NECTAR CONCENTRATION PREFERENCE AND WATER BALANCE IN A FLOWER VISITING BAT, *GLOSSOPHAGA SORICINA ANTILLARUM*

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Abstract. Nectar concentration preference of flower visiting bats *Glossophaga soricina antillarum* was studied in the laboratory. In a series of dual choice tests using sugar concentrations from 10 to 80 %, the bats preferred the higher nectar concentration up to a maximum of 50 %. When presented simultaneously with nectar and additional water, the bats took more water, depending on the nectar concentration offered. The regular distribution of nectar and water intake during the activity period resulted in a roughly constant mean ingested concentration. Its maximal value agreed with that of the preferred nectar concentration, indicating that water needs played a role in determining the upper limit of an acceptable concentration. In preliminary experiments with ad libitum-feeders, sugar intake rate was maximal at 60 % despite the high viscosity at this concentration. Results are discussed in relation to the evolution of nectar concentration in bat-pollinated flowers.

Key words: Nectar concentration, water balance, food selection, bat, Glossophaga soricina antillarum.

INTRODUCTION

The adaptive significance of floral nectar production is generally considered to lie in its attractiveness to pollinators, for which it represents an energy source. Nectar characteristics are seen as adaptations to increase the frequency of visits of the potential pollinators, their fidelity to the source or both. As part of a dynamic, coevolving system, a flower should provide sufficient reward to attract pollinators, but it should limit this reward so that animals move on to visit other plants of the same species, ensuring cross-pollination. Thus, nectar concentration and the rate of nectar production of a plant may have remarkable adaptive significance since they primarily determine the energetic reward available to a forager. On the other side of this coevolving system, preferences of nectarivorous foragers are expected to have selective influence on the evolution of nectar characteristics (Heinrich & Raven 1972).

From the point of view of a flower visitor it is conceivable to delimit a lower and an upper limit of an acceptable nectar concentration. The lower limit may be influenced by: 1) the minimal energetic needs of the visitor, since the sugar content of the reward found in a flower should cover at least its energy expenditure for the visit (Schuchmann & Jakob 1981); 2) osmoregulatory constraints (Calder & Hiebert 1983), and 3) digestive speed and capacity (Diamond *et al.* 1986).

In the upper concentration range, on the other hand, the dramatic increase in viscosity at high concentrations may be of significance since viscosity reduces fluid ingestion rate (Heyneman 1983; Kingsolver & Daniel 1983), resulting in an increased time or energy investment to take a certain quantity of sugar. In addition, water requirements may influence the acceptance of highly concentrated nectars because the water budget must also be balanced. This latter aspect has not been investigated to date for flower visitors.

In this study we first measured the nectar concentration preference of a flower-visiting bat, *Glossophaga soricina antillarum*, over a wide range of concentrations. In a second experimental series we recorded the intake of additional water in relation to the concentration ingested by the bat, addressing the question whether water needs play a role in determining an upper limit for an acceptable nectar concentration. Furthermore, we studied the effects of nectar concentration on volume and sugar intake rates at *ad libitum*-feeders.

MATERIAL AND METHODS

Concentration preference

Concentration preferences of two adult females of *Glossophaga soricina antillarum* were determined in a flight tunnel (7 m x 1 m x 1 m) in the laboratory, under a LD cycle of 12 h : 12 h (light

