

Mechanics of nectar feeding in the orchid bee *Euglossa imperialis*: pressure, viscosity and flow

Brendan J. Borrell

Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA

e-mail: bborrell@cal.berkeley.edu

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Summary

The orchid bee *Euglossa imperialis* sucks nectars through a slender proboscis. I tested how nectar properties influence this suction pressure and whether ambient air pressure sets the upper limit for suction feeding. Nectar intake rate was measured as a function of sucrose concentration (5–75% w/w), nectar viscosity (2–80 mPa s), and ambient pressure (101–40 kPa). Intake rate declines from about $1.2 \mu\text{l s}^{-1}$ to $0.003 \mu\text{l s}^{-1}$ as sucrose concentration increases from 15% to 65% sucrose. When sucrose concentration is held at 25% while viscosity increases from 2 to 80 mPa s, intake rate declines. When viscosity is held at 10.2 mPa s (the viscosity of 50% sucrose) while sucrose concentration increases from 5% to 50%, intake rate remains constant. Intake rate was limited

by a reduction in ambient pressure at all nectar concentrations. Assuming a rigid proboscis, the Hagen–Poiseuille equation suggests that suction pressure increases with viscosity from 10 kPa at 5% sucrose to 45 kPa at 65% sucrose. However, because intake rate declined by the same fraction under hypobaria (40 kPa) at all sucrose concentrations, the euglossine bee proboscis may be better described as a collapsible tube: expanding or collapsing depending on the flow rate, the pressure gradient along the proboscis, and circumferential forces imposed by the proboscis walls.

Key words: Apidae, Euglossini, foraging, energetics, biomechanics, tongue.

Introduction

Suction feeding is the primary modality by which insects ingest a liquid diet and requires a tightly sealed proboscis, along with one or several fluid pumps (Borrell and Krenn, 2006; Kingsolver and Daniel, 1995). In many insect taxa, the fluid pump is formed from a muscular expansion of the cibario-pharyngeal chamber (henceforth, cibarium) located in the anterior region of the head (Snodgrass, 1993). As the airtight pump chamber expands *via* contraction of pump dilator muscles, a pressure gradient develops along the length of the proboscis. Because the external pressure of the atmosphere is greater than the internal pressure of the pump, fluid is forced through the feeding canal to fill the expanding pump chamber.

Butterflies, pollen wasps, long-tongued bee-flies and orchid bees are among the nectar feeding insects that have independently evolved a purely suctorial feeding mode (Borrell and Krenn, 2006). Because viscosity of floral nectars varies exponentially with sucrose concentration and ranges over more than an order of magnitude (Heyneman, 1983), a principal goal of biomechanical studies has been to understand how viscosity influences the dynamics of suction feeding (Daniel et al., 1989; Josens and Farina, 2001; Kingsolver and

Daniel, 1979; May, 1985; Smith, 1979). An early model of suction feeding assumed that butterflies produce a constant pressure drop at all viscosities (Kingsolver and Daniel, 1979), and a modification of the Hagen–Poiseuille equation was used to relate this pressure drop to the rate of nectar flow in the tapering proboscis. Subsequently, May (May, 1985) falsified this model by measuring nectar intake rate Q ($\text{m}^3 \text{s}^{-1}$) in two butterfly species, along with the length L (m) and radius R (m) of the proboscis, in order to calculate the pressure drop P (Pa):

$$P = \frac{8\mu LQ}{\pi R^4}, \quad (1)$$

where viscosity μ (Pa s) is a function of sucrose concentration. The pressure drop increased nonlinearly with sucrose concentration, suggesting a complex relationship between fluid flow and the physiology of the suction pump (May, 1985; Pivnick and McNeil, 1985). Indeed, an earlier study on the operation of the suction pump in the blood-sucking bug *Rhodnius prolixus* demonstrated that at low viscosities, flow rate departs from the linear dependence on viscosity predicted by the Hagen–Poiseuille equation (Smith, 1979). Smith

hypothesized that as the speed of pump contraction increases, the tension of the muscle will be too low to produce a constant pressure drop (Smith, 1979). In quantitative terms, the inverse relationship between the speed of contraction V (m s^{-1}) and the amount of force developed by a muscle, F_{muscle} (N), is traditionally described by the Hill equation (Hill, 1938):

