

Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences

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Summary

1. Specialised nectar-feeding birds tend to prefer sucrose over glucose-fructose mixtures or to be indifferent when tested at concentrations close to 584 mmol L^{−1} sucrose equivalents. The role of the potential interaction between sugar composition and concentration on the sugar preferences of these birds, however, has not been explored.

2. We tested the hypothesis that sugar preferences in nectar-feeding birds are concentration-dependent. We predicted that at high concentrations they would prefer sucrose over hexoses, whereas at low sugar concentrations they would prefer hexoses. We expected birds to show differences in food intake that matched their sugar preferences when they fed on equicaloric solutions of sucrose and 1 : 1 mixture of glucose and fructose. Consequently, the curves describing the relationship between food intake and sugar concentration for these two sugar solutions should cross. We tested these hypotheses in two species of nectar-feeding birds: the Cinnamon-bellied Flowerpiercer, *Diglossa baritula* (Wagler) and the Magnificent Hummingbird, *Eugenes fulgens* (Swainson).

3. The sugar preferences of both species were concentration dependent. At lower concentrations they preferred hexoses, whereas at higher concentrations they shifted their preference to sucrose. However, these concentration-dependent preferences were not matched by parallel differences in intake.

4. Although nectar composition and concentration are often discussed as two different floral traits, our results show that they have a synergistic effect on the sugar preferences of nectar-feeding birds.

Key-words: Animal–plant coevolution, nectar composition, osmotic concentration, physiological constraints, sugar assimilation

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Introduction

Floral nectars vary among plant species in both sugar concentration and composition (Pyke & Waser 1981; Baker & Baker 1983; Baker, Baker & Hodges 1998). Sugar concentration varies from 5% to about 66% (weight/volume or 146–1927 mmol L^{−1} sucrose equivalents), and some plants produce nectar rich in hexoses whereas others tend to produce nectar rich in the disaccharide sucrose (reviewed by Nicolson 2002). Flowers pollinated by hummingbirds and specialised nectar-feeding passerines tend to secrete sucrose rich nectars (Baker 1975; Stiles & Freeman 1993; Baker

et al. 1998; Nicolson 2002), whereas those pollinated by generalist nectar-feeding passerines tend to secrete nectars that contain primarily glucose and fructose (Baker & Baker 1983; Baker *et al.* 1998). Recently, Nicolson (2002) uncovered a perplexing pattern: in the nectar of some plant genera there is a positive correlation between sucrose content and concentration.

Two complementary explanations have been posed to explain these patterns in nectar composition and concentration. Because of their emphasis on either plants or birds, we call these explanations the phyto-centric and ornithocentric hypotheses. The phyto-centric explanation posed by Nicolson (2002) assumes that osmotic effects explain the correlation between sucrose dominance and high sugar concentration in nectar. Sucrose rich phloem sap is either hydrolysed by acid invertase or secreted without prior hydrolysis in nectaries. Sucrose hydrolysis increases nectar osmolality, causing water to move from the nectary walls

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into nectar. Nicolson's (2002) hypothesis explains the correlation between sugar concentration and sucrose dominance in nectar, but does not explain why some plants produce dilute hexose-dominated nectars instead of concentrated sucrose-rich ones. The ornithocentric hypothesis attempts to explain this dichotomy.

The ornithocentric hypothesis assumes that the sugar preferences of floral visitors act as a selective pressure on floral traits (Martínez del Río & Karasov 1990; Martínez del Río, Baker & Baker 1992). This hypothesis has two components: it predicts that bird species differ in sugar preferences, and that physiological differences among bird taxa should explain these preference differences. As predicted, bird species differ in both sugar preferences and in the ability to assimilate sucrose. Although most species tested can assimilate glucose and fructose efficiently, the ability to assimilate sucrose varies widely. A large monophyletic clade of birds that includes thrushes (Muscicapidae), starlings (Sturnidae) and mockingbirds (Mimidae) does not express sucrase, the intestinal enzyme responsible for sucrose hydrolysis (Martínez del Río & Stevens 1989; Martínez del Río 1990a; Martínez del Río & Karasov 1990). These birds not only prefer glucose and fructose over sucrose, but when fed on sucrose they develop an aversion for it. Some fruit-eating birds have intestinal sucrase activity, but their fast food passage rates seem to hinder the assimilation of a substrate such as sucrose that needs to be hydrolysed before it is absorbed (Afik & Karasov 1995). Finally, many groups of birds, including hummingbirds (Trochilidae), orioles (Icteridae), sunbirds (Nectarinidae) and honeyeaters (Meliphagidae), assimilate sucrose as efficiently as they assimilate glucose and fructose. When tested at a 20% concentration (weight/volume) many of them prefer sucrose and a few are indifferent (Lotz & Nicolson 1996; Jackson, Nicolson & Lotz 1998; Martínez del Río 1990a; Martínez del Río 1990b; Martínez del Río *et al.* 1992). Although the ornithocentric hypothesis has been quite successful at explaining why some birds prefer glucose and fructose over sucrose, it is incomplete. It has failed to provide a physiological mechanism that can account for the sucrose preference of many specialised nectar-feeding birds.

