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Sugars are complementary resources to ethanol in foods consumed by Egyptian fruit bats

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SUMMARY

Food resources are complementary for a forager if their contribution to fitness is higher when consumed together than when consumed independently, e.g. ingesting one may reduce the toxic effects of another. The concentration of potentially toxic ethanol, [EtOH], in fleshy fruit increases during ripening and affects food choices by Egyptian fruit bats, becoming deterrent at high concentrations (≥1%). However, ethanol toxicity is apparently reduced when ingested along with some sugars; more with fructose than with sucrose or glucose. We predicted (1) that ingested ethanol is eliminated faster by bats eating fructose than by bats eating sucrose or glucose, (2) that the marginal value of fructose-containing food (food+fructose) increases with increasing [EtOH] more than the marginal value of sucrose- or glucose-containing food (food+sucrose, food+glucose), and (3) that by increasing [EtOH] the marginal value of food+sucose is incremented more than that of food+glucose. Ethanol in bat breath declined faster after they ate fructose than after eating sucrose or glucose. When food [EtOH] increased, the marginal value of food+fructose increased relative to food+glucose. However, the marginal value of food+sucrose increased with increasing [EtOH] more than food+fructose or food+glucose. Although fructose enhanced the rate at which ethanol declined in Egyptian fruit bat breath more than the other sugars, the bats treated both fructose and sucrose as complementary to ethanol. This suggests that in the wild, the amount of ethanol-containing fruit consumed or rejected by Egyptian fruit bats may be related to the fruit's own sugar content and composition, and/or the near-by availability of other sucrose- and fructose-containing fruits.

Key words: fructose, frugivory, glucose, marginal value of food, sucrose, toxins.

INTRODUCTION

When foraging on plant parts or products such as fruits, leaves or nectar, animals not only may be rewarded in terms of nutrients, but also may be deterred by toxins produced by the plant itself, or by micro-organisms in the food (Jakubska et al., 2005; Janzen, 1977). These toxins may reduce the nutritional value of food and therefore affect food selection (Cipollini, 2000; Harborne, 1993). However, the toxicity of these compounds may also be reduced by nutrients present in the same, or in a different, food item. Indeed, the increase in palatability of toxin-containing food brought about by certain nutrients might be one of the explanations why herbivores function better when offered combinations of different foods than when fed single-food diets (Freeland and Janzen, 1974). Thus, toxins in plants and those nutrients that reduce their toxicity should be treated as complementary resources (Rapport, 1980; Tilman, 1980) by herbivores because, by ingesting these resources together, a forager would earn more towards its fitness than by ingesting the toxin separately.

Ethanol is a potentially toxic compound often encountered by frugivorous bats in their food. Ethanol occurs ubiquitously in fleshy fruit as a by-product of the alcoholic fermentation of sugars mainly by micro-organisms, but also by the fruit itself (Battcock and Azam-Ali, 1998; van Waarde, 1991). Ethanol content increases as fruit ripens (Dominy, 2004; Dudley, 2004; Sánchez et al., 2004), suggesting that obligate frugivores, such as fruit bats, may consume significant amounts of this alcohol. For example, ripe fruit eaten by Egyptian fruit bats (*Rousettus aegyptiacus*, E. Geoffroy 1810) may contain ~0.1 to 0.7% ethanol, whereas unripe and overripe fruit may contain lower and higher concentrations, respectively (Sánchez et al., 2004). Our research on the effects of the presence of ethanol in artificial food on the foraging behaviour of captive Egyptian fruit bats indicated that at low concentrations (<1%) ethanol does not affect food selection, whereas at high concentrations (≥1%) ethanol deters the bats (Sánchez et al., 2004; Sánchez et al., 2006). In addition, the ingestion of artificial food containing 1% ethanol can impair the flight skills of the bats (F.S., unpublished observations), i.e. food containing ≥1% ethanol can be considered as aversive for these bats. Thus, micro-organisms, *via* the ethanol they produce, may have negative effects on fruit bat–plant interactions due to the toxic effects of ethanol. Nevertheless, the presence of nutrients in fruit that complement ethanol could modify its effects on food selection by fruit bats.

For example, sugars such as fructose, glucose and sucrose, all common in fleshy fruits (Baker et al., 1998) and, coincidentally, in the natural diet of Egyptian fruit bats (Biner et al., 2007; Nazif, 2002; Van Handel et al., 1972), reduce the toxic effects of ethanol in rats and humans (Berman et al., 2003; Mascord et al., 1991; Roberts et al., 1999). Moreover, independent experiments in rats and humans showed that, in both species, elimination of ethanol from the blood was faster after ingestion of fructose than after ingestion of sucrose or glucose (Jones, 1983; Parlesak et al., 2004).

In light of the above, we hypothesized that Egyptian fruit bats treat sugars and ethanol as complementary resources, but the degree

of complementarity differs among sugars, being higher between fructose and ethanol than between either sucrose or glucose and ethanol. Since sucrose is hydrolysed into fructose and glucose, we further hypothesized that the complementarity between sucrose and ethanol would be higher than that between glucose and ethanol. Hence, we predicted that ethanol would be eliminated faster when the bats ate food containing fructose than when their food contained either sucrose or glucose.

Patch use theory can be used to determine whether a forager treats food resources as complementary. In food patches where foragers experience diminishing returns, the amount left in the patch after foraging, the giving-up density (GUD), is related to the forager's quitting harvest rate (Brown, 1988). The quitting harvest rate is sensitive to the marginal value of the food patch, i.e. the fitness value of an additional food item ingested by a forager (Brown, 1992). Therefore, the lower the GUD, the higher the marginal value of the food in the patch, and the higher its expected contribution to fitness. Thus, GUD can be used to estimate the marginal value of food to a forager, and also to evaluate nutritional relationships among foods (Schmidt et al., 1998). For example, Schmidt et al. (Schmidt et al., 1998) showed that when resources are perfectly substitutable, their marginal values remain constant independent of their consumption, and they may be valued by the forager depending only on energy content and handling time (Pulliam, 1974). In contrast, the marginal value of complementary food resources depends on the relative amount of each that is consumed. Indeed, when two resources are mutually complementary, the ingestion of one increases the marginal value of the other, and *vice versa*. Nonetheless, in the case of toxin ingestion, complementarity may not be mutual because by ingesting a toxin the nutrient that complements it increases its marginal value, but not *vice versa* (Whelan et al., 1998).