$$F_{\text{muscle}} = \frac{T_0(V_{\text{max}} - V)}{V_{\text{max}} + 4V}, \quad (2)$$

where T_0 is the maximum isometric tension of the pump dilator muscle (N), and V_{max} is the maximum shortening velocity (m s^{-1}). By assuming an equivalence between the pressure drop in the Hagen–Poiseuille equation and the pressure drop developed in the cibarium, Daniel et al. (Daniel et al., 1989) combined Eqn 1 and Eqn 2 to develop a general expression for the mechanics of suction feeding:

$$Q = \frac{\pi R^4 [T_0(V_{\text{max}} - Q/A_{\text{cib}})]}{8\mu L [A_{\text{cib}}(V_{\text{max}} + 4Q/A_{\text{cib}})]}, \quad (3)$$

where A_{cib} is the cross-sectional area of the cibarial pump (m^2). Energy intake rate during feeding, E_{max} (W) is then:

$$E_{\text{max}} = eS\rho, \quad (4)$$

where e is the energy equivalent of nectar, 15.4 J mg^{-1} , S is sucrose concentration (% w/w), and ρ is nectar density (kg m^{-3}). The Daniel et al. model (Daniel et al. 1989) predicts that energy intake rate will be maximized for all suction feeders drinking nectars between 35–40% sucrose, but the concentration that maximizes the mechanical power output of the suction pump depends on the configuration of the nectar feeding system.

Although predictions of the Daniel et al. model (Daniel et al., 1989) concur with optima derived from laboratory feeding trials with butterflies (Boggs, 1988; May, 1985; Pivnick and McNeil, 1985), hawkmoths (Josens and Farina, 2001), orchid bees (Borrell, 2004), bee-flies (Kingsolver and Daniel, 1995), and ants (Paul and Roces, 2003), the predicted relationship between the pressure drop produced in the cibarium and fluid flow through the proboscis has never been experimentally validated (Kingsolver and Daniel, 1995). The relationship between pressure and flow depends crucially on values of V_{max} and T_0 , which are not easily measured. Furthermore, a study with hawkmoths has demonstrated that nectar intake rates depend on behavioral motivation: the sweetness of ingested solutions influences intake rate independent of viscosity (Josens and Farina, 2001). Finally, a third challenge to linking pressure and flow in feeding mechanics comes from the morphological diversity of the insect proboscis and the difficulty in distilling morphology into two fixed parameters: radius and length (Krenn et al., 2005). In the present study, I used the orchid bee, *Euglossa imperialis*, to test the hypothesis that the pressure drop increases with fluid viscosity alone and that the maximum pressure drop is limited by ambient air pressure.

Materials and methods

Study site and animal

Euglossa imperialis Cockerell was captured with a hand net at chemical baits of cineole and methyl salicylate from populations on Barro Colorado Island, Panama and at La Selva Biological Station in Costa Rica. Immediately following capture, bees were brought to a screened enclosure for feeding trials. Experiments were conducted between June 2002 and April 2004.

Proboscis morphology

Digital photographs were taken of the proboscis components from three *Eg. imperialis*. These images were scaled to a height of 1500 pixels and the width of overlapping and non-overlapping components of the galeae and labial palps were measured along their length. The sum of these widths represents the outer circumference of the proboscis at a given distance, and the overall radius was calculated by treating the total cross-section as a circle. In order to estimate resistance to flow in a pipe with an annular cross-section, the ratio k of the inner radius to the outer radius is used to calculate a correction factor that can be multiplied by the radius in the Hagen–Poiseuille equation:

$$\text{Correction} = (1 - k^4) - \frac{(1 - k^2)^2}{\ln(1/k)}, \quad (5)$$

for $Re < 2000$ (Bird et al., 2002). Because the proboscis tapers towards the tip and the resistance of pipes in series is additive, hydraulic resistance should be calculated from the numerical integration of $\text{Correction} \times r^4$ from the proximal end of the feeding tube to the distal end (Pivnick and McNeil, 1985). For simplicity, I refer to the fourth root of this value as the effective radius because it can be substituted for r in Eqn 1 and Eqn 3.