This study explores a potential explanation for this preference. We examined the hypothesis that sugar preferences in specialised nectar-feeding birds are concentration dependent. We predicted that at high sugar concentrations these birds would prefer sucrose over hexoses, whereas at low sugar concentrations they would prefer the hexoses glucose and fructose (Fig. 1). Following conventional thinking in foraging ecology, we assumed that preferences are shaped by relative energy assimilation rates. Briefly, we expected energy assimilation rates to be dependent on both sugar concentration and sugar type. Specifically, we predicted that the curves describing the relationship between volumetric intake and sugar concentration for sucrose

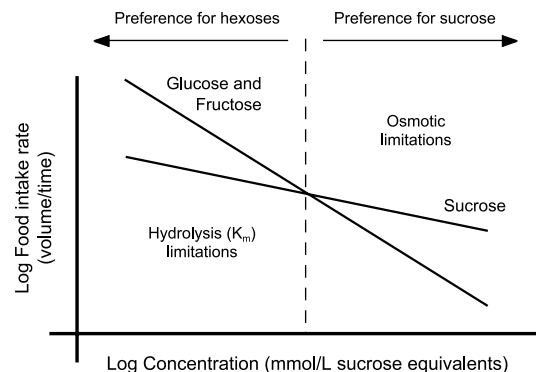


Fig. 1. We hypothesized that sugar concentration and composition interact to determine food intake rate. We predicted higher intake rates of 1 : 1 glucose-fructose mixtures when birds were offered solutions at low concentrations and higher intake rates of sucrose at high concentrations. A corollary of this hypothesis is that nectar-feeding birds would prefer the hexose mixture at low concentrations, but prefer sucrose at high concentrations. The justification for this hypothesis is described in text. We used log-log axes in this figure because the intake response of birds to sugar concentration is adequately described by power functions.

and a 1 : 1 mixture of glucose and fructose should cross. We invoked two complementary mechanisms to explain this hypothesised shift in sugar intake, and thus preferences with increased sugar concentrations. First, food intake, stomach emptying rate, and gut motility are negatively affected by the concentration of ingested food (Duke 1982; Carter & Grovum 1990; Savory 1999; Denbow 2000). Sucrose has twice the molecular weight of glucose and fructose, and hence produces half the osmotic concentration for a given amount of energy. Birds choosing between equicaloric solutions of sucrose and hexoses at high concentrations should prefer to ingest sucrose because its lower osmotic concentration will increase the speed at which they can deliver food from the crop/stomach to the intestine (Beuchat, Calder & Braun 1990). Secondly, at low concentrations the rate at which sucrose is hydrolysed may be limiting. The high sucrase K_m (Michaelis–Menten constant) values exhibited by hummingbirds (36.4 ± 5 mM) and orioles (21.2 ± 3.8 mM; Martínez del Río 1990a) suggest that their ability to digest sucrose will be reduced when feeding on low concentration nectars. Consequently, feeding on a glucose and fructose mixture may yield a higher intestinal sugar absorption rate when nectar is dilute (McWhorter & Martínez del Río 2000).

In this paper we examined the effect of sugar concentration and chemical identity on the sugar preferences of two species of New World nectar-feeding birds: the Cinnamon-bellied Flowerpiercer, *Diglossa baritula* Wagler (Thraupidae), and the Magnificent Hummingbird, *Eugenes fulgens* Swainson (Trochilidae). *Eugenes fulgens* is a pollinator of a variety of plant species, whereas *Diglossa baritula* is a specialised nectar robber of the same plant species at our study site in Mexico (Arizmendi, Domínguez & Dirzo 1996).

Materials and methods

BIRD CARE AND HOUSING

Birds were captured using mist-nets (10 individuals of *Diglossa baritula* and six individuals of *Eugenes fulgens*) in secondary vegetation areas of Las Joyas Research Station, Sierra de Manantlán Biosphere Reserve, Jalisco, Mexico. Birds were housed individually (60 × 30 × 60 cm cages) under ambient temperature and photoperiod (12 h light). Ambient temperature varied from a minimum of 7 °C (± 2 °C) in the early morning to a maximum of 22 °C (± 2 °C) at 14:00. During experiments, temperature varied from 16 °C (± 2 °C) to 20 °C (± 2 °C). Birds were allowed to acclimate to experimental cages for 2–3 days before the experiments began. Between experiments and during the acclimation period, birds were fed Roudybush Nectar 3 for adult hummingbirds (Roudybush, Templeton, CA, USA). Birds maintained body mass while in captivity (mean body mass (g) ± SD (n) for *D. baritula* = 7.74 ± 0.52 (10), and for *E. fulgens* = 7.26 ± 0.62 (6)). At the end of the experiments all birds were banded and released at the site of capture.

SUGAR PREFERENCES

To test the hypothesis that sugar selection is concentration-dependent, we presented individuals of *D. baritula* and *E. fulgens* with pairwise combinations of equicaloric solutions of sucrose and a 1 : 1 mixture of glucose and fructose at three concentrations: 146, 584 and 1168 mmol L⁻¹ sucrose equivalents. Note that we standardized the sugar concentration units to 'sucrose equivalents' to emphasise that the concentrations of hexoses and sucrose were equicaloric. In reality, the molarity of the hexose solutions was double that of the sucrose solutions. We presented the two sugar solutions simultaneously to each bird using glass feeding tubes. Both tubes were at the same distance (approximately 30 cm) from a single 'resting' perch and 15 cm apart from each other. Hummingbirds fed while hovering. Flowerpiercers fed while perching on a rod located in front of the feeders. The solutions were prepared with distilled water and reagent grade sugars (Sigma, St. Louis, Missouri, USA). The order in which the three concentrations were presented to each bird was determined randomly. Trials lasted five hours and were conducted from 07:00 to 12:00. After the trials we fed the birds maintenance diets for the rest of the day (12:00–19:00). To separate the effects of food position and food attributes on food selection we used an *ad libitum* feeding design suggested by Jackson, Nicolson & Lotz (1998). At the beginning of each trial the position of the tubes was randomised. Each bird was tested on each concentration during four consecutive days, and the position of the solutions was reversed every day. By comparing the individual preferences during the four days of trials, we were able to rule out

