

Sex differences in food intake and digestive constraints in a nectarivorous bird

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Summary

Sex-specific foraging behaviour might be influenced by digestive constraints. However, evidence for sex differences in digestive performance is limited. Various physiological traits are known to be body size dependent. Therefore, we hypothesized that body size differences between male and female birds may lead to differences in their digestive characteristics. We predicted that if food intake and digestive functions are only governed by body mass, then males that are heavier than females would have higher food intake, food assimilation efficiency and gut transit time, but not after controlling for the effect of body mass.

We fed a diet of equicaloric solutions of sucrose and a 1:1 mixture of glucose and fructose (hexose mixture) solutions to Palestine sunbirds (*Nectarinia osea*). When fed sucrose solutions, males had longer transit times but similar absorption efficiencies as females. Transit times,

corrected for differences in body mass and food intake, were still longer in males than in females when fed on sucrose solutions. The sex-specific differences in transit time disappeared when the birds were fed the hexose mixture.

Our results suggest that males take longer to digest than females when fed on sucrose-rich nectars as opposed to hexose-rich nectars, and therefore can allow themselves a relatively lower digestive capacity. This may suggest sex-specific co-evolution of sunbirds within mixed plant communities, which have both sucrose- and hexose-rich nectar-producing plants. Furthermore, future studies on digestion in birds may pay attention to sex-specific differences.

Key words: absorption efficiency, foraging, Palestine sunbird, *Nectarinia osea*, sexual dimorphism, transit time.

Introduction

The energy requirements and food intake of male and female vertebrate species may differ throughout the year (Robbins, 1990). Individuals of both sexes may satisfy different energy requirements by adopting different foraging strategies (e.g. Durell et al., 1993; Clarke et al., 1998; Markman et al., 2002; Markman et al., 2004) and food selection (e.g. Forero et al., 2002). Sex differences in digestive functions, such as nutrient extraction, should be more easily detected when males and females do not differ in the proportion and amount of various consumed food items, as was found in American robins (*Turdus migratorius*) (Wheelwright, 1986).

Differences in digestive functions among species are frequently explained by differences in body size (Ricklefs, 1996). Although sexual size dimorphism is widespread among birds (Dunn et al., 2001), there is a lack of knowledge concerning its potential effect on the differences in digestive attributes between sexes within a bird species. Such differences may have implications for the foraging behaviour, and therefore on the life history traits, of a species.

In the present study, we specifically addressed the question of whether there are differences between male and female birds in their food intake and digestive traits. If so, can these differences be fully explained by differences in body mass between the sexes? We predicted that if food intake and digestive functions are only governed by body mass, then after controlling for body mass there would be no differences between the sexes with regard to volumetric food intake, food assimilation efficiency and gut transit time.

Nectar is a simple liquid food source (Roxburgh and Pinshow, 2002) containing various proportions of sucrose and hexoses (Nicolson, 2002), and no apparent sex-specific external processing of it, for example by different bill shapes, is known to be needed. Therefore, sex-specific digestive traits are ideal to be studied in nectarivorous bird species. Hence, we chose Palestine sunbirds (*Nectarinia osea*), which show sexual dimorphism in body mass, to test for possible sex-specific differences in digestion.

We primarily fed the sunbirds with sucrose solution because it is a common sugar in the nectar of many plant species that

sunbirds readily feed on (Lotz and Nicolson, 1996). Furthermore, sunbirds will need to use their intestinal disaccharidase sucrase, which must hydrolyze sucrose into its components glucose and fructose before absorption can occur (Martínez del Rio, 1990; Martínez del Rio et al., 2001; Karasov et al., 1986). Therefore, we further predicted that if there are sex-specific differences in digestion of sucrose, for example due to sucrase activity, it will be seen when feeding the birds sucrose and not when feeding them with a 1:1 mixture of glucose and fructose of the same concentration.