A minimum effective radius was derived functionally by plotting viscosity versus the pressure drop (calculated from Eqn 1) for radii ranging from 0.02 mm to 0.06 mm. The viscosity at which nectar intake rate approaches zero corresponds to the maximum pressure that can be developed in the cibarium. By assuming that ambient pressure equals the limiting pressure drop, one may calculate a minimum estimate of the radius.

Feeding protocol

Experimental nectar solutions were placed in 200 μl micropipette tips sealed at the small end using a drop of cyanoacrylate. Nectar intake rate was then measured with either starved bees introduced to nectar solutions or from previously trained bees freely visiting flowers. In short, the mass gain of the bee ($\pm 1 \text{ mg}$) was divided by the time spent feeding (Borrell, 2004). Volumetric rate of nectar intake is mass intake rate divided by density of the sucrose solution (Mathlouthi and Génotelle, 1995), and sucrose intake rate is mass intake rate multiplied by sucrose concentration.

The temperature of the nectar solution was controlled

between 29 and 31°C, by warming it before feeding trials using a 15 W halogen lamp. Ambient temperature could not be controlled, and only trials in which ambient temperature was within 2°C of the experimental nectar solution were included in these analyses.

Viscosity experiments

To independently test the effects of sweetness and viscosity on nectar intake rates, low concentrations of tylose (Coquinox, Panama City, Panama), an inert sugar, were used to augment fluid viscosity without significantly affecting density or energy content. Artificial nectar solutions were prepared using either laboratory grade or commercially available sucrose, distilled water, and tylose (Josens and Farina, 2001). A Brookfield Desktop Viscometer (Middleboro, MA, USA) was connected to a constant temperature water bath to measure the viscosity of test solutions at 30°C. In the constant sucrose experiment, sucrose content was held at 25%, while viscosity was increased to the equivalent of 35, 45, 55 and 65% sucrose. Viscosity of experimental solutions matched true sucrose solutions ($R^2=0.999$). In the constant viscosity experiment, viscosity was held at the equivalent of 50% sucrose, nominally 10.21 mPa s, at five sucrose concentrations, 5%, 15%, 25%, 35% and 50%. For pure sucrose feeding trials, 5%, 15%, 25%, 35%, 45%, 55%, 65% and 75% solutions were prepared. The concentration of all solutions was checked with a pocket refractometer before each trial.

Hypobaric experiments

To estimate the maximum pressure drop produced by *Eg. imperialis*, nectar intake rates were measured as bees fed inside a vacuum chamber (Nalge Nunc International, Rochester, NY, USA) under reduced ambient pressure. Rubber housing connected the chamber to an AC powered pump and a series of three-way valves were employed to switch between pump suction and ambient pressure. Chamber pressure was monitored using a catheter-style transducer (Millar Instruments, Houston, TX, USA), which was inserted through a small, tight-fitting hole at the base of the chamber. The output of the pressure transducer along with that of a thermistor thermometer were digitized at 10 samples s^{-1} with a 12 bit A-D converter (Vernier Instruments, Beaverton, OR, USA).

At the start of each trial, artificial flowers were filled with either 25% or 45% sucrose solution. The 45% solution was selected as the high viscosity treatment because intake rates were high enough that they could be easily measured under reduced ambient pressure. Immediately after bees began to feed, data acquisition was initiated, the pump was switched on, the chamber was placed over the bee, and valves were slowly adjusted to a previously determined set point nominally lowering the pressure in the chamber to 80, 60, 50 or 40 kPa. The pressure reduction required 5–15 s to reach a stable final pressure. Control trials were conducted at ambient pressure (101 kPa) with the pump running but not connected to the chamber. Additional nectar intake rate measurements were made as bees fed from 15%, 35% and 55% sucrose solutions

under reduced ambient pressure (40 kPa), but control trials were not conducted for these solutions.

Nectar feeding model

Morphological and kinematic data for orchid bees were used to parameterize the Daniel et al. model (Daniel et al., 1989), and the Hill constants V_{\max} and T_0 along with the cibarium height, H_{cib} , were estimated by minimizing the sums-of-squares error between the model and the nectar intake rate data. Radius and length of the proboscis were held at 0.045 mm and 17.5 mm, respectively, as measured in the present study. The maximum flow rate, $1.2 \mu\text{l s}^{-1}$, was divided by the estimated pump frequency of 2.5 Hz to obtain a cibarium volume of $0.48 \mu\text{l}$. Cross-sectional area of the cibarium was calculated by dividing the volume by the height.

