Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird

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Summary

1. The nectars of hummingbird- and passerine-pollinated plants differ in both sugar type and concentration. Firstly, hummingbird nectars tend to be more concentrated than passerine nectars. Secondly, sucrose dominates hummingbird nectars, whereas glucose and fructose (hexose sugars) are the prevalent sugars in nectar of almost half the passerine-pollinated plants. It has been commonly assumed that these differences can be attributed to selection pressure from birds, largely determined by avian sugar preferences and digestive physiology.

2. Intake and preferences of Whitebellied Sunbirds *Nectarinia talatala* (A. Smith) and Broadtailed Hummingbirds *Selasphorus platycercus* (Swainson) were examined across a range of sucrose and equicaloric hexose solutions at two ambient temperatures. Hummingbird energy balance was not affected by sugar type. Sunbird energy balance was only influenced by sugar type on a very dilute diet ($0{\cdot}1$ mol l^{-1} sucrose equivalents, 'SE'), when the birds ingested 12% more sucrose than hexoses. Sunbirds and hummingbirds showed similar patterns in sugar preferences. Sunbirds preferred hexoses when offered dilute diets ($0{\cdot}1$ mol l^{-1} SE at 21 °C) and showed slight preference for sucrose when offered more concentrated diets (significant at $0{\cdot}75$ mol l^{-1} SE). Hummingbirds showed slight (non-significant) hexose preference when offered a dilute diet ($0{\cdot}25$ mol l^{-1} SE at 10 °C), but, in contrast to previous findings, hummingbirds showed no significant sucrose preference.

3. Our findings for Whitebellied Sunbirds and Broadtailed Hummingbirds do not support an ornithocentric explanation for nectar composition. Plant physiology and opportunist nectar feeders may also be influencing nectar sugars. To further address this question we discuss methodological considerations for researchers investigating how avian preferences and physiology may affect nectar composition.

Key-words: Digestive constraint, nectar sugars, plant–pollinator coevolution, sucrose hydrolysis

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Introduction

Why plants pollinated by different orders of birds produce nectars with different concentrations and composition has intrigued pollination ecologists. The nectars of hummingbird- and passerine-pollinated plants (hereafter termed hummingbird and passerine or sunbird nectars) differ in both concentration and predominant sugar type (Baker & Baker 1983; Baker, Baker & Hodges 1998; reviewed by Nicolson & Fleming 2003b). Almost half (47%) of 259 sunbird-pollinated species produce hexose-dominated nectar with <10% of the sugar present in the form of sucrose, while almost a quarter (23%) produce sucrose-dominated nectars with

>80% sucrose (Nicolson & Fleming 2003b). The sucrose–hexose dichotomy can even be distinguished within genera (Barnes, Nicolson & van Wyk 1995; Nicolson & van Wyk 1998). This pattern contrasts with the unimodal data for hummingbird nectars, where an average of 64 ± 19 SD% of sugar is present as sucrose, and only 2% of hummingbird-pollinated plant species produce hexose-dominant nectar (<10% sucrose, Nicolson & Fleming 2003b).

'Conventional ecological wisdom' suggests an ornithocentric explanation for this dichotomy, where differences in nectar composition reflect past selective pressures resulting from the sugar preferences and physiological abilities of bird pollinators (Martínez del Rio, Baker & Baker 1992; Schondube & Martínez del Rio 2003). Firstly, it has been assumed that nectar concentration reflects bird concentration preferences,

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although laboratory studies indicate that both hummingbirds and sunbirds prefer solutions that are more concentrated than natural nectars (Pyke & Waser 1981; Tamm & Gass 1986; Roberts 1996; Nicolson & Fleming 2003a). Secondly, it has also been assumed that hummingbird nectars contain more sucrose than passerine nectars due to sucrose preference in hummingbirds and hexose preference or sucrose avoidance in passerines (e.g. Martínez del Rio *et al*. 1992). Despite the frequent hexose-dominance of passerine nectars, sucrose preference has also been documented in passerines (Lotz & Nicolson 1996; Jackson, Nicolson & van Wyk 1998b; Schondube & Martínez del Rio 2003).

The disaccharide sucrose must be broken down (hydrolysed) to its hexose components, glucose and fructose, before absorption; this is accomplished by the intestinal enzyme sucrase. Sucrose hydrolysis rate in the small intestine has previously been suggested to limit sugar assimilation rate, thereby affecting bird preferences (Martínez del Rio *et al*. 1992; McWhorter & Martínez del Rio 2000; Martínez del Rio *et al*. 2001). Hexose absorption rates may also limit feeding rate (Karasov 1996). Little comparative data are available for the rates of digestion and absorption of different sugar types in hummingbirds and sunbirds, but both show equally high assimilation efficiencies for sucrose, glucose and fructose (Hainsworth 1974; Martínez del Rio 1990b; Lotz & Nicolson 1996; and summary table in Jackson *et al*. 1998b).