We predicted that the marginal value of fructose-containing food (food+fructose) increases with ethanol concentration, [EtOH], more than with sucrose- or glucose-containing food (food+sucrose; food+glucose). We further predicted that the marginal value of food+sucrose increases with [EtOH] more than that of food+glucose. We assumed that following changes in the marginal value of foods relative to their [EtOH] would reveal whether the bats treat sugars and ethanol as complementary resources, and used the difference between marginal values of sugar-containing foods with and without ethanol for this purpose (Table 1).

In addition, we expected that the preference for fructose relative to sucrose or glucose would increase with [EtOH] in food, and the preference for sucrose relative to glucose would increase with [EtOH] in food.

Table 1. Predictions of the effects of [EtOH] on the marginal value of foods containing different sugars for Egyptian fruit bats

Ethanol-free food		Ethanol-rich food
$(MV_{Frc}–MV_{Scr})$	<	$(MV_{Frc}–MV_{Scr})$
$(GUD_{Frc}–GUD_{Scr})$	>	$(GUD_{Frc}–GUD_{Scr})$
$(MV_{Frc}–MV_{Glc})$	<	$(MV_{Frc}–MV_{Glc})$
$(GUD_{Frc}–GUD_{Glc})$	>	$(GUD_{Frc}–GUD_{Glc})$
$(MV_{Scr}–MV_{Glc})$	<	$(MV_{Scr}–MV_{Glc})$
$(GUD_{Scr}–GUD_{Glc})$	>	$(GUD_{Scr}–GUD_{Glc})$

The marginal value of fructose-containing food (food+fructose) will increase with [EtOH] relative to that of sucrose- or glucose-containing food (food+sucrose, food+glucose). Also, the marginal value of food+sucrose will increase with [EtOH] relative to that of food+glucose. MV_{Frc} and GUD_{Frc} are the marginal value and giving-up density for food+fructose; MV_{Scr} and GUD_{Scr} are the marginal value and giving-up density for food+sucrose; MV_{Glc} and GUD_{Glc} are the marginal value and giving-up density for food+glucose.

MATERIALS AND METHODS

Experimental animals

We tested our predictions using adult, non-reproductive, Egyptian fruit bats (Chiroptera, Pteropodidae) from a colony maintained on the Sede Boqer Campus of Ben-Gurion University of the Negev, Israel. Females weighed 120–140 g and males 130–170 g. The females and males were kept in separate outdoor flight cages that had sides covered with plastic mesh shading material (~90% shade). Between experiments, we offered the bats commercially produced fleshy fruit, such as melons, watermelons, bananas and apples, *ad libitum*. [EtOH] in commercial fruits is highly variable, and depends on fruit variety, conditions of storage and display at the market. [EtOH] in these fruits is between 0.01% and 0.8% (Ke et al., 1991; Liu and Yang, 2002; Senesi et al., 2005) [see also Stevens, cited by Milton (Milton, 2004)], although higher values have been found. For example, only 2 days after optimum commercial maturity, some varieties of melon contain ~4% ethanol (Senesi et al., 2005).

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Ethanol in bat breath

Breath analysis has been widely used as a non-invasive technique to estimate blood-ethanol in humans. Indeed, breath analysis provides a similar pharmacokinetic profile of [EtOH] to that measured in venous blood. Namely, these methods show similar patterns of ethanol elimination and their measurements are highly correlated (Jones and Anderson, 2003). This is because, after consumption, ethanol distributes itself completely in body water, and well-vascularized organs, such as brain, liver and lungs, may rapidly establish an equilibrium between extra- and intracellular [EtOH] (Eckardt et al., 1998). In addition, after oral ingestion of ethanol and before equilibration among all tissue and extracellular compartments, [EtOH] in the brain and arterial blood are higher than in less-vascularized tissues such as muscle and peripheral veins (Eckardt et al., 1998). Therefore, breath [EtOH] more accurately reflects the level of central nervous system exposure to this alcohol than [ETOH] in venous blood, particularly at the outset of distribution of ethanol in blood and other tissues (Eckardt et al., 1998). Thus, we considered breath ethanol content as a relevant indicator to assess the effects of ingested sugar on ethanol elimination in Egyptian fruit bats. Furthermore, given that the method of analysis is non-invasive, it avoids the trauma of serial blood sampling, which might harm the bats.

We prepared mixtures containing 1% ethanol, and fructose, sucrose or glucose (200 g) in 1 l of distilled water. The mixtures were prepared no more than 5 min before the beginning of each trial and were administered orally to five adult males. We assumed that, as in humans, the likelihood of ethanol intoxication in bats may be estimated based on blood volume (Garriot, 2003). Therefore, the volume of ethanol given to each bat was proportional to its total blood volume, which is ~7.2% of body mass (Noll, 1979). Unfortunately, to our knowledge, there is no information on ethanol metabolism in fruit bats; thus, we also assumed that ethanol kinetics in humans and Egyptian fruit bats was similar when estimating the intoxicating dose for each bat. For example, for a 70 kg human with a 5 l blood volume, 9000 ml of a 1% mixture of ethanol in water is necessary to increase blood alcohol content to a level of intoxication, ~0.15 g 100 ml^{-1} (Morris et al., 2006); thus a 0.14 kg

fruit bat would require 18.5 ml of the same mixture to achieve a similar blood alcohol content.

We measured ethanol levels in fruit bat breath with a gas chromatograph (GC; Scentoscreen, Sentex Systems Inc., Fairfield, NJ, USA) using argon as the carrier gas, and a column temperature of 130°C. Before the trials, we determined its retention time with ethanol standards and then measured ethanol levels in the breath of bats before administering a dose of ethanol and 5, 30, 50, 70, 90, 110 and 130 min thereafter. Between measurements, two samples of clean, dry air were run to ensure that no ethanol traces remained in the GC column. Breath samples were taken by placing the bat's snout at the end of a funnel connected to the GC sampling tube. The GC was programmed to pump samples for 10 s each time. We used the integrated area under the peak of the retention time of ethanol as a relative measurement of the ethanol content in the breath.

