Sugar preferences of avian nectarivores are correlated with intestinal sucrase activity

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Abstract

Nectar-feeding birds generally demonstrate preference for hexose solutions at low sugar concentrations, switching to sucrose/no preference at higher concentrations. Species vary in the concentration at which the switch from hexose preference occurs; this could reflect physiological constraints that would also influence nectar selection when foraging. We recorded concentration-dependent sugar type preferences in three opportunistic/generalist Australian nectarivorous species: *Dicaeum hirundinaceum*, *Zosterops lateralis* and *Lichenostomus virescens*. All three preferred hexoses up to sugar concentrations of 0.25 $mol \cdot L^{-1}$ and switched to sucrose/no preference for higher concentrations. Using these and literature records, we investigated physiological mechanisms that may explain the concentration-dependence of sugar type preferences and compared diet preference data with foraging records. We measured sucrase activity in *Z. lateralis* and *L. virescens* as well as three specialized nectarivorous species (*Anthochaera carunculata, Phylidonyris novaehollandiae* and *Trichoglossus haematodus*) for comparison with published concentration-dependent sugar preference data. Sucrase activity varied between these species ($P=0.003$). The minimum diet concentration at which birds show no sugar preference was significantly correlated with sucrase activity for the eleven species analyzed ($P=0.005$). Birds with the lowest sucrase activity showed hexose preference at higher diet concentrations and birds with the greatest sucrase activity either showed no hexose preference or hexose preference on only the most dilute diets. Foraging data compiled from the literature also support the laboratory analyses, e.g. *T. haematodus* (preference for hexose over a wide range of diet concentrations, low sucrase activity) also feed primarily on hexose nectars in the wild. Intestinal sucrase activity is likely to contribute to diet selectivity in nectarivorous bird species.

Key-words: fructose, glucose, hexoses, honeyeater, hummingbird, intestinal enzymes, maltase, nectar, sucrose, sunbird.

Introduction

Nectar and fruit are an important carbohydrate-rich food source for many bird species. The disaccharide sucrose and its monosaccharide components glucose and fructose (i.e. hexoses, which are similar in chemical structure and in energy content per unit mass) are among the most common carbohydrates in nectar and fruit (Levey and Martinez Del Rio 2001). The composition and concentration of sugars in nectar and fruit pulp varies widely amongst plant species (Whiting 1970; Pyke and Waser 1981; Baker and Baker 1982; Baker and Baker 1983; Baker et al. 1998; Nicolson and Van Wyk 1998; Nicolson 2002; Wilson and Downs 2012). Fruit pulp tends to be hexose-dominant, with sucrose content averaging only 8% of total sugars in fruits consumed by passerines (Martinez del Rio et al. 1992; Baker et al. 1998). Nectar may be sucrose-dominant, hexose-dominant, or contain a mixture of both sucrose and hexoses (Nicolson and Fleming 2003a; Johnson and Nicolson 2008). Many nectarivorous and frugivorous bird species exhibit distinct preferences for these sugars (see review by Lotz and Schondube 2006), although past studies were commonly conducted using a single sugar concentration and so the role that energy density may play in sugar selection is not clear. These past studies also used a wide variety of experimental methodologies, which can make comparing results among different studies difficult (Brown et al. 2008).

The potential physiological mechanisms underlying the sugar preferences of birds and the extent to which the sugar composition of natural nectars reflects selection by birds have long been debated. Dramatic differences in the composition of sugars in nectar were first reported by Baker and Baker (1982; 1983). While these differences in plant nectar sugar composition were first thought to reflect selective pressures from their hummingbird (sucrose-dominant nectars) or passerine (hexose-dominant nectars) pollinators (Martinez del Rio 1990a; Martinez del Rio et al. 1992), subsequent studies on the digestive enzymes of various avian lineages have shown that both hummingbirds and nectar-specialist passerines

are capable of efficient digestion and assimilation of sucrose (see review by Lotz and Schondube 2006). The intestinal enzyme sucrase-isomaltase is responsible for the hydrolysis of sucrose into its monosaccharide hexose components. Most specialist and occasional nectarivores and frugivores are able to efficiently assimilate both sucrose and hexoses (Lotz and Schondube 2006; Fleming et al. 2008; Napier et al. 2008), with the exception of frugivores in the Sturnidae-Muscicapoidea lineage that lack sucrase (Martínez del Rio and Stevens 1989; Brugger et al. 1993; Sabat and Gonzalez 2003; Gatica et al. 2006; Brown et al. 2012). Some occasional nectarivores, however, exhibit lower apparent assimilation efficiencies for both sucrose and hexoses (Brown et al. 2010b, 2010a) and some occasionally nectarivorous and frugivorous passerines exhibit sucrose assimilation efficiency that is significantly lower than that for hexoses (Lane 1991; Odendaal et al. 2010). These patterns are consistent with findings presented by Johnson and Nicolson (2008), who demonstrated that nectars of plants pollinated by specialist nectarivorous passerines are strongly convergent with those of plants pollinated by hummingbirds. Specifically, plants pollinated by specialist avian nectarivores tend to have small volumes of concentrated, sucrose-dominant nectars, while those pollinated by generalists tend to have large volumes of dilute, hexose-dominant nectars.

One important finding of recent studies is that sugar type preference varies with sugar concentration. Nectarivorous birds tested using a range of concentrations of 'equicaloric' (Fleming et al. 2004) sucrose or hexose diets generally demonstrate preference for hexose solutions at low sugar concentrations (i.e. energy densities), with a switch to sucrose or no preference at higher concentrations. This has been demonstrated in specialist nectarivores including sunbirds, hummingbirds, honeyeaters and lorikeets (Schondube and Martinez del Rio 2003; Fleming et al. 2004; Lotz and Schondube 2006; Fleming et al. 2008; Brown et al. 2010c), and occasional nectarivores such as *Colius striatus* (speckled mousebird) and *Ploceus*

cucullatus (village weaver) (Brown et al. 2010a; Odendaal et al. 2010, see Table 1). Although these species demonstrate a similar pattern in sugar preferences, they differ in the concentration at which the switch from hexose preference to no preference occurs. Most specialist nectarivores prefer hexoses at extremely dilute diets only, e.g. *Anthochaera carunculata* (red wattlebird), *Phylidonyris novaehollandiae* (New Holland honeyeater), *Cinnyris talatala* (white-bellied sunbird), *Nectarina famosa* (Malachite sunbird), *Eugenes fulgens* (magnificent hummingbird) and *Diglossa baritula* (cinnamon-bellied flowerpiercer) (Table 1). Some opportunistic nectar feeders (*C. striatus* and *P. cucullatus*) prefer hexoses up to slightly higher concentrations than these specialized nectarivores, yet *Pycnonotus tricolor* (dark-capped bulbul, a nectar generalist), and *Trichoglossus haematodus* (rainbow lorikeet, a nectar specialist) prefer hexoses at much higher sugar concentrations (Table 1). Brown and colleagues suggested that these findings help to explain the dichotomy reported by Johnson and Nicolson (2008); however, aside from the work by Brown et al. (2010a, 2010b) and Odendaal et al. (2010), little comparative data on sugar preferences in generalist nectar feeders has been available to date. Compared with nectarivores, we know far less about the concentration-dependence of sugar preferences of opportunistic or generalist avian frugivores.

Compensatory feeding, where birds increase volumetric intake rate as food energy density decreases, allows birds to deal with variations in nectar concentration (Martinez del Rio et al. 2001; Nicolson and Fleming 2003b). Lotz and Schondube (2006) and Fleming et al. (2008) have hypothesized that the concentration-dependence of sugar preferences in nectarivorous birds may be attributed to varying levels of sucrase activity and the need for constant energy assimilation (i.e. compensatory feeding). Birds that exhibit a lower capacity to hydrolyze sucrose are more likely to show preference for hexoses over sucrose solutions on dilute diets in this scenario, because digesta transit rates will be faster and substrate

concentration for the sucrase enzyme will be lower, limiting the hydrolysis rate (McWhorter and Martinez del Rio 2000). In this study, we have tested this prediction with new and available published data. We investigated sugar preferences and apparent assimilation efficiency in three opportunistic/generalist Australian nectarivorous species: *Dicaeum hirundinaceum* (mistletoebird), *Zosterops lateralis* (silvereye) and *Lichenostomus virescens* (singing honeyeater). We also analyzed the activity of the intestinal enzymes sucrase-isomaltase (EC 3.2.1.48, hereafter 'sucrase') and maltase-glucoamylase (EC 3.2.1.20, hereafter 'maltase') in *Z. lateralis* and *L. virescens,* as well as three specialized nectarivorous species (*Ph. novaehollandiae*, *T. haematodus* and *A. carunculata*) for comparison with published sugar preference data for these species (Fleming et al. 2008). Finally, we compiled foraging data for these species and Australian nectar sugar compositions, where available, from the literature. We predicted that:

1) specialized nectarivorous species would exhibit greater apparent assimilation efficiencies for both hexoses and sucrose than generalist nectarivores;

2) the degree of preference for hexose over sucrose solutions would be correlated with variation in the capacity to hydrolyze sucrose; and

3) specialist nectarivores should preferentially forage on sucrose-rich nectars compared with generalist species.

Materials and methods

Birds and their maintenance

Dicaeum hirundinaceum is a specialized frugivore that feeds primarily on mistletoe fruit (Richardson and Wooller 1988), but also includes nectar and insects in its diet (Reid 1990). *Zosterops lateralis* is a generalist, feeding on fruit, nectar and insects (Wilkinson 1931; Thomas 1980; Richardson and Wooller 1986). *Lichenostomus virescens* is a nectarivore that also ingests a relatively high proportion of insects (Collins and Morellini 1979; Richardson and Wooller 1986); both *L. virescens* and *Z. lateralis* have more muscular gizzards than specialized nectarivores due to their ingestion of insects (Richardson and Wooller 1986), therefore we have classified these species as generalist nectarivores (Table 1).