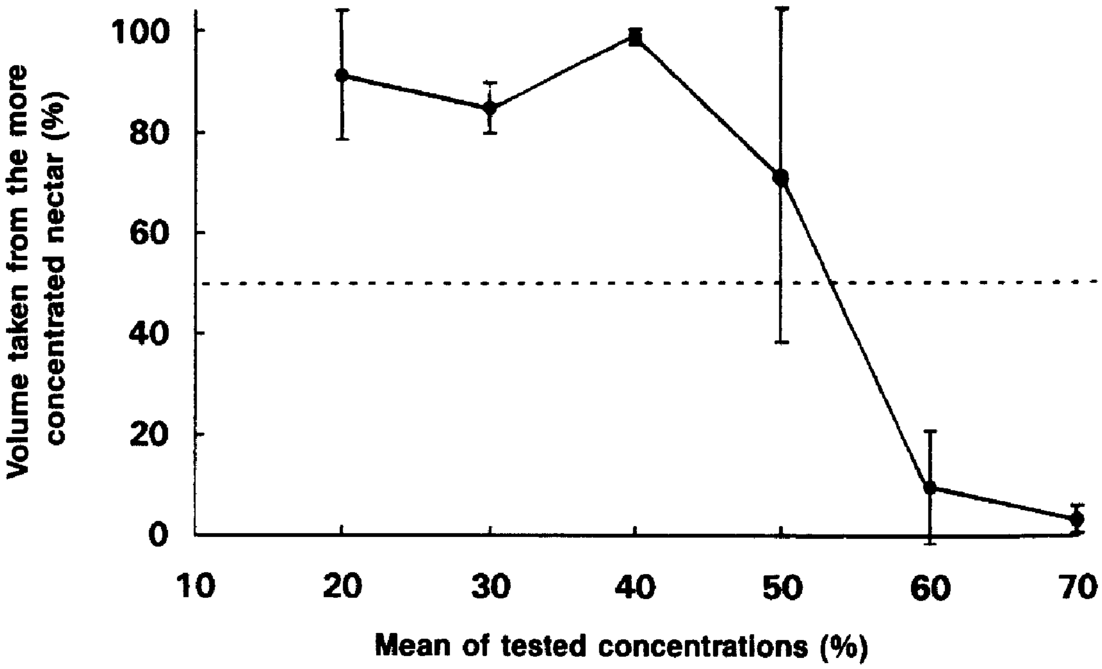

FIG. 1. Concentration preference of the bat *Glossophaga soricina antillarum*. Percentage of total volume taken from the more concentrated nectar (mean + SD) is plotted against mean concentration of the pair tested (e.g., results for the 10 % and 30 % pair are plotted at 20 %) (N = 16).

induced at 05.00 a.m.) Bats were presented with a choice between two artificial flowers mounted 15 cm apart at one end of the tunnel, centered in relation to the tunnel's longest axis.

Artificial flowers consisted of a glass tube (diameter: 15 mm; depth: 38 mm) mounted on a metal stem. Sugar solutions (henceforth: 'nectar') were made by diluting honey with water, and their concentrations were determined with an Atego refractometer to the nearest 0.2 % (sugar concentrations referred to in this study are percentage sugar equivalents on a solute mass/total mass basis; Bolten *et al.* 1979). A computarized arrange allowed the supply of controlled quantities of sugar solution. The number and temporal distribution of the bat visits were automatically recorded by interruption of an infrared light beam at each flower.

To determine concentration preferences, we presented individuals with a simultaneous choice between two nectars of different concentrations. At each visit the bat received a fixed volume of nectar (14 μl when lower concentrations were tested and 7 μl when higher concentrations were tested). This was only ca. 30—15 % of the volumes taken at ad libitum-feeders and ensured that the amount provided was completely taken by the bat during the visit. Rewards found at the two flowers during an experiment, therefore, differed in concentration but not in volume.

We used a total of eight concentrations between 10 % and 80 % (10 percentage points of concentration apart). Preference experiments were performed by presenting nectars 20 percentage points of concentration apart, i.e. 10 % *vs* 30 %, 20 % *vs* 40 % and so on. Each one lasted for one night. Five experiments were performed consecutively, and the pair to be tested during each one was selected at random. In between experiments the bat was provided with its regular diet. Each assay was replicated two or three times and the position of a given concentration was alternated to cancel the effect of preference for the left or right side. A total of 16 assays was performed. The volumes taken from each flower were then compared.