The marginal value of food containing ethanol and sugars

We used feeders as artificial food patches from which the bats experienced diminishing returns (Sánchez, 2006), and measured the GUD of liquid food containing ethanol and one of three different sugars. The liquid food contained 3 g of soy protein infant formula (Isomil; Abbot Laboratories, Hoofddorp, The Netherlands), 0.66 g NaCl, 0.84 g KCl, and 0.584 mol sucrose (200 g) or 2×0.584 mol (210.5 g) of fructose or glucose, all dissolved in 1 l of distilled water.

The feeders were made of a cylindrical, plastic container (base diameter 6 cm; height 10 cm) with an opening big enough (diameter 6 cm) for the bats to feed from, as described previously (Sánchez, 2006). The feeders were attached to the walls of the flight cages, and filled with liquid food. To induce diminishing returns from the feeder, we placed an inedible substrate made of 39 pieces of latex hose (each 20 mm long, 10 mm outer diameter, 7 mm inner diameter) strung on fishing line in the feeder. The fishing line was anchored to the bottom of the feeder to prevent removal of the rubber pieces by the bats. The interference caused by the rubber pieces forced the bats to work harder and harder as they removed food from the feeder and had to push down on the rubber pieces in order to obtain more food while going deeper into the feeder (Sánchez, 2006). We used this type of feeder in all experiments.

In these trials, we put five female bats at a time in one of the cages, and placed two feeders, containing different sugars, at each of three stations, i.e. a total of six feeders in the cage. At the same time, in another cage, we placed two feeders containing different sugars at each of four stations, i.e. a total of eight feeders in the cage, and introduced eight male bats at a time. In the cage of females, each feeder was filled with 75 ml of food, whereas in the males' cage each feeder contained 100 ml of food. We used different numbers of female and male bats because those were the numbers of each in the colony. The amount of food available for an individual male (8 feeders×100 ml/8 bats=100 ml per bat) was slightly greater than that for a female (6 feeders×75 ml/5 bats=90 ml per bat) to compensate for their larger size.

We offered the bats food containing fructose, sucrose or glucose, and tested all pair-wise comparisons when the patches also contained either 0% or 1% ethanol, i.e. we did six pair-wise comparisons. The order of presentation of each comparison was chosen randomly, and we repeated each experiment three times. We provided the food shortly before sunset (~18:30 h) and measured the amount of food left in the feeders, i.e. the GUD, shortly after sunrise (~06:30 h). We did these experiments during the spring of 2006.

Estimated daily energy expenditure

Because we previously found that daily energy expenditure (DEE) affects GUD in Egyptian fruit bats kept in outdoor cages (Sánchez et al., 2008), we assessed the possible influence of DEE here as well. Since the cages were protected from the sun and wind, we assumed that the effects of direct solar radiation and of convection were negligible, and used air temperature, T_a, to estimate the metabolic rate of resting (day) and active (night) bats with the equations of Noll (Noll, 1979) (see below). We observed that our bats were active for about 11 h per night and, of that, they were in flight for some 8 min (Sánchez et al., 2008). Therefore, to estimate DEE, we assumed that the bats rested for 13 h during their daytime, inactive phase, rested for 10 h 52 min in their night-time, active phase, and flew for 8 min at a metabolic rate 14 times the active resting rate (Thomas, 1975).

We measured T_a in the cages to ±0.5°C using two Thermochron iButtons (DS1921 Maxim/Dallas Semiconductor Corp., Sunnyvale, CA, USA), hanging 20 cm from the roof of the cages. We set the iButtons to record T_a at 10 min intervals, and averaged the measurements. Based on each average, we estimated the oxygen consumption ($\dot{V}_{O_2}$, in ml g^{-1} h^{-1}) by male and female bats whose body masses were 130 and 150 g, respectively, during each 10 min interval using empirically derived equations for bats acclimated to 15°C at rest during the day ($\dot{V}_{O_2}$=2.63–0.054T_a), and active at night ($\dot{V}_{O_2}$=6.73–0.134T_a) [see table 2, p. 82 of Noll (Noll, 1979)]. To obtain an estimate of total daily $\dot{V}_{O_2}$, which we converted to DEE, we summed all estimates for 10 min intervals of a diurnal cycle and added 3 ml O_2 per gram of body mass for the 8 min of flight.

Statistical analyses

We analysed the measurements of ethanol in bat breath by repeated-measures analysis of variance (RM-ANOVA), using breath ethanol content as the dependent variable, bat as a random effect, and sugar type and time as fixed effects. We used contrasts with a Bonferroni correction in a multiple comparison procedure (Neter et al., 1996). We used analysis of covariance (ANCOVA) to examine the effect of [EtOH] on the marginal value of food containing different sugars for the bats, with GUD_{Frc}–GUD_{Scr}, GUD_{Frc}–GUD_{Glc} or GUD_{Scr}–GUD_{Glc} as the dependent variable, [EtOH] as a fixed effect, food station as a blocking factor, and thermoregulatory costs entered as a covariant. We also examined the sugar preferences of the bats using 95% confidence intervals based on Student's one-tailed *t*-tests. As an index of preference we used $GUD_{Sugar\ A}/GUD_{Sugar\ A}+GUD_{Sugar\ B}$. $\alpha \leq 0.05$ was chosen as the minimal acceptable level of significance.

RESULTS

Ethanol in bat breath

Ethanol levels in bat breath, before administration of the ethanol–food mixtures, were similar to those of fresh air, and increased considerably after ingestion (Fig. 1). RM-ANOVA indicated that the main effect sugar type did not significantly affect ethanol levels in bat breath [mean square (MS)=3.4×10^{12}, $F_{2,8.01}$=2.71, P=0.126], whereas both time and bat did (MS=1.1×10^{12}, $F_{6,24.1}$=10.5, $P<0.001$, and MS=1.4×10^{12}, $F_{4,11.5}$=8.27, P=0.002, respectively). The analysis also showed that the interaction between sugar type and time was significant (MS=1.8×10^{12}, $F_{12,47}$=3.02, P=0.003), and from 70 min on, ethanol content in breath was lower after bats ingested fructose than after ingesting either of the other sugars (contrasts $P<0.05$, Fig. 1). In addition, there were no significant differences between ethanol in bat breath 90 min after they ingested an ethanol mixture containing

