Supplemental Information for: The mechanics of nectar offloading in the bumblebee *Bombus terrestris* and implications for optimal concentrations during nectar foraging.

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Supplemental Information 1. Study animals and general experimental setup

Bees were obtained from Biobest (supplied by Agralan, Ashton Keynes, UK) and housed in plastic nest boxes of approximate size 292 x 225 x 240 mm (all dimensions here are length x width x height). The plastic mesh lid of the nest box was replaced by a clear acrylic lid during experiments. The nest boxes were connected via a gated tube to a 1.12 x 0.75 x 0.30 m flight arena constructed from wood with a clear acrylic lid. The gates in the connecting tube were used to control which bee entered and left the arena. In between experimental trials, the colonies were provided with sucrose solution of concentration circa. 30 % w/w. Colonies were supplied with pollen (honeybee-collected pollen pellets) *ad libitum*. For the experiments we selected motivated workers, which were those observed to be completing successful foraging bouts. Bees were individually marked on the thorax, either with water-based paints (Thorne, Rand, Market Rasen, UK), or with numbered tags (Abelo, Full Sutton, York, UK), using a resin-based glue (obtained from Thorne, Rand, UK). Room temperature varied from 22 to 23.2 °C. To ensure selected bees were motivated, and to familiarise them with the setup, each bee was allowed at least four foraging bouts before measurements started. There is evidence that bumblebees foragers spend slightly longer in the nest between their first three to four foraging bouts than they do on subsequent bouts, in order to recruit additional foragers [1]. By starting recording after the fourth bout, we also avoided any potential confound from this effect.

Supplemental table 1. Description of parameters recorded for each foraging bout.

Parameter	**Description**
Drinking time	The time the bee spent with her proboscis in contact with the sucrose solution*.
Extra foraging time	The time spent by the bee in the foraging arena without the proboscis in contact with the sucrose solution*.
Offloading time	Once the bee had foraged for nectar, she returned to the nest, then searched for a honeypot in which to offload the sucrose solution. Offloading was recorded as the time the bee spent with her head in a honeypot, visibly contracting her abdomen†.
Extra colony time	The total time spent in the nest box excluding offloading time (above).
The number of independent offloading events	Bees sometimes offloaded into more than one honey pot. This measure is the number of honeypots in which the bee offloaded at the end of each foraging bout.

NB. Time was recorded using a stopwatch.

*Bees occasionally extended their proboscis into the solution to taste it but did not drink. To exclude incidences where the bee was tasting the solution, we discounted any proboscis contact with the sucrose solution for which the duration was less than 5 s. Similarly, bees occasionally withdrew their proboscis while drinking. To simplify recording, rests of duration < 5 s were not recorded.

†Occasionally a bee offloaded at a honeypot that was out of view of the observer. If this occurred, then measurements from that foraging bout were discounted and a further foraging bout was recorded.

Supplemental Information 2. Measuring the effect of water loss on sucrose concentration

We conducted an additional experiment to measure whether water loss due to evaporation may affect the sucrose concentration of the solution offered to the bees. A 48-well PCR plate was filled with one of three sucrose solutions (35 %, 50 % and 65 % w/w) in the same manner as for the main experiment and placed in the flight arena. We also recorded the humidity in the flight arena during this experiment, which was 65 %. We measured the effects of any water loss by recording the mass of the PCR plate before starting and after one hour (which is a little longer than the time taken to record 10 foraging bouts for the average bee). The sucrose concentration was also directly recorded using a handheld refractometer (Bellingham and Stanley) before and after this experiment. We carried out three replicates for each sucrose concentration.

Using the refractometer there was no detectable difference in concentration from the start to the end of the experiment. On recalculating the concentration based on the water loss from each solution, the mean concentration of each solution after one hour was 35.29 %, 50.38 %, and 65.37 % w/w for the 35 %, 50 % and 65 % solutions respectively. If we assume that the mean concentration when measuring loading/offloading rate is halfway between the value at the start and end of an hour, (i.e. 35.14 %, 50.19 % and 65.18 % respectively) the effect of any evaporation only has a minimal effect on our models of the relationship between viscosity and flow rate. We therefore do not include any effects of evaporation in our models.

Supplemental information 3. Calculations for volume and mass of solution transferred; viscosity; and temperature during offloading.

