Intake Responses in Nectar Feeding Birds: Digestive and Metabolic Causes, Osmoregulatory Consequences, and Coevolutionary Effects[1]

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SYNOPSIS. Nectar-feeding vertebrates respond to variation in nectar sugar content by modulating volumetric intake. In some nectar feeding animals, the intake response to sugar concentration can be accurately predicted from simple mathematical models that rely on knowledge of gut morphology, *in vitro* rates of sugar digestion, and daily energy expenditures. Because most of the floral nectars consumed by vertebrates are dilute, these animals ingest large amounts of water while feeding. The water turnover rates of hummingbirds feeding on dilute nectar are more similar to those of amphibious and aquatic organisms than to those of terrestrial vertebrates. Dilute nectars can pose osmoregulatory challenges for nectarivores. Nectarivorous birds exhibit renal traits that are well suited to dispose of large water loads and that appear inadequate to produce concentrated urine. Nectar-feeding birds prefer concentrated over dilute sugar solutions. However, the concentration difference that they can discriminate is smaller at low than at high concentration. We hypothesize that this pattern is a consequence of the functional form of intake responses that often results in decelerating sugar intakes with increasing sugar concentration. The diminishing returns in floral attractivity that may result from increased nectar concentration may be one of the reasons why the nectars of hummingbird pollinated flowers are dilute in spite of the preference of birds for higher concentrations. The intake responses of nectar-feeding birds capture the integration of a behavioral response with the physiological processes that shape it. Because the behavior of nectar-feeding birds can have consequences for the plants that they visit, the intake response may also have coevolutionary effects.

INTRODUCTION

Floral nectar is arguably the simplest food on earth. With a few notable exceptions (*e.g.*, floral oily secretions, Simpson and Neff, 1983), nectar is a dilute aqueous sugar solution containing trace amounts of amino acids and electrolytes (Baker and Baker, 1990). Although nectar is a relatively simple food, it shows significant heterogeneity in composition (reviewed by Baker *et al.*, 1998) and concentration (Pyke and Waser, 1981). For example, bird-pollinated plants can secrete floral nectars containing the disaccharide sucrose, the monosaccharides glucose and fructose, or mixtures of these three sugars (Martínez del Rio *et al.*, 1992). Vertebrate-pollinated plants also differ in the concentration of sugars secreted. Floral nectars can vary several-fold in sugar concentration (Pyke and Waser, 1981; Stiles and Freeman, 1993; Fig. 1). Although the ecological, and evolutionary correlates of sugar composition in nectar have received considerable attention (Martínez del Rio *et al.*, 1992; Jackson *et al.*, 1998; Herrera, 1999), the significance of nectar concentration for nectar-feeding vertebrates has been relatively unexplored. Nicolson (1998) reviewed the consequences of nectar concentration for insect pollinators. The purpose of this paper is to identify the behavioral, physiological, and ecological consequences
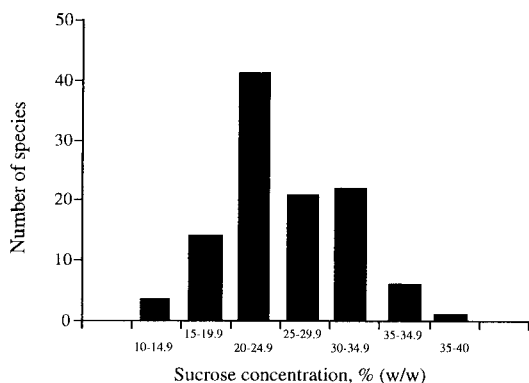
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FIG. 1. Sugar concentration varies four-fold in a sample of 108 Costa Rican plant species pollinated by hummingbirds (data from Table 1. In Stiles and Freeman, 1993).

FIG. 2. Intake response of 6 Cinammon Flower-piercer (*D. baritula*, Emberizidae) individuals (body mass ± SD = 7.7 ± 0.51, n = 6). Birds were fed on sucrose solutions spanning the range of concentrations found in natural flowers (from 10 to 35% mass/volume, Fig. 1). Each bird received solutions containing 290, 438, 584, 730, 876, and 1,022 mM·liter⁻¹. The relationship between volumetric intake and sucrose concentration was well described by power functions (r^2 ranged from 0.89 to 0.97) with exponents that ranged from −0.77 to −0.97. The average exponent was significantly smaller than −1 ($t = 4.0$, $P < 0.01$).

of variation in nectar concentration for nectar-feeding vertebrates.

When sugar concentration in food is increased, many nectar-feeding bird species decrease food intake (Collins, 1981; Downs, 1997; López-Calleja *et al.*, 1997; Fig. 2). A negative relationship between nutrient density and food intake is not exclusive to nectar-feeding birds. Similar relationships have been observed in many animal species (Montgomery and Baumgardt, 1965; Castle and Wunder, 1995). This relationship between intake and food quality has been named the "intake response" (Castle and Wunder, 1995).