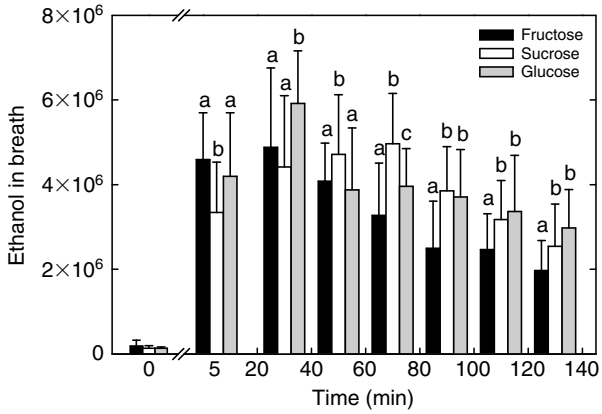

Fig. 1. Changes in ethanol levels (relative values) in Egyptian fruit bat breath after they ingested mixtures containing 1% ethanol and fructose, sucrose or glucose. Ethanol in breath is equal to the integrated area under the peak of the retention time of ethanol, measured by gas chromatography. Ethanol levels were affected by the interaction of sugar type and time (RM-ANOVA, $P<0.01$). After 70 min, ethanol levels were significantly lower when bats ingested food+fructose than for either food+sucrose or food+glucose. Values at time 0 were recorded before the mixtures were administered. Error bars are 1 s.d. Different letters above the bars denote significant differences (contrasts with Bonferroni correction, $P<0.05$).

sucrose or glucose (contrasts $P>0.05$). Thus, the bats apparently eliminated ethanol faster when food contained fructose than when it contained either sucrose or glucose.

[EtOH] and the marginal value of sugar-containing foods

Ethanol concentration affected the marginal value of the sugar-containing foods for both female and male bats (Table 2), i.e. GUD increased with the addition of 1% ethanol to the food (Fig. 2). Moreover, the marginal value of foods containing different sugars changed owing to the increase in [EtOH]. Indeed, by increasing [EtOH] in the food, the marginal value of food+fructose decreased relative to food+sucrose (Fig. 2A,B) and increased relative to food+glucose (Fig. 2C,D). The marginal value of food+sucrose was higher than that of food+glucose when [EtOH] increased (Fig. 2E,F).

Only in males was the interaction between DEE and [EtOH] significant (Table 2B) when the bats were offered food+fructose and food+glucose. Likewise, only in females offered food+sucrose and food+glucose was the effect of DEE significant (Table 2C).

[EtOH] and sugar preferences

In general, female and male bats appeared to prefer sucrose over fructose and glucose, and fructose over glucose, and when food contained ethanol these preferences were accentuated (Fig. 3). When food did not contain ethanol, the bats did not show significant differences in their preference for fructose or sucrose (Student's one-tailed t-test, $P>0.05$; Fig. 3A). However, when ethanol was added, they significantly preferred sucrose over fructose (Student's one-tailed t-test, $P<0.05$). The bats preferred fructose over glucose when food contained either 0% or 1% ethanol (Student's one-tailed t-test, $P<0.05$; Fig. 3B), although for the females there was no significant difference with no ethanol (Student's one-tailed t-test, $P>0.05$). Finally, the bats preferred sucrose over glucose when food contained either 0% or 1% ethanol (Student's one-tailed t-test, $P<0.05$; Fig. 3C).

DISCUSSION

In support of our predictions, we found that the elimination of ethanol from bat breath was faster when ethanol was administered together with fructose than when it was administered with sucrose or glucose. However, in contrast to our predictions, ethanol elimination was not faster when sucrose was added to ethanol-containing food than when glucose was added. Thus, these results indicate that the complementary relationship between fructose and ethanol was stronger than that between sucrose or glucose and ethanol for Egyptian fruit bats.

In general, sugars affect ethanol kinetics by reducing gastric emptying, which slows ethanol absorption (Schwartz et al., 1996), and in doing so reduces the speed at which ethanol reaches the

Table 2. Results of the ANCOVAs used to test the effect of ethanol concentration on the marginal value of food containing different sugars for female and male Egyptian fruit bats

	Females				Males			
Source	d.f.	MS	F-ratio	P	d.f.	MS	F-ratio	P
A. Fructose–sucrose								
Ethanol	1	2138.06	5.36	**0.038**	1	4090.64	9.76	**0.006**
DEE	1	1142.01	2.86	0.114	1	480.82	1.15	0.298
Station	2	275.56	0.69	0.519	3	309.05	0.74	0.543
Error	13	398.78			18	419.06		
B. Fructose–glucose								
Ethanol	1	1462.83	6.16	**0.028**	1	2457.14	4.28	**0.05**
DEE	1	218.60	0.92	0.355	1	8.41	0.02	0.905
Ethanol×DEE	–	–	–	–	1	3050.68	5.31	**0.034**
Station	2	94.69	0.40	0.679	3	661.33	1.15	0.357
Error	13	237.507			17	574.61		
C. Sucrose–glucose								
Ethanol	1	2715.05	7.70	**0.016**	1	8085.20	18.85	**<0.001**
DEE	1	1955.05	5.55	**0.035**	1	65.58	0.15	0.70
Station	2	39.02	0.11	0.896	3	123.50	0.29	0.833
Error	13	352.47			18	428.88		

We did independent analyses for each pair-wise comparison of sugars (A, fructose *vs* sucrose; B, fructose *vs* glucose; C, sucrose *vs* glucose), and for each sex. In all comparisons the factor Ethanol (ethanol concentration) was significant. MS, mean square; DEE, estimated daily energy expenditure. Significant values, $P<0.05$, are presented in bold.