The volume of sucrose solution transferred during drinking and offloading for each foraging bout was calculated by dividing the mass of solution by the concentration-specific density ρ_c (in g mL^{-1}), which we calculated using the formula in Prŷs-Jones and Corbet [2] where:

$$\rho_c = 0.9988603 + 0.0037291c + 0.0000178c^2. \qquad (1)$$

The mean flow rates for drinking were 1.28, 1.17, and 0.71 µL s^{-1} for 35, 50 and 65 % w/w sucrose respectively. The mean flow rates for offloading were 23.4, 15.2, and 4.65 µL s^{-1} for 35, 50 and 65 % w/w respectively.

The mass of sucrose (and thus energy content) transferred was calculated by multiplying the mass of solution by the concentration (% w/w) / 100.

For calculating the viscosity of sucrose solutions at varying concentrations and temperatures, we used the Génotelle equation ([Equation 2] and also see Longinotti and Corti [3]). This provides a good approximation of the viscosity μ in mPa s of sucrose solutions at mole fractions of sucrose x and temperature T (in °C); and at the temperatures and concentrations considered here, gives reasonable agreement with published values of viscosity of sucrose solutions [e.g. 4,5]:

$$\log_{10}\frac{\mu}{\mu^*} = a_1 + a_2 x + \Phi(b_1 + b_2 x^n), \qquad (2)$$

where μ^* = 1 mPa s. We used the values a_1 = -0.114, a_2 = 22.46, b_1 = 1.1, b_2 = 43.1, n = 1.25 for the coefficients [3]. Φ is a reduced temperature:

$$\Phi = \frac{(30-T)}{(91+T)}. \qquad (3)$$

We calculated the mole fraction x of sucrose at concentration c (% w/w) using:

$$x = \frac{(c\ /\ 342.3)}{\big((100-c)\ /\ 18.02\big) + (c\ /\ 342.3)}. \qquad (4)$$

For offloading, we assumed that sucrose solution was at abdominal temperature, for which we used 27 °C. We based this value on measurements of abdominal temperatures of foraging bumblebees [6]. Bumblebees store nectar in the honeycrop, which is located in the abdomen [7]. Unlike thoracic temperature, abdominal temperatures of foraging bumblebees are typically correlated with air temperature [6,8,9]. Using $T_{air} = 23\,°C$ (average lab temperature) and the regression equation $T_{abdominal} = 16.8 + 0.438\,T_{air}$ from Heinrich and Vogt [6], we estimated abdominal temperature to the nearest degree as 27 °C. We also made the assumption that there was no change in sucrose concentration in the honeycrop between drinking and offloading.

Supplemental table 2. Fitted model parameters for regressions of $\log_{10}$(viscosity in mPa s) versus $\log_{10}$(volumetric flow rate in µL s^{-1}) for 10 bees foraging on sucrose solutions of concentration 35, 50, and 65 % w/w. Flow rates for each bee were calculated from 10 foraging bouts, with regressions performed both on the mean and maximum flow rates for each bee. For drinking, viscosity was calculated assuming a temperature of 23 °C yielding viscosities of 4.00, 13.78, and 120.9 mPa s for the three concentrations respectively. For offloading, viscosity was calculated assuming a temperature of 27 °C (see above), yielding respective viscosities of 3.51, 11.56, and 92.80 mPa s.

	Intercept [-95 % CI, +95 % CI]	**Slope [-95 % CI, +95 % CI]**
Mean flow rates		
Drinking	0.236 [0.192, 0.280]	-0.180 [-0.211, -0.148]
Offloading	1.652 [1.534, 1.770]	-0.502 [-0.590, -0.413]
Max flow rates		
Drinking	0.288 [0.237, 0.340]	-0.183 [-0.219, -0.146]
Offloading	1.759 [1.635, 1.883]	-0.512 [-0.605, -0.418]

Supplemental information 4. The effect of body mass on regressions of viscosity versus volumetric flow rate.

As our experimental design resulted in an equal distribution of bee masses for the different concentrations, and body mass has previously been shown not to interact with viscosity in its effect on volumetric flow rates [10], we chose not to include body mass in our linear models of viscosity versus flow rate. However, for completeness, we give the models here. For drinking rate there was no significant interaction between mass and viscosity ($t_{26} = 2.640$, $p > 0.99$); however, body mass does influence drinking rate as a main effect (Supplemental Table 3). Both of these findings are in agreement with Harder [10].