Lichenostomus virescens (n=8) and *Z. lateralis* (n=8) were captured on the grounds of Murdoch University, Perth, Western Australia (WA; 32°04′S, 115°50′E) by mist-netting in May 2009 and January 2010, respectively. There is no measure for sexual dimorphism in plumage for either species. *D. hirundinaceum* (four male and two female) were captured on private property at York, WA (31°50′S, 116°44′E), in December 2010, January and March 2011. All birds were acclimated to captive conditions for at least two weeks before the commencement of experimental trials.

Birds were housed in individual outdoor aviaries (116 x 160 x 210 cm), but were confined to smaller cages (47 x 54 x 41 cm) placed within each aviary for the experiments. During the period of captivity, all three species were fed a maintenance diet of Wombaroo® nectarivore mix (Wombaroo Food Products, South Australia), which contains sucrose as the main sugar type, supplemented with additional sucrose or equal parts of glucose and fructose for a total sugar content of *c.* 25% w/w dry matter. Birds fed through a small hole (*c*. 1-1.5 mm diameter) from plastic, stoppered syringes hung on the sides of the cage. The

frugivorous *Z. lateralis* and *D. hirundinaceum* were also fed a variety of fleshy fruits (e.g. mistletoe fruit, watermelon, grapes, apricots) daily. Martinez del Rio (1990a) reported that measured sugar preferences in hummingbirds were not correlated with the sugar type of their maintenance diet. All animal care procedures and experimental protocols adhered to Murdoch University Animal Ethics Committee regulations (R1137/05 and R2175/08). Birds were collected under permits issued by the Western Australian Department of Environment and Conservation (DEC).

Apparent assimilation efficiency (AE*)

Lichenostomus virescens (n=8), *Z. lateralis* (n=8) and *D. hirundinaceum* (3 male, 2 female) fed *ad libitum* from sucrose and hexose solutions at three concentrations (0.25, 0.5, 1 $mol \cdot L^{-1}$) for 24 h. Each bird fed from each sugar solution at each diet concentration, with sugar type and concentration randomized. Trials commenced within 30 min after sunrise (0500 to 0716 WST). Maintenance diet was removed one hour before sunrise to ensure all previously ingested food (i.e from the previous day) was voided before trials commenced. Trays were placed under experimental cages to collect excreta, and liquid paraffin was placed in containers directly beneath feeders to collect any diet spilt. Food intake was recorded over 24 h by weighing feeders (0.01 g). Excreta produced over 24 h were allowed to evaporate and were then reconstituted and collected with a known volume of dH_2O and stored at -20 °C until analysis.

Glucose assays. Two replicates of each excreta sample (100 µl) were incubated at room temperature (~21 °C) for 15 min with 500 µl of hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay reagent (G3293, Sigma Aldrich). Absorbance was then measured at 340 nm relative to distilled water by spectrophotometry (UV mini 1240, Shimadzu Scientific Instruments, Balcatta, WA, Australia).

Fructose assays. Two replicates of each excreta sample (45 µl) were incubated at room temperature (~21 °C) for 15 min with 650 µl of hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay reagent (G3293, Sigma Aldrich) and 5 µl phosphoglucose isomerase from baker's yeast (F2668, Sigma Aldrich). Absorbance was then measured at 340 nm relative to distilled water by spectrophotometry.

Sucrose assays. Two replicates of each excreta sample (25 µl) were incubated at room temperature (~21 °C) for 10 min with 25 µl invertase from baker's yeast sucrose assay reagent (S1299, Sigma Aldrich). 650 µl of hexokinase-glucose-6-phosphate dehydrogenase enzymatic assay reagent (G3293, Sigma Aldrich) was then added, and samples incubated for a further 15 min. Absorbance was then measured at 340 nm relative to distilled water by spectrophotometry.

Apparent assimilation efficiency (AE*) was estimated separately for sucrose, glucose and fructose as:

$$AE^* = (sugar_{in}\text{-}sugar_{out}) / (sugar_{in})$$

where $sugar_{in}$ (g) is calculated as the concentration ($g \cdot L^{-1}$) of sugar in the ingested diet multiplied by the volume of solution ingested (L), and $sugar_{out}$ (g) is the sugar concentration ($g \cdot L^{-1}$) in the total volume of excreta plus rinse water (L).

AE* data were arcsine square root transformed (Zar 1999) before analysis. Differences in AE between sugar type, sugar concentration, species and total sugar intake were assessed by ANCOVA with total sugar intake as a covariate and Tukey-Kramer *post hoc* tests for unequal sample sizes as required. Additional data for sucrose AE* (excluding species from the sub-family Muscicapoidea) were obtained from Fleming et al. (2008) and differences between specialist (n=21 species) and generalist (n=13 species) nectarivores assessed by Mann-Whitney U test.

Sugar preference trials

Lichenostomus virescens (n=8), *Z. lateralis* (n=8) and *D. hirundinaceum* (four males) participated in sugar preference trials which, following the methodology of Fleming et al (2008) for consistency, lasted for 6 h, commencing within 30 min of sunrise (0535 to 0705 WST). Sugar preferences were examined by comparing the intake of seven paired concentrations of sucrose and energetically equivalent hexose (1:1 glucose:fructose) solutions: 0.075, 0.1, 0.25, 0.5, 0.75, 1 and 2 mol·L^{-1} Sucrose Equivalents (SE). Hexose diets were equicaloric with, but had approximately twice the osmolality of sucrose solutions (Fleming et al. 2008). Birds were simultaneously presented with pairs of feeders containing sucrose and hexose concentrations of the same SE molarity in random order. To account for potential sources of side bias (Jackson et al. 1998b; Jackson et al. 1998a), the start position of each feeder was random, with the positions of the feeders switched half way though each trial. Each concentration was also tested on each bird twice, with the starting position of the feeders reversed on the second trial. Liquid paraffin was placed in containers directly below feeders to collect any diet spilt. Sugar intake was determined by weighing the feeders before and after trials (0.01 g) and calculating the mass of sugar ingested by taking into account the density of each diet. Trials were conducted approximately every second day, with at least one day of rest and maintenance diet between trials. Trials were repeated for a third time in the instance of low diet intake (a few individuals did not drink when first offered the lowest concentration of 0·075 mol·L^{-1} SE, but increased intake during subsequent trials). The average intake over all trials for each diet was used to calculate a sugar preference index, with hexose intake expressed as a proportion of total sugar intake (H/(H+S), where a value of 0.5 indicates no preference whilst a value close to 1 indicates a strong hexose preference).

Average food intake (g sugar in 6 h of each trial) was analyzed via one-way ANOVA for each species, with diet sugar concentration as the independent factor and Tukey's Honest Significant Differences (HSD) *post-hoc* tests as required. Preference data were arcsine

square root transformed (Zar 1999) before analysis by one-way ANOVA for each species, with diet sugar concentration as the independent factor and Tukey's HSD tests as required. Differences in preferences between species and diet concentrations were assessed via two-way ANOVA with Tukey-Kramer *post hoc* tests for unequal sample sizes as required. For each species, sugar preference at each concentration was analyzed by one-sample t-tests (Sokal and Rohlf 1995) comparing the arcsine-transformed square root of preference indices against 0.5 (no preference).

Intestinal enzymes

Study species and dissection. *Anthochaera carunculata* (n=3), *L. virescens* (n=7), *Z. lateralis* (n=4), *Ph. novaehollandiae* (n=9) and *T. haematodus* (n=7) were captured by mist- or cannon-netting near Perth, Western Australia, between 2007 and 2011 (see Appendix A for details). We did not have access to mistletoebirds for this part of the study. Birds were not fasted prior to euthanasia. Birds were euthanized via Isoflurane overdose or a 1:1 sodium pentobarbital:distilled H_2O solution injected into the heart. Sex was determined by examination of reproductive organs upon dissection. The intestines were removed from stomach to cloaca within 10 min of euthanasia, dissected length-wise, cut into three sections (proximal, medial and distal) and measured (length and width to calculate nominal surface area, cm^2). The intestinal sections were then rinsed in 0.75 mol NaCl, blotted, and weighed (0.001 g). Each section was then frozen in liquid nitrogen and stored at -80 °C until enzyme activity analysis (<12 months after euthanasia). All animal care procedures and experimental protocols adhered to Murdoch University Animal Ethics Committee regulations (R1137/05). Birds were collected under permits issued by DEC. Some tissues were kindly provided by Joao Coimbra (The University of Western Australia Animal Ethics Committee RA/3/100/927 and DEC permit SF007556).

Disaccharidase assays. Intestinal samples were thawed at room temperature (21±2 °C) and homogenized (Heidolph 'DIAX 600', Heidolph Instruments, Schwabach, Germany) in 0.3 $mol \cdot L^{-1}$ mannitol in 0.001 $mol \cdot L^{-1}$ HEPES/KOH pH 7.5 buffer (99 to 128 mg $intestine \cdot mL^{-1}$ of homogenate). Aliquots of homogenates were immediately diluted in 0.3 $mol \cdot L^{-1}$ mannitol in 1.0 $mmol \cdot L^{-1}$ HEPES/KOH pH 7.5 buffer (1:40 for sucrase, 1:300 for maltase) and frozen in liquid nitrogen and stored at -80 °C until disaccharidase (sucrase and maltase) assays were performed.

Disaccharidase activity was measured according to Dahlqvist (1984) as modified by Martinez del Rio et al. (1995). Diluted intestinal homogenates (30 µL) were incubated with 30 µL of 0.056 $mol \cdot L^{-1}$ sugar substrate (maltose or sucrose) solutions in 0.1 $mol \cdot L^{-1}$ maleate NaOH pH 6.5 buffer at 40 °C for 20 min. 400 µL of a stop/develop reagent was then added, and samples were vortexed and incubated at 40 °C for a further 30 min. Stop/develop reagent was made by dissolving one bottle of Glucose oxidase/peroxidase reagent (G3660, Sigma Aldrich) in 19 mL 0.5 $mol \cdot L^{-1}$ phosphate buffer (NaH_2PO_4/Na_2HPO_4) pH 7.0 plus 19 mL 1 $mol \cdot L^{-1}$ Tris/HCl pH 7.0, plus 2 mL O-dianisidine solution (2.5 mg O-dianisidine dihydrochloride [D3252, Sigma Aldrich] per mL dH_2O). Lastly, 400 µL $12NH_2SO_4$ was added and the absorbance read at 540 nm. Maltase and sucrase activity ($\mu mol \cdot min^{-1}$) was measured for each section of intestine and summed together to calculate 'total activity' for each individual. Total enzyme activity for each individual bird was then adjusted to optimal pH, and then standardized for nominal gut surface area ($\mu mol \cdot min^{-1} \cdot cm^2$).