Materials and methods

Bird care and housing

Sunbirds (*Nectarinia osea* Bonaparte 1856) were caught by mist nets in the campus of Oranim, Haifa University, north of Israel. The birds (eight males and six females) were colour ringed for individual identification and housed separately in cages (40×60×80 cm) in a controlled temperature room (24°C). For maintenance, birds were fed (1) a 0.6 mol l⁻¹ (~20% w/w) sucrose solution and (2) a 2.3% protein (Isomil formula; Promedico Ltd, Zwolle, The Netherlands) in a 0.6 mol l⁻¹ (~20% w/w) sucrose solution, which were presented to the birds in commercial feeders *ad libitum*. In addition, the birds were offered fruit flies (*Drosophila* sp.) twice a week. A week prior to the experiments, each bird was housed separately in an experimental cage (40×30×20 cm) for acclimatization. During that time they received maintenance solutions (see above). The experiments were carried out during the end of winter 2003; therefore, the birds were not in a breeding state.

Food intake and gut transit time

Hexose solutions have twice the total molarity of the sucrose solutions, but values of experimental solutions are given throughout for the sucrose equivalent (SE) solution to emphasise that the concentrations of hexoses and sucrose were equicaloric (see Fleming et al., 2004). Birds were fed diets, one diet at a time, of 0.3 mol l⁻¹ SE (~10% w/w), 0.6 mol l⁻¹ SE (~20% w/w), 1 mol l⁻¹ SE (~35% w/w) and 1.5 mol l⁻¹ SE (~50% w/w) sucrose or 1:1 equivalent glucose:fructose (hexose) solutions. The birds did not receive any protein-sucrose solution or free water during this period. In order to allow the birds to adjust to a given sugar solution, birds were offered the solution for two days before the experiment started. To quantify the food intake by the birds, two 5 ml syringes were offered as an *ad libitum* solution every morning at the same time. The syringes were weighed before being placed in the cages, and again one hour later. Later, at noon, the two syringes were removed and another two 5 ml syringes were weighed before being offered to the birds, and again one hour later. The order of the diets was randomised and all birds were fed all diets. Body mass of each bird was recorded at the beginning of the experiments.

Immediately after measuring the food intake of each bird on any given sugar solution in the morning session, the birds

were deprived of food for 30 min. At the end of the food deprivation period, a 5 ml syringe with a red dye solution (0.001 g E122 Carmoisine dye powder, a synthetic red azo dye, per ml of sugar solution) was placed in the cages for 10 min, after which the clear sugar solution was resumed. The transit time was measured as the time passed from the beginning of drinking the dye solution to the first appearance of red-coloured excreta on a piece of white paper placed at the bottom of the cage. These time points were determined while observing the birds through eye-size openings in a curtain, without apparent disturbance to the birds, and timing each event with a stopwatch. Transit time was repeated again at noon of the same day (following Downs, 1997) immediately after the noon food intake session. As there were no significant differences in the transit times between morning and noon sessions (for sucrose, males $F_{1,48}=0.95$, females $F_{1,32}=0.67$; for hexose, males $F_{1,40}=0.57$, females $F_{1,32}=1.68$; all $P>0.05$), we used an average transit time per bird for further analysis.

Apparent sugar absorption efficiency

While feeding the birds each of the four sucrose solutions, excreta were collected over a period of two hours into trays filled with mineral oil, which were placed beneath the cages. The trays were emptied and the excreta were separated from the oil using centrifugation (Sorvall RC 5B plus, Wilmington, DE, USA; 4068 g, 3 min). Sugar concentration in the nectar and the excreta was measured by using a temperature-compensated refractometer (Atago ATC-1E, Tokyo, Japan, 0–32%). The apparent sugar absorption efficiency (AE^*) was calculated as follows:

$$AE^* = \{S_{\text{nectar}} - [S_{\text{excreta}} (V_{\text{excreta}}/V_{\text{nectar}})]\} / S_{\text{nectar}} \times 100,$$

where S is the sugar concentration (%), and V is the volume (ml) (following Roxburgh, 2001).