Water and nectar intake

A second experimental series was performed to measure the intake of additional water in relation to the sugar concentration of the presented nectar. The experimental arrange was the same as in the previous experiment, but here a bat was presented with a choice between water in one flower and nectar in the other. A different concentration was presented in each experimental night (from 35 to 80 %, nectars 5 percentage points of concentration apart).

Ad libitum intake rates

To investigate the effect of nectar concentration on intake rate we measured the volume intake rate of bats taking nectar from an unlimited-volume feeder. The feeder consisted of a glass tube (diameter: 15 mm; depth: 128 mm) mounted on an electronic balance (Mettler PM-100). It was filled with nectar to 30 mm below its rim. The volume taken during each bat visit was determined to the nearest 1 mg by reading the balance immediately after each visit. Hovering time was recorded with a resolution of 10 ms by interruption of an infrared light beam positioned at the tube rim.

Assays were performed in a room where a total of twenty *G. soricina* bats (*G. s. antillarum* and *G. s. soricina*; differences in intake rates be-

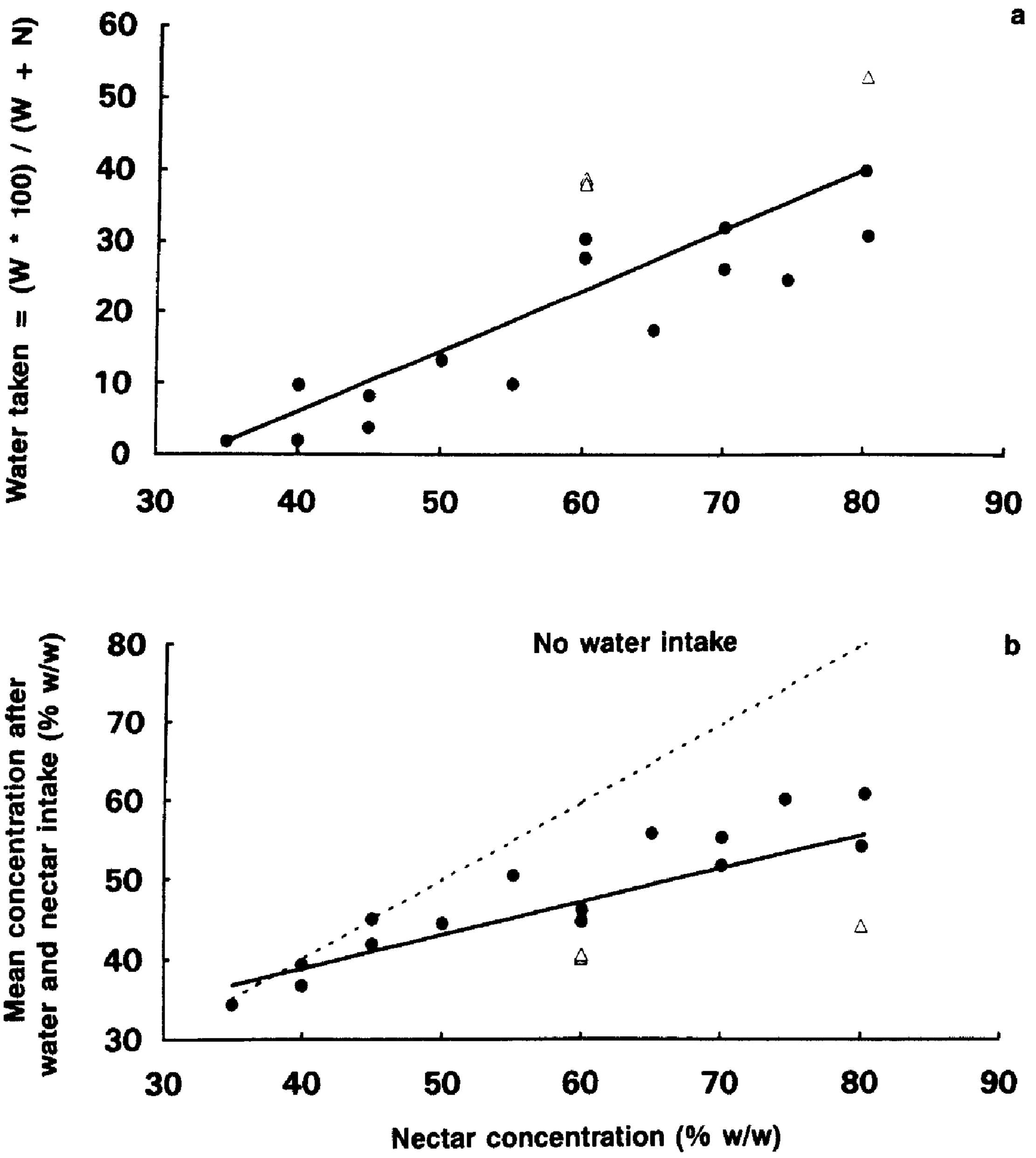

FIG. 2a. Additional intake of water as a function of the concentration of the offered nectar, expressed as percentage of the total fluid ingested. *W*: water intake (ml). *N*: nectar intake (ml). *Circles* and *triangles* correspond to different individuals. Regression equation of the pooled data: $Y = 0.85X - 28.05$; $r^2 = 69$ %; $N = 18$; $p < 0.01$. The absolute volumes of nectar taken during each assay depended on the concentration presented. At 40 % sugar concentration, for instance, the bat ingested ca. 9 ml of nectar during a night.

2b. Mean concentration for the whole night that resulted from additional water and nectar intake, as a function of the offered nectar concentration. Regression equation of the pooled data: $Y = 0.42X + 21.92$; $r^2 = 61$ %; $N = 18$; $p < 0.01$). For comparison, a situation in which there is no water intake during the night is presented (*dotted line*).