Given the similarities in sugar preferences and overall assimilation efficiencies between hummingbirds and passerines, why is nectar composition different? A potential explanation lies in the methodology of previous intake and preference studies, which have been conducted under mild conditions (ambient temperatures T_a 18–22 °C) using average nectar concentrations (around 20% w/w or ~0·64 mol l^{-1} SE) (Hainsworth & Wolf 1976; Pyke & Waser 1981; Martínez del Rio 1990b; Lotz & Nicolson 1996; Jackson, Nicolson & Lotz 1998a). These conditions are not physiologically demanding and subtle differences in digestive physiology are unlikely to influence assimilation rates. Acute exposure to cold increases metabolic costs because of immediate increases in thermogenic demands (Dawson & O'Conner 1996). Also, when birds are physiologically challenged by lower nectar concentrations, especially when energy demands are high, their preferences for different nectar sugars may change. Of the avian preference studies conducted to date, few have considered more than one sugar concentration (Stiles 1976; Schondube & Martínez del Rio 2003) and the concentrations chosen (varying from 15 to 30% among studies) have always been at the high end of the range of nectar concentrations exploited by wild birds. When fed extremely dilute nectars, sunbirds and hummingbirds cannot increase intake enough to maintain energy balance. Both taxa consume up to five times their body mass per day on dilute diets (Lotz 1999; McWhorter & Martínez del Rio 1999; Nicolson & Fleming 2003a), and yet still fail

to maintain energy balance (Lloyd 1991; Lotz 1999; Nicolson & Fleming 2003a; C. A. Beuchat, personal communication).

We examined sugar preferences and daily energy intake in two unrelated nectar-feeding birds, Whitebellied Sunbirds *Nectarinia talatala* (A. Smith) (Nectariniidae) and Broadtailed Hummingbirds *Selasphorus platycercus* (Swainson) (Trochilidae), over a range of sugar concentrations and at two ambient temperatures. Whitebellied Sunbirds are resident in southern Africa; Broadtailed Hummingbirds breed at high elevations in the central Rocky Mountains of the United States and overwinter in Mexico.

In order to determine if sucrose hydrolysis *alone* limits digestive function, we examined energy intake on sucrose and hexose solutions under both mild and physiologically demanding conditions. Consuming hexoses rather than sucrose would save the delay involved in sucrose hydrolysis (Martínez del Rio, Karasov & Levey 1989), and enable immediate absorption of hexose sugars. Birds would then increase hexose intake, but not sucrose intake, in response to physiologically demanding circumstances. We also assessed sugar preferences of these two species in order to determine whether the Broadtailed Hummingbirds prefer sucrose and Whitebellied Sunbirds prefer hexoses, as predicted by the sucrose and hexose dominance of hummingbird and sunbird nectars, respectively.

Materials and methods

BIRD CAPTURE AND MAINTENANCE

Seven male Whitebellied Sunbirds (mean mass = 9·27 ± 0·98 SD g) and eight male Broadtailed Hummingbirds (3·17 ± 0·30 g) were mist-netted in Pretoria, South Africa, and Albany County, Wyoming, USA, respectively. Birds were housed in individual cages (sunbirds: 27 × 31 × 21 cm; hummingbirds: 61 × 61 × 61 cm) in constant environment rooms maintained at 21 ± 1 °C on a 13L:11D photoperiod.

While in captivity, sunbirds were fed a maintenance diet of 0·6 mol l^{-1} (20% w/w) sucrose and a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa). Hummingbirds were maintained on a diet of 13% (w/w) Nektar-Plus® (Günter Enderle, Pforzheim, Germany) and a 4% (w/w) vitamin supplement (Nekton-S®, Günter Enderle). Maintenance diets were provided *ad libitum* in inverted, stoppered syringes. Feeding necessitated hovering by hummingbirds. Energy intake and preference trials were conducted during March–August 2002. Differences in experimental protocols (i.e. experimental diets and trial schedule) were the consequence of species' tolerances.

ENERGY INTAKE

Experimental diets for analysis of energy intake were 'dilute' (0·1 mol l^{-1} SE: *sunbirds* or 0·25 mol l SE^{-1}:

hummingbirds) and 'moderate' (1 mol l^{-1} SE) sucrose and equivalent glucose–fructose (1 : 1, 'hexose') solutions. Hexose solutions have twice the total molarity of the sucrose solutions, but values are given throughout for the sucrose equivalent solution ('SE'). The dilute experimental diet for hummingbirds was designated as 0·25 mol l^{-1} SE because they were unable to cope on 0·1 mol l^{-1} SE solutions (entered torpor during the photophase; B. Hartman Bakken, unpublished observations). Energy intake was examined at 10 and 21 °C and birds were weighed pre- and post-trial (±0·001 g). Temperature was reduced for the acclimation and test day for sunbirds, and on the test day only for hummingbirds (the 15 min while weighing the birds at the commencement of each trial was sufficient for the environmental chambers to drop to 10 °C from the initial 21 °C). Energy intake was measured over 24 h and is expressed in kJ g^{-1} day^{-1} (5648 kJ mol^{-1} sucrose or half this for each mole of hexose sugar). Following the completion of a trial, the maintenance diet was returned and T_a was reset at 21 °C if altered. Sugar concentration, T_a and sugar type were randomized for each bird. Sunbirds were allowed to adjust to their experimental diet for 24 h before a trial. Preliminary experiments suggested that this was unnecessary in hummingbirds: when birds were switched to carbohydrate-only diets there was no difference in intake between the first day and subsequent days. Sunbirds spent 3 days between energy intake trials; for hummingbirds, energy intake trials were conducted every second day.