the existence of a position effect on sugar selection. At the end of each trial, consumption of solutions was measured to the nearest millimetre and then converted to volume units. Preference for sugar A over sugar B was calculated as the ratio of the consumption of sugar A divided by the total consumption (A + B). The interindividual variance in preference (i.e. the variance among individual means) for each concentration was used to construct a confidence interval to test the null hypothesis that preference was not significantly different from 0.5 (the indifference point where the consumption of both sugars tested equal) using one sample *t*-tests on arcsine square root transformed preference values (Sokal & Rohlf 1995). Magnitude of preference indexes were compared using paired *t*-tests. As an index of preference strength we used the absolute value of the difference between observed preferences and 0.5 (i.e. preference strength = $\left| \frac{\text{sucrose}}{\text{sucrose} + (\text{glucose} + \text{fructose})} - 0.5 \right|$). All experimental trials on the two species were run concurrently (i.e. using the same solutions).

SUGAR ABSORPTION

During sugar preference and intake response trials we collected the excreta from individuals of both species of birds. One hour after the trials were started we positioned a plastic sheet under the bird's perch and waited 30 min to collect excreta. Sugar concentration (in °BRIX) of excreta was measured with a hand-held refractometer (Reichert 10431 0–50 °Brix temperature compensated, Leica, Buffalo, NY, USA). Because solutes other than sugars bias refractometer readings (Inouye *et al.* 1980; Hiebert & Calder 1983), our measurements of sugar concentration in excreta were used only to assess relative differences between birds in this study.

INTAKE RESPONSE

Six birds of each species were placed in individual cages and fed one of two diets: sucrose or a 1 : 1 mixture of glucose and fructose. Diets varied in sugar concentration (292, 438, 584, 730, 876 and 1022 mmol L⁻¹ in sucrose equivalents). Each bird received each one of the six concentrations for the entire active phase of the day (12 h of light). Birds were assigned to concentrations using a 6 × 6 Latin Square design. Trials on the two species were run concurrently. At the end of the 12 h experimental period, we quantified the total amount of food ingested and removed the feeding tubes from the cages. Birds were fed maintenance diets for a day between trials. Intake response data were analysed using regression and analysis of covariance routines for Latin Square experimental designs (Kirk 1982). Because intake vs. concentration data are well described by power functions (Martínez del Río *et al.* 2001), regression analyses for intake responses were performed on log-transformed data.

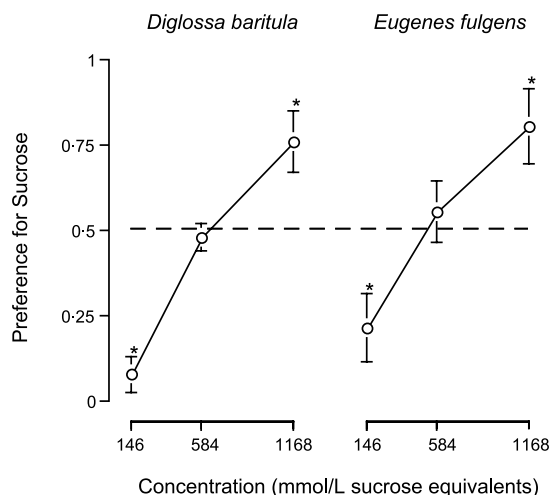


Fig. 2. Sugar preferences of *D. baritula* and *E. fulgens* as a function of sugar concentration. Preference was defined as the ratio of sucrose to the total amount of solutions consumed. At low concentrations (146 mmol L⁻¹ sucrose equivalents) both species preferred the 1 : 1 glucose and fructose mixture over sucrose, whereas at high concentrations (1168 mmol L⁻¹ sucrose equivalents) they preferred sucrose. At intermediate concentrations (584 mmol L⁻¹ sucrose equivalents) both species were indifferent to sugar composition. Groups marked with an asterisk (*) were statistically different from 0.5 (the indifference point where the consumption of both sugars tested equal). Values are means $\pm$ 95% confidence intervals.

Results

SUGAR PREFERENCES

Sugar preferences were concentration-dependent in *E. fulgens* and *D. baritula*. At low concentrations (146 mmol L⁻¹ sucrose equivalents) birds preferred glucose and fructose over sucrose ($t_9 = -12.18$, $P < 0.001$, $n = 10$ for *D. baritula* and $t_5 = -5.49$, $P = 0.003$, $n = 6$ for *E. fulgens*; Fig. 2). At intermediate concentrations (584 mmol L⁻¹ sucrose equivalents) they showed no preference ($t_9 = -0.37$, $P = 0.71$ for *D. baritula* and $t_5 = 1.54$, $P = 0.18$ for *E. fulgens*), and at high concentrations (1168 mmol L⁻¹ sucrose equivalents) they preferred sucrose over glucose and fructose ($t_9 = 6.27$, $P < 0.001$ for *D. baritula* and $t_5 = 5.67$, $P = 0.002$ for *E. fulgens*; Fig. 2). For intermediate and high sugar concentrations (584 and 1168 mmol L⁻¹ sucrose equivalents) the volume of hexose solution ingested was negatively correlated with the volume of sucrose solution ingested ($r = -0.96$, $P < 0.001$ at 584 mmol L⁻¹ sucrose equivalents and $r = -0.63$, $P = 0.02$ at 1168 mmol L⁻¹ sucrose equivalents for *D. baritula*, and $r = -0.95$, $P = 0.001$ at 584 mmol L⁻¹ sucrose equivalents and $r = -0.92$, $P = 0.008$ at 1168 mmol L⁻¹ sucrose equivalents for *E. fulgens*, Fig. 3). The slopes of these linear relationships did not differ significantly from -1 ($t_9 = -0.82$, $P = 0.43$ at 584 mmol L⁻¹ sucrose equivalents and $t_9 = 2.12$, $P = 0.08$ at 1168 mmol L⁻¹ sucrose equivalents for *D. baritula*; $t_5 =$