tween these subspecies are unlikely and were not considered) are currently maintained. These bats had not previously been exposed to this type of feeder. Four different series were performed using nectar concentrations of 20, 40, 60 and 80 %, and correlations between volume taken (data converted using relative density of aqueous sucrose solutions: Wolf *et al.* 1978) and hovering time were then calculated for each concentration.

RESULTS

Concentration preference

The bat took more from the more concentrated nectar at and below 50 % mean concentration, and more from the more dilute nectar above this value (Fig. 1). The proportion of the volumes taken deviates statistically from the ratio 1 : 1 (Gp-test for goodness of fit, Sokal & Rohlf 1981), thus indicating a preference for the more concentrated nectar up to 50 % mean concentration and for the more dilute one at and above 60 % mean concentration.

Water and nectar intake

The proportion of the water ingested during a night increased with the concentration of the nectar presented. There was a significant positive regression ($p < 0.01$) between these two variables (Fig. 2a).

The mean concentration for the whole night, resulting from nectar and water intake, is presented in Fig. 2b as a function of the offered nectar concentration. There was a significant positive regression ($p < 0.01$) between these variables. Although bats took significantly more water when presented with high nectar concentrations (Fig. 2a), mean concentration for the whole night increased with nectar concentration, but it did not exceed a value around 50—60 % (Fig. 2b).

An example of the temporal distribution of visits to the water-flower and to the nectar-flower during the night is depicted in Fig. 3a. After a high rate of water ingestion at the beginning of the night, the proportion of nectar-visits to water-visits remained roughly constant. This is shown in fig. 3b where the mean concentration resulting from water and nectar intake was calculated in 30 minute intervals. If the high water intake at the beginning of the night is not considered, a simple linear regression fitted the obtained data (Fig. 3b). The null hypothesis that its slope equals zero could not be rejected (two-tailed t-test; $t = 1.71$; $0.1 < p < 0.2$). Such an approximately regular distribution of visits to water and nectar was observed during all nights, resulting in a nearly constant mean ingested concentration throughout the activity period as presented in Fig. 3b (highest t-value obtained after all comparisons: $t = 2.05$; $0.05 < p < 0.1$).