This paper is organized around three interrelated motifs, each of which emphasizes a different aspect of the intake response in nectar-feeding birds. We first describe the intake response and propose a theoretical framework that permits characterization of the factors that shape it. We show that intake responses are well described by power functions and propose a model that integrates the interaction between the supply of energy provided by the gastrointestinal tract with the demands imposed by metabolic expenses. Decreasing power relationships between volumetric intake and sugar concentration imply high water intakes at low sugar concentrations. The second topic of this paper is an analysis of how nectar-feeding birds cope with the osmoregulatory challenges of a watery diet. Nectar-feeding birds are plant pollinators. Their behavior
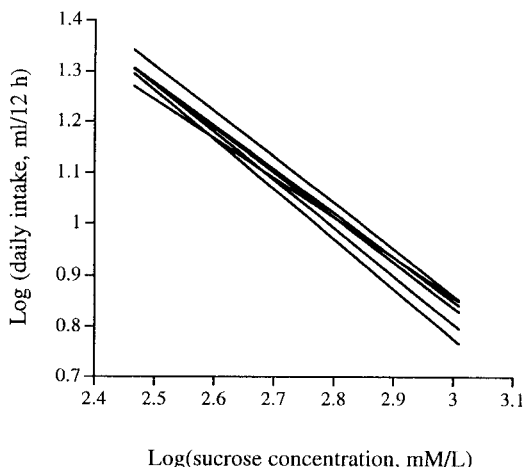
influences pollination success and can influence the evolution of floral traits (Martínez del Rio *et al.*, 1992). The last subject of this paper is the potential effect of the intake response on the interaction between birds and plants.

THE INTAKE RESPONSE

Intake responses: two complementary hypotheses

The widespread occurrence of intake responses is often attributed to compensatory feeding (Simpson *et al.*, 1989). According to this explanation, animals regulate food intake to maintain a constant flux of assimilated energy or nutrients (Montgomery and Baumgardt, 1965; Slansky and Wheeler, 1992). If the energy/nutrient density in food is decreased, animals compensate by increasing intake. Indeed, the negative relationship between volumetric intake and sugar concentration exhibited by nectar-feeding birds can lead to relatively invariant sugar intake (Levey and Martínez del Rio, 1999). By modulating volumetric intake in

response to sugar concentration, some birds seem to defend a constant rate of energy intake (López-Calleja *et al.*, 1997). An alternative hypothesis to compensatory feeding is that intake is constrained by the ability of animals to process the nutrients contained in food (Levey and Martínez del Rio, 1999). How can compensatory feeding be differentiated from physiological constraint? Here we describe two complementary approaches: One is descriptive and relies on examining the functional structure of the intake response. The other is experimental and relies on determining the effect of changing energetic demands on the intake response. We claim that compensatory feeding and physiological constraint are complementary mechanisms that shape intake responses. We also identify the conditions under which each of these mechanisms is likely to have dominant importance.

Intake responses: constraint or compensatory feeding?

The relationship between volumetric intake and sugar concentration is well described by power functions of the form

$$V = aC^{-b}, \qquad (1)$$

where V equals volumetric intake, C equals sugar concentration and a and b are empirically derived constants (McWhorter and Martínez del Rio, 1999, 2000; Fig. 2). Throughout this paper we will refer to the equation (eq. 1) that describes the relationship between intake and concentration as the intake response. Because volumetric intake (V) decreases as a power function of concentration (C), the amount of sugar ingested (A) is also a power function of sugar concentration ($A = aC^{-b}C = aC^{1-b}$). Animals exhibiting values of b equal to 1, show "perfect" compensation and sugar intake that is independent of concentration ($1 - b = 0$). In contrast, animals with values of the exponent b smaller than 1, show a positive relationship between sugar ingested and sugar concentration in food. Most intake responses reported to date have exponents that range from 0.65 to 1 (reviewed in McWhorter and López-Calleja, 2000). Here

we examine a single example in some detail.

Cinnamon Flower-piercers (*Diglossa baritula*, Emberizidae) are nectar robbers (*sensu* Inouye, 1980) of hummingbird flowers. Figure 2 shows their volumetric intake when feeding on sucrose solutions of varying concentration in the laboratory. The average exponent of their intake responses was significantly smaller than 1 (Fig. 1). Consequently the amount of sugar that they assimilated increased with sugar concentration in food. The average exponent of the power function relating daily sugar ingestion (in grams per 12 hr) with sucrose concentration was significantly positive ($1 - b = 0.12 \pm 0.06$, one-sample $t = 4.2$, $P < 0.01$, n = 6). Because the value of this exponent is relatively low, the difference in daily sucrose intake between the lowest and the highest concentration was small (about 10%). In contrast, McWhorter and López-Calleja (2000) reported intake responses of four species of hummingbirds (*Lampornis clemenciae*, *Eugenes fulgens*, *Archilochus alexandrii*, and *Selasphorus platycercus*) with exponents that ranged from 0.71 to 0.77. In these species, sugar ingestion was more than 30% higher at the highest (1,020 mM·liter^{-1}) than at the lowest concentration (290 mM·liter^{-1}). Lloyd (1991) found a similar result in the Double-collared Sunbird (*Nectarinia afra*). In these birds the intake response had an exponent equal to 0.82 and hence daily caloric intake increased by 50% from the lowest (125 mM·liter^{-1}) to the highest experimental concentration (1,000 mM·liter^{-1}).