0.40, $P = 0.7$ at 584 mmol L⁻¹ sucrose equivalents and $t_5 = 1.57$, $P = 0.15$ at 1168 mmol L⁻¹ sucrose equivalents for *E. fulgens*) indicating that hexose and sucrose solutions were energetically equivalent at these concentrations. At low concentrations, however, there was no relationship between the volumetric ingestion of hexoses and that of sucrose ($r = -0.14$, $P = 0.56$ and $r = 0.25$, $P = 0.63$ for *D. baritula* and *E. fulgens*, respectively, Fig. 3).

In *D. baritula*, the preference for hexoses was significantly stronger at low concentrations than was the preference for sucrose at high concentrations ($t_9 = 4.74$, $P = 0.001$). In contrast, *E. fulgens* seemed to prefer hexoses at low and sucrose at high concentrations with the same strength ($t_5 = -0.053$, $P = 0.78$). It is noteworthy that at low concentrations, *D. baritula* appeared to prefer hexoses over sucrose more strongly than did *E. fulgens*. Mean sugar content in excreta was low (0.31 ± 0.31 °Brix, $n = 10$ for *D. baritula*, and 0.3 ± 0.32 °Brix, $n = 6$ for *E. fulgens*) and independent of sugar concentration ($r = 0.11$, $P = 0.54$ and $r = -0.09$, $P = 0.7$ for *D. baritula* and *E. fulgens*, respectively) and sugar preferences ($r = 0.23$, $P = 0.2$ and $r = -0.07$, $P = 0.79$ for *D. baritula* and *E. fulgens*, respectively).

EFFECT OF SUGAR COMPOSITION ON INTAKE RESPONSES

Because we found no significant effects of individual birds in our analyses, we dropped this term from our linear model. The relationship between volumetric intake and concentration was well described by power functions in both species, with volumetric intake decreasing as a function of concentration (Fig. 4). However, contrary to our predictions, the interaction between sugar type and concentration was not significant (Fig. 4). The intake responses for hexoses and sucrose had the same slope and hence did not cross as predicted by our model. Indeed, the slopes of the log-log relationship between volumetric intake and concentration were statistically indistinguishable ($F_{(\text{slope})1,68} = 0.05$, $P = 0.81$ and $F_{(\text{slope})1,68} = 1.09$, $P = 0.3$ for *D. baritula* and *E. fulgens*, respectively; Fig. 4). Unexpectedly, at all concentrations *D. baritula* individuals ingested about 10% more of the hexose solution than the sucrose solution ($F_{(\text{intercept})1,69} = 16.97$, $P < 0.0001$). The intake response experiments on *E. fulgens* did not show this sugar effect ($F_{(\text{intercept})1,69} = 1.15$, $P = 0.28$). At all concentrations, *E. fulgens* ingested the same amount of hexose and sucrose solutions.

Discussion

Our results demonstrate that sugar preferences of specialised nectar feeding birds are concentration-dependent. At lower concentrations birds preferred the hexose mixture, whereas at higher concentrations they shifted their preference to sucrose. However,