Supplemental Table 3. Model parameter estimates and 95 % CI for a linear model with $\log_{10}$(drinking rate in µL s^{-1}) as response, $\log_{10}$(viscosity in mPa s) and $\log_{10}$(bee mass in g; minimum unladen) as predictors, with no interaction term.

Parameter	**Estimate [-95% CI, +95 %CI]**
Intercept	0.638 [0.417, 0.859]
Viscosity	-0.181 [-0.206, -0.155]
Bee mass	0.506 [0.232, 0.781]

Given that mass did not interact with viscosity, the effect of body mass on drinking rate did not affect our estimates of optimum concentrations for maximising energy transfer rate during drinking, and adding this term into our model of the optimum concentration for maximising energy return to the nest had a negligible effect on the predictions given in figure 5. Proboscis length also affects drinking speed [10]. We did not measure proboscis length; however, as is the case with body mass, proboscis length does not influence the relationship between flow rate and viscosity. Additionally, as proboscis length strongly correlates with body size [11] any potential effect of proboscis length would be captured in the models including bee mass, described in this section.

In contrast to drinking rate, body mass did not affect offloading rate at all, neither as an interaction with viscosity (t_{26} = 0.217, p = 0.8302), nor as a main effect (t_{27} = 1.191, p = 0.244).

Supplemental information 5. Models of volumetric flow rate and calculation of overall energy transfer rates

Volumetric transfer rates of sucrose solution were modelled for drinking and offloading using linear models of $\log_{10}$ viscosity versus $\log_{10}$ flow rate. This gives a volumetric flow rate Q_{drink} (in µL s^{-1}) for drinking of:

$$Q_{drink} = 10^{0.236} \times \mu^{-0.180}, \quad (5)$$

and a volumetric flow rate Q_{off} for offloading of:

$$Q_{off} = 10^{1.652} \times \mu^{-0.502}, \quad (6)$$

where µ is viscosity in mPa s.

Volumetric flow rates were converted into energy transfer rates using the rate (S) of sucrose transferred by mass in mg s^{-1} as a proxy for energy transfer, by multiplying the respective volumetric transfer rates Q by the sucrose concentration c (% w/w) and the concentration-specific density ρ_c [Equation 1], such that:

$$S = \frac{Qc\rho_c}{100}. \quad (7)$$

For Figure 4b. we standardised energy transfer rates for drinking and offloading by expressing them as percentage of the respective maximum rate.

Supplemental information 6. Rate of energy return across a complete foraging trip

We model the rate of energy return in J s^{-1} back to the nest for a whole foraging trip as the difference between energy gain and energy used divided by the total time spent on the foraging trip. As well as drinking and offloading time, total time includes travel time, search for flowers, flower handling as well as other activities between foraging trips. Our model is based on that used by Harder [10]. We calculate the energy return rate (ERR) as:

$$ERR = \frac{\frac{V\rho_c ec}{100} - \frac{1}{2}\left(m+\left(m+\frac{V\rho_c}{1000}\right)\right)(M_d t_d + M_{off} t_{off} + M_f t_f + M_{other} t_{other})}{t_d + t_{off} + t_f + t_{other}}, \quad (8)$$

where V is the volume of sucrose collected in a foraging trip in µL, ρ_c is the concentration-specific density of sucrose (calculated as above), c is the sucrose concentration in % w/w, e is the energy