Not all birds studied have shown values of b lower than 1. Green-backed Firecrowns (*Sephanoides sephanoides*, Trochilidae) and Cedar Waxwings (*Bombycilla cedrorum*, Bombycillidae) exhibited intake responses with exponents that were statistically indistinguishable from 1 (López-Calleja *et al.*, 1997; Levey and Martínez del Rio, 1999). In these two species, sugar intake did not vary with sucrose concentration. The presence of values of b lower than 1, provides circumstantial evidence for the existence of physiological constraints on feeding intake in nectar-feeding birds, at least under some conditions and in some species. In a sub-

sequent section we will identify with more precision the conditions that may lead to the existence of physiological constraints on intake in nectar-feeding.

A low exponent provides only inferential evidence of constraints on intake. McWhorter and Martínez del Rio (2000) provided experimental evidence for a physiological constraint on intake. They presented broad-tailed hummingbirds with sucrose solutions at four concentrations (292, 584, 876, and 1,168 mM·liter^{-1}) and exposed them to two environmental temperatures (10°C and 22°C). As expected, birds exhibited intake responses and decreased volumetric food intake in response to sugar concentration. However, when they were exposed to a relatively sudden drop in environmental temperature, and hence to an acute increase in thermoregulatory energy expenditures, they did not increase their rate of energy consumption and lost mass. These results support the existence of a physiological constraint on feeding intake (McWhorter and Martínez del Rio, 2000).

Other experimental studies that have exposed nectar-feeding birds to low temperatures acutely have revealed considerable interspecific variation. Beuchat et al. (1979) compared the food intake rates of rufous (Selasphorus rufus) and Anna's hummingbirds (Calypte anna) at several temperatures and found that these two species exhibited different responses. Between 0 and 20°C, Selasphorus rufus, like the S. platycercus studied by McWhorter and Martínez del Rio (2000), exhibited relatively constant energy intake rates. In contrast, Calypte anna showed the negative correlation between energy intake and environmental temperature that is expected in animals that compensate for increased expenditures by increasing energy intake. Beuchat et al. (1979) hypothesized the existence of a digestive limitation to explain the absence of a temperature effect on intake rate in S. rufus. Gass et al. (1999) provided additional support for the existence of a physiological constraint in S. rufus. Birds exposed to 5°C lost mass at low but not high sugar concentrations. In a recent study, Lotz (1999) exposed lesser double-collared sunbirds (Nectarina chalybea) to variable temperatures and fed them on two sucrose concentrations (400 and 1,200 Mm·liter^{-1}). These birds increased intake with decreased sucrose concentration, exhibited a linear decrease in sugar intake with increased temperature over a large range of temperatures (from 10 to 30°C), and maintained body mass. The amount of energy assimilated by N. chalybea increased with decreasing ambient temperature with the same slope as the amount of energy used for thermoregulation. We interpret Lotz's (1999) results as unambiguous evidence for compensatory feeding.

Three main inferences can be gleaned from the responses of nectar-feeding birds to sugar concentration and ambient temperature: 1) birds consistently exhibited intake responses that are well described by power functions; 2) there was variation in the exponent of intake responses: some birds showed compensation and constant sugar intake over a range of concentrations whereas others show increased sugar intake with increased sugar concentration; and 3) some bird species responded to decreased ambient temperature by increasing intake, but others did not. These three conclusions lead to two questions. First, why are intake responses well described by power functions? Second, what are the conditions that determine whether compensatory feeding or physiological constraint shape the intake response? The next section provides a framework to answer these questions.

A simple model for the intake response in nectar-feeding animals

Perfect compensatory feeding in which volumetric intake is regulated to yield constant energy intake generates a power function with exponent equal to 1 (i.e., $A = aC^{1-b}$ is constant only if $b = 1$). McWhorter and Martínez del Rio (2000) present a simple mathematical model that predicts a power relationship between volumetric intake and sugar concentration. This model assumes that sucrose hydrolysis in the small intestine is the limiting step in sugar assimilation in nectar-feeding birds. Here we briefly describe this model and elaborate on it to identify the conditions under which birds should exhibit compensatory feeding

or constraint. We also describe the experiments needed to refine and test the model.

McWhorter and Martínez del Rio (2000) assumed that the intestine of nectar-feeding birds functions as a plug-flow chemical reactor (Penry and Jumars, 1987). Thus, their model makes two crucial assumptions: 1) digesta flows unidirectionally (Jumars and Martínez del Rio, 1999) and 2) the rate at which sucrose is hydrolyzed in the intestine $(-r_s)$ follows simple Michaelis-Menten kinetics:

$$-r_S = S_{max}C_s(K_m + C_s)^{-1} \qquad (2)$$

where S_{max} equals the rate of hydrolysis along the intestine (in $\mu moles\ min^{-1}\ \mu l^{-1}$), K_m is sucrase's Michaelis constant (in $\mu mole\ \mu l^{-1}$), and C_s is the concentration of sucrose (in $\mu mole\ \mu l^{-1}$) down the intestine or with time (Jumars and Martínez del Rio, 1999). Equation (1) can be integrated to yield the throughput time (τ) required to reduce the initial sucrose concentration (C_{s0}) to a given final value (C_{sf}):

$$\tau = (S_{max})^{-1}(K_m Ln(C_{s0}C_{sf}^{-1})$$
$$+ (C_{s0} - C_{sf})) \qquad (3)$$