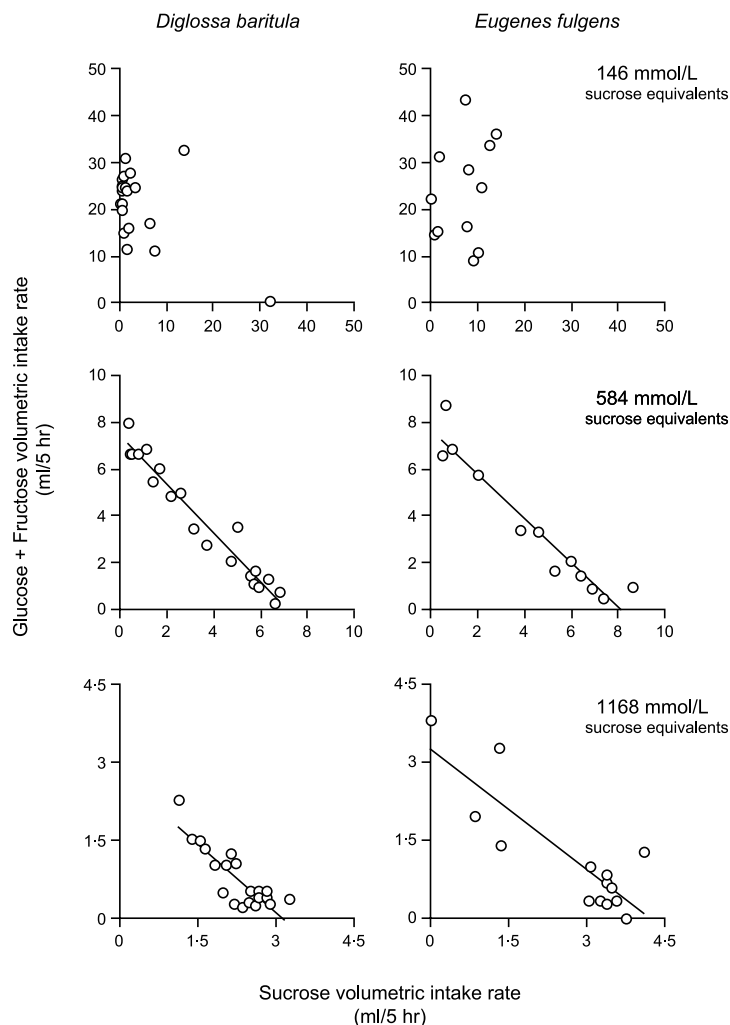


Fig. 3. At intermediate and high sugar concentrations (567 and 1168 mmol L⁻¹ sucrose equivalents; centre and lower panels) the volume of hexose solution ingested was negatively correlated with the volume of sucrose solution. The slopes of these linear relationships did not differ significantly from -1, indicating that hexoses and sucrose solutions were energetically equivalent. At low concentrations (146 mmol L⁻¹ sucrose equivalents; upper panels) there was no relationship between the volumetric ingestion of hexoses and that of sucrose because birds ingested very little of the sucrose solution.

contrary to our predictions, these concentration-dependent preferences were not matched by relative differences in intake. Although in both species volumetric intake declined with sugar concentration, the form of these intake responses was not that predicted *a priori*. At any given concentration, *E. fulgens* individuals ingested indistinguishable amounts of the hexose and sucrose solutions. At all concentrations, *D. baritula* individuals ingested more hexose than sucrose solution. Although our results on sugar preferences provide a potential explanation for the association between sucrose dominance and high concentration in floral nectars, they also pose a perplexing question: why do birds exhibit concentration-dependent preferences? In this discussion we first attempt to explain why our intake response results did not support our model (Fig. 1). Second, we compare the energetic value of both sugar solutions. Then, we discuss the difference in hexose and sucrose intake

exhibited by *D. baritula*. Finally we consider the ecological and evolutionary implications of concentration-dependent sugar selection by specialised nectar-feeding birds.

INTAKE RESPONSES AND CONCENTRATION-DEPENDENT SUGAR PREFERENCES

Dietary preferences are often explained using analogies from economics that assume that animals evaluate benefits in relation to costs (Hainsworth 1974; Gass & Montgomerie 1981; Collins, Grey & McNee 1990; Dunning 1990; Boggs 1992; Hainsworth & Hamill 1993; Guglielmo, Karasov & Jakubas 1996). A large theoretical literature with considerable empirical support suggests that animals should exhibit preferences for the items that offer them higher net benefits (gross benefits obtained minus costs; Huey & Pianka 1981; Heyneman 1983; Mangel & Clark 1986; Heinemann

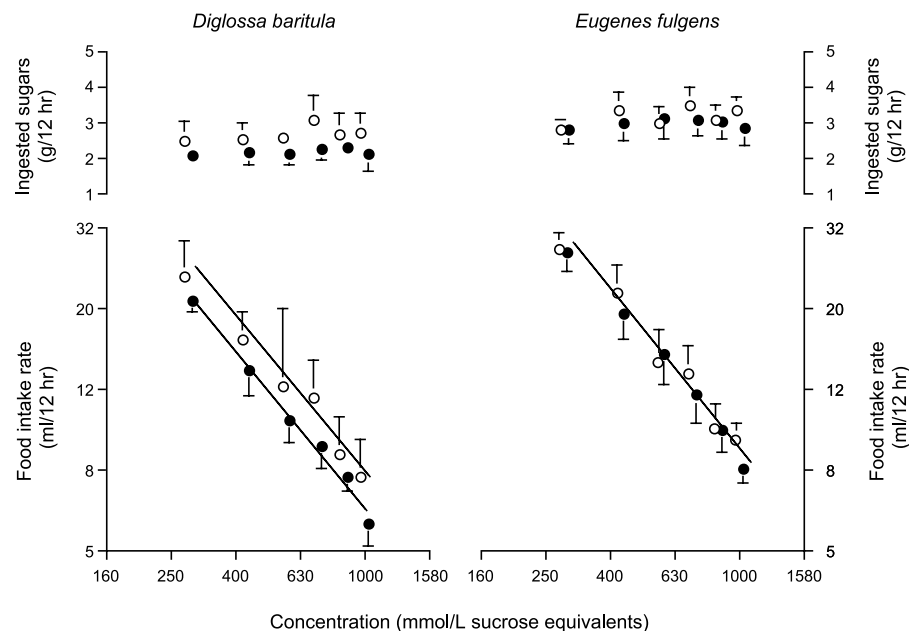


Fig. 4. Volumetric intake (lower panel) and sugar ingestion (upper panel) of sucrose and hexose solutions as function of sugar concentration. The relationship between volumetric intake of sucrose (●) and a 1 : 1 glucose-fructose mixture (○) and concentration (C) were adequately described by power functions for both species. The intakes responses for sucrose and the hexose mixture did not cross as predicted by our model (Fig. 1). Volumetric intakes of sucrose and hexose solutions were the same for *E. fulgens* (intake = $255.09 C^{-0.94}$), but differed for *D. baritula*. At all concentrations *D. baritula* ingested about 10% less sucrose (intake = $200.45 C^{-0.94}$) than hexoses (intake = $221.36 C^{-0.94}$). Values are means $\pm$ 95% intervals of confidence. Note that values for both axis in the lower panels are on logarithm scales.