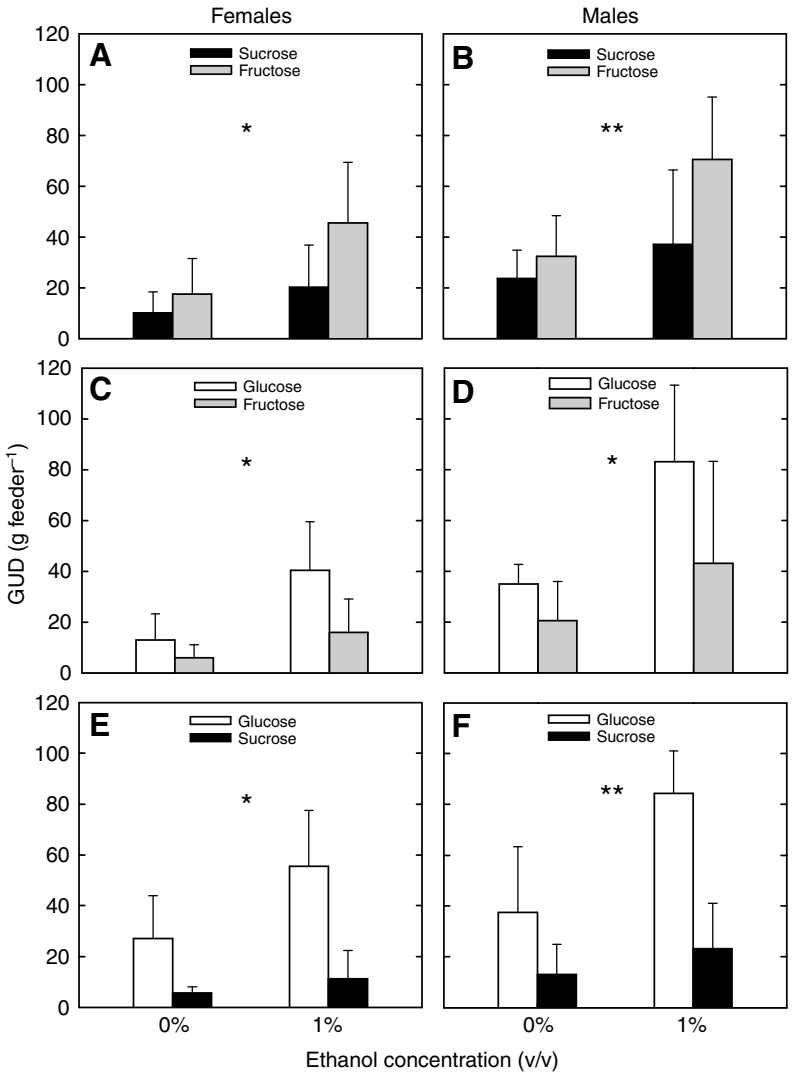

Fig. 2. Effects of ethanol concentration on the marginal value of food containing fructose, sucrose or glucose for female (left panels) and male (right panels) Egyptian fruit bats. Marginal values were estimated using giving-up density (GUD). The difference between the GUD of the sugar-containing foods tested was significantly greater when the foods contained 1% ethanol than when they were ethanol free (one and two asterisks indicate significance at the $P<0.05$ and $P<0.01$ level, respectively). By increasing ethanol concentration in the food, the marginal value of food+fructose decreased relative to food+sucrose (A,B) and increased relative to food+glucose (C,D). The marginal value of food+sucrose increased relative to that of food+glucose with ethanol abundance (E,F). Error bars are 1 s.d.

bloodstream (Kricka and Clark, 1979). The 'fructose effect', i.e. faster elimination of ethanol in the presence of fructose than in presence of other sugars, has been recognized for more than 50 years [Stuhlfauth and Neumaier, 1951, cited in Tygstrup et al. (Tygstrup et al., 1965)]; nonetheless, the underlying mechanism is not clearly understood. During ethanol catabolism NAD is reduced to NADH, and the supply of NAD might limit ethanol oxidation. It has been proposed that during fructose metabolism NADH is transformed into NAD, and thus fructose increases ethanol oxidation (Scholz and Nohl, 1976; Thieden et al., 1972; Tygstrup et al., 1965). However, this idea is not fully accepted yet (Mascord et al., 1991). Egyptian fruit bats assimilate fructose faster than glucose (Keegan, 1977), and although there is no information on sucrose assimilation in these bats, it is possible that differences in the rate of assimilation of sugars may help explain their effect on ethanol elimination.

The results of the present study suggest that Egyptian fruit bats are less efficient at metabolizing ethanol than wild, frugivorous birds. For instance, European starlings, *Sturnus vulgaris,* administered oral doses of ethanol of 3 g kg^{-1} eliminated 100% of the alcohol within 130 min (Prinzinger and Hakimi, 1996). Waxwings, *Bombycilla garrulous*, administered intravenous doses of ethanol of 2 g kg^{-1} eliminated almost all the alcohol in 120 min (Eriksson and Nummi, 1982). In contrast, the breath of Egyptian fruit bats that had been given a relatively small oral dose of ethanol (~0.13 g kg^{-1}) still contained three orders of magnitude more ethanol than before administration after 130 min [before, 152681±85032, mean ± s.d.; after 130 min, 2.495($\times10^6$)±918123].

Even though there is little information in the literature on the ethanol content of fruits consumed by birds and bats, at least in Israel, it seems that birds and bats consume fruits that have a similar [EtOH] (Sánchez et al., 2004). So, there does not appear to be an *a priori* reason to expect that frugivorous birds should metabolize ethanol more efficiently than frugivorous bats. The fact that birds apparently do so suggests that they have an advantage over bats when high quality, ripe fruit is scarce. This may be because when ripe fruit is hard to find, frugivores may be in relatively poor body condition and are, therefore, more likely to take the risk of ingesting ethanol-rich fruit (Sánchez et al., 2008).

In support of our predictions, the increase of [EtOH] in food raised the marginal value of food+fructose and food+sucrose relative to food+glucose, indicating that Egyptian fruit bats treated ethanol and sucrose, and ethanol and fructose as complementary resources (Schmidt et al., 1998). However, contrary to our predictions, incrementing [EtOH] increased the marginal value of food containing sucrose relative to food containing fructose. These results suggest that, when confronted with ethanol-rich food, the amount ingested by the bats depends, at least in part, on the sugar content of the food item.

Although the increase in [EtOH] did not modify the rank order of sugar preferences by Egyptian fruit bats, it augmented their preference for the sugar with a higher marginal value when food did not contain ethanol. The Egyptian fruit bats' preference for sucrose over fructose, and for fructose over glucose, is similar to that of other Old World fruit bats, and this pattern has been explained by the lower threshold for tasting sucrose than fructose or glucose in these bats (Herrera et al., 2000). The pattern of sugar preferences shown by female and male bats was very similar; however, females did not appear to find differences between fructose and glucose in ethanol-free mixtures, whereas males did. The power of this particular comparative test for females was close to 0.5, suggesting that to ascertain whether female and male bats really do have different preferences for fructose and glucose requires that the sample size be increased.