Differences in standardized sucrase activity between the five species were assessed by one-way ANOVA followed by Tukey-Kramer *post hoc* tests for unequal sample sizes. Least squares linear regression was also used, with data averaged for species to assess relationships between log body mass (m_b) and log gut nominal surface area and log total sucrase and maltase activity.

As maltose may be hydrolyzed by both sucrase and maltase (Alpers 1987; Martinez del Rio 1990b), the activity of both disaccharidases were measured. The slope of the relationship between sucrase and maltase indicates the amount of maltase activity relative to sucrase activity and the y-intercept provides an estimate of maltase activity that occurs in the absence of sucrase (Martinez del Rio 1990b). The relationship between standardized sucrase and maltase activities was therefore examined using least squares linear regression.

Least squares multiple linear regression was also used to assess the relationship between hexose preference (scored as the minimum diet concentration at which birds show no sugar preference) and standardized sucrase activity with data averaged for all individuals for eleven species (Table 1). Studies that have used only a few diet concentrations may not yield accurate information in this regard, but the use of the minimum no-preference concentration is a conservative estimate of sugar type preference. Minimum no-preference concentration values also allowed inclusion of species that do not exhibit hexose preference, e.g. *Cynanthus latirostris* (broad-billed hummingbird) and *Selasphorus platycercus* (broad-tailed hummingbird). *Onychognathus morio*, like other starlings, lacks the intestinal enzyme sucrase and therefore has non-detectable levels of sucrase activity (Bizaare et al. 2012). We then included *O. morio* in the analyses with a sucrase activity value of 0.

Phylogenetic analyses. As phylogenetic relationships may confound the inferences of allometric analyses (Garland et al. 1992; Garland and Adolph 1994; Rezende and Diniz-Filho 2012), these conclusions were corroborated using phylogenetically-independent contrasts. Felsenstein's (1985) independent contrasts method was used in the computer program PDAP (Garland et al. 1992; Garland et al. 1993; Garland et al. 1999; Garland and Ives 2000) running through Mesquite (Version 2.75) (Midford et al. 2009). Phylogenetically-independent contrasts (PIC) of dependent and independent variables were calculated and standardized utilising the branch length transformation (Garland et al. 1992). Evolutionary

relationships (Figure 1) were determined using the phylogenetic tree of Ericson et al. (2006) as a 'backbone' with sets of pseudo-prosterior samples of the dated phenologies built by Jetz et al. (2012) subsampled and then pruned for our full set of species. Regressions were fitted to standardized PIC values, forcing the data through the origin (Garland et al. 1992).

Foraging data and Australian nectar composition

Foraging data for *Z. lateralis, L. virescens*, *Ph. novaehollandiae*, *A. carunculata* and *T. haematodus* were compiled from the Western Australian Pollination Database (Brown et al. 1997). As the foraging records for *T. haematodus* in Western Australia (Brown et al. 1997) were rather limited (due to their recent introduction in the 1960s and subsequent establishment as a pest species in Perth, WA), detailed foraging records for *T. haematodus* were also compiled from the Queensland-New South Wales border region (Cannon 1984). Nectar compositions of Australian plants were compiled from published and unpublished sources (Baker and Baker 1982; Paton 1982; Gottsberger et al. 1984; McFarland 1985; Nicolson 1994; Davis 1997; Baker et al. 1998; Nicolson and Van Wyk 1998; Hölscher et al. 2008; Morrant et al. 2010; S.W. Nicolson and B.-E. Van Wyk, pers. comm.). The ratio of hexoses to sucrose was calculated as H/(H+S), and nectars classed as hexose-dominant (>0.8), hexose-rich (0.6-0.8), mixed (0.4-0.6), sucrose-rich (0.2-0.4) or sucrose-dominant (<0.2). Ratios were adapted from Baker and Baker (1982), with new classifications developed for this study. To examine the relationship between nectar type and foraging preference of the five species, a contingency table was constructed for foraging data for each species and the five nectar classifications (excluding plants for which we lack information on the nectar composition – classified as 'unkown') and analysed for significance by Pearson's χ^2 square analysis (with Bonferroni correction applied).

General statistical analysis

Data are reported as means±1SD throughout, with n referring to the number of animals. Statistical analyses were performed with Statistica (StatSoft Inc 2007) and SPSS (SPSS, Inc, Chicago, IL, USA). Statistical significance was accepted for $\alpha<0.05$.

Results

Apparent assimilation efficiency (AE*)

Zosterops lateralis, L. virescens, and *D. hirundinaceum* displayed high assimilation efficiencies for all three sugar types (>97.5%, Table 1). AE* was not different between sugar concentrations ($F_{1,191}$=2.56, P=0.111) but varied with sugar intake ($F_{1,191}$=10.20, P=0.002), where AE* increased as sugar intake decreased. AE* varied between species ($F_{2,191}$=7.19, P=0.001), being greatest for *L. virescens* and least for *Z. lateralis* overall. AE* also varied between sugars ($F_{2,191}$=65.54, P<0.001), being greatest for glucose and least for fructose. The significant sugar type by species interaction ($F_{4,191}$=11.54, P<0.001) demonstrated that *Z. lateralis* assimilated less fructose than *D. hirundinaceum* and *L. virescens*, and *L. virescens* assimilated more sucrose than *D. hirundinaceum* and *Z. lateralis*.

AE* for sucrose differed significantly between generalist (86.24±16.21%, n=13) and specialist nectarivores (98.09±1.25%, n=21) (U=64, Z=2.62, P=0.0093). Comparable data for hexoses were not available.

Sugar preferences

Zosterops lateralis, L. virescens, and *D. hirundinaceum* all failed to consume sufficient volumes to maintain energy balance on the most dilute diets, with significantly lower intakes of sugar compared with the more concentrated diets. Compensatory feeding (identified here as diet concentrations where sugar intake was not significantly different from the most

concentrated diets) was observed for *L. virescens* for diets ≥0.25 mol·L^{-1}, but only for diets ≥0.5 mol·L^{-1} in *D. hirundinaceum* and *Z. lateralis* (Figure 2). Sugar preferences were influenced by sugar concentration ($F_{6,279}$=36.17, P<0.001), with all three species showing significant preferences for hexose solutions at low sugar concentrations (Figure 3). Sugar preferences differed significantly between the species ($F_{2,279}$=4.460, P=0.012), with *Z. lateralis* displaying significant preferences for sucrose diets at the higher concentrations (i.e. ≥0.75 mol·L^{-1}; Figure 3).

Intestinal enzymes

Body mass, gut nominal surface area, total sucrase activity and kinetic parameters for *T. haematodus*, *Z. lateralis, L. virescens*, *Ph. novaehollandiae* and *A. carunculata* are summarized in Table 1, with data for additional species reported from the literature. Total maltase activity and associated kinetic parameters are detailed in Appendix B. Sucrase and maltase activity, as a function of substrate concentration, followed Michaelis-Menten kinetics. Sucrase and maltase activities were highest in proximal sections of the intestine and decreased distally (data not shown). There were species differences in standardized sucrase activity (one-way ANOVA: $F_{5,26}$=4.87, P=0.003); *T. haematodus* and *Z. lateralis* had significantly lower sucrase activity than *A. carunculata* (post hoc: P=0.012, P=0.032). When comparing data averaged for each species, gut nominal surface area increased with body mass ($F_{1,9}$=120.88, P<0.001, R^2=0.94; Figure 4a). This result was confirmed by PIC analysis of $\log_{10}$body mass^{PIC} and $\log_{10}$gut nominal $\text{surface area}^{PIC}$ ($F_{1,8}$=62.53, P<0.001, R^2=0.887). Total sucrase activity was also significantly correlated with body mass ($F_{1,9}$=12.46, P=0.008, R^2=0.61, Figure 4b; confirmed by PIC analysis: $F_{1,8}$=15.08, P=0.006, R^2=0.653). Total maltase activity showed a borderline correlation with body mass, which was not upheld by PIC analysis ($F_{1,8}$=5.97, P=0.04, R^2=0.43, Figure 4c; PIC: $F_{1,8}$=0.432, P=0.532, R^2=0.051). Standardized maltase activity was not significantly correlated with standardized sucrase

activity ($F_{1,9}$=0.18, P=0.686, R^2=0.02; Figure 4d), which was confirmed by PIC analysis ($F_{1,8}$=4.113, P=0.082, R^2=0.340).

For eleven species tested, hexose preference (the minimum no-preference concentration) was significantly correlated with standardized sucrase activity ($F_{1,10}$=13.44, P=0.005, R^2=0.60; Figure 4e). Phylogenetically-corrected analysis confirmed this result ($F_{1,9}$=18.0, P=0.003, R^2=0.667). Birds with the lowest standardized sucrase activity showed hexose preference at more concentrated diets (i.e. greater minimum no-preference concentration), and birds with the greatest standardized sucrase activity either showed no hexose preference (e.g. hummingbirds *C. latirostris* and *S. platycercus*) or hexose preference on only the most dilute diets.