The muscle parameters V_{\max} and T_0 are each the products of two implicit parameters: V_{\max} is the product of the length of the muscle and length-specific V_{\max} ($L s^{-1}$); T_0 is the product of the maximum isometric stress of the muscle (kPa) and the cross-sectional area of the pump-dilator muscle, A_{muscle} . Length-specific V_{\max} tends to vary widely among animal taxa from 1 to $20 L s^{-1}$ (Medler, 2002), although $5 L s^{-1}$ is probably the upper limit for the cibarium dilator (Josephson, 1993). The maximum isometric stress of muscle tends to be relatively conserved among taxa, ranging from 5 to 12 kN m^{-2} (Medler, 2002). To avoid redundant simulations, maximum isometric stress was fixed at 8 kPa (Daniel et al., 1989) and L_{muscle} was fixed at 1 mm (twice the final estimate of H_{cib}). A total of 250 simulations were performed where the unknown parameters were varied as follows: H_{cib} (0.0002–0.002 mm), V_{\max} ($1\text{--}5 L s^{-1}$), and A_{muscle} ($0.5\text{--}0.9 \text{ mm}^2$). The five models with the lowest sums-of-squares error were examined for convergence.

All analyses and simulations were conducted in JMP (v. 4.04, SAS Institute, Cary, NC, USA) and Matlab (v. 7.1, MathWorks, Natick, MA, USA). Statistical tests were performed using analysis of variance (ANOVA), analysis of covariance (ANCOVA), and ordinary least-squares regressions. Error bars are presented as 95% confidence intervals (CI). Sample sizes varied with treatment but typically ranged from 5–10 individuals.

Results

Proboscis morphology

Proximally, the overall radius of the proboscis is 0.11 ± 0.02 mm (mean \pm 95% CI), diminishing to 0.042 ± 0.006 mm at the tip. The mean value for k averaged over the proboscis length was 0.75 ± 0.05 . Numerically integrating the radius and the correction factor over the length of the proboscis, gave an effective radius of the proboscis of 0.050 ± 0.012 mm.

The maximum pressure drop recorded from freely foraging bees occurred at 65% sucrose. If the cibarial pump were able to produce an absolute vacuum under these conditions, then the mean radius would be 0.039 ± 0.002 mm. Orchid bees have been

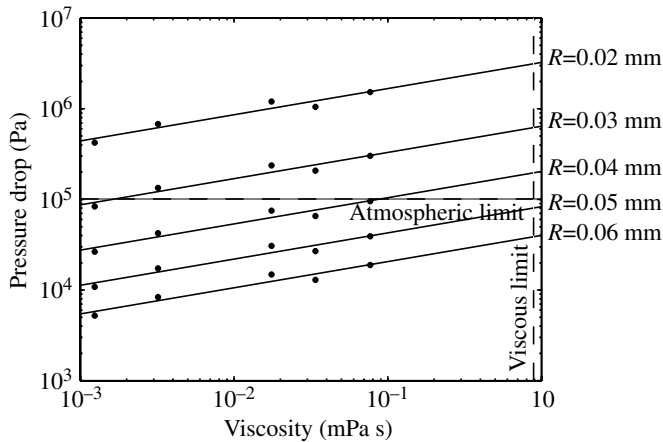


Fig. 1. Calculated pressure drop *versus* nectar intake rate for proboscis radii ranging from 0.02 to 0.06 mm. The atmospheric pressure line (broken horizontal line) represents the putative upper limit of the pressure drop that can be developed in the cibarium. The viscous limit line (broken vertical line) represents the most viscous sucrose solution from which orchid bees have been observed feeding.

recorded feeding or attempting to feed at 75% sucrose under laboratory conditions. Using this value as the maximum viscosity, then the minimum radius becomes 0.047 mm (Fig. 1).

The effective radius of the proboscis likely falls between 0.04 and 0.05 mm. For subsequent calculations, I used 0.045 mm as the standard proboscis radius which, for the data in Fig. 1, gave a mean pressure drop of 597 ± 121 kPa at 65% sucrose and a predicted pressure drop of 1225 kPa at 75% sucrose.