Another apparent difference between male and female bats was related to the effect of DEE on the marginal value of sugar-

containing foods. In females the effect of DEE was significant for sucrose *vs* glucose, whereas for males it was also significant for fructose *vs* glucose, but in interaction with [EtOH]. No other comparison for either sex was significant. Thus, it is unclear whether there are differences between the sexes for the effect of DEE on the marginal value of sugar-containing foods in the presence of ethanol. In addition, in a previous experiment, designed to determine the effect of [EtOH] on the marginal value of artificial food in male Egyptian fruit bats, the effect of DEE was also significant (Sánchez et al., 2008). This experiment was done in summer, thus the effect of DEE, and its interaction with [EtOH], on the marginal value of food for the bats may also be related to season.

How does ethanol modify the marginal value of sugar-containing foods for Egyptian fruit bats? In humans, the perception of sweet, bitter and irritant taste in mixtures containing different concentrations of sucrose varies with [EtOH] (Nurgel and Pickering, 2006). Also, the perception of sweet and sour by humans in mixtures with different [EtOH] changes with fructose concentration (Zamora et al., 2006). In light of this, since Egyptian fruit bats use flavour, i.e. taste and odour, to assess food quality (Korine et al., 1996; Sánchez et al., 2006), it is likely that the changes in the marginal value of the foods containing fructose, sucrose and glucose with the increase in [EtOH] for the bats were a result of ethanol interacting differently with each of the sugars and affecting the flavour of the foods in which they occurred.

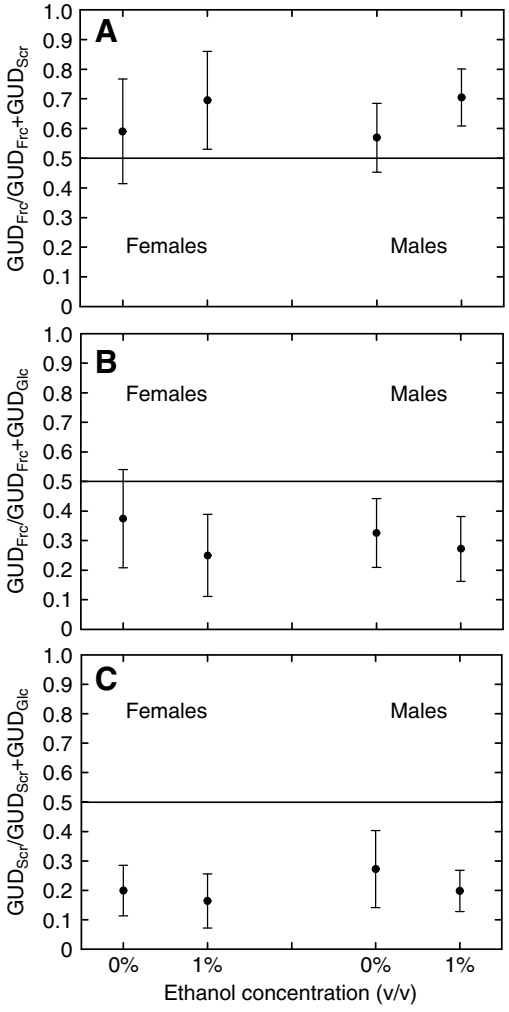

Fig. 3. Sugar preferences of female and male Egyptian fruit bats when food contained either 0% or 1% ethanol. Preferences are expressed as $GUD_{Sugar\ A}/GUD_{Sugar\ A}+GUD_{Sugar\ B}$. Values smaller than 0.5 denote preference for the sugar in the numerator (Sugar A). The filled circles are means, and the error bars are 95% confidence intervals. Means with error bars above or below the 0.5 line indicate significant differences (one-sample tests, $P<0.05$). Frc, fructose; Scr, sucrose; Glc, glucose.

The odour and taste of ethanol may be used by Egyptian fruit bats to identify overripe, unpalatable fruit (Sánchez et al., 2008; Sánchez et al., 2006). Thus, another explanation for our results is that the bats might perceive fruit rich in ethanol and sucrose as food with lower levels of toxins than fruit rich in ethanol and fructose or glucose. It is also possible that sucrose-rich fruit contain other compounds that improve ethanol elimination, that are not associated with fructose or glucose content. For example, Lisander et al. (Lisander et al., 2006) showed that in humans, infused amino acid mixtures improved ethanol elimination better than infused equicaloric mixtures of glucose.

Our results on ethanol in bat breath, and on the marginal value of food containing ethanol and sugars, intimate a contradiction between physiology and behaviour. Specifically, although fructose enhanced ethanol elimination in Egyptian fruit bat breath more than the other sugars, the bats treated sucrose and ethanol as being 'more' complementary than fructose and ethanol. This discord implies that the bats did not identify the potentially beneficial effects of ingesting fructose when food contained ethanol. Nonetheless, the notable preference for sucrose-rich fruit by Egyptian fruit bats may be advantageous with regard to ethanol consumption, since many wild fruits consumed by pteropodid bats are rich in sucrose, and also have a higher content of fructose than glucose (Baker et al., 1998). In Israel, Egyptian fruit bats consume domestic (e.g. persimmon *Diospyros kaki*, loquat *Eriobotrya japonica*) and wild (e.g. date *Phoenix dactylifera*, carob *Ceratonia silicua*) fruits (Korine et al., 1998), which may have a high sucrose and fructose content (Baker et al., 1998; Biner et al., 2007; Clark and MacFall, 2003; Van Handel et al., 1972). Thus, the preference for food+sucrose by Egyptian fruit bats could still be beneficial for these bats when they eat ethanol-rich food.