Ad libitum intake rates

Despite the high variability in the data, there was a significant positive correlation ($p < 0.01$, data fitted with Y-intercept equal zero) between the volume taken per visit and the hovering time for each concentration tested except at 80 % (Fig. 4). The slope for 20 % differed statistically from 40 and 60 % (Tukey multiple comparison-test: 20 % vs 40 %, $q = 6.1$, $p < 0.05$; 20 % vs 60 %, $q = 5.7$, $p < 0.05$). Those for 40 and 60 % were not statistically different ($q = 1.2$, NS).

Calculations of the volume and sugar intake rates from the slopes of the obtained correlation equations are presented in Fig. 5. While volume intake rate decreased with concentration, sugar intake rate peaked at 60 %.

DISCUSSION

When presented with a choice of fixed-volume rewards of nectar differing in 20 percentage points of concentration, the flower-visiting bat *Glossophaga soricina antillarum* preferred the higher concentration but only to a maximum of 50 %. Why did the bat not select the highest concentration to obtain the maximum energy intake per visit? Two factors are likely to play a role in determining a preference for sugar concentrations not higher than 50 %: water might be required to balance the animal's daily budget and to facilitate food transit through the digestive tract, and a high viscosity might seriously impede fluid intake.

The idea that water requirements indeed play a role in setting an upper limit for an acceptable nectar concentration is supported by the results from our experiments where the bat could choose between a 'water flower' and a 'nectar flower'. During their first hour of activity all bats ingested a considerable amount of water (Fig. 3), probably for rehydration. After this initial phase, a bat took more water, depending on the nectar concentration offered (Fig. 2a), thus 'diluting' the ingested nectar. The resulting mean