SUGAR PREFERENCES

Sugar preferences were examined at 10 °C (*hummingbirds only*) and 21 °C. For each preference trial, birds were simultaneously offered matched pairs of sucrose and energetically equivalent hexose solutions of 0·1 (*sunbirds only*), 0·25, 0·5, 0·75 and 1 mol l^{-1} SE. Preference trials were 6 h in duration; to avoid side biases (Jackson *et al.* 1998a), feeders were switched mid-way through each trial. Sugar preference indices are expressed as the ratio of hexose consumption to total sugar consumption.

Sunbirds received each sugar pair twice (total of 10 trials), in random order, with the starting order reversed on the second trial of each pair since sunbirds have been shown to vary feeding with time of day (Lotz 1999; Fleming, Gray & Nicolson 2004) and side bias could confound results (data were then averaged). Sunbird preference trials started at the same time of day (07.00–07.30 h). For the eight hummingbird preference trials, start time (07.30–12.30 h), initial feeder side (left *vs* right), concentration and T_a were randomized for each bird. Feeding was not affected by time of day (repeated-measures analysis of variance 'RM-ANOVA': $F_{1,7} = 0.95$, $P = 0.335$) in hummingbirds. Following each trial, birds were allowed to feed on the maintenance diet *ad libitum*; for hummingbirds, T_a was restored to 21 °C when necessary. For sunbirds, preference trials

were conducted every second day; for hummingbirds there was no maintenance day between trials.

STATISTICAL ANALYSES

For energy intake trials, volumetric consumption, energy intake, and mass change (surrogate measure of energy balance) were analysed by three-way RM-ANOVA followed by Tukey Honest Significant Difference ('Tukey') tests. Concentration, T_a and sugar type served as independent variables. Sugar preferences were analysed using one-way RM-ANOVA followed by Tukey tests. Sugar preferences at each concentration were determined by comparing the arcsine-transformed square root of preference indices (Zar 1996) against 0·5 (no preference) by one-sample *t*-test (Sokal & Rohlf 1995). Data are reported as means ± 1 SD throughout.

Results

ENERGY INTAKE

There was no significant difference in the energy balance (body mass) of either Whitebellied Sunbirds or Broadtailed Hummingbirds fed sucrose or hexose diets. The only effect of sugar type was that Whitebellied Sunbirds consumed more sucrose than hexose on the dilute diets. Acute exposure to low temperature resulted in increased energy intake in birds fed a moderate diet (1 mol l^{-1} SE), but none in those fed dilute diets (0·1 and 0·25 mol l^{-1} SE for sunbirds and hummingbirds, respectively). Both species lost mass when fed dilute solutions. The responses of the two species are discussed in detail below.

Whitebellied Sunbirds

Volumetric food consumption (ml g^{-1} day^{-1}) was affected by concentration and sugar type, but not by T_a (Fig. 1a; concentration: $F_{1,6} = 256.12$, $P < 0.001$; T_a: $F_{1,6} = 0.88$, $P = 0.385$; sugar: $F_{1,6} = 7.86$, $P = 0.031$). Sunbirds consumed 5·9 times the volume of dilute solutions (0·1 mol l^{-1} SE) compared with the moderate (1 mol l^{-1} SE) diet (Tukey, $P < 0.001$). On the dilute diet, they also consumed 11·7% more sucrose than hexose (Tukey, $P = 0.042$).

However, a different pattern emerged when energy intake (kJ g^{-1} day^{-1}) was analysed. Birds consumed more of the moderate diet and consequently had a greater energy intake, whereas T_a and sugar type influences on energy intake were not significant (Fig. 1b; concentration: $F_{1,6} = 292.80$, $P < 0.001$; T_a: $F_{1,6} = 5.37$, $P = 0.060$; sugar: $F_{1,6} = 5.23$, $P = 0.062$). Decreased T_a resulted in only a 1% increase in energy intake on the 0·1 mol l^{-1} SE diets (Tukey, $P = 0.998$) and a 18% increase on 1 mol l^{-1} SE diets (Tukey, $P = 0.056$) in response to the increased thermogenic costs.