Diet mixing in herbivores may have its roots in non-additive interactions, e.g. complementarity, between the resources they consume (Bjorndal, 1991). Fruits are chemically complex resources (Cipollini, 2000), and this complexity is increased by the activity of frugivorous micro-organisms, which may explain why some fruits are treated as complementary by frugivorous vertebrates. Since the basis of nutritional complementarity between resources, such as fruit, lies in the non-additive interactions of particular compounds, examining these interactions may help to explain patterns of food selection observed in herbivores. One of the possible consequences of frugivores perceiving fruits as complementary resources is the occurrence of 'neighbourhood effects' (Whelan et al., 1998). This may be because food selection by a frugivorous forager, and the time spent exploiting a patch, may depend on the diversity of fruiting plants occurring in an area and their nutritional relationships. Thus, one might expect that the amount of an ethanol-rich fruit ingested, or its rejection, by Egyptian fruit bats could be related to the proximity of sucrose- and fructose-containing fruits.

In summary, previous studies have recognized that micro-organisms can reduce food quality and deter frugivores, and thus affect seed dispersal (Borowicz, 1988; Buchholz and Levey, 1990). We have gone a step further to show that ethanol, a potential toxin produced by fermentative micro-organisms, and sugars produced in fleshy fruit can interact and affect food selection by Egyptian

fruit bats. Our research suggests that the influence of micro-organisms on the interactions between fruit-bearing plants and frugivores is complex, and may depend on the individual effects of compounds produced by micro-organisms on frugivorous animals and on the interaction between these compounds and nutrients in the fruits.

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REFERENCES

Baker, H. G., Baker, I. and Hodges, S. A. (1998). Sugar composition of nectars and fruits consumed by birds and bats in the Tropics and Subtropics. *Biotropica* **30**, 559-586.

Battcock, M. and Azam-Ali, S. (1998). *Fermented Fruits and Vegetables: A Global Perspective* (*FAO Agricultural Services Bulletin No. 134*). Rome: Food and Agriculture Organization of the United Nations.

Berman, P. A. M., Baumgarten, I. and Viljoen, D. L. (2003). Effect of oral fructose on ethanol elimination from the bloodstream. *S. Afr. J. Sci.* **99**, 47-50.

Biner, B., Gubbuk, H., Karhan, M., Aksu, M. and Pekmezci, M. (2007). Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey. *Food Chem.* **1000**, 1453-1455.

Bjorndal, K. A. (1991). Diet mixing: nonadditive interactions of diet items in an omnivorous freshwater turtle. *Ecology* **72**, 1234-1241.

Borowicz, V. A. (1988). Do vertebrates reject decaying fruit? An experimental test with *Cornus amomum* fruits. *Oikos* **53**, 74-78.

Brown, J. S. (1988). Patch use as an indicator of habitat preference, predation risk, and competition. *Behav. Ecol. Sociobiol.* **22**, 37-47.

Brown, J. S. (1992). Patch use under predation risk. I. Models and predictions. *Ann. Zool. Fenn.* **29**, 301-309.

Buchholz, R. and Levey, D. J. (1990). The evolutionary triad of microbes, fruits, and seed dispersers: an experiment in fruit choice by cedar waxwings, *Bombycilla cedrorum*. *Oikos* **59**, 200-204.

Cipollini, M. L. (2000). Secondary metabolites of vertebrate-dispersed fruits: evidence for adaptive functions. *Rev. Chil. Hist. Nat.* **73**, 421-440.

Clark, C. J. and MacFall, J. S. (2003). Quantitative magnetic resonance imaging of 'Fuyu' persimmon fruit during development and ripening. *Magn. Reson. Imaging* **21**, 679-685.

Dominy, N. J. (2004). Fruits, fingers, and fermentation: the sensory cues available to foraging Primates. *Integr. Comp. Biol.* **44**, 295-303.

Dudley, R. (2004). Ethanol, fruit ripening, and the historical origins of human alcoholism in primate frugivory. *Integr. Comp. Biol.* **44**, 315-323.

Eckardt, M. J., File, S. E., Gessa, G. L., Grant, K. A., Guerri, C., Hoffman, P. L., Kalant, H., Koob, G. F., Li, T. K. and Tabakoff, B. (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcohol. Clin. Exp. Res.* **22**, 998-1040.

Eriksson, K. and Nummi, H. (1982). Alcohol accumulation from ingested berries and alcohol metabolism in passerine birds. *Ornis Fenn.* **60**, 2-9.

Freeland, W. J. and Janzen, D. H. (1974). Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* **108**, 269-289.

Garriot, J. C. (2003). *Medical-legal Aspects of Alcohol*. Tucson, AZ: Lawyers and Judges.

Harborne, J. B. (1993). *Introduction to Ecological Biochemistry*. London: Academic Press.

Herrera, L. G., Leblanc, D. and Nassar, J. M. (2000). Sugar discrimination and gustatory thresholds in captive-born frugivorous Old World bats. *Mammalia* **64**, 135-143.

Jakubska, A., Przado, D., Steininger, M., Aniol-Kwiatkowska, J. and Kadej, M. (2005). Why do pollinators become "sluggish"? Nectar chemical constituents from *Epipactis helleborine* (L.) Crantz (Orchidaceae). *Appl. Ecol. Environ. Res.* **3**, 29-38.

Janzen, D. H. (1977). Why fruits rot, seeds mold, and meat spoils. *Am. Nat.* **111**, 691-713.

Jones, A. W. (1983). Effects of fructose, glucose, and mixed sugars on ethanol detoxification and blood glucose response in rats. *Med. Biol.* **61**, 319-323.

Jones, A. W. and Anderson, L. (2003). Comparison of ethanol concentrations in venous blood and end-expired breath during a controlled drinking study. *Forensic Sci. Int.* **132**, 18-25.

Ke, D., Rodriguez-Sinobas, L. and Kader, A. A. (1991). Physiology and prediction of fruit tolerance to low-oxygen atmospheres. *J. Am. Soc. Hortic. Sci.* **116**, 253-260.

Keegan, D. J. (1977). Aspects of the assimilation of sugars by *Rousettus aegyptiacus*. *Comp. Biochem. Physiol.* **58A**, 349-352.

Korine, C., Arad, Z. and Arieli, A. (1996). Nitrogen and energy balance of the fruit bat *Rousettus aegyptiacus* on natural fruit diets. *Physiol. Zool.* **69**, 618-634.