Statistical analysis

The unit for analysis was always a data point for each bird, namely its: (1) food intake per hour, (2) food transit time and (3) apparent sugar absorption efficiency. Prior to analysis, apparent absorption efficiencies, which are given as percentages, were normalized using an arcsine-square-root transformation.

A two-sample *t*-test was performed to test for differences between male and female body mass. We used Spearman correlations to test the effect of sucrose and hexose mixture concentration on the volumetric food intake and transit time for each sex. As birds were repeatedly fed on all the concentrations, repeated-measures ANOVAs were applied to test for the effects of sex and sugar concentration (of sucrose or hexose mixture solutions) on the volumetric food intake and absorption efficiency (Sokal and Rohlf, 1997). However, in order to compare the non-linear curves of volumetric food intake between the sexes we used the method offered by Motulsky and Ransnas (1987). Repeated-measures ANCOVAs were used to control for the effect of body mass (i.e. log body

mass used as a covariate) when testing for the effect of sex and sugar concentration on the volumetric food intake and transit time. Repeated-measures ANCOVAs were also used to control for the effect of allometric body mass [\log body mass^{0.25} (see Karasov, 1990); used as a covariate] when testing for the effect of sex and sugar concentration on transit time.

We used three-way ANCOVA to control for the effect of food intake (i.e. as a covariate) when testing for the effects of sex, type of sugar (i.e. sucrose and 1:1 hexose mixture solutions) and sugar concentration on transit time.

Results

Body mass

Mean body mass of males (6.9 ± 0.3 g, $N=8$) was 28% higher than that of females (5.4 ± 0.4 g, $N=6$) ($t_{12}=7.82$, $P<0.001$).

Food intake

The relationships between food intake and sugar concentration were well described by similar power functions for both sexes (Fig. 1A,B). Sucrose concentration had a significant effect on food intake (repeated-measures ANOVA: $F_{3,30}=92.96$, $P<0.001$), with volumetric food intake decreasing significantly with sucrose concentration in both sexes (Fig. 1A; for males $r_s=-0.84$, $N=28$, $P<0.001$; for females $r_s=-0.80$, $N=23$, $P<0.001$). There were no significant differences in the volumetric intake of sucrose solutions per hour between males and females, as detected by comparing the two curves (Fig. 1A; $F=0.86$, $P>0.05$) following Motulsky and Ransnas (1987).

Hexose mixture concentration had a significant effect on food intake (repeated-measures ANOVA: $F_{3,27}=189.96$, $P<0.001$), with both sexes consuming less food as hexose concentration increased (Fig. 1B; for males $r_s=-0.86$, $N=24$, $P<0.001$; for females $r_s=-0.91$, $N=20$, $P<0.001$). Males consumed more food per hour than females, but this difference was only marginally significant (Fig. 1B; $F=3.08$, $P=0.057$). When log body mass was applied as covariate, sex differences in hexose consumption were not significant (repeated-measures ANCOVA: $F_{1,8}=1.79$, $P=0.218$).

Transit time

The transit time for digesta was not affected by intake rate (Table 1), when applied as a covariate. There was a significant effect of sex on transit time (Table 1), with males having longer transit times than females on sucrose (58% longer when averaged across the four sucrose concentrations; Fig. 2A; repeated-measures ANOVA, $F_{1,10}=73.175$, $P<0.001$) but having similar transit times to females when feeding on the hexose mixture (Fig. 2B; repeated-measures ANOVA: $F_{1,9}=0.05$, $P=0.82$). Type of sugar (i.e. sucrose or hexose mixture) had a significant effect on transit time (Table 1). There was a significant interaction term between sex and type of sugar (Table 1), reflecting the fact that although the females had shorter transit times when feeding on sucrose as compared with the hexose mixture, in males it was the other way around with shorter transit times on the hexose mixture, except for the

