differences in feeding behavior. For instance, species that feed predominantly from substrates, such as rotting fruit, will have more opportunity to flex the portion of their proboscises exposed to fluid than would species that feed chiefly on floral nectar. *Papilio glaucus* and *J. coenia* are nectar feeders, but males of *P. glaucus* also drink from damp soil (Arms et al. 1974). Analyses of interlegular spacing in relation to feeding habits, especially on floral versus nonfloral sources, are needed.

Uptake rates are significantly faster in males than in females of *P. glaucus* when their proboscises are fully submersed in 1% sucrose. Multiple factors influence uptake rates, such as body size, age, fluid viscosity, and ambient temperature; yet a faster drinking rate for males is common in butterflies (Pivnick and McNeil 1985, Boggs 1988). Forces, such as capillarity and friction, exerted by the fluid source (e.g., floral corolla or damp soil) also influence uptake rates (Monaenkova et al. 2012). Given the positive relation that exists between cibarial pump dimensions and proboscis length (Karolyi et al. 2013), differential feeding rates might be expected if the cibarial pump is larger in males; however, proboscis lengths of males and females in our sample of *P. glaucus* did not differ significantly. The gender difference might be related to unexplored factors, such as possible dynamic differences in legular spacing between males and females, particularly in species with male-specific puddling habits.

The ability of butterflies to exert control over fluid acquisition along the length of the proboscis reinforces the conclusions that the lepidopteran proboscis is more complex than a drinking straw (Monaenkova et al. 2012, Lehnert et al. 2014, Tsai et al. 2014). The additional complexity provides an enhanced range of functionality, allowing physical mechanisms of fluid acquisition and delivery of saliva to be adjusted behaviorally, such as by bending the proboscis. The ability to manipulate interlegular spacing by bending and straightening the proboscis would allow butterflies greater control over fluid uptake and saliva expression, and might permit more accurate predictions of feeding habits based on proboscis structure and behavior.

Acknowledgments

We thank the staff at the Clemson University Electron Microscope Facility for technical advice, S. Aultman and M. Williamson (Department of Plant Industry, Clemson University) for assistance in the permitting process to ship specimens, and two anonymous reviewers for suggestions to improve the manuscript. This is Technical Contribution No. 6218 of the Clemson University Experiment Station, and is based on work supported in part by National Institute of Food and Agriculture–U.S. Department of Agriculture (NIFA–USDA) under project number SC-1700433, and by the National Science Foundation, Award No. 1305338.

References Cited

Adler, P. H. 1982. Soil- and puddle-visiting habits of moths. J. Lepid. Soc. 36: 161–173.

Arms, K., P. Feeney, and R. C. Lederhouse. 1974. Sodium stimulus for puddling behavior by tiger swallowtail butterflies, *Papilio glaucus.* Science 185: 372–374.

Bauder, J.A.S., N. R. Lieskonig, and H. W. Krenn. 2011. The extremely long-tongued Neotropical butterfly *Eurybia lycisca* (Riodinidae): proboscis morphology and flower handling. Arthr. Struct. Dev. 40: 122–127.

Bauder, J.A.S., S. Handschuh, B. D. Metscher, and H. W. Krenn. 2013. Functional morphology of the feeding apparatus and evolution of proboscis length in metalmark butterflies (Lepidoptera: Riodinidae). Biol. J. Linn. Soc. 110: 291–304.

Boggs, C. L. 1988. Rates of nectar feeding in butterflies: effects of sex, size, age and nectar concentration. Funct. Ecol. 2: 289–295.

Borrell, B. J., and H. W. Krenn. 2006. Nectar feeding in long proboscid insects, pp. 85–212. *In* A. Herrell, T. Speck, and N. P. Rowe (eds.), Ecology and Biomechanics: A Mechanical Approach to the Ecology of Animals and Plants. CRC, Boca Raton, FL.

Corbet, S. A. 2000. Butterfly nectaring flowers: butterfly morphology and flower form. Entomol. Exp. Appl. 96: 289–298.

Davis, A. R. 2001. Searching and breeding for structural features of flowers correlated with high nectar-carbohydrate production, pp. 107–121. *In* P. Benedek and K. W. Richards (eds.), Proceedings: 8th Pollination Symposium. 10–14 July 2000, Mosonmagyaróvár, Hungary. Acta Horticulturae 561, Leuven, Belgium.

Eastham, L. E., and Y.E.E. Eassa. 1955. The feeding mechanism of the butterfly *Pieris brassicae* L. Phil. Trans. R. Soc. Biol. Sci. 239: 1–43.