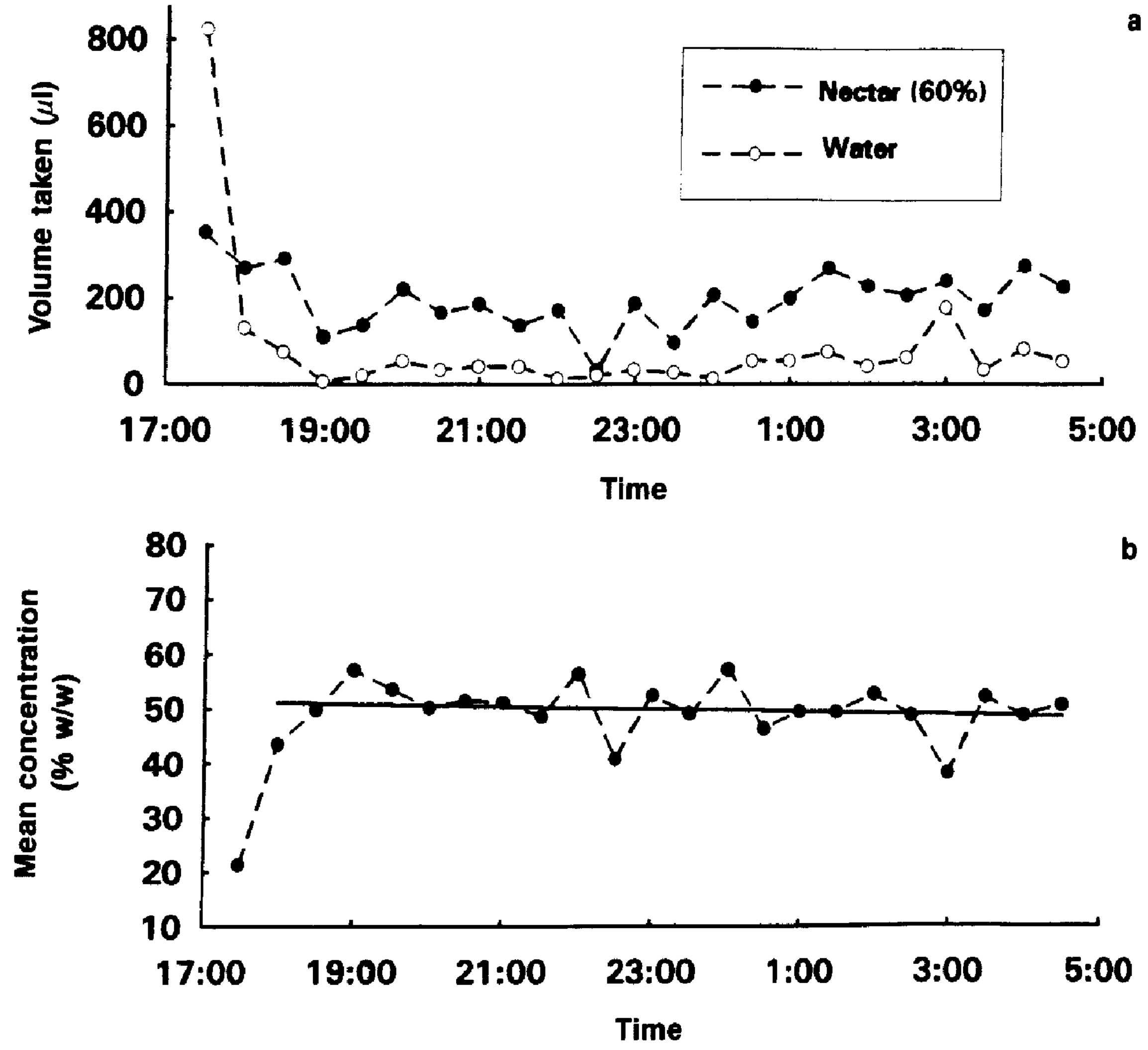

FIG. 3 a. Example of the temporal distribution of water and nectar intake throughout the bat's activity period. Data were pooled in 30 min intervals (under the given experimental conditions, the bat usually visited 20 to 30 times in 30 min the nectar-flower). Scotophase: 17:00 to 5:00 h.
3 b. Average ingested concentration after nectar and additional water intake, for the same intervals.

concentration of this 'diluted' nectar did not exceed 50—60 % (Fig. 2 b), which coincided with the preferred concentration in the choice experiments (Fig. 1). In addition, the regular temporal distribution of water and nectar intake throughout the bat's activity period suggests the participation of a regulatory system which enables the bat to maintain a roughly constant value of sugar concentration. It is interesting to note that this mean sugar concentration remained constant during each specific night although on different nights it could vary even for the same nectar concentration (e.g., 80 % in Fig. 2b). This might be due to differences in the physiological condition of the bats or to inter-individual variability (see Fig. 2, circles and triangles).

It is unlikely that bats of this size need a large amount of water. As nectar from chiropterophilous flowers is very diluted (v. Helversen, *this volume*) nectar-feeding bats have, during evolution, adapted to a high water intake. Correspondingly, the medulla of glossophagine kidneys is very poorly developed (Studier *et al.* 1983), which might make it impossible to produce concentrated urin in order to save water.

The second factor that might determine an upper limit for concentration preference is viscosity. Because it increases with sugar concentration, the energetic cost of nectar extraction should also increase (Heyneman 1983; Kingsolver & Daniel 1983). In our measurements the highest sugar intake rate at unlimited-volume feeders was obtained at 60 % (Fig. 5). Comparable measurements for hummingbirds peaked in the 30—50 % range (Tamm & Gass 1986). Different feeding techniques between hummingbirds