There was no difference in mass change (%) for sunbirds fed either sucrose or hexose sugars (Fig. 1c, sugar: $F_{1,6} = 0.12$, $P = 0.736$). Mass losses were significantly
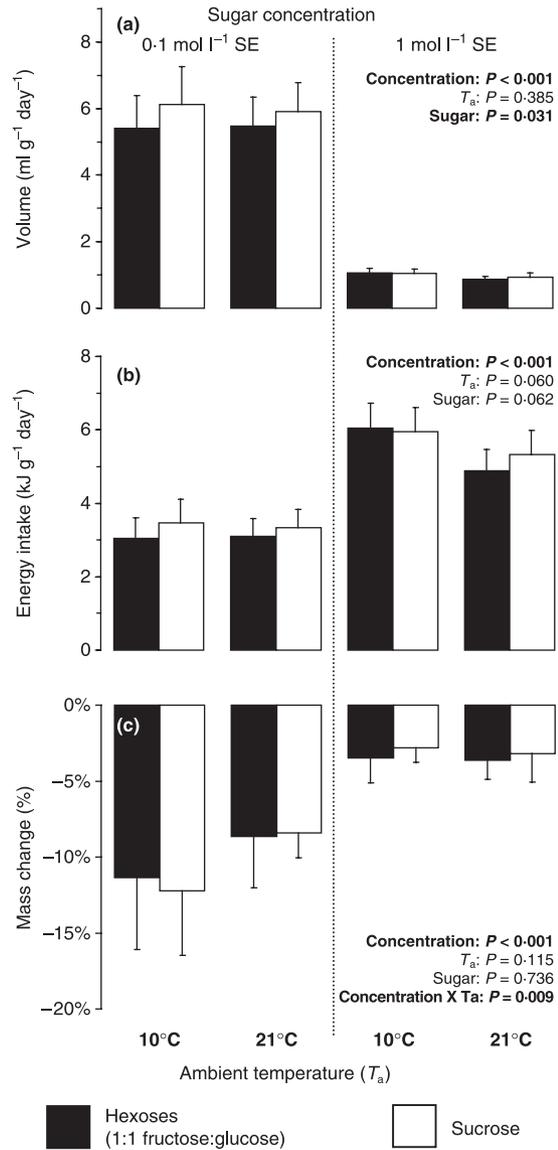
Fig. 1. Volumetric consumption (a), energy intake (b) and change in body mass (c) for Whitebellied Sunbirds *Nectarinia talatala* (*n* = 7) fed two concentrations (sucrose equivalent 'SE') of sucrose (open bars) and hexose (black bars) at 10 and 21 °C. Values are means ± 1 SD. *P*-values are for the results of repeated-measures ANOVA.
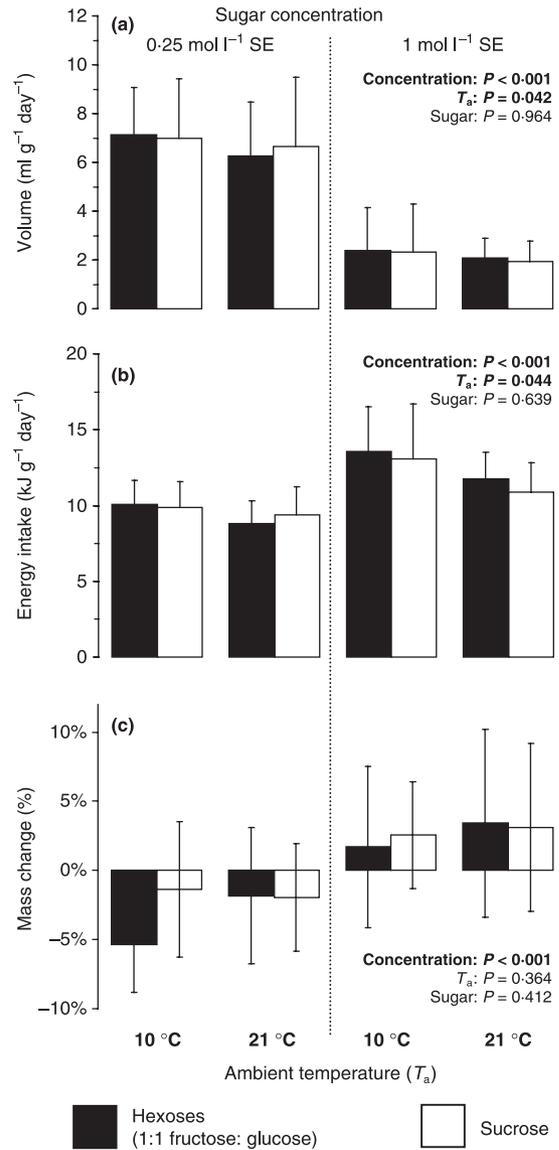
Fig. 2. Volumetric consumption (a), energy intake (b) and change in body mass (c) for Broadtailed Hummingbirds *Selasphorus platycercus* (*n* = 8) fed two concentrations (sucrose equivalent 'SE') of sucrose (open bars) and hexose (black bars) at 10 and 21 °C. Values are means ± 1 SD. *P*-values are for the results of repeated-measures ANOVA.

affected by concentration (concentration: $F_{1,6} = 251 \cdot 03$, $P < 0 \cdot 001$): on $0 \cdot 1$ mol l^{-1} SE diets, sunbirds lost about $10 \cdot 2\%$ of their initial mass compared with only $3 \cdot 1\%$ on 1 mol l^{-1} SE diets (Tukey, $P < 0 \cdot 001$). Although the effect of T_a was not significant (T_a: $F_{1,6} = 3 \cdot 39$, $P = 0 \cdot 115$), a significant interaction term between T_a and concentration reflected the poor ability of sunbirds to cope with both dilute diet and low temperature (interaction concentration × T_a: $F_{1,6} = 14 \cdot 57$, $P = 0 \cdot 009$).