1992; Karasov 1992; Houston 1995). Our hypothesis (Fig. 1) assumes that the costs of feeding on hexose and sucrose solutions at different concentrations are equal, and consequently that birds use sugar ingestion rate as their only decision-making currency while choosing between sugar solutions. Our model ignores potential concentration-dependent feeding/foraging costs that could explain the concentration-dependent shift in sugar preferences that we found. The mismatch between the food intake of birds to different sugars and their preferences indicate that our model is incorrect. This mismatch also suggests that foraging costs may be the missing component in potential explanations for concentration-dependent preferences in nectar-feeding birds.

Our experiments revealed that nectar composition had only a small effect on volumetric intake in *D. baritula*, and no effect in *E. fulgens*. The next question that must be asked is, does sugar composition have an effect on feeding frequency and hence on the cost of acquiring food? Because volumetric intake is the product of feeding frequency and meal size (Heyneman 1983; Tiebout 1991; Wolf, Hainsworth & Gill 1975), total feeding costs will depend on how sugar composition and concentration affects these variables. Exploring the interplay between sugar type, sugar concentration, and both foraging frequency and meal size can lead to a better understanding of the mechanisms that lead to concentration-dependent sugar selection in birds.

ARE DIFFERENT SUGARS ENERGETICALLY EQUIVALENT FOR NECTAR-FEEDING BIRDS?

When animals are given a choice between nutrients with different energy contents, they often maintain a constant energy intake by combining them in appropriate ratios (Simpson & Raubenheimer 1997; Raubenheimer & Simpson 1999). The results depicted in Fig. 3 are a good example of this phenomenon. In preference experiments, we found that the slopes of the regression lines of sucrose vs. hexoses intake were statistically indistinguishable from -1 for both *D. baritula* and *E. fulgens* when birds were offered 567 and 1168 mmol L⁻¹ sucrose equivalent solutions (Fig. 3). These results suggest that at these concentrations, nectar-feeding birds perceived sucrose and hexoses as energetically equivalent. Ingesting 1 mL of sucrose solutions reduced the consumption of an equicaloric solution of hexoses by 1 mL. At low concentrations (146 mmol L⁻¹ sucrose equivalents), however, there was no significant negative correlation between the intake of hexose and sucrose solutions. The birds clearly preferred the hexoses and appeared not to reduce hexose consumption even when ingesting significant amounts of sucrose solutions. At low concentrations, the ingestion of sucrose solutions did not seem to inhibit the ingestion of hexoses. Because birds appeared to assimilate sucrose efficiently at all concentrations, differences in sucrose digestion do not explain this outcome.

A comparison of Figs 3 and 4 reveals a perplexing inconsistency. In intake response trials, *D. baritula* individuals ingested approximately 10% more hexose than sucrose solution at all concentrations. However, in the 567 and 1168 mmol L⁻¹ sucrose equivalent concentration preference experiments, the lines relating hexose and sucrose volumetric intake had slopes that did not differ significantly from -1. Assuming a 10% difference in intake, the slope of these lines should have been approximately -1.1 (the *y* intercept should have been 10% higher than the *x* intercept). A possible explanation for this discrepancy is that the slope of the hexose against sucrose intake slope was indeed -1.1, but that this value was too close to -1 to distinguish them statistically. This is indeed possible for the 584 mmol L⁻¹ preference trials. The 95% confidence interval for the slope included -1.1 (slope $\pm$ 95% CI = 1.04 $\pm$ 0.12). However, for the 1168 mmol L⁻¹ trials, the confidence interval included -1 but did not include -1.1 (slope $\pm$ 95% CI = 0.72 $\pm$ 0.29).

WHY DID *D. BARITULA* INGEST GREATER VOLUMES OF THE HEXOSE THAN THE SUCROSE SOLUTION?

We found a significant effect of sugar composition on the intake response of *D. baritula* but not of *E. fulgens*. Although the effect was small, *D. baritula* ingested about 10% more hexose than sucrose solution at any given concentration. This difference between hummingbirds and flowerpiercers may be the result of differences in digestive function. Biochemical measurements of intestinal sucrase activity revealed that the hydrolytic capacity of *E. fulgens* (total intestinal sucrase activity = 9.9 μ mol min⁻¹) are about three times higher than those of *D. baritula* (total sucrase activity = 3.2 μ mol min⁻¹; J. E. Schondube unpubl. data). These measurements suggest that *D. baritula* individuals feeding on sucrose were ingesting an amount of sucrose ($\approx$ 2 g 12 h⁻¹) that was very close to their maximal predicted digestive capacity ($\approx$ 2 g 12 h⁻¹). In contrast, *E. fulgens* individuals appeared to have significant spare capacity to hydrolyse sucrose ($\approx$ 3 g 12 h⁻¹ ingested vs. a maximum predicted digestive capacity of $\approx$ 4 g 12 h⁻¹; see Martínez del Río *et al.* 2001 for the method used to estimate maximum hydrolytic capacity). Note that in spite of a three-fold difference in the capacity to hydrolyse sucrose, *E. fulgens* only ingested about 40% more sucrose than *D. baritula* (Fig. 4). Anecdotal evidence gathered during experiments supports the notion that flowerpiercers feeding on sucrose were hydrolysis-limited. Several *D. baritula* individuals entered torpor (defined as 'a state of inactivity and reduced responsiveness to external stimuli'; Blight & Johnson 1973) the morning after the day when they fed on dilute sucrose solutions (< 584 mmol L⁻¹ sucrose equivalents). In contrast, we never observed them enter torpor when feeding on glucose and fructose. Although *D. baritula* is a

specialised robber of hummingbird flowers (Arizmendi *et al.* 1996; Arizmendi 2001), its capacity to feed on sucrose-rich nectars appears not to match that of hummingbirds of similar body mass (J. E. Schondube unpubl. data).