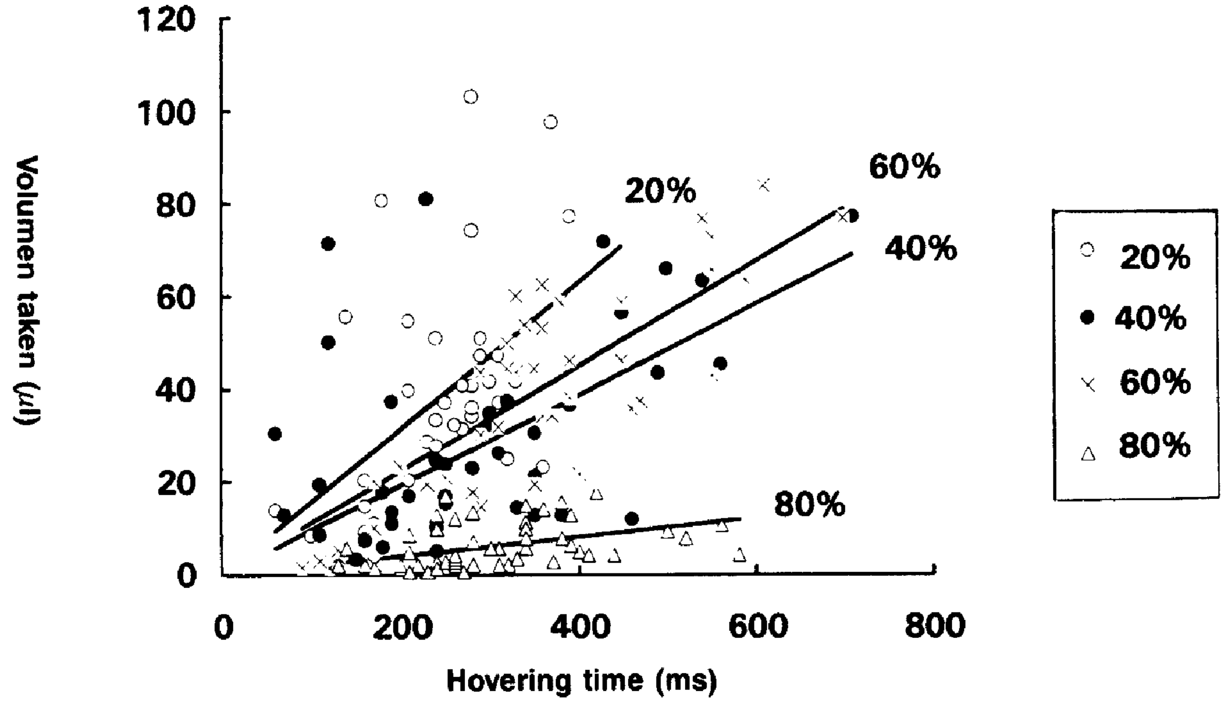

FIG. 4. Simple linear correlations between hovering time and volume taken from an unlimited-volume feeder. The equations were calculated with Y-intercept equal zero.
20 %: $Y = 0.156X$ ($r^2 = 31$ %; SE of the slope = 0.012; N = 39; $p < 0.01$). 40 %: $Y = 0.100X$ ($r^2 = 48$ %; SE of the slope = 0.008; N = 42; $p < 0.01$). 60 %: $Y = 0.112X$ ($r^2 = 71$ %; SE of the slope = 0.005; N = 42; $p < 0.001$). 80 %: $Y = 0.020X$ ($r^2 = 11$ %; SE of the slope = 0.002; N = 50; NS).