Eberhard, S. H., and H. W. Krenn. 2005. Anatomy of the oral valve in nymphalid butterflies and a functional model for fluid uptake in Lepidoptera. Zool. Anz. 243: 305–312.

Gosse, P. H. 1993. Letters from Alabama (US), chiefly relating to natural history. University of Alabama Press, Tuscaloosa, AL. (Originally published in 1859, Morgan and Chase, London, United Kingdom).

Heinrich, B. 1975. Energetics of pollination. Annu. Rev. Ecol. Syst. 6: 139–170.

Hirota, T., and Y. Obara. 2000. Time allocation to the reproductive and feeding behaviors in the male cabbage butterfly. Zool. Sci. 17: 323–327.

Karolyi, F., L. Morawetz, J. F. Colville, S. Handschuh, B. D. Metscher, and H. W. Krenn. 2013. Time management and nectar flow: flower handling and suction feeding in long-proboscid flies (Nemestrinidae: Prosoeca). Naturwissenschaften 100: 1083–1093.

Kim, W., T. Gilet, and J.W.M. Bush. 2011. Optimal concentrations in nectar feeding. Proc. Natl. Acad. Sci. U.S.A. 108: 16618–16621.

Kingsolver, J. G., and T. L. Daniel. 1995. Mechanics of food handling by fluid-feeding insects, pp. 32–74. *In* R. G. Chapman, and G. de Boer (eds.), Regulatory Mechanisms in Insect Feeding. Springer, New York, NY.

Knopp, M.C.N., and H. W. Krenn. 2003. Efficiency of fruit juice feeding in Morpho peleides (Nymphalidae, Lepidoptera). J. Insect Behav. 16: 67–77.

Krenn, H. W. 1998. Proboscis sensilla in *Vanessa cardui* (Nymphalidae, Lepidoptera): functional morphology and significance in flower-probing. Zoomorphology 118: 23–30.

Krenn, H. W. 2010. Feeding mechanisms of adult Lepidoptera: structure, function, and evolution of the mouthparts. Annu. Rev. Entomol. 55: 307–327.

Krenn, H. W., K. P. Zulka, and T. Gatschnegg. 2001. Proboscis morphology and food preferences in Nymphalidae (Lepidoptera, Papilionoidea). J. Zool. Lond. 253: 17–26.

Kunte, K. 2007. Allometry and functional constraints on proboscis lengths in butterflies. Funct. Ecol. 21: 982–987.

Lehnert, M. S., D. Monaenkova, T. Andrukh, C. E. Beard, P. H. Adler, and K. G. Kornev. 2013. Hydrophobic-hydrophilic dichotomy of the butterfly proboscis. J. R. Soc. Interface 10: 20130336. (doi:10.1098/rsif.2013.0336).

Lehnert, M. S., C. P. Mulvane, and A. Brothers. 2014. Mouthpart separation does not impede butterfly feeding. Arthr. Struct. Dev. 43: 97–102.

Miles, C. I., and R. Booker. 1998. The role of the frontal ganglion in the feeding and eclosion behavior of the moth *Manduca sexta.* J. Exp. Biol. 201: 1785–1798.

Milliken, G. A., and D. E. Johnson. 2001. Analysis of messy data Volume III: Analysis of Covariance. CRC, Boca Raton, FL.

Molleman, F., H. W. Krenn, M. E. Van Alphen, P. M. Brakefield, P. J. Devries, and B. J. Zwaan. 2005. Food intake of fruit-feeding butterflies: evidence for adaptive variation in proboscis morphology. Biol. J. Linn. Soc. 86: 333–343.

Monaenkova, D., M. S. Lehnert, T. Andrukh, C. E. Beard, B. Rubin, A. Tokarev, W. Lee, P. H. Adler, and K. G. Kornev. 2012. Butterfly proboscis: combining a drinking straw with a nanosponge facilitated diversification of feeding habits. J. R. Soc. Interface 9: 720–726.

Pivnick, K. A., and J. N. McNeil. 1985. Effects of nectar concentration on butterfly feeding: measured feeding rates for *Thymelicus lineola* (Lepidoptera: Hesperiidae) and a general feeding model for adult Lepidoptera. Oecologia 66: 226–237.

Rusterholz, H. P., and A. Erhardt. 2000. Can nectar properties explain sex-specific flower preferences in the Adonis blue butterfly *Lysandra bellargus*? Ecol. Entomol. 25: 81–90.

Tsai, C.-C., D. Monaenkova, C. E. Beard, P. H. Adler, and K. G. Kornev. 2014. Paradox of the drinking-straw model of the butterfly proboscis. J. Exp. Biol. 217: 2130–2138.

Received 11 February 2014; accepted 29 May 2014.