Broadtailed Hummingbirds

For hummingbirds, sugar type exerted no influence over volumetric consumption (ml g^{-1} day^{-1}), whereas volumes of diet consumed were significantly influenced by concentration and T_a (Fig. 2a; concentration: $F_{1,7} =$ $833 \cdot 62$, $P < 0 \cdot 001$; T_a: $F_{1,7} = 6 \cdot 13$, $P = 0 \cdot 042$; sugar: $F_{1,7} = 0 \cdot 00$, $P = 0 \cdot 964$). Hummingbirds consumed approximately three times the volume of dilute diet ($0 \cdot 25$ mol l^{-1} SE) relative to the moderate diet (1 mol l^{-1} SE, Tukey, $P < 0 \cdot 001$).

When energy intake (kJ g^{-1} day^{-1}) was analysed, the relationship was consistent with volumetric intakes: concentration and T_a significantly affected energy intake, but there was no effect of sugar type (Fig. 2b; concentration: $F_{1,7} = 45 \cdot 80$, $P < 0 \cdot 001$; T_a: $F_{1,7} = 6 \cdot 01$, $P = 0 \cdot 044$; sugar: $F_{1,7} = 0 \cdot 24$, $P = 0 \cdot 639$). At 10 °C, hummingbirds increased energy intake by an average of 9% on the dilute diet (Tukey, $P = 0 \cdot 784$) and 17% on the 1 mol l^{-1} SE diets (Tukey, $P = 0 \cdot 227$) (these values are not significant at $P < 0 \cdot 05$ since multiple *post-hoc* tests do not have the power of the main analysis).

The ability of hummingbirds to maintain body mass was significantly affected by concentration, but not T_a and sugar type (Fig. 2c; concentration: $F_{1,7} = 14.25$, $P < 0.001$; T_a: $F_{1,7} = 0.94$, $P = 0.364$; sugar: $F_{1,7} = 0.76$, $P = 0.412$). When fed moderate solutions hummingbirds gained an average of 2.7% of initial mass, while on dilute solutions they lost an average of 2.6% of their initial mass (Tukey, $P = 0.007$).

SUGAR PREFERENCES

For sunbirds, there was a significant effect of diet concentration on sugar preference (Fig. 3a, $F_{4,20} = 8.02$, $P < 0.001$). Sunbirds offered 0.1 mol l^{-1} SE solutions showed a strong preference for hexose, consuming about five times more of this than the sucrose solution (one-sample t-test: $t_5 = 3.07$, $P < 0.05$). For solutions ≥ 0.25 mol l^{-1} SE, sunbirds drank nearly the same volumes of sucrose and hexose. A slight preference for sucrose solutions was significant only at 0.75 mol l^{-1} SE (one-sample t-test: $t_5 = -2.60$, $P < 0.05$).

Unlike sunbirds, hummingbirds showed no significant sugar preferences at 21 °C (Fig. 3b, $F_{3,21} = 0.12$, $P = 0.945$). Their slight preference for hexoses was not significant. However when challenged by reduced T_a, hummingbirds showed a significant effect of concentration on sugar preference ($F_{3,21} = 7.40$, $P = 0.001$). Slight (non-significant) hexose preference was found at 0.25 mol l^{-1} SE (one-sample t-test: $t_7 = 1.85$, $P < 0.20$).

Discussion

To understand how our experimental findings relate to the differences between hummingbird and sunbird nectars, we first discuss how physiology may affect behavioural preferences. We then examine how these preferences may be tied to the patterns of nectar concentration and composition observed in the field. We also discuss methodological considerations for researchers investigating intake and preferences of bird species that will bring more clarity to our understanding of plant–bird coevolution.

CONCENTRATION AND TEMPERATURE EFFECTS ON SUGAR INTAKE

This study does not support the hypothesis that sucrose hydrolysis *alone* constrains the energy intake of Whitebellied Sunbirds or Broadtailed Hummingbirds. If this were so, we would have expected birds, under physiologically stressful conditions, to increase their intake of hexose compared with sucrose solutions or show improved ability to maintain energy balance (body mass) on hexoses. There is ample evidence in the literature that indicates that both sucrase and glucose transporter capacities may be coadjusted in response to dietary load of sucrose (Weiss, Lee & Diamond 1998).