In plug flow reactors if one knows τ and the volume of gut contents (G in μl), intake rate $(\dot{v}_0$ in $\mu l\ min^{-1})$ can be estimated as:

$$\dot{v}_0 = G\tau^{-1} \qquad (4)$$

McWhorter and Martínez del Rio (2000) applied their model to Broad-tailed hummingbirds. Here we use it to investigate the intake response of Magnificent Hummingbirds (*Eugenes fulgens*). The model uses sucrase activity values measured *in vitro* and data on intestinal morphology to predict intake rates as a function of experimental sucrose concentrations. The parameter values used in the model were determined for Magnificent Hummingbirds studied by J. Schondube (unpubl. data) in Las Joyas Research Station, Jalisco, Mexico. His methods followed Martínez del Rio *et al.* (1995) and McWhorter and Martínez del Rio (2000). Maximal sucrase activity $(S_{max} \pm SD)$ averaged along the intestine's length equaled $0.18 \pm 0.06\ \mu mole\ min^{-1}\ \mu l^{-1}$. The apparent Michaelis constant $(K_m^* \pm SE)$ equaled $0.0524 \pm 0.0012\ \mu mole\ \mu l^{-1}$, and intestinal

volume $(G \pm SD)$ equaled $121.5 \pm 27\ \mu l$ (data on 3 individuals). Following McWhorter and Martínez del Rio (2000) we assumed that 99.6% of sucrose was hydrolyzed, and thus that C_{sf} was equal to 0.004 C_{s0}. The model also assumes that birds have 12 hr of activity during which they can feed.

Figure 3 compares the intake predicted by the model with observed intakes at 6 sucrose concentrations. Both the model's output and the observations were well described by power functions (Fig. 3). However, the exponent of the predicted intake response (0.822) was significantly lower than that of the observed relationship between volumetric intake and concentration (0.942 ± 0.047, $t = 3.0$, $P < 0.05$, n = 6) suggesting that under the experimental conditions, birds were exhibiting compensatory feeding. They appeared to possess digestive "spare capacity" (Diamond, 1991). Following Diamond and Hammond (1992), we use the term safety factor for the ratio of capacity (estimated by our model) to load (*i.e.*, the amount of nutrient ingested, Weiss *et al.*, 1998). Safety factors for *E. fulgens* were modest and ranged from 1.09 to 1.26 from the lowest (290 Mm·liter^{-1}) to the highest (1,022 mM·liter^{-1}) sucrose concentration. McWhorter and Martínez del Rio (2000) also reported moderately small safety factors for Broad-tailed Hummingbirds (*S. platycercus*).

The existence of small safety factors in hummingbirds exposed to the relatively mild conditions of the laboratory (with unlimited food availability and steady temperature) highlights the profound importance of the ability of these animals to enter into nocturnal torpor (Hiebert, 1993 and references there). In nature, diurnal energy demands are unpredictable and can sometimes increase as a result of low ambient temperatures and/or low flower densities that force birds to increase flight distances (reviewed by Calder, 1994). Under these conditions if digestive characteristics limit the supply of energy, balancing the energy budget requires reducing energy outputs at night. The metabolic strategies of hummingbirds appear to be inextricably linked to their digestive abilities.

The model presented here suggests that increasing energy demands can reduce the
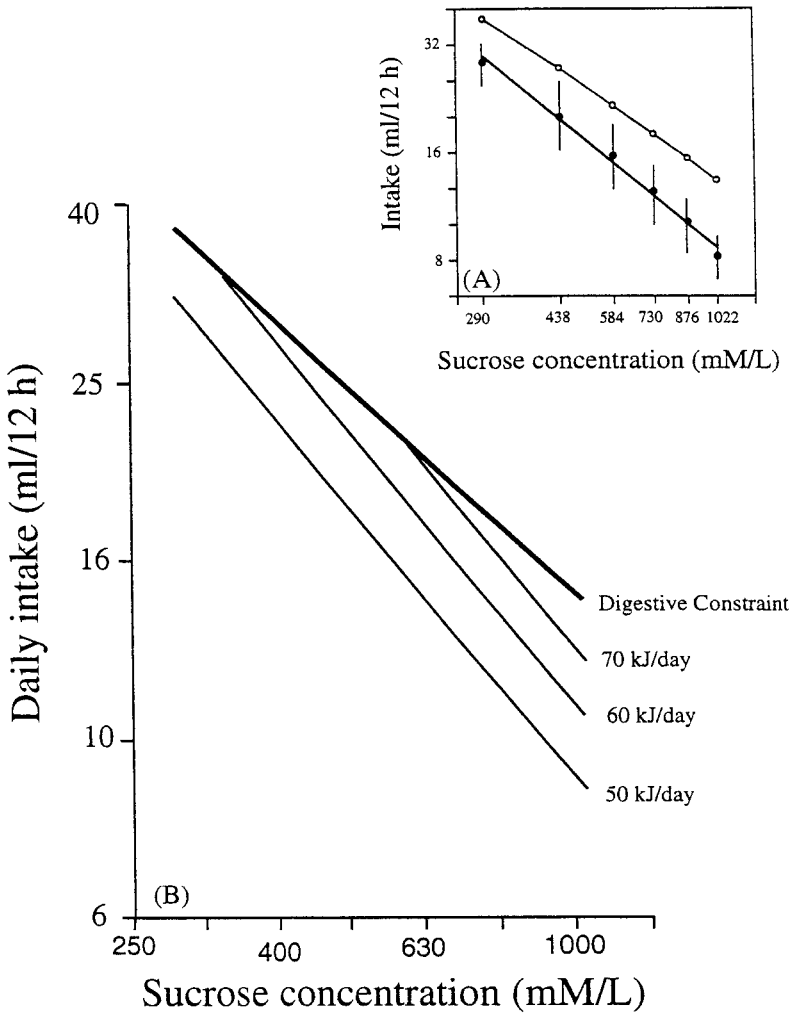
FIG. 3. (A) Comparison of the intake response of Magnificent Hummingbirds with intake predicted from a chemical reactor model of gut function. Both the model and the observed results were adequately described by power functions. However the exponent of the relationship predicted by the model ($y = 4030x^{-0.82}$) was lower than that of a power function fitted to observed data ($7229x^{-0.94}$, $r^2 = 0.98$, the regression line was fitted through means using a non-linear fitting procedure). (B) Hypothetical relationship between daily food intake, sucrose concentration in food, and energy expenditures of Magnificent Hummingbirds. The heavy line represents maximal food intake predicted by a model of gut function. Other lines represent intake responses for 3 levels of daily energy expenditure (DEE). At the lowest level of DEE (50 kJ/day), birds are in neutral energy balance and the relationship between intake and concentration can be described by a single power function with exponent equal to 1. At higher levels of energy expenditures (60 and 70 kJ/day), we predict "broken" intake responses that are described by two power functions with different slopes.