Foraging data and Australian nectar composition

Foraging data (Cannon 1984; Brown et al. 1997) are summarized in Figure 5. Foraging records indicate that all of the focal species have a diverse diet, including multiple plant taxa in their diets (Figure 5a). Nectar composition was available for 16 Australian genera (Figure 6, Appendix C). There was a significant association between bird species and nectar type (χ^2_{16}=532.77, P<0.001; Figure 5b), with *T. haematodus* avoiding sucrose and mixed nectars in favor of hexose-rich nectars and the three honeyeater species avoiding hexose nectars in favour of sucrose-dominant, -rich and mixed nectars. The foraging preferences of *Z. lateralis* were not very clear, which may reflect few foraging records (n=44) for this species.

Discussion

We investigated physiological mechanisms that may explain the concentration-dependence of sugar type preferences using data obtained from laboratory trials and literature records, and compared diet preference data with foraging records.

Supporting our first prediction, we found that specialized nectarivorous species exhibited greater apparent assimilation efficiencies for sucrose than generalist nectarivores when comparing broadly using data available from this study (Table 1) and the literature (Fleming et al. 2008). Not enough information for glucose and fructose assimilation was available for generalist nectarivores (n=3) so we were unable to make this broader comparison for hexoses. However, the Australian generalist nectarivore species studied exhibited high apparent assimilation efficiencies (AE*) for sucrose, glucose and fructose (all >97.5%) comparable with specialist nectarivores. These results suggest that these Australian generalist nectarivores should be as capable of feeding on both sucrose- and hexose-rich nectars as specialist nectarivores.

In terms of our second prediction, both specialist and generalist nectarivores demonstrated concentration-dependent sugar preferences. The degree of preference for hexose over sucrose solutions on dilute diets (assessed as the minimum no-preference concentration) was negatively correlated with the capacity to hydrolyze sucrose. For example, *T. haematodus*, a specialist nectarivore, had one of the lowest sucrase activity levels and correspondingly preferred hexose diets over a broad range of diet concentrations. Hummingbirds, with the greatest sucrase activity levels, showed no preference for hexose over sucrose.

Our third prediction was that diet preferences would match foraging records. While some specialist nectarivores (e.g. *Ph. hovaehollandiae*) preferentially foraged on sucrose nectars over hexose nectars in the wild, others (e.g. *T. haematodus*) preferred hexose-rich

nectars to mixed and sucrose-rich and sucrose-dominant nectars. These data therefore do not support a simplistic differentiation in diet preferences between specialist and generalist nectarivores and indicate that the digestive physiology of each species is more closely correlated with its diet preferences (measured in the laboratory or foraging records in the field) than broad classifications have lead us to expect.

Are there differences in apparent assimilation efficiency between sugar types?

Although all three Australian generalist nectarivore species assessed show high apparent assimilation efficiencies of sucrose and hexoses, there were some differences between these sugar types. AE* was greatest for glucose and least for fructose, and varied by species. Greater AE* for glucose over sucrose has been noted in studies of other species (Table 1) and may reflect the direct assimilation of glucose, but the need for hydrolysis of sucrose before its constituent monosaccharides can be assimilated. Fructose absorption (by GLUT5 transporters) appears to be more concentration-dependent than the absorption of D-glucose (Holdsworth and Dawson 1964; Rand et al. 1993), therefore the lower AE* for fructose may reflect the availability of GLUT transporters and reliance on facilitated diffusion (rather than secondary active transport via SGLT1 transporter proteins as for glucose).

Our data revealed a clear distinction between specialist and generalist nectarivores in terms of their AE*for sucrose. Specialist nectarivores uniformly have high AE* for sucrose, while many generalist nectarivores have lower AE* which could reflect lower sucrase activity. We could only compare AE* between generalist and specialist nectarivores for sucrose, due to lack of available data for the other sugars. However, because AE* values for Australian generalist and specialist nectarivores feeding on all three sugars are >97.5%, these differences are not likely to be functionally significant or impact the sugar preferences or foraging choices of these birds.

Can we explain hexose preferences on dilute diets?

We examined whether hexose preference on dilute diets could be influenced by the amount of intestinal sucrase activity. Across eleven bird species, hexose preference (minimum no-preference concentration) was significantly negatively correlated with sucrase activity (Figure 4e). Birds with lesser capacities to digest sucrose show a significant preference for hexose solutions at higher sugar concentrations. For example, the lorikeet, *T. haematodus*, assessed in this study does not have the same sucrose digestive capacity shown by other specialist nectarivores, with only one third the sucrase activity of *A. carunculata*, a similar-sized honeyeater (Figure 4d,e). *T. haematodus* prefered hexose solutions up to 0.75 $mol \cdot L^{-1}$. By contrast, birds with greater capacities to digest sucrose showed hexose preference on only the most dilute diets or no preference over the range of concentrations tested. We included data for two hummingbird species (*C. latirostris* and *S. platycercus*) which show no sugar type preference at room temperature for the minimum diet concentrations they have been tested on (0.146 and 0.25 $mol \cdot L^{-1}$ diets, respectively). When tested at lower diet concentrations (0.1 $mol \cdot L^{-1}$), *S. platycercus* resorted to torpor rather than feeding on the dilute solutions (Fleming et al. 2004). Challenging them with colder ambient temperatures (i.e. increasing their metabolic demands; Fleming et al. 2004) may be the only way to test for evidence of a hexose preference in hummingbirds. These data, together, demonstrates that preference for hexose at low diet concentrations reflects the digestive capacity of bird species.

Most specialist nectarivores prefer hexoses at only the most dilute diet concentrations tested, while many species of generalist nectarivores (e.g. *Py. tricolor* and *O. morio*) show hexose preference across a greater range of diet concentrations (Table 1). However, *T. haematodus* (a specialist nectarivore) shows significant hexose preference for more concentrated diets than other specialist nectarivores. Furthermore, the simplistic

categorization of honeyeater species as specialist or generalist, in itself, may also be problematic. These data therefore do not support a simplistic distinction between specialist and generalist nectarivores across all avian lineages. Compared to specialist avian nectarivores, we know far less about the concentration-dependence of sugar preferences of avian frugivores (see Table 1).

Can we explain sucrose preference on concentrated diets?

A number of species have now been shown to switch over to preference for sucrose solutions at high diet concentrations (Table 1). Significant sucrose preference has been somewhat puzzling, since these solutions have similar energetic value compared with the hexose equivalents, and sucrose solutions require sucrose hydrolysis before assimilation. As sucrose-dominant nectars tend to be more concentrated than predominantly hexose nectars (Nicolson 1998), birds may prefer sucrose at high concentrations and hexose at low diet concentrations as this reflects the pattern found in natural floral nectars (Lotz and Schondube 2006).

It has also been suggested that the preference for sucrose on high sugar concentrations could reflect taste preferences. By human tastes, fructose is 1.3x sweeter than sucrose while glucose has only 0.7x the sweetness of sucrose (Harborne 1993). Birds may also show discrimination in sugar tastes. A recent study demonstrated that *C. latirostris* perceives glucose, fructose and sucrose differently and is able to detect fructose at ~30% lower concentrations than sucrose or ~20% lower than glucose, indicating that fructose has a more intense flavor for this hummingbird (Medina-Tapia et al. 2012). These authors suggested that hummingbirds were selecting sugar solutions in relation to their relative sweetness, and that gustatory thresholds may play an important role in determining sugar selection at least for

more dilute diets (Medina-Tapia et al. 2012). The role of taste in sugar type preference for concentrated diets remains to be tested.

Do laboratory results reflect foraging preferences in the wild?

The three honeyeater species examined (*Ph. novaehollandiae*, *A. carunculata,* and *L. virescens*) feed preferentially on sucrose nectars, avoiding hexose nectars; these foraging data reflect their preferences for hexoses only on very dilute sugar concentrations when tested in the laboratory. By contrast, *T. haematodus* feed predominantly on hexose nectars, avoiding sucrose nectars; again, these data reflects preferences of these birds for hexoses at much higher sugar concentrations under laboratory conditions. We have few foraging data for *Z. lateralis* to date, therefore it is difficult to make any conclusions about their foraging preferences.

We have been limited by several constraints in our comparison between laboratory sugar type preferences and foraging choices in the wild. Firstly, there are very few data available on nectar sugars of Australian plants. While foraging observations are identified to plant species, the nectar composition data for these same plant species are often unavailable. We therefore present nectar composition data for plant genera rather than species (even so, we still lack data on nectar composition data for plant genera accounting for an average of 15% of foraging records for the five bird species examined for this measure). Secondly, these bird species also forage widely at plant species outside of Western Australia (we have not found comparative data of foraging observations for the rest of the country). Finally, where nectar data are available for multiple species of a plant genus, averaging values for sugar composition obscures the fact that some genera (notably *Banksia* and *Grevillea*), show a dichotomy in nectar composition, with some species having sucrose nectars and other species hexose nectars (Nicolson and Van Wyk 1998). Many species included in this data set

(e.g. *Grevillea* spp.; Appendix C) may be not be primarily bird-pollinated, although birds may visit their flowers on an opportunistic basis.

Conclusions

In the Americas and Africa, nectar-feeding birds are relatively easily categorized as specialized (hummingbirds and sunbirds, respectively) or generalist (all other bird taxa) due to distinctions between bird lineages. However, there are ~180 species of Australasian honeyeaters (Family Meliphagidae) which exhibit a range of diets, from predominantly nectar through to predominantly insect diets. This makes a simplistic dichotomy between specialized and generalist/opportunistic nectarivores difficult for Australian honeyeaters.

We have identified that sucrase activity is likely to be a key digestive constraint directly influencing the concentration-dependence of sugar type preferences shown in birds. To our knowledge, this is the first study to compare sugar preferences assessed in the laboratory with both aspects of digestive physiology and wild foraging observations. We suggest that further comparative work on generalist and specialist nectarivores, particularly in larger birds such as lorikeets, takes a similarly multi-faceted approach by incorporating avian ecology and behavior with digestive physiology.

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Table 1: Summary of sugar type preferences, Apparent Assimilation Efficiency (AE*) and digestive capacity.