In this study, diet dilution (and resulting preformed water load) proved to be more limiting than the rate of sucrose hydrolysis in restricting intake. Diet dilution was clearly the primary factor affecting energy balance in both Whitebellied Sunbirds and Broadtailed Hummingbirds: both species lost considerable body mass on the dilute diets. Physiological limitations to drinking dilute solutions may occur for a number of reasons. Limitations on the volume of water that the kidneys are able to process daily (McWhorter & Martínez del Rio 1999; Nicolson & Fleming 2003a), possible electrolyte losses (Fleming & Nicolson 2003) and dramatically increasing costs of warming food to body
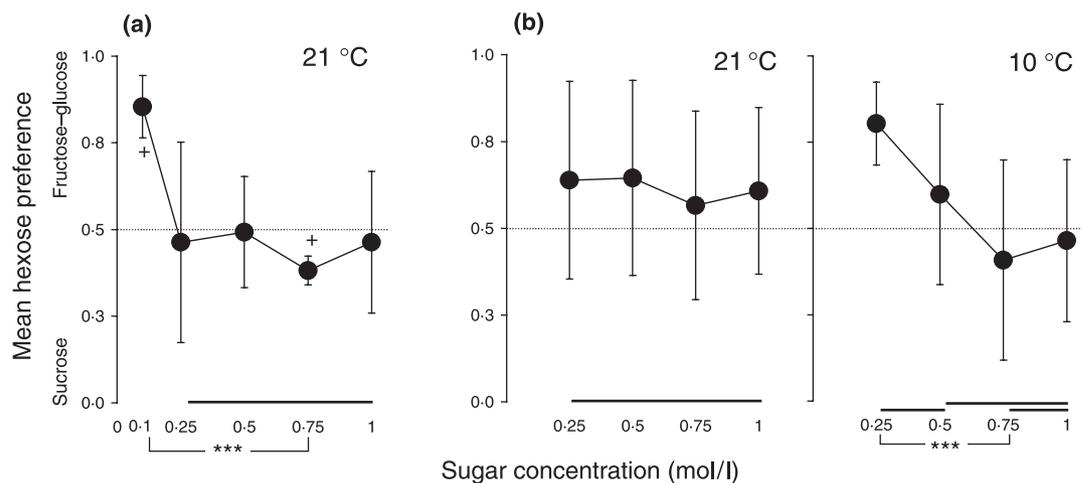

Fig. 3. Concentration-dependent sugar preferences of (a) Whitebellied Sunbirds and (b) Broadtailed Hummingbirds. Birds were offered pairs of sucrose and hexose (fructose/glucose) solutions of varying concentrations: 0.1 (3.6% w/w, *sunbirds only*), 0.25 (8.3%), 0.5 (15.8%), 0.75 (23.1%) and 1 (30%) mol l^{-1} SE. Hummingbirds resorted to diurnal torpor on the most dilute solutions (0.1 mol l^{-1}) and so were challenged by testing their preferences at 21 and 10 °C. Values are means ± 1 SD. Bold lines link concentrations that were not significantly different (RM-ANOVA followed by Tukey tests; different groups indicated by ***$P \leq 0.001$). + indicates concentrations for which a distinct sugar preference was exhibited (i.e. hexose preference at low concentrations, sucrose preference at higher concentrations, one-sample t-tests: + $P < 0.05$).

temperature (Lotz & Nicolson 2002; Lotz, Martínez del Rio & Nicolson 2003) may occur in birds fed dilute solutions. Furthermore, the fast passage rates of nectarivorous and frugivorous birds feeding on dilute diets (Downs 1997; Levey & Martínez del Rio 1999) may not provide adequate processing time to hydrolyse sucrose (Martínez del Rio *et al.* 1989; Martínez del Rio *et al.* 1992). Absorption of glucose and fructose, either passively or via transporters (Diamond *et al.* 1986; Karasov & Cork 1994), could be seriously retarded by extremely dilute gut contents (Karasov *et al.* 1986; Karasov & Cork 1994; Caviedes-Vidal & Karasov 1996; Witmer 1999). It may be informative to examine changes in the sugar concentration gradient along the length of the intestine in nectarivores, particularly because sunbirds (but not hummingbirds) have been shown to be capable of modulating water absorption in response to diet dilution (McWhorter & Martínez del Rio 1999; McWhorter, Martínez del Rio & Pinshow 2003) and because glucose and water absorption may be coupled (Loo *et al.* 1996).

Osmolality of the very dilute sucrose diet fed to sunbirds (0.1 mol l^{-1} SE sucrose is ~100 mOsmol kg^{-1}) is substantially below that of bird plasma concentrations of between 320 and 370 mOsmol kg^{-1} (Skadhauge 1981; Goldstein & Braun 1988; Goldstein & Braun 1989; Goldstein & Skadhauge 2000). It seems likely that birds feeding on extremely dilute diets are suffering osmotic limitations on their digestive ability (Levey & Martínez del Rio 1999); this is also supported by the increased total solute excretion observed on very dilute diets (Fleming & Nicolson 2003). In the present study, the only difference in handling of sugars was that sunbirds ingested 12% more sucrose than hexoses on the dilute (0.1 mol l^{-1} SE) diets. In contrast, sugar preference trials indicated that sunbirds (and hummingbirds) switched to hexose preference when solutions were very dilute. Both observations would be consistent with osmotic limitations on gut processing affecting intake and preferences, since the osmolality of the hexose diet will be almost twice the value of the equivalent sucrose diet.

Acute exposure to low T_a resulted in both species increasing their energy intake. Whitebellied Sunbirds increased energy intake by 18% on the 1 mol l^{-1} SE diet, and subsequently maintained body mass, in response to the additional thermogenic costs (at 10 °C). Although hummingbirds increased their energy intake in response to reduced T_a, the 17% increase (on 1 mol l^{-1} SE diets) was far short of the calculated 200% increase in metabolic rate expected for an 11 °C reduction in T_a (Calder & Calder 1992). However, we recorded negligible body mass losses, suggesting that energy-saving behavioural changes such as the use of torpor, decreased flying time or reduced perching metabolic rate (Beuchat, Chaplin & Morton 1979; Hiebert 1991; Tiebout 1991) may have been employed in response to the increased thermogenic costs. Similar increased intake in response to acute cold exposure has also been demonstrated in the Lesser Doublecollared Sunbird *Nectarinia chalybea*

(L.) (Lotz 1999) and Anna's Hummingbird *Calypte anna* (Lesson) (Beuchat *et al.* 1979). However, previous studies on Broadtailed and Rufus (*S. rufus* (Gmelin)) Hummingbirds report that they are unable to increase energy intake to meet increased thermogenic demands so that they lost body mass or employed extensive torpor (Beuchat *et al.* 1979; McWhorter & Martínez del Rio 2000).