safety margin and even force birds to shift from an intake response that is the result of compensatory feeding to one that is shaped by digestive constraints. Figure 3 shows the hypothetical effect of increasing energy demands on the intake response. The daily energy expenditures (DEEs) shown in the graph are in the range of those estimated for *E. fulgens* in the highlands of Costa Rica by Wolf *et al.* (1976) and by Powers (1996) for birds in the Chiricahua Mountains of Arizona. Even the highest DEE depicted in Figure 3 is within the range that *E. fulgens* individuals incur in the field

(Powers, 1996). According to our model, at modest daily energy expenditures (50 kJ/day) birds can remain in neutral energy balance and exhibit an intake response with an exponent equal to 1. When energy demands are increased (60 and 70 kJ/day), the model predicts a "broken" intake response. At low concentrations its slope is dictated by the digestive constraint (0.82 in *E. fulgens*), whereas at high concentrations the slope equals 1 and reflects compensation. At low concentrations birds are unable to meet energy expenditures unless they are reduced through torpor.

The model has the useful feature of generating precise predictions about the form of the intake response based on energy expenditures and the magnitude of physiological traits (sucrase activity and intestinal volume). The model also allows making predictions on the concentrations that will, and will not, permit neutral or positive energy balances. We point here at a methodological nuance that should guide efforts that depend on varying thermoregulatory costs to test our model. Chronic cold exposure can be accompanied by increased digestive and metabolic capacities (Konarzewski and Diamond, 1994; McWilliams and Karasov, 1998). Thus, testing between constraint and compensatory feeding requires that animals be exposed to the cold under short-term, acute conditions (see López-Calleja *et al.*, 1997).

The speculations presented in this section would be idle if they were not testable. We present them because all the ingredients to test them are available under both field and laboratory conditions: daily energy expenditures can be measured using standard methods (Powers and Nagy, 1988; Tiebout and Nagy, 1991), digestive capacities can be estimated from physiological measurements and estimates of sugar concentration of floral nectars, and finally, statistical techniques are available to examine whether a data set is best described by two, rather than a single relationship (Neter *et al.*, 1996). Nectar-feeding birds present an unparalleled opportunity to explore the interaction between gut and metabolic function, and to test the notion that digestive constraints
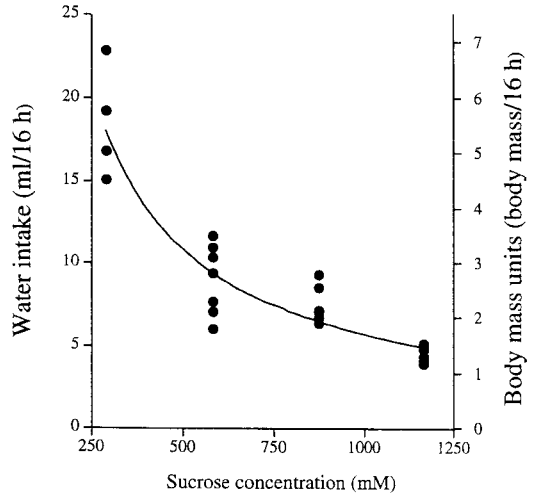

FIG. 4. Effect of sucrose concentration on water intake in Broad-tailed hummingbirds. Water intake and sucrose concentration are related by a power function ($y = 3651x^{-0.93}$, $r^2 = 0.84$). Note that at low sucrose concentrations birds ingest about 5 times their body mass per day (McWhorter and Martínez del Rio, 1999).

have ecological consequences for animals under natural conditions.

CONSEQUENCES OF THE INTAKE RESPONSE FOR OSMOREGULATION

Coping with a watery diet

The maintenance of fluid and electrolyte balance under the desiccating conditions that characterize their environments is a perpetual challenge facing terrestrial animals. Over the last four decades osmoregulatory research has focused to a large extent on the challenges to water balance faced by animals living under conditions of limited water availability (Schmidt-Nielsen, 1964). The emphasis on desert animals may have led to an unbalanced view of the water relationships of terrestrial animals in general. Nectar-eating animals exemplify the other end of the osmoregulatory spectrum. Because floral nectars contain large amounts of water, little protein, and relatively high osmotic concentrations (Nicolson, 1998), they can pose special osmoregulatory challenges (Beuchat *et al.*, 1990).