Viscosity experiments

Nectar intake rate declined nonlinearly with increasing sugar concentration from a maximum of $1.2 \mu\text{l s}^{-1}$ to less than $0.1 \mu\text{l s}^{-1}$ (Fig. 2). The intake rate for starved bees was linearly related to intake rate measured from freely foraging bees ($R^2=0.994$, $F_{1,2}=343.3$, $P=0.0029$) with a slope of 1.39 ± 0.08 and an intercept not significantly different from zero ($P=0.2801$).

When sucrose concentration was held at 25%, nectar intake rate declined with increasing viscosity of tylose treatments (Fig. 3). Nectar intake rate declined with viscosity according to the power relationship: $Q = -1.3 \mu^{-0.64}$ ($R^2=0.890$, $F_{1,31}=253.4$, $P<0.0001$) When viscosity was held constant while sucrose concentration was varied from 5% to 50%, nectar intake rate remained constant (Fig. 3)

Hypobaria experiments

The mean pressure drop calculated for intake of 25% and 45% sucrose solutions (Eqn 1) in the pressure chamber at ambient pressure (control) was not significantly different than values recorded during typical laboratory feeding trials (Fig. 4, ANOVA: $F_{1,56}=1.244$, $P=0.2695$). There was no interaction between sucrose concentration and method

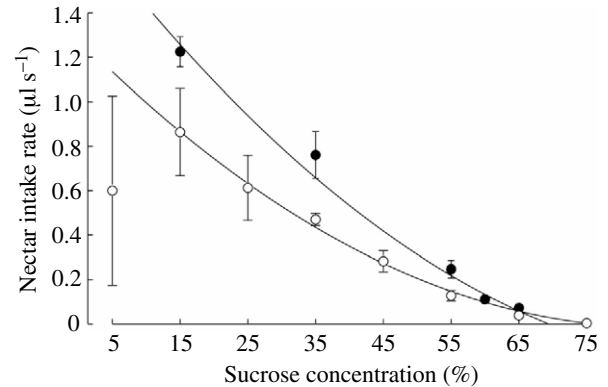


Fig. 2. The relationship between nectar intake rate and sucrose concentration in *Euglossa imperialis* depends on experimental conditions. Nectar intake rate was measured from trained bees visiting artificial flowers (filled circles) and starved bees accepting sucrose solutions in the laboratory (open circles).

($P=0.4517$). Pressure drop declined with decreasing sucrose concentration and reduced ambient pressure (sucrose: $F_{1,56}=9.612$, $P=0.0030$; pressure: $F_{1,56}=27.06$, $P<0.0001$). However, the interaction effect between sucrose concentration and ambient pressure was not significant ($P=0.6387$), suggesting that the pressure drop may not vary with sucrose concentration.

By incorporating data at all ambient pressure levels (40, 50, 60, 80 and 101 kPa), it was possible to test for linear trends. Pressure drop declined linearly with ambient pressure at both 25% and 45% sucrose concentrations (ANCOVA: $F_{1,85}=41.34$, $P<0.0001$). The pressure drop during feeding from a 45% sucrose

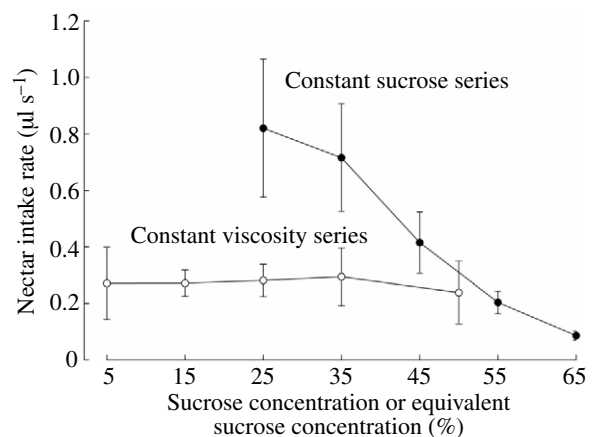


Fig. 3. Nectar intake rate depends on nectar viscosity not nectar sweetness in laboratory feeding trials with *Euglossa imperialis*. Constant sucrose experiment (filled circles): sucrose concentration was held at 25% and solution temperature was maintained 30°C as viscosity was increased to the equivalent of a 65% sucrose solution. Constant viscosity experiment (open circles): viscosity was held at the equivalent of 50% sucrose (10.21 mPa s) and solution temperature was maintained at 30°C as sucrose concentration ranged from 5–50%. Sucrose concentration has no effect on nectar intake rate ($F_{1,28}=0.2301$, $P=0.6352$).