ECOLOGICAL AND EVOLUTIONARY CONSEQUENCES OF CONCENTRATION-DEPENDENT SUGAR PREFERENCES

Nectar composition and concentration are often discussed as two different floral traits (Pyke & Waser 1981). Our results showing concentration-dependent preferences and Nicolson's (2002) phytocentric hypothesis suggest that these two variables may in fact be linked. Nicolson (2002) noted a relationship between sugar composition and sugar concentration in the nectar secreted by two bird-pollinated plant genera and posed a mechanistic ('proximate', *sensu* Mayr 1961) hypothesis to explain it. She hypothesised that hydrolysis of sucrose delivered by the phloem increases both the nectar's hexose content and its osmolality, causing water to move from the nectary walls into nectar. Thus, hexose-rich nectars should be copious and more dilute than sucrose-rich nectars. Our results suggest that sugar preferences in hummingbirds and flowerpiercers are concentration dependent. These birds preferred hexoses when food was dilute and sucrose when it was concentrated. Our observations link the ornithocentric evolutionary ('ultimate' *sensu* Mayr 1961) hypothesis with Nicolson's proximate hypothesis to explain the sugar composition of floral nectars.

Our results and Nicolson's (2002) hypothesis point to clear gaps in our understanding of bird sugar preferences, their interaction with nectar concentration, and the consequences of this interaction for the evolution of nectar traits in plants. On the plants' side of the interaction, Nicolson's (2002) phytocentric hypothesis reminds us that we must not assume that plants are passive subjects of the birds' needs and preferences. The effects of the potential selective pressure of birds on nectar composition and concentration are probably mediated by the mechanisms that plants use to secrete it. In spite of decades of descriptive research on nectar sugar concentration and composition, and in spite of abundant data on variation in these two traits, the physiological mechanisms that produce this variation remain obscure (Nicolson 2002 and references therein).

On the birds' side of the interaction, research on feeding behaviour has ignored the potentially complex interaction between sugar concentration and composition (see Nicolson 1998 and McWhorter & Martínez del Río 1999). Laboratory studies that control variables, such as sugar concentration, may have neglected their potential importance for feeding and sugar preference patterns. Consequently, our understanding of the effects of concentration and composition on bird feeding patterns and sugar preferences is still

rudimentary. Our data includes only two species in two of the several radiations of nectar-feeding birds. The generality of our results for other nectar-feeding species such as sunbirds (Nectariniidae), honeyeaters (Meliphagidae), Hawaiian honeycreepers (Drepanididae) and lorikeets (Psittacidae, Loriinae) is unknown.