The osmoregulatory challenges posed by a sugary and watery diet are well illustrated by the intake response (Fig. 4). Broad-tailed

hummingbirds (3.3 ± 0.08 g, n = 10) ingesting food containing 290 mM·liter^{-1} (≈10% w/vol) ingested from 4 to 6 times their body mass in water in a 16 hr day (McWhorter and Martínez del Rio, 1999; Fig. 4). As shown in Figures 2 and 3, extremely high daily intake rates were also shown by Cinammon Flower-piercers (7.7 ± 0.5 g, n = 10 individuals, J. Schondube, unpubl. data) and Magnificent Hummingbirds (7.2 ± 0.6, n = 10) feeding at low sugar concentrations. It is apparent that some, and perhaps all, nectar-feeding birds can ingest prodigious amounts of water with apparent impunity (see Lloyd, 1991). This ability does not appear to be the norm among terrestrial vertebrates. In humans, rats, domestic pigeons, and gray parrots, overingestion of water is accompanied by "water intoxication" (DeLeon *et al.*, 1994; Gebel *et al.*, 1989; Gevaert *et al.*, 1991; Lumeji and Westerhof, 1988). Water overingestion leads to negative effects because it can be accompanied by plasma dilution, hyponatraemia (low plasma sodium), and rupture of red-blood cells due to osmotic swelling (Faenestil, 1977). Water intoxication occurs when excessive water intake and absorption overwhelm healthy, intact osmoregulatory processes (Gebel *et al.*, 1989).

How do nectar-feeding birds cope with apparent polydipsia (excessive water intake)? To explain the ability of hummingbirds to process large volumes of dietary water, Beuchat *et al.* (1990) hypothesized that perhaps only a small fraction of ingested water is absorbed from the intestine, leaving the rest to pass quickly and directly to the cloaca. McWhorter and Martínez del Rio (1999) used a mass balance model to test Beuchat *et al.*'s (1990) conjecture. Their results did not lend support to the hypothesis that the bulk of dietary water passes through the intestine unabsorbed in nectar-feeding birds. Application of the model to broad-tailed hummingbirds suggested that about 80% of ingested water was absorbed in the gastrointestinal tract and hence must be processed by the kidneys.

Their data set also revealed that fractional and total water turnover (water flux, *sensu* Nagy and Peterson, 1988) increased lin-
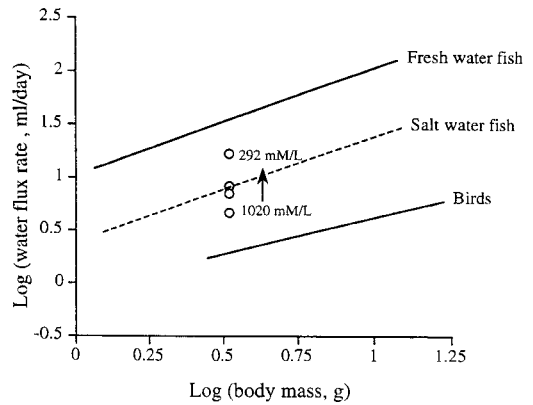

FIG. 5. Water fluxes in broad-tailed hummingbirds were higher than those expected for a bird of their mass (lower allometric line, y = 0.87x$^{0.69}$). At low sucrose concentrations (292 Mm·liter^{-1}, lower point), they were higher than those of salt water telesot fish (dashed allometric line, y = 2.5x$^{0.99}$, Nagy and Peterson, 1988) and they approached those of fresh water fish (upper allometric line, y = 11.0x$^{0.99}$; Nagy and Peterson, 1988). Birds were fed on solutions containing 292, 584, 876, and 1,168 mM·liter^{-1}. Water fluxes were inversely ranked with sucrose concentration in food.

early with water ingestion. Thus, the intake response can lead to astounding water fluxes. The daily water fluxes experienced by broad-tailed hummingbirds feeding on dilute nectar are much higher than those expected for birds of their size (Nagy and Peterson, 1988; Fig. 5). They are even higher than those experienced by salt-water teleosts and aquatic amphibia (Nagy and Peterson, 1988; Pruett *et al.*, 1991), and approach those of fresh-water bony fishes (Karnaky, 1998; Fig. 5). Broad-tailed hummingbirds, and probably other nectar-feeding birds (Rooke *et al.*, 1983), can face water fluxes typical of aquatic and amphibious organisms.

Hummingbird gastrointestinal and renal morphology support the conclusion that dietary water is more or less completely absorbed. In *S. platycercus* the diameter of the small intestinal lumen decreases dramatically distally, from 1.59 mm at the pyloric-duodenal junction to 0.64 mm at the distal ileum, indicating that digesta volume must decrease distally (McWhorter and Martínez del Rio, 2000). The kidneys of the three hummingbird species that have been ex-

amined also support the notion that water is absorbed in the GI tract and then excreted (Johnson and Mugaas, 1970). Most (90%) of the kidney's tissue is occupied by cortex and only 2% by medulla (Casotti *et al.*, 1993), a very large percentage (99%) of nephrons are the so-called "reptilian-type" (Casotti *et al.*, 1998), and although the size of glomeruli is slightly smaller than expected, the number of glomeruli is about twice that anticipated from allometric considerations (Beuchat *et al.*, 1999). Hummingbird kidneys appear to be designed to filter large amounts of plasma and to recover the valuable solutes contained in it rather than to concentrate urine (Goldstein and Skadhauge, 2000).