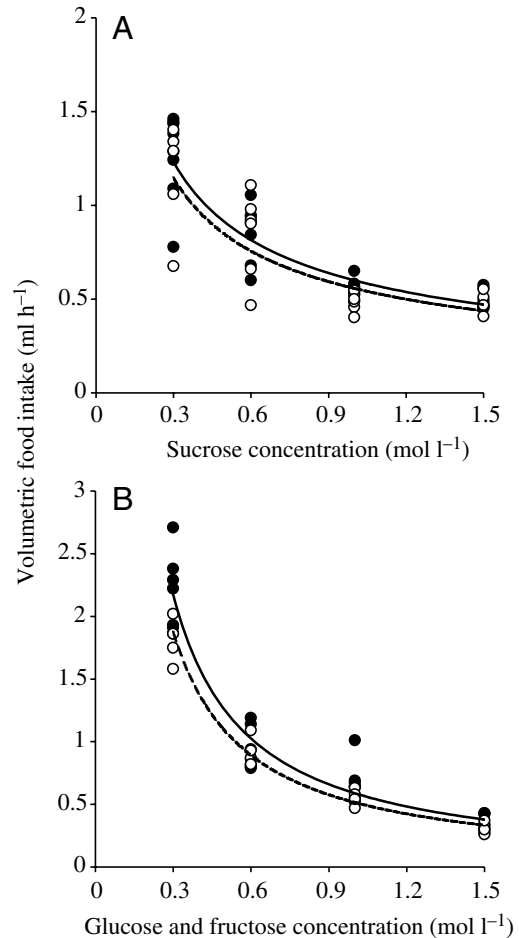


Fig. 1. The relationship between volumetric food intake in male and female Palestine sunbirds (males represented by filled circles and solid regression line; females represented by open circles and broken regression line) and (A) sucrose concentration (sucrose equivalents) of their diet (males, $y=4.85x^{-0.59}$, $r^2=0.83$; females, $y=4.52x^{-0.59}$, $r^2=0.72$) or (B) glucose + fructose concentration (sucrose equivalents) of their diet (males, $y=25.32x^{-1.07}$, $r^2=0.91$; females, $y=21.28x^{-1.06}$, $r^2=0.96$).

Table 1. ANCOVA of gut transit times in Palestine sunbirds

Source	d.f.	MS	F	P
INTAKE	1	8.7	0.239	0.626
SEX	1	1126.6	35.306	0.000
SUG	1	365.0	11.438	0.001
CON	3	1031.0	32.309	0.000
SEX × SUG	1	896.8	28.106	0.000
SEX × CON	3	82.1	2.572	0.060
SUG × CON	3	410.7	12.872	0.000
SEX × SUG × CON	3	24.4	0.766	0.517
Error	78	31.9		

The effects of the volumetric food intake (INTAKE; as a covariate), bird sex (SEX), sugar type (SUG) and sugar concentration (CON) of the diets of the birds are given, along with interaction terms.

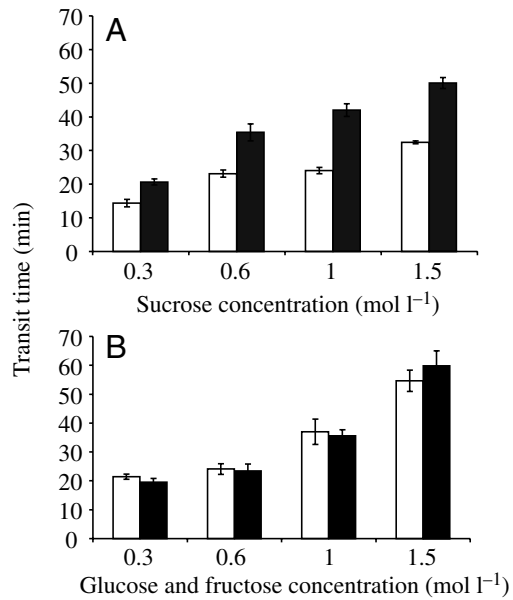


Fig. 2. The relationship between gut transit time (mean \pm s.e.m.) in male (filled columns) and female (open columns) Palestine sunbirds and the concentration of their (A) sucrose solution (sucrose equivalents) or (B) glucose + fructose solution (sucrose equivalents) diets.