				Sugar type preferences							Apparent Assimilation Efficiency (AE*, %)			Digestive capacity (sucrase)				
				Diet Concentration (Sucrose Equivalents, mol•L-1)														
Bird species	**Diet**	**Body mass (g)**	**Ref**	**0.075**	**0.1**	**0.25**	**0.5**	**0.75**	**1**	**2**	**Sucrose**	**Glucose**	**Fructose**	**n**	**Gut nominal surface area** (cm^2)	**Total activity** (µmol·min^{-1})	**V_{max}** (µmol·min^{-1})	**Km** (mmol)
Trichoglossus haematodus (rainbow lorikeet)	**SN**	137±14	1,2,3	H	H	H	H	H	ns	S	>98	99.7±0.1	-	7	31.6±7.1	25.8±12.1	42.8±21.7	21.7, 16.7†
ylidonyris novaehollandiae (New Holland honeyeater)	**SN**	20.5±3.4	1,2	H	H	ns	ns	ns	ns	ns	>99	-	-	9	6.0±1.7	12.6±7.9	25.3 ±15.3	39.0, 25.0†
Anthochaera carunculata (red wattlebird)	**SN**	105±3	1,2	H	ns	ns	ns	ns	S	S	>99	99.8±0.1	-	8	17.3±6.0	41.7±23.6	77.9±37.0	42.9, 20.2†
Cinnyris talatala (white-bellied sunbird)	**SN**	9.0±1.4	4,7,8	-	H	ns	ns	S	ns	-	99.8±0.05	99.7±0.2	99.7±0.1	4	3.3±0.5	8.3±2.2	12.7±3.1	15.4±4.5
Selasphorus platycercus (broad-tailed hummingbird)	**SN**	3.3±0.1	4,6,7,18	-	-	ns	ns	ns	ns	-	95.0±0.02	-	-	3	2.1±0.4	4.8±1.6		
Zosterops lateralis (silvereye)	Fr, GN	9.0±0.4	1	H	H	H	ns	S	S	S	98.7±0.3	99.9±0.1	97.7±0.5	4	6.2±1.1	4.1±1.5	7.5±2.8	22.9
Lichenostomus virescens (singing honeyeater)	GN	28.9±4.1	1	H	H	H	ns	ns	ns	ns	99.6±0.2	99.9±0.1	99.3±0.3	7	8.3±1.7	13.6±8.6	23.7±14.5	24.9, 20.0†
Dicaeum hirundinaceum (mistletoebird)	Fr, GN	~8	1	H	H	H	ns	ns	ns	ns	98.4±1.4	99.8±0.2	99.3±0.4		-	-	-	-
				0.146			**0.584**		**1.168**									
Eugenes fulgens (magnificent hummingbird)	**SN**	7.1±0.2	5,6	H			ns		S		99	99	99	3	3.5±0.5	21.4±4.2	-	-
Diglossa baritula (cinnamon-bellied flowerpiercer)	**SN**	8.1±0.2	5,6	H			ns		S		99	99	99	4	3.7±0.2	3.3±0.6	10.2±1.9	59.5
				0.146			**0.73**		**1.022**									
Cynanthus latirostris (broad-billed hummingbird)	**SN**	2.9±0.2	16,17	ns			ns		ns		99±2.4	97±4.9	98±2.4	3	1.7	5.6±0.9	-	-
				0.146	**0.29**	**0.438**	**0.584**	**0.73**				**'Hexoses'**						
Nectarina famosa (malachite sunbird)	**SN**	~16	11,14	H	ns	ns	ns	S			>99	>99						
Ploceus cucullatus (village weaver)	GN	36.7±2.8	12	H	H	ns	ns	ns			~90-94	~96-98						
Colius striatus (speckled mousebird)	GN	~47	9	H	H	ns	ns	S			~84-87	~89-93						
Pycnonotus tricolor (dark-capped bulbul)	GN	~37	10	H	H	H	H	H			~65-85	~75-95						
Onychognathus morio (red-winged starling)	GN	~126	15,19	H	H	H	H	ns			0	~64-73				nd		
				0.193			**0.643**											
Tauraco corythaix (Knysna tauraco)	Fr	~260	13	S(vs G)			ns											
Tauraco porphyreolophus (purple-crested tauraco)	Fr	~250	13	ns			ns											

Note. – indicates not measured or not available. **Diet:** Specialist nectarivore (SN, bold), generalist nectarivore (GN), frugivore (Fr). **References:** [1] this study; [2] Fleming *et al.* (2008) Napier *et al.* (2008); [4] Fleming *et al.* (2004); [5] Schondube and Martinez del Rio (2003); [6] Schondube and Martinez del Rio (2004); [7] McWhorter *et al.* (unpublished), [8]Köhler *et al.* (20 Brown *et al*. (2010a), [10]Brown *et al*. (2010b), [11] Brown *et al*. (2010c), [12] Odendaal *et al*. (2010), [13] Wilson and Downs (2011), [14]Downs (1997) [15]Brown *et al.* (2012) [16]Martinez del Ri (1990a) [17]Martinez del Rio (1990b), [18]McWhorter and Martinez del Rio (2000), [19]Bizaare *et al.*(2012). **Sugar preferences:** dark grey: hexose (H) or glucose (G), light grey: sucrose (

no significant preference. **Total activity:** nd: not detectable. **Km and pH optima:** Kinetic parameters obtained using at least n=1 tissue homogenate (proximal intestinal section): † t sets for birds caught in 2010-2012 (n=1) or 2006-2007 (n=1).

Figure legends

Figure 1: Phylogenetically independent contrast values were calculated using the evolutionary phylogenetic tree of Ericson et al. (2006) as a backbone, with sets of pseudo-posterior samples of the dated phenologies built by Jetz et al. (2012) subsampled and then pruned for our full set of species (http://birdtree.org).

Figure 2: Concentration-dependent total sugar intake of *Dicaeum hirundinaceum* (circle), *Zosterops lateralis* (triangle) and *Lichenostomus virescens* (square) offered paired sucrose and hexose (fructose + glucose) solutions of varying concentrations: 0·075, 0·1, 0·25, 0·5, 0·75, 1 and 2 $mol \cdot L^{-1}$ sucrose equivalents (SE). Diets where birds did not achieve energy balance (statistically lower intake than the maximal sugar intake) are indicated with increasingly lighter shaded symbols (one-way ANOVA and Tukey's HSD test). n refers to number of individuals.

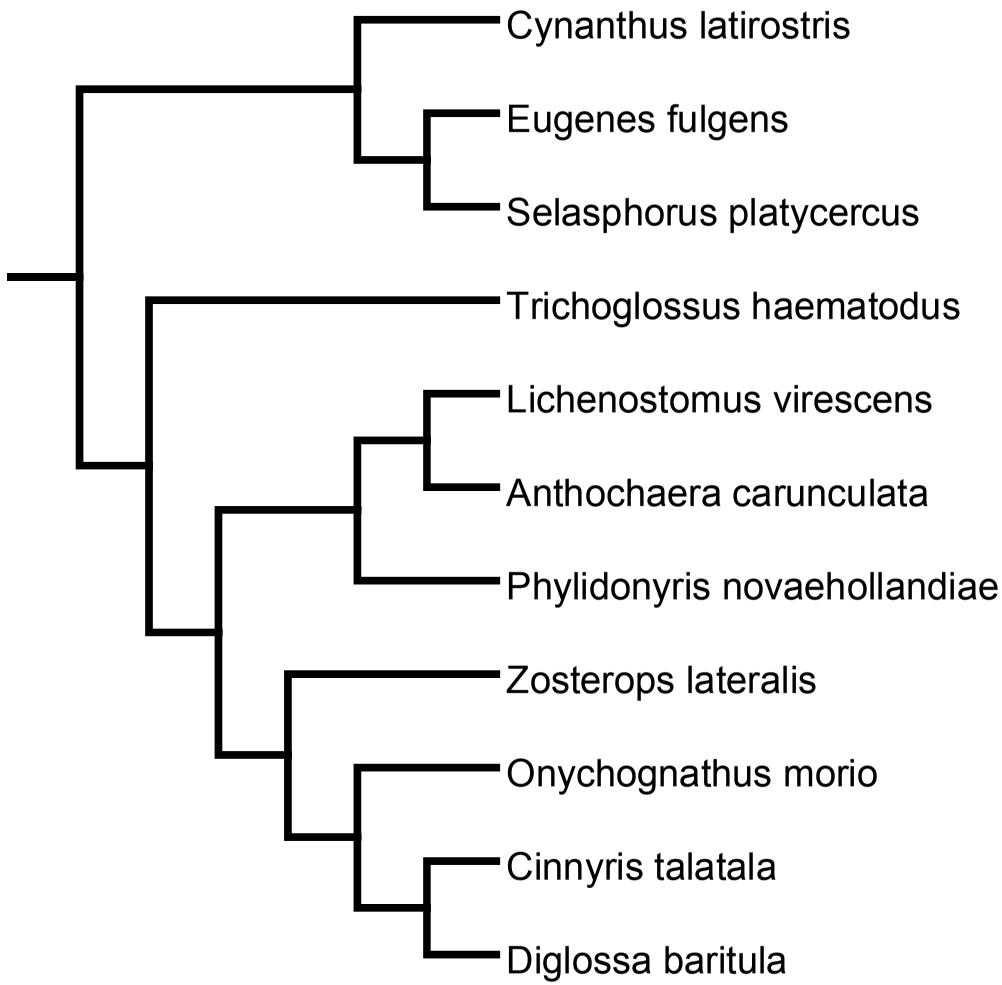
Figure 3: Concentration-dependent sugar preferences of a) *Dicaeum hirundinaceum* (circle), b) *Zosterops lateralis* (triangle) and c) *Lichenostomus virescens* (square) offered paired sucrose and hexose (fructose + glucose) solutions of varying concentrations: 0·075, 0·1, 0·25, 0·5, 0·75, 1 and 2 $mol \cdot L^{-1}$ sucrose equivalents (SE). Diets where birds did not achieve energy balance (statistically lower intake than the maximal sugar intake; Figure 2) are indicated with increasingly lighter shaded symbols. Letters indicate diets that are statistically different from each other (one-way ANOVA with Tukey's HSD test). Asterisks indicates concentrations where there was a significant preference for either hexose or sucrose diets (one-sample t-test). n refers to number of individuals.