Functional relationships between digestive, metabolic, and renal function

So far we have emphasized the large water loads that nectar-feeding birds can experience. Although nectar-feeding birds can experience extremely high rates of fluid intake, there are also situations in which they must deal with potential dehydration. Here we discuss how they might cope with situations in which water conservation is necessary. Because nectar-feeding birds are small and maintain high metabolic rates, their rates of evaporative water loss are high even at modest ambient temperatures (Lasiewski, 1964; Powers, 1992). Moreover, when nectar-feeding birds are feeding on concentrated nectars at high environmental temperatures, the amount of nectar that they require for energy balance can be insufficient to maintain positive water balance (Calder, 1979). Under these circumstances, birds must be able to minimize urinary water loss through renal or post-renal mechanisms (Casotti *et al.*, 1998). Urinary water losses can be minimized by producing smaller volumes of more concentrated urine.

In birds, the process of urinary concentration involves dilution of glomerular filtrate by the reabsorption of sodium chloride in the thick ascending limb of looped nephrons (Nishimura, 1993). The reabsorbed solutes produce an osmotic gradient in the medullary interstitium that drives water reabsorption and concentration of excreted

urine (Nishimura, 1993). The structures required to produce concentrated urine appear to be poorly developed or absent in hummingbird kidneys (Beuchat *et al.*, 1999). The unique renal morphology of hummingbirds prompted Casotti *et al.* (1998) to speculate that for nectar-feeding birds, the ability to produce a concentrated urine may be less critical than the capacity to process large water loads while conserving valuable solutes (see also Casotti *et al.*, 1993).

Casotti *et al.* (1998) appear to have identified an interesting tradeoff. The kidneys of hummingbirds and other nectar-feeding birds may be well suited to process large amounts of water while recovering solutes, but they may be incapable of producing concentrated urine (Calder and Hiebert, 1983). We predict that urine osmolality will decrease with water load in nectar-feeding birds. However, we predict that under conditions of water deficit, nectar-feeding birds will not produce urine with higher osmolality than plasma ($\approx$350 mosm, reviewed in Beuchat *et al.*, 1999). In summary, we predict that under dehydration, nectar-feeding birds will reduce water losses by reducing glomerular filtration rate and by increasing fractional water recovery (Osono and Nishimura, 1994), but not by making their urine hyperosmotic.

The hypothetical account of osmoregulation in hummingbirds that we have depicted resembles, with some significant differences (*e.g.*, hummingbirds lack urinary bladders!), that of renal function in amphibians. Like amphibians, hummingbirds can presumably produce abundant urine that is very dilute and thus conserve electrolytes while excreting excess water. We have also hypothesized that in hummingbirds, like in amphibians, urine production declines rapidly under dehydration (Shoemaker, 1987, 1992). In amphibians under dehydrating conditions, water conservation takes precedence over renal excretion, and if water deprivation is prolonged, nitrogenous wastes accumulate in body fluids (Shoemaker and McClanahan, 1980). In some amphibian species there is a shift from ammonotelism to ureotelism under dehydrating conditions (Shoemaker, 1987). Curiously, an analogous shift may be present in

hummingbirds. Preest and Beuchat (1997) documented an increase in the excretion of ammonia, relative to uric acid, in response to increased water loads in Anna's hummingbirds. Although it is unclear if this shift occurs in all species of nectar-feeding birds, the hypothesis is functionally plausible. The costs of reducing renal excretion may be ameliorated by exceedingly low rates of nitrogenated end-product production and excretion (Brice and Grau, 1991).

WHY DO BIRD-POLLINATED PLANTS SECRETE DILUTE NECTARS?

Our discussion of the intake response has been almost completely ornithocentric. We have focused on how birds respond to a floral trait (nectar concentration) without considering the role that hummingbirds can play in shaping it. Because nectar-feeding birds are members of a mutualistic partnership, their physiological and behavioral traits can have consequences for plants (Martínez del Rio *et al.*, 1992). By shaping the preferences of birds, the intake response can have coevolutionary consequences. In this section we briefly review the concentration preferences of nectar-feeding birds and the factors that may shape them. We then consider their potential influence on the evolution of sugar concentration in floral nectars.

Concentration preferences in nectar-feeding birds

Research on the concentration preferences of nectar-feeding birds has emphasized the factors that determine intake rate before nectar is swallowed and assimilated (reviewed by Roberts, 1996). Nectar viscosity increases with concentration, and therefore the rate at which a meal of a given volume can be harvested ("licked") decreases with increasing concentration (Gass and Roberts, 1992). Because energy ingestion equals the product of volumetric intake and concentration, most models of nectar licking in nectar-feeding birds predict a relationship between sugar intake and sugar concentration with a distinct optimum (Kingsolver and Daniel, 1983). Optimal nectar concentrations have been predicted by a plethora of models that differ in detail and realism (re-

viewed by Gass and Roberts, 1992). These optimal nectar concentrations have one important common feature: They are invariably lower than those chosen by birds. Nectar-feeding birds consistently choose the highest concentration offered (Roberts, 1996).