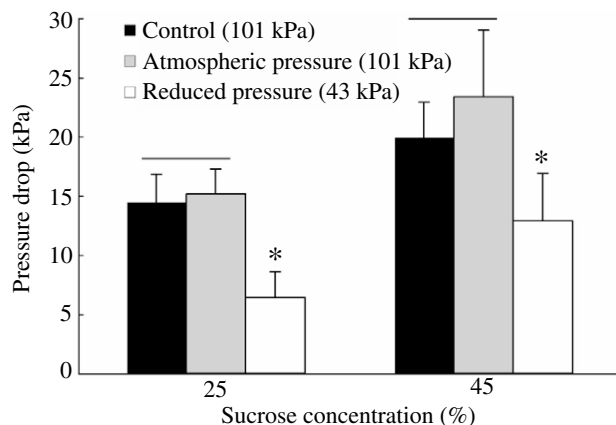


Fig. 4. Hagen–Poiseuille pressure drop for *Euglossa imperialis* depends on sucrose concentration ($F_{1,56}=9.612$, $P=0.0030$) and ambient air pressure ($F_{1,56}=27.06$, $P<0.0001$). Asterisks indicate significant differences.

solution was twice that for the 25% solution at all ambient pressures ($F_{1,85}=44.97$, $P<0.0001$). The interaction between ambient pressure and sucrose concentration was not significant ($P=0.1860$), suggesting again that a reduction in ambient pressure affects the pressure drop in the same way for both high and low concentrations. Although there was some indication of a threshold effect in the 45% data set, where the pressure drop begins to decline at ambient pressures below 60 kPa, a quadratic term added to the model was not significant ($P=0.0531$).

At all concentrations, nectar intake rate recorded at an ambient pressure of 43 kPa was on average 46% of the nectar intake rate measured at 101 kPa. Although an ambient pressure reduction should have the greatest impact at high concentrations (when the pressure drop is highest), for 15%, 25%, 35% and 45% solutions, mean intake rate was between 38 and 42% of standard values. For the 55% solution, intake rate was at 68% of the standard value. Thus, the relative reduction in intake rate showed no relationship with sucrose concentration ($P>0.05$).

Suction feeding model

The sums-of-squares error was minimized when H_{cib} was 0.5 mm, V_{max} was $4.5 L s^{-1}$ and A_{muscle} was $0.5 mm^2$. Although a range of values can lead to a good fit of the model, it was clear that V_{max} must be greater than $1 L s^{-1}$ in order to be compatible with the data from either starved bees or trained bees. The best fit of the model to the data is shown in Fig. 5.

Using optimal model parameters, the simulation provided estimates of the pump characteristics of the cibarium. At zero pressure, the flow rate of the pump would be about $3.8 \mu l s^{-1}$. At zero flow, the maximum pressure drop is about 42 kPa (Fig. 5A). Mechanical power of the pump was calculated as nectar flow rate times the pressure drop and is about $1.7 \times 10^{-5} W$ when pumping nectars from 5% to 35% sucrose, dropping to zero as concentration increases to 75% sucrose (Fig. 5B).