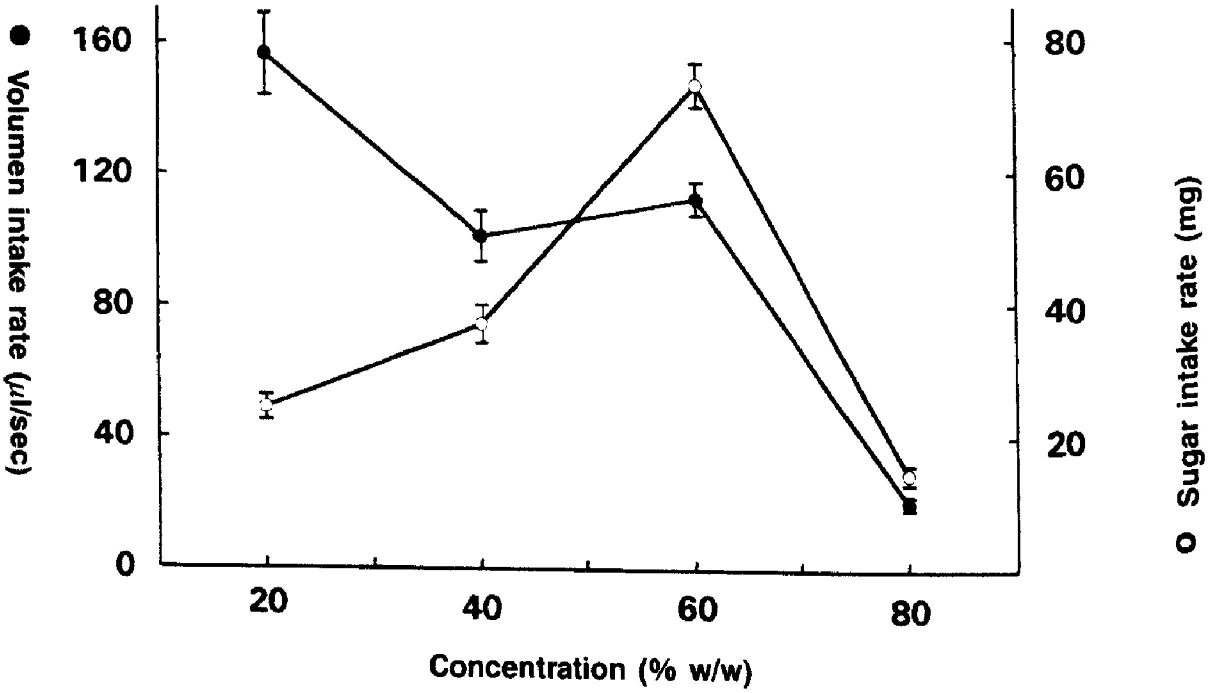

FIG. 5. Volume (μl/s) and sugar intake rates (mg/s) at different concentrations, calculated from the equations presented in Fig. 4 (Mean + SE of the slope).

and bats may be responsible for this result. The considerable reduction of both volume and sugar intake rates recorded at 80 % may be attributed to the dramatic increase in viscosity (viscosity of a sucrose solution at e.g., 74 % is 857 times higher than at 20 %, Wolf *et al.* 1978).

The nectars of chiropterophilous flowers, with mean sugar concentrations between 9 and 29 % (v. Helversen, *this volume*) are similarly or even more dilute than those of hummingbird-pollinated flowers. Since nectars of bee-pollinated flowers are much more concentrated (Baker 1975), it was proposed that dilute nectars have evolved not to attract hummingbirds but to deter diurnal nectar-robbing bees (Bolten & Feinsinger 1978). It appears unlikely, however, that bees had a significant influence on the nocturnal chiropterophilous flowers. Several other reasons may, however, be responsible for such low concentrations. If the carbohydrate that is lost via the nectar sugar has an influence on a plant's seed set as shown by Pyke (1991), a plant should reduce sugar investment for pollinator attraction to the necessary minimum. Most rainforest plants should be energy limited rather than water limited. In this situation the water fraction of the nectar should merely function as 'packaging material' for the limited amount of sugar that a plant is willing to invest. In such a case a low nectar concentration may be adaptive even from the pollinator's point of view as shown by the experiments of Mitchell & Paton (1990). They showed that honeyeaters feeding from artificial flowers that contained small nectar rewards which differed in concentration but were constant in sugar content attained their maximal sugar intake rates at 20 %. In general one should expect a conflict of interest between plant and pollinator with the plant trying to maximise the rate of visits and the pollinator trying to maximise the energy gain per visit (cf. Heinrich & Raven 1972).

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