CONCENTRATION AND TEMPERATURE EFFECTS ON SUGAR PREFERENCES

An ornithocentric explanation for nectar concentration requires that nectar reflects bird concentration preferences. However, both sunbirds and hummingbirds *prefer* solutions that are more concentrated than natural nectars (Pyke & Waser 1981; Tamm & Gass 1986; Roberts 1996; Nicolson & Fleming 2003a). Other reasons for dilute bird nectars could lie with flower structure (Plowright 1987) or nectary physiology (Nicolson 1998, 2002), viscosity and ingestion rate relationships (Baker 1975), discouraging bee pollinators (Bolten & Feinsinger 1978) or encouraging more bird visits (Collins & Clow 1978; Martínez del Rio *et al.* 2001). While water turnover of wild nectar-feeding birds greatly exceeds the values predicted allometrically (nectar-feeding birds consume 0.9–2.4 times their body mass of water daily, Weathers & Stiles 1989; Williams 1993; Goldstein & Bradshaw 1998), it has also been proposed that dilute nectars may have evolved to meet the water requirements of birds (Baker 1975; Calder 1979).

Selection for higher sugar concentration in hummingbird nectars relative to sunbird nectars may be a function of differences in body size, metabolism and/or renal function between these birds. In particular, differences in body size and therefore mass-specific metabolic rates and fat stores may have a substantial effect on nectar preferences. Sunbird body mass averages 9.5 ± 3.2 g (102 species, Cheke, Mann & Allen 2001), compared with 5.1 ± 2.3 g for hummingbirds (250 of 331 hummingbird species, Dunning 1992; Cotton 1996) (Fig. 4, ANOVA $F_{1,349} = 210.89$, $P < 0.001$). The high energetic demands of hovering flight (Suarez 1992), compounding the already extreme thermoregulatory costs associated with small body size (Pearson 1950; Lasiewski 1964), may result in hummingbirds selecting for more energetically concentrated nectars. Moreover, more dilute sunbird nectars may reflect the sunbirds' ability to modulate water load by shunting up to 60% of water through their gut (McWhorter *et al.* 2003). Hummingbirds on the other hand, are constrained to absorb most of their large water intake and process it in the kidneys (McWhorter & Martínez del Rio 1999).

Evidence from the present study contradicts simple explanations for nectar composition, since Whitebellied Sunbirds and Broadtailed Hummingbirds exhibited similar preferences. Although we observed sucrose preference in Whitebellied Sunbirds (0.75 mol l^{-1} SE),
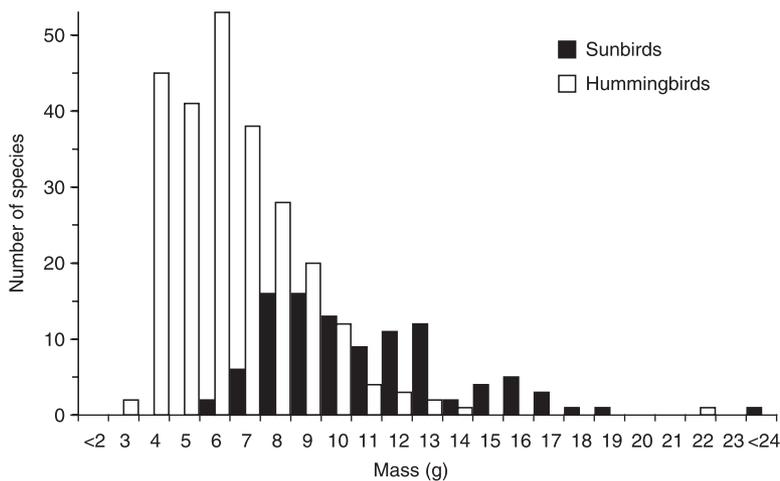
Fig. 4. Frequency histogram of the distribution of body mass values for 102 sunbird species (Cheke *et al*. 2001) and 250 hummingbird species (Dunning 1992).

corroborating previous studies on Lesser Double-collared Sunbirds *Nectarinia chalybea* (Lotz & Nicolson 1996; Jackson *et al*. 1998a), Broadtailed Hummingbirds demonstrated no significant sucrose preference. This finding for hummingbirds contrasts with previous studies that have demonstrated sucrose preference in several hummingbird species (Stiles 1976; Martínez del Rio 1990b; Martínez del Rio *et al*. 1992). However, we believe that these findings may be due to a methodological bias from using solutions prepared on a percentage weight basis (see below).