Figure 4: Relationships between body mass and **a)** gut nominal surface area; **b)** total sucrase activity and **c)** total maltase activity. **d)** Relationship between maltase and sucrase activity (both standardized by gut nominal surface area). **e)** Relationship between degree of hexose preference (i.e. minimum no-preference concentration) and standardized sucrase activity. Data are averaged for each species. White symbols denote generalist nectarivores, grey symbols denote specialized nectarivores. See Table 1 for details of references, numbers of individuals and diet categories.

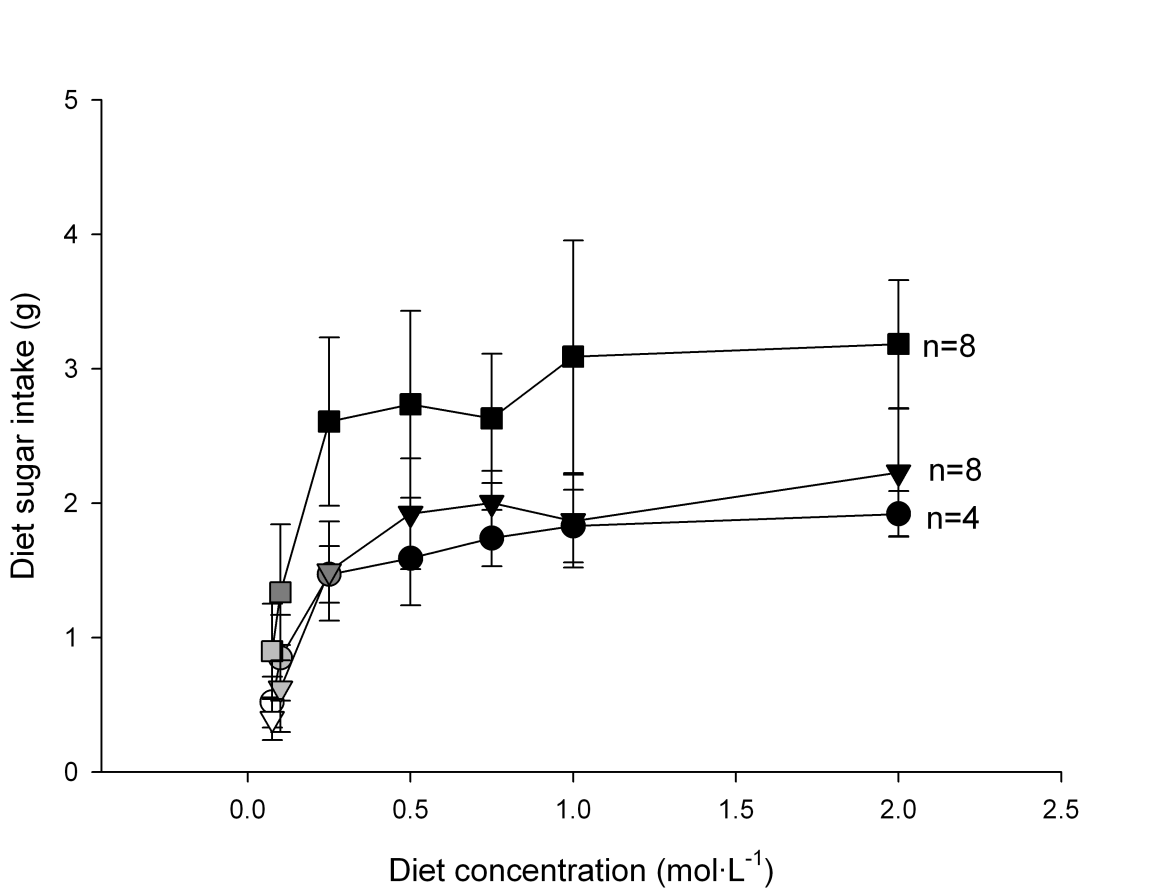
Figure 5: Feeding observations for *Phylidonyris novaehollandiae, Anthochaera carunculata, Lichenostomus virescen* and *Zosterops lateralis* in Western Australia (Brown et al. 1997) and *Trichoglossus haematodus* in Western Australia and the Queensland-New South Wales

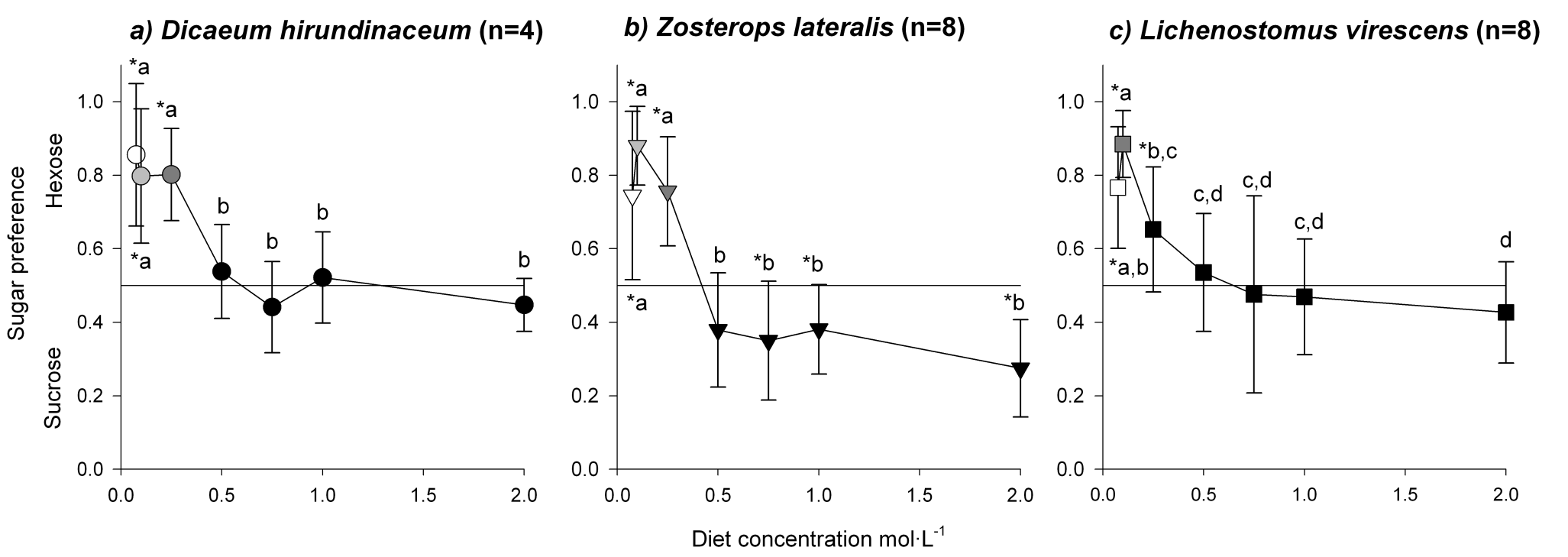
border region (Cannon 1984; Brown et al. 1997). **a)** Feeding observations grouped by plant genus. White lines indicate hexose dominant and rich nectars, solid light grey indicates mixed sugars, dark grey lines indicates sucrose dominant and rich nectars. Dots indicate unknown sugar composition, solid white indicates other genera comprising <2% of feeding observations (including *Agonis, Adansonia, Astroloma, Billardiera, Blancoa, Bombax, Bossiaea, Braxychiton, Brachysema, Chasmanthe, Chorilaena, Cosmelia, Crotalaria, Darwinia, Diplolaena, Eremophilia, Erythina, Gastrolobium, Hardenbergia, Hybanthus, Jacksonia, Jansonia, Kunzea, Leptosema, Leptospermum, Loranthus, Lysiana, Macropidia, Microcorys, Muiriantha, Nematolepis, Nicotiana, Nutysia, Pimelea, Pittosporum, Psoralea, Regelia, Temletonia,* and *Xanthorrea*). In parentheses: total number of foraging observations, number of plant genera. **b)** Feeding observations grouped by nectar composition; see text for definitions of nectar categories. Asterisks denote significant preference (P) or avoidance (A) of nectar categories as determined by χ^2 analysis with Bonferroni correction applied (P<0.05*, P<0.01**, P<0.001***).

Figure 6: Average nectar composition from 16 Australian plant genera (mean fructose, glucose, sucrose). In parentheses: number of species sampled for each genus. Fructose is represented by white bars, glucose by grey and sucrose by black. Hexose-dominant nectars include: *Sternocarpus, Hakea, Corymbia, Anigozanthos, Amyema, Telopea, Callistemon, Erythrina* and *Adenanthos*; hexose-rich nectars: *Eucalyptus* and *Melaleuca;* mixed nectars: *Banksia* (including former *Dryandra* species); sucrose-rich nectars: G*revillea* and *Calothamnus;* sucrose-dominant nectars: *Lambertia* and *Macadamia.*



Modified from tree 9462


Diet sugar intake (g)
Diet concentration (mol·L-1)
n=8
n=8
n=4
0
1
2
3
4
5
0.0
0.5
1.0
1.5
2.0
2.5


a) Dicaeum hirundinaceum (n=4)
b) Zosterops lateralis (n=8)
c) Lichenostomus virescens (n=8)
Sugar preference
Hexose
Sucrose
Diet concentration mol·L-1
*a
*a
*a
b
b
b
b
*a
*a
*a
b
*b
*b
*b
*a,b
*b,c
c,d
c,d
c,d
d
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.5
1.0
1.5
2.0