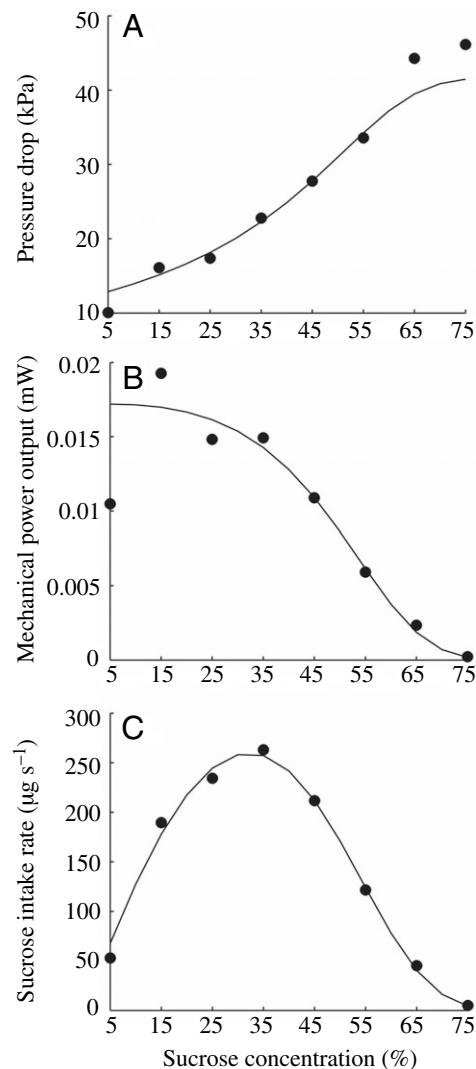


Fig. 5. The fit of a mathematical model of suction feeding (Daniel et al., 1989) to measurements from *Euglossa imperialis*. These data points were measured from starved bees, but were multiplied by a correction factor of 1.39 to be applicable to freely foraging bees. (A) Pressure drop versus sucrose concentration assuming a fixed radius of 0.045 mm; (B) mechanical power output versus sucrose concentration; (C) sucrose intake rate versus sucrose concentration.

Discussion

Viscosity and flow

Nectar intake rate in *Eg. imperialis* is determined solely by nectar viscosity not by nectar sweetness. This result contrasts with experiments on the hawkmoth *Macroglossum stellatarum* (Josens and Farina, 2001). These authors found that even when viscosity was held constant using tylose, energy intake rate still peaked at 34% sucrose, suggesting that gustatory input influences the dynamics of cibarial pumping in those insects. Honeybees have been shown to respond independently to changes in viscosity and sweetness during trophallactic (regurgitation) behavior (Tezze and Farina, 1999), and in nectar choice experiments *Eg. imperialis* discriminates among

constant sucrose solutions of variable viscosity (B. J. Borrell, unpublished observation). Sensors on both the glossa and gut measure the sweetness and quantity of ingested fluids allowing bees to monitor the profitability of nectar resources (Blatt and Roces, 2002). It seems, however, that such behavioral assessments do not influence cibarial pump contraction in *Eg. imperialis*. Indeed, if foraging bees are attempting to maximize energy intake, then there is little reason to feed at sub-maximal rates simply because resources are deemed non-optimal.

As has been previously noted (Kingsolver and Daniel, 1995; Smith, 1979), flow rate does not exhibit the linear relationship with viscosity as predicted by the Hagen–Poiseuille equation. Instead, when ingesting low viscosity solutions with high frequency contractions of the cibarium, insects may not be producing adequate force. Owing to the nonlinearity of the force–velocity relationship, nectar viscosity ranges over nearly two orders of magnitude, but actual flow rates exhibit a far smaller of a spread. If the radius of the proboscis varies with flow rate or pressure (see below), then this interaction could prove to be more complex.

Pressure and flow

The pressure drop appears to be limited by ambient air pressure, but it is unclear whether it actually increases with viscosity. At all sucrose concentrations nectar intake rate was significantly affected by a reduction in ambient pressure. In fact, the relative effect of the pressure reduction was similar for 15%, 25%, 35% and 45% solutions, and the relative reduction in nectar intake rate was directly proportional to the relative reduction in ambient pressure.

Flow through a rigid tube depends on the pressure difference along the length of the tube, not the absolute pressure at either end of the tube (Vogel, 1994). Consequently, nectar intake rate and the associated pressure drop should be independent of a reduction in ambient pressure until the absolute pressure inside the cibarium approaches the vapor pressure of the ingested fluid (i.e. a perfect vacuum), or some negative value if orchid bees are able to avoid fluid cavitation (Bennet-Clark, 1963). Calculations using the Hagen–Poiseuille equation indicate that the pressure drop for bees feeding on a 25% sucrose solution should be about 15 kPa. If the ambient pressure is lowered to 43 kPa, then the absolute pressure inside the cibarium will be 28 kPa, meaning that cavitation should not be an issue. Even if the instantaneous pressure drop peaks at twice the average value, the absolute pressure inside the cibarium will still be 13 kPa. Because backflow was not taken into account, these calculations are likely to be underestimates of the true pressure drop. Quantitative errors, however, cannot explain why intake of nectar solutions declined by the same fraction at all concentrations.