1.5 mol l⁻¹ SE where their transit time was shorter on the sucrose solution (Fig. 2A,B).

Both sexes significantly increased their transit time with increasing concentrations of both sugars (Table 1; once feeding on sucrose solution – males $r_s=0.89$, $N=28$, $P<0.001$, for females $r_s=0.90$, $N=23$, $P<0.001$; once feeding on hexose mixture solution – males $r_s=0.88$, $N=24$, $P<0.001$, for females $r_s=0.85$, $N=20$, $P<0.001$) in a similar way, as reflected by the non-significant interaction term between sex, sugar type and sugar concentration. There was a significant interaction term between type of sugar and sugar concentration, probably because while feeding on sucrose the increase in transit time was steady, as opposed to the hexose mixture, where at low concentrations (0.3 mol l⁻¹ SE and 0.6 mol l⁻¹ SE) transit time was almost constant but at higher concentrations (1 mol l⁻¹ SE and 1.5 mol l⁻¹ SE) it remarkably increased.

The transit times in males, while feeding on sucrose solutions, were significantly longer than in females even after using body mass as a covariate (repeated-measures ANCOVA, $F_{1,9}=103.3$, $P<0.0001$) or allometric body mass (body mass^{0.25}) as a covariate (repeated-measures ANCOVA, $F_{1,9}=103.8$, $P<0.0001$). The transit time in males was not significantly different from that of females while feeding on the hexose mixture solutions, once we used either body mass (repeated-measures ANCOVA, $F_{1,8}=0.335$, $P=0.579$) or allometric body mass as a covariate (repeated-measures ANCOVA, $F_{1,8}=0.305$, $P=0.596$).

Absorption efficiency

Sunbirds of both sexes absorbed 98% of the sucrose, irrespective of the concentration of the four solutions. There

were no significant differences in absorption efficiency between the males and females (repeated-measures ANOVA, $F_{1,12}=1.63$, $P=0.226$) or while the birds consumed different sucrose concentrations (repeated-measures ANOVA, $F_{3,36}=2.25$, $P=0.099$). Previous work by Roxburgh and Pinshow (2002) using Palestine sunbirds as a model showed that their digestion efficiency on both 10% w/w (~ 0.3 mol l⁻¹ SE) and 50% w/w (~ 1.5 mol l⁻¹ SE) of the hexose mixture was 99.2%; therefore, we did not find a justification to test the digestive efficiency of our birds while feeding on the hexose mixture.

Discussion

Both male and female Palestine sunbirds regulated the rate of sugar intake while consuming lower amounts from the more concentrated sugar solutions, which may support the energy regulation and/or the physiological food intake limit hypotheses, as suggested by Lloyd (1991), which tested his predictions using greater double-collared sunbirds (*Nectarinia afra*). Similar apparent patterns of food regulation by nectarivorous birds were previously reported (e.g. Downs, 1997; Martinez del Rio et al., 2001; Nicolson and Fleming, 2003), most recently in Palestine sunbirds (McWhorter et al., 2003).

Both sexes seemed to be limited by digestion processes, as transit time increased with increasing sugar concentration (see Downs, 1997). The fact that both sexes consumed the same amounts of sucrose solutions, contrary to the fact that the males were significantly heavier and therefore expected to have higher energy intake, may suggest that males were limited in their ability to digest sucrose as compared with females. Indeed, males had longer transit times on sucrose compared with females, even after correcting for food intake and body mass. This means that the difference between transit times of males and females cannot be explained fully by either allometry or food intake rates, demonstrating sex-specific digestive responses.