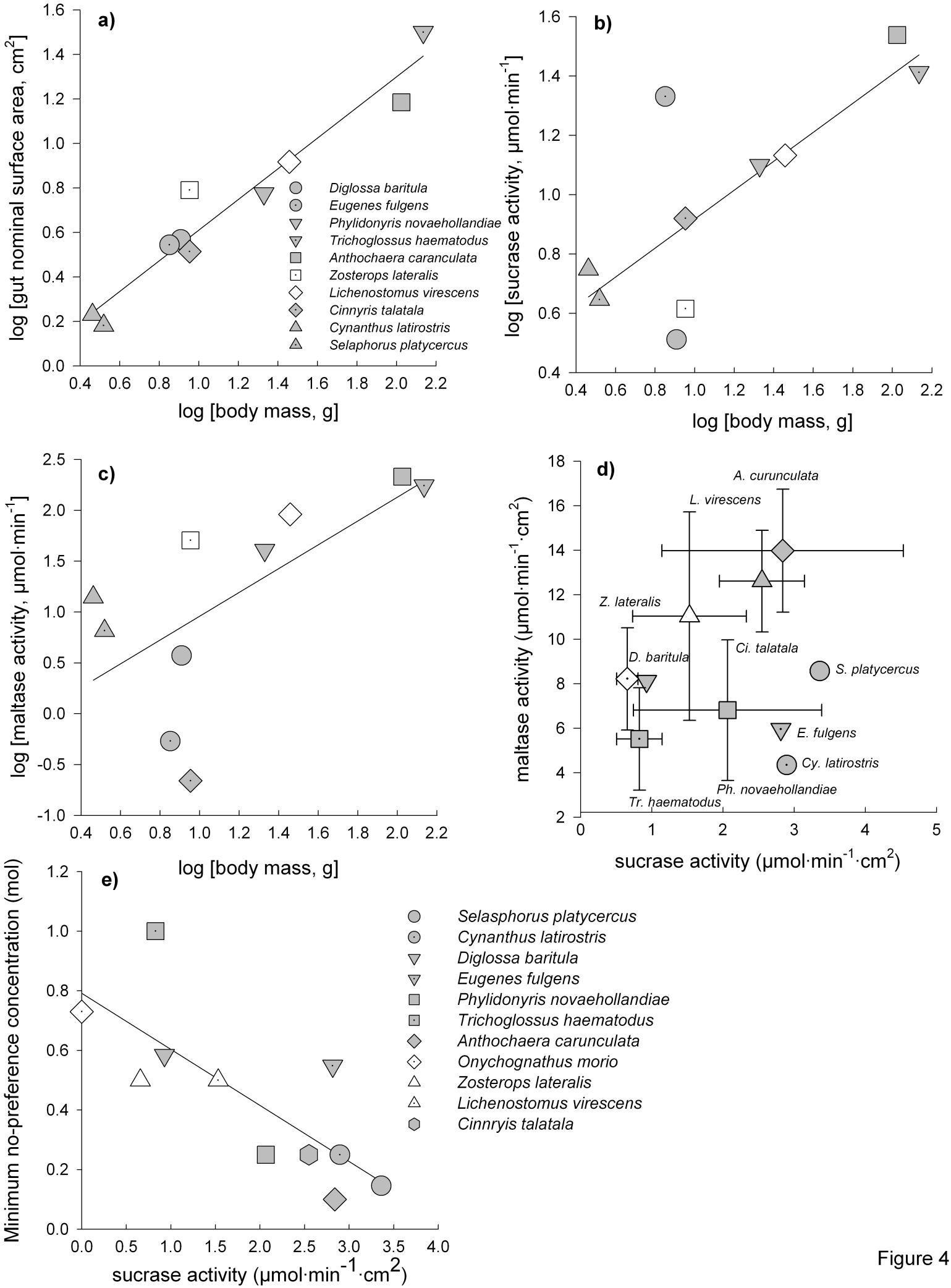
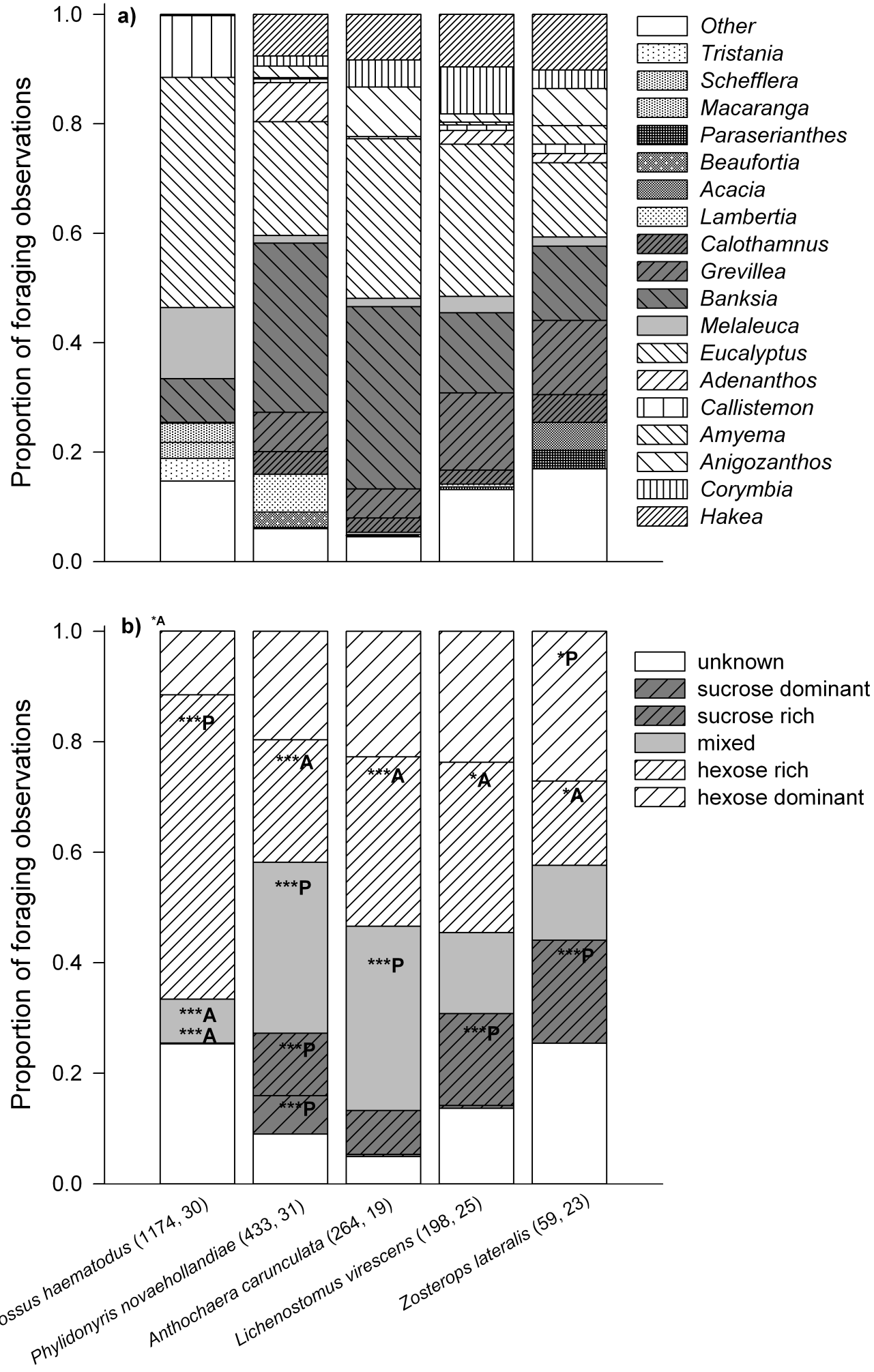

a)
log [gut nominal surface area, cm^2]
log [body mass, g]
Diglossa baritula
Eugenes fulgens
Phylidonyris novaehollandiae
Trichoglossus haematodus
Anthochaera caranculata
Zosterops lateralis
Lichenostomus virescens
Cinnyris talatala
Cynanthus latirostris
Selaphorus platycercus
b)
log [sucrase activity, µmol·min^{-1}]
log [body mass, g]
c)
log [maltase activity, µmol·min^{-1}]
log [body mass, g]
d)
A. curunculata
L. virescens
Z. lateralis
D. baritula
Ci. talatala
S. platycercus
E. fulgens
Cy. latirostris
Ph. novaehollandiae
Tr. haematodus
maltase activity (µmol·min^{-1}·cm^2)
sucrase activity (µmol·min^{-1}·cm^2)
e)
Minimum no-preference concentration (mol)
sucrase activity (µmol·min^{-1}·cm^2)
Selasphorus platycercus
Cynanthus latirostris
Diglossa baritula
Eugenes fulgens
Phylidonyris novaehollandiae
Trichoglossus haematodus
Anthochaera carunculata
Onychognathus morio
Zosterops lateralis
Lichenostomus virescens
Cinnryis talatala

Figure 4

Figure 5


a)
Proportion of foraging observations
1.0
0.8
0.6
0.4
0.2
0.0
Other
Tristania
Schefflera
Macaranga
Paraserianthes
Beaufortia
Acacia
Lambertia
Calothamnus
Grevillea
Banksia
Melaleuca
Eucalyptus
Adenanthos
Callistemon
Amyema
Anigozanthos
Corymbia
Hakea
b)
*A
***P
***A
***A
***P
***P
***P
***P
***A
***A
***P
***P
*P
*A
unknown
sucrose dominant
sucrose rich
mixed
hexose rich
hexose dominant
Trichoglossus haematodus (1174, 30)
Phylidonyris novaehollandiae (433, 31)
Anthochaera carunculata (264, 19)
Lichenostomus virescens (198, 25)
Zosterops lateralis (59, 23)