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References

- Afik, D. & Karasov, W.H. (1995) The trade-offs between digestion rate and efficiency in warblers and their ecological implications. *Ecology* **76**, 2247–2257.
- Arizmendi, M.C. (2001) Multiple ecological interactions: nectar robbers and hummingbirds in a highland forest in Mexico. *Canadian Journal of Zoology* **79**, 997–1006.
- Arizmendi, M.C., Domínguez, C.A. & Dirzo, R. (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. *Functional Ecology* **10**, 119–127.
- Baker, H.G. (1975) Sugar concentrations in nectars from hummingbird flowers. *Biotropica* **7**, 37–41.
- Baker, H.G. & Baker, I. (1983) Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. *Biochemical Aspects of Evolutionary Biology* (ed. M.H. Niteki), pp. 131–171. The University of Chicago Press, Chicago.
- Baker, H.G., Baker, I. & Hodges, S.A. (1998) Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* **30**, 559–586.
- Beuchat, C.A., Calder, W.A. III & Braun, E.J. (1990) The integration of osmoregulation and energy balance in hummingbirds. *Physiological Zoology* **63**, 1059–1081.
- Blight, J. & Johnson, K.G. (1973) Glossary of terms for thermal physiology. *Journal of Applied Physiology* **35**, 941–961.
- Boggs, C.L. (1992) Resource allocation: exploring connections between foraging and life history. *Functional Ecology* **6**, 508–518.
- Carter, R.R. & Grovum, W.L. (1990) Factors affecting the voluntary intake of food by sheep 5. The inhibitory effect of hypertonicity in the rumen. *British Journal of Nutrition* **64**, 285–299.
- Collins, B.G., Grey, J. & McNee, S. (1990) Foraging and nectar use in nectarivorous bird communities. *Studies in Avian Biology* **13**, 110–121.
- Denbow, D.M. (2000) Gastrointestinal anatomy and physiology. *Sturkie's Avian Physiology* (ed. G.C. Whittow), pp. 299–325. Academic Press, London.
- Duke, G.E. (1982) Gastrointestinal motility and its regulation. *Poultry Science* **61**, 1245–1256.
- Dunning, J.B. Jr (1990) Meeting the assumptions of foraging models: an example using tests of avian patch choice. *Studies in Avian Biology* **13**, 462–470.
- Gass, C.L. & Montgomerie, R.D. (1981) Hummingbird foraging behavior: decision making and energy regulation. *Foraging Behavior: Ecological, Ethological and Physiological Approaches* (eds A.C. Kamil & T.D. Sargent), pp. 159–199. Garland STPM, New York.
- Guglielmo, C.G., Karasov, W.H. & Jakubas, W.J. (1996) Nutritional costs of a plant secondary metabolite explain selective foraging by ruffed grouse. *Ecology* **77**, 1103–1115.
- Hainsworth, F.R. (1974) Food quality and foraging efficiency. *Journal of Comparative Physiology* **88**, 425–431.
- Hainsworth, F.R. & Hamill, T. (1993) Foraging rules for nectar: food choices by painted ladies. *American Naturalist* **142**, 857–863.
- Heinemann, D. (1992) Resource use, energetic profitability, and behavioral decisions in migrant rufous hummingbirds. *Oecologia* **90**, 137–149.
- Heyneman, A.J. (1983) Optimal sugar concentrations of floral nectars dependence on sugar intake efficiency and foraging costs. *Oecologia* **60**, 198–213.
- Hiebert, S.M. & Calder, W.A. III (1983) Sodium, potassium, and chloride in floral nectars: energy-free contributions to refractive index and salt balance. *Ecology* **64**, 399–402.
- Houston, A.I. (1995) Energetic constraints and foraging efficiency. *Behavioral Ecology* **6**, 393–396.
- Huey, R.B. & Pianka, E.R. (1981) Ecological consequences of foraging mode. *Ecology* **62**, 991–999.
- Inouye, D.W., Favre, N.D., Lanum, J.A., Levine, D.M., Meyers, J.B., Roberts, M.S., Tsao, F.C. & Wang, Y.-Y. (1980) The effects of nonsugar nectar constituents on estimates of nectar energy content. *Ecology* **61**, 992–996.
- Jackson, S., Nicolson, S.W. & Lotz, C.N. (1998) Sugar preferences and 'side bias' in Cape Sugarbirds and Lesser Double-collared Sunbirds. *Auk* **115**, 156–165.
- Karasov, W.H. (1992) Daily energy-expenditure and the cost of activity in mammals. *American Zoologist* **32**, 238–248.
- Kirk, R.E. (1982) *Experimental Design: Procedures for the Behavioral Sciences*. Brooks/Cole Publishing Co, Belmont, California.
- Lotz, C.N. & Nicolson, S.W. (1996) Sugar preferences of a nectarivorous passerine bird, the lesser double-collared sunbird (*Nectarinia chalybea*). *Functional Ecology* **10**, 360–365.
- Mangel, M. & Clark, C.W. (1986) Towards a unified foraging theory. *Ecology* **67**, 1127–1138.
- Martínez del Río, C. (1990a) Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. *Physiological Zoology* **63**, 987–1011.
- Martínez del Río, C. (1990b) Sugar preferences in hummingbirds: the influence of subtle chemical differences on food choice. *Condor* **92**, 1022–1030.
- Martínez del Río, C., Baker, H.G. & Baker, I. (1992) Ecological and evolutionary implications of digestive processes: bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia* **48**, 544–551.
- Martínez del Río, C. & Karasov, W.H. (1990) Digestion strategies in nectar- and fruit-eating birds and the sugar

- composition of plant rewards. *American Naturalist* **136**, 618–637.
- Martínez del Río, C., Schondube, J.E., McWhorter, T.J. & Herrera, L.G. (2001) Intake responses in nectar feeding birds: Digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *American Zoologist* **41**, 902–915.
- Martínez del Río, C. & Stevens, B.R. (1989) Physiological constraint on feeding behavior: intestinal membrane disaccharides of the starling. *Science* **243**, 794–796.
- Mayr, E. (1961) Cause and effect in biology. *Science* **134**, 1501–1506.
- McWhorter, T.J. & Martínez del Río, C. (1999) Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? *Journal of Experimental Biology* **202**, 2851–2858.
- McWhorter, T.J. & Martínez del Río, C. (2000) Does gut function limit hummingbird food intake? *Physiological and Biochemical Zoology* **73**, 313–324.
- Nicolson, S.W. (1998) The importance of osmosis in nectar secretion and its consumption by insects. *American Zoologist* **38**, 418–425.
- Nicolson, S.W. (2002) Pollination by passerine birds: why are the nectars so dilute. *Comparative Biochemistry and Physiology B* **131**, 645–652.
- Pyke, G.H. & Waser, N.M. (1981) The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* **13**, 260–270.
- Raubenheimer, D. & Simpson, S.J. (1999) Integrating nutrition: a geometrical approach. *Entomologia Experimentalis et Applicata* **91**, 67–82.
- Savory, C.J. (1999) Temporal control of feeding behaviour and its association with gastrointestinal function. *Journal of Experimental Zoology* **283**, 339–347.
- Simpson, S.J. & Raubenheimer, D. (1997) Geometric analysis of macronutrient selection in the rat. *Appetite* **28**, 201–213.
- Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*. W.H. Freeman, New York.
- Stiles, F.G. & Freeman, C.E. (1993) Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica* **25**, 191–205.
- Tiebout, H.M. III (1991) Daytime energy management by tropical hummingbirds: responses to foraging constraint. *Ecology* **72**, 839–851.
- Wolf, L.L., Hainsworth, F.R. & Gill, F.B. (1975) Foraging efficiencies and time budgets in nectar-feeding birds. *Ecology* **1**, 117–128.

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