The present experiments are more likely to reflect biomechanical effects on the pumping apparatus rather than physiological effects of limiting oxygen availability. Post-flight resting metabolic rate of honeybees exhibits a slight increase as ambient pressure is reduced from 101 kPa to 24 kPa

(Withers, 1981). However, resting metabolic rate is still less than 1/40th of flight metabolic rate in free flight, and even flight metabolic rate is not affected by a reduction in oxygen partial pressure down to 10 kPa, the equivalent of 60 kPa total pressure (Joos et al., 1997). The power requirements of the suction pump are only a small fraction of the power requirements of flight.

Radial expansion: a hypothesis

One possible explanation for the results from the pressure reduction experiment is that the euglossine proboscis functions as a ‘collapsible’ tube. The butterfly proboscis is sealed *via* series of hook-shaped structures and overlapping cubacula plates, forming a food canal that is permanently interlocked (Hepburn, 1971). The circumferential stress imposed by an atmosphere of pressure is insufficient to trigger collapse of the proboscis (Kingsolver and Daniel, 1979). By contrast, in long-tongued bees, the food canal is sealed *via* a series of tongue-and-groove junctions that permit proboscis components to slide with respect to each other and even disconnect (Borrell and Krenn, 2006). The proboscis is extended *via* contraction of muscles at its base in addition to an increase in haemolymph pressure within proboscis components, particularly the glossa (Paul et al., 2002). It is unclear how the euglossine proboscis, which is normally in a flattened configuration, remains open during feeding. Some critical pressure must be developed before flow is initiated, a phenomenon that is apparent during the first moments after the proboscis has contacted a nectar solution. Bees attempting to feed from 75% sucrose solutions often extend their tongues repeatedly before giving up, suggesting that flow rates were insufficient for proboscis expansion. During pressure reduction experiments, bees feeding on all concentrations occasionally retracted their tongues as ambient pressure fell below 50 kPa, re-extending them when ambient pressure rose again. The critical pressure required for opening of the feeding canal must be greater than the pressure drop required during steady flow. Once fluid is flowing inside the proboscis, the feeding canal may still be susceptible to partial or total collapse if the external pressure is sufficient to overcome supporting forces. In collapsed, flexible tubes, either the flow rate can become pressure drop independent or the pressure drop can be flow rate independent (Bertram, 1995). These nonlinear phenomena, which are common in the circulatory system, may be important if fluid forces counterbalance structural properties of the proboscis during feeding.

Because of the high degree of overlap of proboscis components and the low Reynolds number of the system, the proboscis will tend to expand in response to a pressure drop (Vogel, 1994). The hooks on the galeae and labial palps set a minimum radius of the proboscis, but the maximum radius depends on the degree of overlap required to maintain an airtight seal. Although the morphological estimate of the radius was in good agreement with the functionally derived estimate, small differences in radius can have a large effect on the pressure drop. For instance, a 10% decrease in overlap will cut

pipe resistance in half, thereby halving the required pressure drop for a given flow rate. Radial expansion may even prevent proboscis collapse by maintaining a constant pressure gradient independent of flow rate. Radial expansion would also explain why flow rate declined with ambient pressure at all sucrose concentrations.

I performed a series of simulations to see whether a variable radius could be compatible with both the empirical flow data and the suction feeding model. Proboscis radius was set to either increase linearly with sucrose concentration from 0.04 mm to 0.054 mm, increase exponentially with sucrose concentration from 0.04 mm to 0.058 mm, or decrease linearly from 0.054 to 0.04 mm with sucrose concentration. In these three simulations, the optimal nectar concentration always matched the empirical data (30–38% sucrose). Not surprisingly, however, the match of the calculated pressure drop to the model depended strongly on chosen values for the Hill parameters. For the case of the linearly increasing radius, the pressure drop will remain nearly constant at all concentrations, and the simulation is capable of replicating these results with reasonable values for the Hill parameters. This analysis shows that optimal nectar concentrations will be largely independent of radial expansion, but the pressure drop cannot be predicted without more detailed knowledge of the physiological properties of the pump dilator muscle or the dependence of radial expansion on flow rate.

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