Sugar preferences in our study were concentration-dependent. Similarly, Magnificent Hummingbirds *Eugenes fulgens* Swainson (Trochilidae) and Cinnamon-Bellied Flowerpiercers *Diglossa baritula* Wagler (Emberizidae) also switch to hexose preference on dilute diets (Schondube & Martínez del Rio 2003). Given the differences in osmolality of hexose and sucrose nectars of the same concentration (Nicolson 2002), there may be a simple reason for hexose preference at low concentrations: birds may be able to distinguish between sucrose and hexose solutions based on the higher osmotic concentrations of the hexoses. Viscosity is also an important consideration in nectarivore preferences, having a significant effect on feeding rates, and the possibility of lower viscosity of sugar mixtures (Boggs 1988) in nectars warrants further attention.

METHODOLOGICAL CONSIDERATIONS FOR SUGAR PREFERENCE TRIALS

In an analysis of the last three decades of literature on nectar sugar composition and bird preferences, a consistent error becomes apparent, one that may have a profound effect on how we perceive nectarivore–plant relationships and that makes published preference data difficult to interpret. Most sugar preference trials have been carried out using sugars mixed up on a percentage weight basis (doubtless because nectar concentrations read by refractometry are in g sugar/100 g

solution, Bolten *et al*. 1979). However, these sucrose and hexose solutions are not equicaloric, since the molecular mass of sucrose (1 mole = 342·3 g) is not exactly twice that of glucose and fructose (for both: 1 mole = 180·2 g). For each sucrose molecule hydrolysed, a water molecule is added; the carbon atoms, the source of energy for the bird, do not change. As a consequence, hexose solutions mixed on a percentage weight basis will have 95% the energy value of sucrose solutions.

This is not inconsequential for the birds. For example, hummingbirds are particularly sensitive to concentration. When provided with concentrations in 5% increments up to 45%, Rufous Hummingbirds invariably drink substantially more of the higher concentration (Tamm & Gass 1986) and they can distinguish concentrations differing by only 1% (Blem *et al*. 2000). The best discrimination occurs between 20 and 30% (the range over which most preference trials have been carried out), when Rufous Hummingbirds will drink about four times the more concentrated diet compared with the dilute diet (Tamm & Gass 1986; Blem *et al*. 2000). Consequently, it is not possible to distinguish between sucrose preference and concentration preference in trials comparing solutions mixed up on a percentage weight basis. This problem also affects conclusions about processing times, which are affected by intake rates.

Although it is entrenched in the literature that hummingbirds show strong sucrose preference, our Broadtailed Hummingbirds offered equicaloric solutions were near-indifferent to sugar type for all concentrations at 21 °C (in fact consuming slightly more hexose solutions), and showed only very slight (not significant) sucrose preference at 10 °C for 0·75 and 1 mol l^{-1} SE solutions. We therefore need to examine sugar preference for a variety of hummingbird species, using equicaloric solutions, to determine whether the lack of sucrose preference we observed in Broadtailed Hummingbirds remains a consistent trend for hummingbirds in general.

OTHER INFLUENCES ON NECTAR COMPOSITION

In the absence of strong differences in sugar preferences, why do sunbird and hummingbird nectars differ so much in composition? One possibility is that the Nectariniidae (sunbirds and sugarbirds) may not be the only birds influencing sunbird nectars: the abundance of opportunistic nectar feeders in southern Africa (Nicolson & Fleming 2003b) may confound selection for nectar sugars. While some New World opportunist nectar feeders have been found to lack sucrase and consequently show sucrose intolerance (Martínez del Rio *et al*. 1988; Martínez del Rio 1990a), such work has not been carried out for opportunist nectar-feeders in southern Africa.

In understanding nectar compositions, we may need to look further at plant physiology and morphology

(Baker *et al*. 1998; Nicolson 2002). Nectar concentration and sucrose content are positively correlated: hexose-dominated nectars are generally more dilute than those dominated by sucrose (Nicolson 2002). The presence of nectary invertase may allow the plants to produce dilute hexose nectars that have a similar attractiveness for their bird pollinators, but at a lower cost to the plants. Consistent with this idea (and with the differences in body mass between sunbirds and hummingbirds), passerine flowers generally produce greater volumes of nectar than hummingbird flowers (Nicolson 2002). Baker *et al*. (1998) suggest that low nectar sucrose is an ancestral condition, shifting towards sucrose dominance due to association with hummingbirds (e.g. in the genera *Puya*, *Fuchsia*, *Campsus* and *Heliconia*). Hexose-rich nectar seems to be the basal condition in the Proteaceae (Nicolson & van Wyk 1998). However, the sucrose–hexose dichotomy, conspicuous within African and Australian plant genera (Barnes *et al*. 1995; Nicolson & van Wyk 1998; Nicolson 2002; Nicolson & Fleming 2003b), can occur in species visited by the same pollinators, which contradicts an immediate ornithocentric explanation. For example, species of *Erica*, *Protea* and *Leucospermum* produce nectar that is either sucrose- or hexose-dominated, but few species have nectar of mixed sugar composition (Barnes *et al*. 1995; Nicolson & van Wyk 1998). In conclusion, the causal mechanism responsible for the secretion of nectar with different types and concentrations of sugars by plants pollinated by different groups of birds remains unresolved.

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