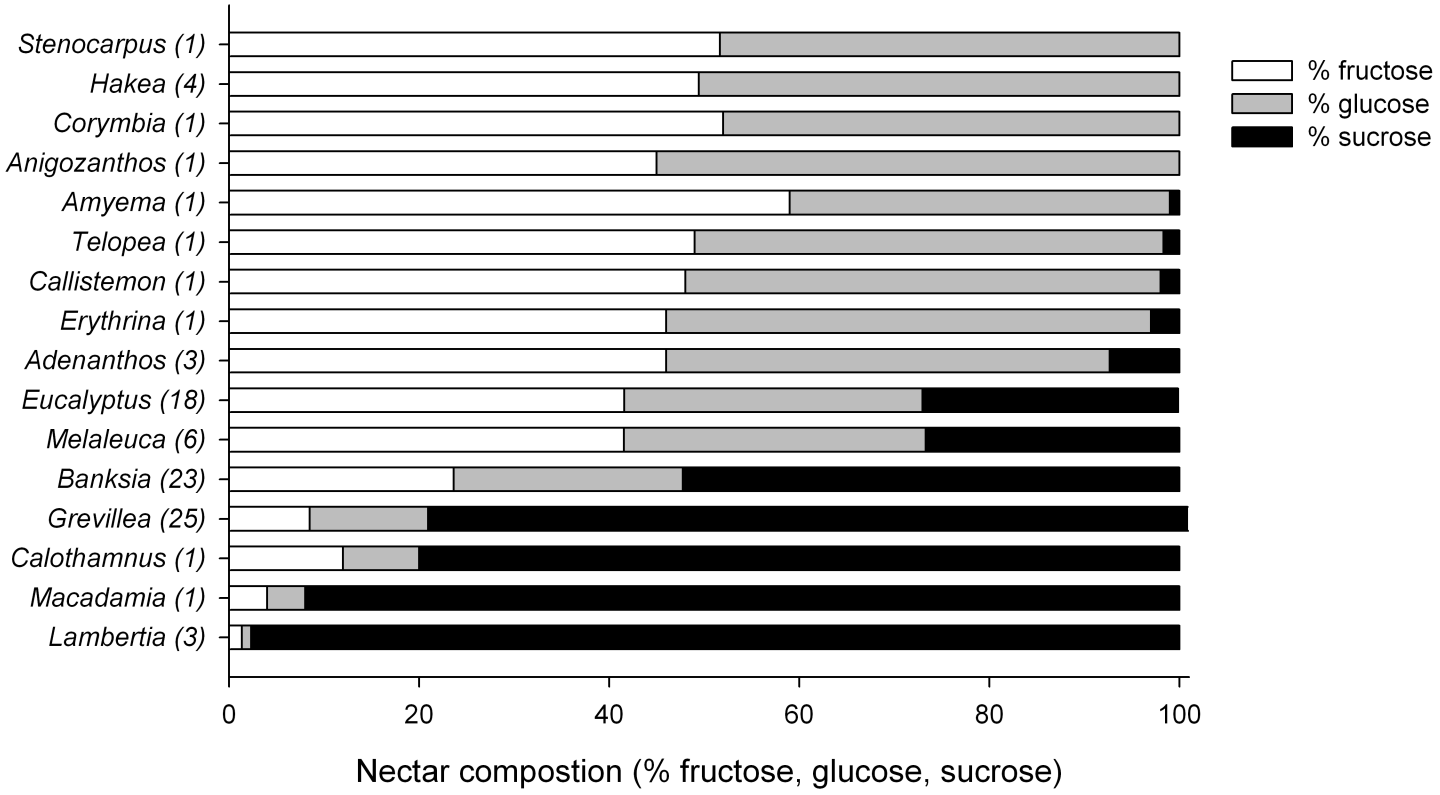

Stenocarpus (1)
Hakea (4)
Corymbia (1)
Anigozanthos (1)
Amyema (1)
Telopea (1)
Callistemon (1)
Erythrina (1)
Adenanthos (3)
Eucalyptus (18)
Melaleuca (6)
Banksia (23)
Grevillea (25)
Calothamnus (1)
Macadamia (1)
Lambertia (3)
0
20
40
60
80
100
Nectar compostion (% fructose, glucose, sucrose)
% fructose
% glucose
% sucrose

Figure 6

Appendix A: Details of birds euthanased for digestive enzymes.

Species	Location of capture*	Year of capture	Method of capture	Sex (M, F)	Period of captivity	Method of euthanasia+
Anthochaera carunculata (red wattlebird)	Nedlands, WA (31°58′S, 115°49′E)	2010	Mist-netting	(3, 0)	< 72 h	Sodium pentobarbital
	Murdoch, WA (32°04′S, 115°50′E)	2007	Mist-netting	(4, 1)	5 mo	Isoflurane
Lichenostomus virescens (singing honeyeater)	Shenton Park, WA (31°57′S, 115°47′E)	2010	Mist-netting	(2, 1)	< 7 h	Sodium pentobarbital
	Murdoch, WA (32°04′S, 115°50′E)	2007	Mist-netting	(4, 0)	5 mo	Isoflurane
Phylidonyris novaehollandiae (New Holland honeyeater)	Roleystone, WA (32°08′S, 116°05′E)	2011	Mist-netting	(3, 1)	< 7 h	Sodium pentobarbital
	Murdoch, WA (32°04′S, 115°50′E)	2007	Mist-netting	(5, 0)	14 mo	Isoflurane
Zosterops lateralis (silvereye)	Roleystone, WA (32°08′S, 116°05′E)	2011	Mist-netting	(3, 1)	< 7 h	Sodium pentobarbital
Trichoglossus haematodus (rainbow lorikeet)	Bentley, WA (32°0′S, 115°53′E)	2012	Unknown	(1, 0)	< 1 h	Natural death
	Wattle Grove, (32°0′S, 115°59′E)	2012	Unknown	(1, 0)	Unknown	Sodium pentobarbital
	Perth Airport, WA (31°55′S, 115°57′E)	2006	Canon-netting	(5, 0)	>12 mo	Isoflurane

*Nedlands- grounds of The University of Western Australia (UWA); Murdoch- grounds of Murdoch University; Shenton Park- grounds of the UWA Shenton Park Field Station; Roleystone- grounds of the Araluen Country Club; Bentley- grounds of Curtin University (bird flew into window and died ~1 h later); Wattle Grove- bird obtained from Wattle Grove Veterinary Clinic after an unknown period in captivity; Perth Airport- grounds of Perth Domestic Airport as part of a Department of Conservation (DEC) culling program. +Birds were euthanased via 1:1 sodium pentobarbital:distilled H_2O solution injected into the heart or by Isoflurane overdose.

Appendix B: Summary of maltase activity.

	ref	Digestive capacity (maltase)			
		Total activity ($\mu mol \cdot min^{-1}$)	V_{max} ($\mu mol \cdot min^{-1}$)	Km (mM)	pH optima
Trichoglossus haematodus (n=7)	1	174±89.7	207.4±103.2	4.5, 5.8	5, 6
Zosterops lateralis (n=4)	1	50.7±20.3	60.5±24.2	5.4	6.5
Lichenostomus virescens (n=7)	1	91.3±47.9	100.6±51.3	3.9, 2.6	5.5, 4.5
Phylidonyris novaehollandiae (n=9)	1	40.6+19.0	50.5±22.0	12.5, 4.3	4.5, 5
*Anthochaera carunculata (*n=8)	1	213.9±119	258.7±130.2	12.6, 4.3	5, 5.5
Selaphorus platycercus (n=2)	6	7.7±1.4	-	-	-
*Eugenes fulgens (*n=3)	6	17.0±3.3	-	-	-
Diglossa baritula (n=4)	6	30.1±4.0	33.2±4.4	2.8	5.5
Cinnyris talatala (n=4)	7	41.0±7.9	44.3±8.5	2.2	5
Cynanthus latirostris (n=3)	17	14.0±2.3	-	-	-

Data are presented as means±SD. – indicates not tested or not available. **References:** [1]Data was obtained from this study; [6] Schondube and Martinez del Rio (2004); [7] McWhorter *et al.* (unpublished), [17]Martinez del Rio (1990). **Km and pH optima:** Kinetic parameters obtained using at least n=1 tissue homogenate (proximal intestinal section): † two data sets for birds caught in 2010-2012 (n=1) or 2006-2007 (n=1).

Appendix C: Nectar composition of 16 Australian plant genera (means±SD, n=total number of species)

Family	Genus	Fructose (%)	Glucose (%)	Sucrose (%)	n	Reference
Fabaceae	*Erythrina*	46	51	3	1	(Baker and Baker 1982)
Haemodoraceae	*Anigozanthos*	45	55	0	1	(Hölscher et al. 2008)
Loranthaceae	*Amyema*	59	40	1	1	(Paton 1982)
Myrtaceae	*Callistemon*	48	50	2	1	S.W. Nicolson and B.-E Van Wyk, unpublished
	Calothamnus	12	8	80	1	S.W. Nicolson and B.-E Van Wyk, unpublished
	Corymbia	52	48	0	1	(Nicolson 1994)
	Eucalyptus	41.6±15.3	31.40±12.15	26.91±21.68	18	(Nicolson 1994; Davis 1997; Baker et al. 1998; Morrant et al. 2010; S.W. Nicolson and B.-E Van Wyk, unpublished)
	Melaleuca	41.5±7.5	31.76±12.95	26.70±19.56	6	(Morrant et al. 2010; S.W. Nicolson and B.-E Van Wyk, unpublished)
Proteaceae	*Adenanthos*	46.0±3.0	46.7±5.1	7.3±8.1	3	(Nicolson and Van Wyk 1998)
	Banksia	23.6±21.0	24.1±21.0	52.2±41.7	23	(McFarland 1985; Nicolson and Van Wyk 1998)
	Grevillea	8.5±16.9	12.5±24.6	82.7±33.7	25	(Gottsberger et al. 1984; Nicolson and Van Wyk 1998)
	Hakea	49.4±1.3	50.6±1.3	0	4	(Nicolson and Van Wyk 1998)
	Lambertia	1.3±0.6	1.0±1.0	97.7±1.5	3	(Nicolson and Van Wyk 1998)
	Macadamia	4	4	92	1	(Nicolson and Van Wyk 1998)
	Stenocarpus	51.7±3.1	48.3±3.1	0	1	(Nicolson and Van Wyk 1998)
	Telopea	49.0±0.0	49.3±1.5	1.7±1.5	1	(Nicolson and Van Wyk 1998)