The fact that the sex differences in transit time disappeared when fed on the hexose mixture may suggest that the differences between the sexes while feeding on sucrose are related to processes associated with the breakdown of sucrose to monosaccharide, such as sucrase enzymatic activity. In light of this, it might be that females possess more sucrase per cm² intestine than do males and/or differ in any conditions that affect sucrase activity, such as pH or temperature in the gut, or gut surface area. Such a conclusion must be supported by further investigation of the sex-specific sucrase activity. However, the combination of the supposedly lower enzyme capacity of males and their longer gut transit time seem to be enough to satisfy sugar digestion rates at normal levels, but if males were pressed to increase their intake for some reason (e.g. higher needs for activity, thermoregulation, etc.) they would have relatively lower capacity to digest their food, as implied by the longer time that they had to keep the food in their digestive system compared with females. Alternatively, it

might be that females are simply designed with relatively higher digestive capacity because it is they, and not males, that must increase intake during egg production (Pendlebury et al., 2004).

Females had shorter transit times while feeding on sucrose compared with when feeding on the hexose mixture, and males had shorter gut transit times when feeding on hexose mixture. As this can affect the capacity of the digestive system, it may mean different abilities to consume food. This can result in a possible difference in the use of sucrose-rich *versus* hexose-rich nectar-producing flowers by males and females. Such a difference between the sexes hints that there might be less competition between males and females if they feed on different types of nectar-producing flowers. This may be selected for in a situation where there are mixed plant communities that comprise both sucrose-rich and hexose-rich nectar-producing plants.

Assimilation efficiency (the proportion of the food or nutrient consumed that is actually digested and absorbed) of nectarivorous birds as a function of sugar concentration was previously studied (Downs, 1997; Jackson et al., 1998; Roxburgh and Pinshow, 2002). Our findings that both male and female Palestine sunbirds assimilated the sugars in their food almost completely, regardless of sugar concentration, fit the results of McWhorter et al. (2003) and Roxburgh and Pinshow (2002), which were obtained from male Palestine sunbirds. The independency of assimilation efficiency from sugar concentration was reported for other nectarivorous birds as well (Karasov et al., 1986; Martínez del Rio, 1990; Jackson et al., 1998).

Assimilation efficiency is dependent on the nutrient content of the food, gut surface area and volume, gut transit time, enzyme activity and the density of transport proteins (Worthington, 1989; Karasov, 1990; Robbins, 1993). In our experiments, the nutrient content of the diet was similar for both sexes. Further, gut transit time should be affected by gut surface and volume. The last two traits are an allometric function of body mass (Worthington, 1989; Karasov, 1990). However, gut transit time of males when fed on sucrose solutions was much longer than that of females even after we controlled for body mass and food intake (see Karasov, 1990). Hence, the much longer food processing in the gastro-intestinal tract of males in order to achieve the same assimilation efficiency as females might be beyond the sexual differences in gut volume or surface area.

As a consequence of these high food intake rates relative to their body mass, high extraction efficiencies and short transit times for digesta, both sexes in Palestine sunbirds probably maximize their net energy gain on diets of low and high sugar concentration solutions, as has also been shown in malachite sunbirds (*Nectarinia famosa*), black sunbirds (*Nectarinia amethystina*) and Gurney's sugarbirds (*Promerops gurneyi*) (Downs, 1997). However, the shorter gut transit time of females while feeding on sucrose solutions, compared with males, raises the question of whether females, which probably have relatively higher digestive capacity when feeding on

sucrose, are better in maximizing their net energy gain while feeding on sucrose-rich nectars. To answer this question, further investigation is needed.

To conclude, the differences between males and females in transit times when fed on sucrose as opposed to when fed on hexose mixture call for: (1) more detailed description of the gastro-intestinal system of both sexes, especially because body mass-independent differences appear between the sexes, and (2) further studies looking into the ecological implications of possible diet segregation between sexes, while feeding on different nectar composition, and their effect on plant pollination. Therefore, we suggest that future studies might use sex as a factor in their analysis of digestive traits in birds.

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