content of sucrose (15.48 J mg^{-1}) [10], m is the mass of the bee in g, M_d, M_{off}, M_f, and M_{other} are the mass specific metabolic rates of a bee in J s^{-1} g^{-1} for drinking, offloading, flight and other activities respectively, and t_d, t_{off}, t_f and t_{other} are the times spent on these respective activities in seconds. Flight time (t_f) is the total (i.e. roundtrip) flight time. We make the simplifying assumption that for half of the time spent on each activity the bee is unloaded i.e. the bee's mass = m; and for the other half of the time the bee is carrying a load of sucrose solution of volume V, such that the bee's mass = $m + \frac{V\rho_c}{1000}$. The volume V was set to 105 µL, which is the mean carried by the bees in our experiment, and m to 0.163 g, the mean of the minimum unladen masses of the bees we used. For M_f and M_d, we used the same values as Harder [10] of 0.435 and 0.034 J g^{-1} s^{-1} respectively. The value for flight originally comes from Heinrich [12]. The exact source that Harder used for M_d is unclear to us; however, Pyke [13] also gives 0.034 J g^{-1} s^{-1}, and cites this as being from Figure 1 of Kammer and Heinrich [14], from which Pyke appears to have obtained the rate of oxygen consumption at a thorax temperature of 37 °C. To simplify our model, we set $M_d = M_{off} = M_{other}$. t_d and t_{off} were calculated from V and the respective volumetric flow rates (Q_{drink} and Q_{off}) for drinking and offloading, assuming an air temperature of 23 °C. Abdominal temperature was calculated as above. t_{other} was set to 84 seconds, which was the mean time the bees in our study spent in the colony on activities other than offloading. We calculated energy return rates for two values of t_f, 100 s and 900 s, representing a short and long foraging trip respectively.

In the full model, we only use one value for V; however, it should be noted that the volume carried will also influence energy return rates. This is not the focus of our study, but briefly, as volume carried increases, the respective optimal concentration for maximising energy return to the nest will decrease, and the rate of energy return at the optimum will increase. Interestingly, in honey bees, nectar load varies with temperature [15]. If the same were true in bumblebees, this would be another way in which temperature could affect our foraging models.

We also use a mean value for bee mass. Both drinking rate (Supplemental information 4) and the maximum volume a bee can carry vary with body mass. Of these two parameters, changing the volume carried has the more substantial effect on our model (described above). Any changes in drinking rate that result from varying bee mass would have a small effect on the optimum concentration, but have a larger effect on the rate of energy return.

In Figure 5, we compare the full model for a flight time of 100 s with a model excluding the viscosity-dependence of flow rate during offloading. For this reduced model we assumed that offloading time is fixed at 7.3 s. This time is calculated using the overall mean offloading rate across all concentrations (14.4 µL s^{-1}) and our mean sucrose solution load of volume V.

Supplemental information 7. The ratio of energy gained to energy used

We calculated the ratio of energy gained to energy used as:

$$Energy\ ratio = \frac{\frac{V\rho_c ec}{100}}{\frac{1}{2}\left(m+\left(m+\frac{V\rho_c}{1000}\right)\right)\left(M_d t_d + M_{off} t_{off} + M_f t_f + M_{other} t_{other}\right)}. \qquad (9)$$

In our calculations of energy ratio and energy return rate, it should be noted that the value we chose for M_d is likely to be lower than the true estimate of metabolic rate during non-flight activities as, depending on ambient conditions, the bee will have to expend energy on maintaining thorax

temperature [9,13]. Although including the energy required for thermoregulation will affect metabolic rates; any such alterations to metabolic rates in the model have a negligible effect on the rate of energy returned to the nest and also to the sucrose concentration which maximises this rate. To avoid overcomplicating the models we therefore chose to exclude costs of thermoregulation. More generally, our model for the rate of energy return to the nest is largely insensitive to the values chosen for metabolic rate. To illustrate this lack of sensitivity we can draw on some implausibly extreme scenarios. For example, for a flight time of 100 s, if we assume the bee is expending energy throughout the whole foraging bout at the rate required for flight (0.435 J g^{-1} s^{-1}), the sucrose concentration which maximises the rate of energy return to the nest is 64.5 %, and the rate of energy return at this concentration is 3.86 J s^{-1}. At the other extreme, if we assume the bee expends no energy at all throughout the foraging bout, the concentration which maximises the rate of energy return to the nest is still 64.5 % and the rate at this concentration is only slightly higher, at 3.96 J s^{-1}. Metabolic rate varies far less than the extreme scenarios illustrated here.

However, the ratio of energy gained to energy used is dependent to the values chosen for metabolic rate. Hence the optimum concentrations predicted for maximising the energy ratio should be treated with some caution. If a high metabolic rate is required to maintain thorax temperature at low ambient temperatures when a bee is not flying, then this will lower the optimum concentration for maximising energy ratio in these situations, potentially leading to similar predictions of the optimum concentration as for energy return rate.

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