Korine, C., Izhaki, I. and Arad, Z. (1998). Comparison of fruit syndromes between the Egyptian fruit-bat (*Rousettus aegyptiacus*) and birds in East Mediterranean habitats. *Acta Oecol.* **19**, 147-153.

Kricka, L. J. and Clark, P. M. S. (1979). *Biochemistry of Alcohol and Alcoholism*. New York: John Wiley and Sons.

Lisander, B., Lundvall, O., Tomner, J. and Jones, A. W. (2006). Enhanced rate of ethanol elimination from blood after intravenous administration of amino acids compared with equicaloric glucose. *Alcohol Alcohol.* **41**, 39-43.

Liu, T.-T. and Yang, T.-S. (2002). Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *J. Agric. Food Chem.* **50**, 653-657.

Mascord, D., Smith, J., Starmer, G. A. and Whitfield, J. B. (1991). The effect of fructose on alcohol metabolism and on the [lactate]/[pyruvate] ration in man. *Alcohol Alcohol.* **26**, 53-59.

Milton, K. (2004). Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? *Integr. Comp. Biol.* **44**, 304-314.

Morris, S., Humphreys, D. and Reynolds, D. (2006). Myth, marula, and elephant: an assessment of voluntary ethanol intoxication of the African elephant (*Loxodonta africana*) following feeding on the fruit of the marula tree (*Sclerocarya birrea*). *Physiol. Biochem. Zool.* **79**, 363-369.

Nazif, N. M. (2002). Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity *Food Chem.* **76**, 77-81.

Neter, J., Kutner, M. H., Nachtsheim, C. J. and Wasserman, W. (1996). *Applied Linear Statistical Models*. Chicago: Irwin Book Team.

Noll, U. G. (1979). Body temperature, oxygen consumption, noradrenaline response and cardiovascular adaptations in the flying fox, *Rousettus aegyptiacus*. *Comp. Biochem. Physiol.* **63A**, 79-88.

Nurgel, C. and Pickering, G. (2006). Modeling of sweet, bitter and irritant sensations and their interactions elicited by model ice wines. *J. Sens. Stud.* **21**, 505-519.

Parlesak, A., Billinger, M. H.-U., Schäfer, C., Wehner, H.-D., Bode, C. and Bode, J. C. (2004). First-pass metabolism of ethanol in human beings: effect of intravenous infusion of fructose. *Alcohol* **34**, 121-125.

Prinzinger, R. and Hakimi, G. A. (1996). Alcohol resorption and alcohol degradation in the European Starling *Sturnus vulgaris*. *J. Ornithol.* **137**, 319-327.

Pulliam, H. R. (1974). On the theory of optimal diets. *Am. Nat.* **108**, 59-74.

Rapport, D. J. (1980). Optimal foraging for complementary resources. *Am. Nat.* **116**, 324-346.

Roberts, A. J., Heyser, C. J. and Koob, G. F. (1999). Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. *Alcohol. Clin. Exp. Res.* **23**, 1151-1157.

Sánchez, F. (2006). Harvest rates and patch use strategy of Egyptian fruit bats in artificial food patches. *J. Mammal.* **87**, 1140-1144.

Sánchez, F., Korine, C., Pinshow, B. and Dudley, R. (2004). The possible roles of ethanol in the relationship between plants and frugivores: first experiments with Egyptian fruit bats. *Integr. Comp. Biol.* **44**, 290-294.

Sánchez, F., Korine, C., Steeghs, M., Laarhoven, L.-J., Cristescu, S. M., Harren, F. J. M., Dudley, R. and Pinshow, B. (2006). Ethanol and methanol as possible odor cues for Egyptian fruit bats (*Rousettus aegyptiacus*). *J. Chem. Ecol.* **32**, 1289-1300.

Sánchez, F., Korine, C., Kotler, B. P. and Pinshow, B. (2008). Ethanol concentration in food and body condition affect foraging behavior in Egyptian fruit bats (*Rousettus aegyptiacus*). *Naturwissenschaften* doi: 10.1007/s00114-008-0359-y.

Schmidt, K. A., Brown, J. S. and Morgan, R. A. (1998). Plant defenses as complementary resources: a test with squirrels. *Oikos* **81**, 130-142.

Scholz, R. and Nohl, H. (1976). Mechanism of the stimulatory effect of fructose on ethanol oxidation in perfused rat liver. *Eur. J. Biochem.* **63**, 449-458.

Schwartz, J. G., Salman, U. A., McMahan, C. A. and Phillips, W. T. (1996). Gastric emptying of beer in Mexican-Americans compared with non-Hispanic whites. *Metabolism* **45**, 1174-1178.

Senesi, E., Di Cesare, L. F., Prinzivalli, C. and Lo Scalzo, R. (2005). Influence of ripening stage on volatiles composition, physiocochemical indexes and sensory evaluation in two varieties of muskmelon (*Cucumis melo* L var *reticulatus* Naud). *J. Sci. Food Agric.* **85**, 1241-1251.

Thieden, H. I. D., Grunnet, N., Damgaard, S. E. and Sestoft, L. (1972). Effect of fructose and glyceraldehyde in ethanol metabolism in human liver and in rat liver. *Eur. J. Biochem.* **30**, 250-261.

Thomas, S. P. (1975). Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii*. *J. Exp. Biol.* **63**, 273-293.

Tilman, D. (1980). Resources: a graphical-mechanistic approach to competition and predation. *Am. Nat.* **116**, 362-393.

Tygstrup, N., Winkler, K. and Lundquist, F. (1965). The mechanism of the fructose effect on the ethanol metabolism of the human liver. *J. Clin. Invest.* **44**, 817-830.

Van Handel, E., Haeger, J. S. and Hansen, C. W. (1972). The sugars of some Florida nectars. *Am. J. Bot.* **59**, 1030-1032.

van Waarde, A. (1991). Alcoholic fermentation in multicelular organisms. *Physiol. Zool.* **64**, 895-920.

Whelan, C., Schmidt, K. A., Steele, B. B., Quinn, W. J. and Dilger, S. (1998). Are bird-consumed fruits complementary resources? *Oikos* **83**, 195-205.

Zamora, M. C., Goldner, M. C. and Galmarini, M. V. (2006). Sourness-sweetness interactions in different media: white wine, ethanol and water. *J. Sens. Stud.* **21**, 601-611.