Gass and Roberts (1992) have pointed to the different time scales at which birds can make optimization decisions. The rate at which energy is imbibed by nectar-feeding birds may not be a process that occurs at the appropriate time scale to have a major effect on intake rate. Hummingbirds, as well as other nectar-feeding birds, spend most of the day perching and spend only a relatively small amount of time actively feeding (Carpenter *et al.*, 1991 and references there). Diamond *et al.* (1986) have hypothesized that the time spent perching is required for the gastrointestinal tract to empty sufficiently to accommodate the next meal. Our consideration of intake responses suggests that this conjecture is correct. Rates of digestion can constrain rates of food intake in nectar-feeding animals. Consequently, the main determinant of intake rate in hummingbirds may be the time required to assimilate a meal rather than the time required to imbibe it. Although hummingbirds spend only a few seconds harvesting a meal (from 2 to 10 sec in 5 hummingbird species), they spend many minutes perching between meals (Wolf and Hainsworth, 1977). It may be that digestive, post-ingestional factors are more important determinants of intake, and hence of preference, than pre-ingestional ones.

Our interpretation of intake responses in nectar-feeding birds indicates that digestive limitations can lead to a positive relationship between sugar concentration in food and sugar intake (see *Intake responses: constraint or compensatory feeding?* and McWhorter and Martínez del Rio, 2000). The exponent of the power function relating sugar intake with sugar concentration is ≤ 1. Therefore, sugar intake rate increases with concentration at a decelerating rate. Because, in general, energy intake rate increases with sugar concentration, the functional form of intake responses predicts that nectar-feeding birds should prefer concen-

trated nectars over dilute nectars. This pre-
diction is consistent with available data.
Preference studies indicate that humming-
birds and other nectar-feeding birds prefer
concentrated sugar solutions (up to 60%)
over dilute ones (see Roberts, 1996 and ref-
erences therein).

Curiously, the nectar concentration usu-
ally found in bird-pollinated flowers is
much lower than that preferred by birds
(Blem et al., 2000). Figure 1 shows that
most hummingbird-pollinated flowers pro-
duce relatively dilute nectars. The average
(±SD) nectar composition in this large
sample of species is 25.6% (±6.1%, w/vol-
ume). About 50% of the plant species sam-
pled secreted nectars with between 20 and
30% sugar, and only ten percent secreted
nectar with a concentration higher than
35% (Fig. 1). This distribution is similar to
that described by other surveys of nectar
concentration (Hainsworth, 1973; Pyke and
Waser, 1981). Consideration of the concen-
tration preferences of nectar feeding birds
and the nectar concentrations found in na-
ture brings forth a question: Why are the
nectars secreted by bird-pollinated flowers
so dilute (Pyke and Waser, 1981)? Rather
than attempting to answer this question, we
will argue that the intake response provides
an important ingredient needed to tackle it.

Is the intake response a missing clue?

Nectar concentration can affect plant fit-
ness through several pathways: 1) it can
change the frequency of return visits to a
plant, 2) it can influence the behavior of
pollinators during the visit to the plant, and
3) it can shape their behavior following the
visit (Pyke, 1981). Because nectar-feeding
birds can learn and remember flower loca-
tions and associate these with rewards
(Brown and Gass, 1993; Sutherland and
Gass, 1995), we will emphasize the effect
of nectar concentration on the first one of
these pathways, which can be characterized
as the "attractiveness" of flowers to polli-
nators. The preferences of nectar-feeding
birds suggest that plants secreting concen-
trated nectar should be more attractive and
hence selected for. However, as Pyke
(1991) has pointed out, nectar secretion can
entail significant costs to plants and the in-

creased attractiveness resulting from the
production of more concentrated nectar
must be weighed against its costs (Pyke,
1981).

The intake responses of birds suggests
that the benefits that birds accrue from feed-
ing on more concentrated nectars increase
rapidly at low concentrations and slowly at
high concentrations. If preferences are me-
diated by the rate at which sugar can be
assimilated, then it can be predicted that
birds should be more discriminating at low
than at high concentrations. Several studies
have demonstrated a concentration prefer-
ence pattern that is consistent with this con-
jecture: In both hummingbirds and sun-
birds, discriminatory ability is more precise
at low that at high concentrations (Hains-
worth and Wolf, 1976; Lloyd, 1989). Ru-
fous hummingbirds in the field discriminate
readily between feeders containing 19%
and 21% sucrose, but do not exhibit signif-
icant preferences between solutions con-
taining 48% and 72% sucrose (Blem et al.,
2000).

We hypothesize that the benefits received
by flowers from secreting concentrated nec-
tars increase with diminishing returns. At
low concentration, a modest increase in
concentration results in higher attractive-
ness and higher visitation rates. At higher
concentrations a large increase in nectar
concentration results in only a small in-
crease in visitation. This observation may
be an important, and so far ignored, com-
ponent in the explanation for the dilute nec-
tars found in bird-pollinated plants. The in-
take response seems to capture the integra-
tion of a behavioral pattern with the phys-
iological process that shape it. Because the
behavior of nectar-feeding birds can have
consequences for the plants that they visit,
the intake response may also have coevo-
lutionary effects.

This paper is dedicated to Bill Calder our mentor in allometry and hummingbirds.

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