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# Sugar and protein digestion in flowerpiercers and hummingbirds: a comparative test of adaptive convergence

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**Abstract** Flowerpiercers are the most specialized nectar-feeding passerines in the Neotropics. They are nectar robbers that feed on the sucrose-rich diet of hummingbirds. To test the hypothesis that flowerpiercers have converged with hummingbirds in digestive traits, we compared the activity of intestinal enzymes and the gut nominal area of cinnamon-bellied flowerpiercers (*Diglossa baritula*) with those of eleven hummingbird species. We measured sucrase, maltase, and aminopeptidase-N activities. To provide a comparative context, we also compared flowerpiercers and hummingbirds with 29 species of passerines. We analyzed enzyme activity using both standard allometric analyses and phylogenetically independent contrasts. Both approaches revealed the same patterns. With the exception of sucrase activity, hummingbirds' digestive traits were indistinguishable from those of passerines. Sucrase activity was ten times higher in hummingbirds than in passerines. Hummingbirds and passerines also differed in the relationship between intestinal maltase and sucrase activities. Maltase activity was two times higher per unit of sucrase activity in passerines than in hummingbirds. The sucrase activity of *D. baritula* was much lower than that of hummingbirds, and not unlike that expected for a passerine of its body mass. With the exception of aminopeptidase-N activity, the digestive traits of *D. baritula* were not different from those of other passerines.

**Keywords** Comparative method · *Diglossa baritula* · Hummingbirds · Sucrase activity · Sugar and protein digestion

**Abbreviations** $K_m$ Michaelis-Menten constant · *PDAP* phenotypic diversity analysis program · *PIC* phylogenetic independent contrast · $V_{max}$ maximal reaction velocity

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## Introduction

Nectarivory evolved independently at least eight times among birds (Ford 1985). In the New World, specialized nectarivory evolved in hummingbirds and within a few clades of the family Thraupidae (Stiles 1981; Burns 1997). Within the Thraupidae, flowerpiercers (genus *Diglossa*) are the most specialized nectarivores (Skutch 1954; Vuilleumier 1969; Stiles 1981; Isler and Isles 1999; Schondube and Martínez del Rio 2003a). Among nectar-feeding birds, flowerpiercers are uniquely specialized for nectar robbing. Their hooked bills are well suited to perforate the corolla and then extract nectar from the tubular flowers of hummingbird-pollinated plants (Schondube and Martínez del Rio 2003a).

Feeding on nectar requires more than the ability to extract nectar from flowers. After ingestion, the sugars in it must be assimilated (Alpers 1987; Semenza and Corcelli 1986), and the water that accompanies it—sometimes in great excess—must be processed (Beuchat et al. 1990; McWhorter and Martínez del Rio 1999; McWhorther et al. 2003). Because nectar contains only trace amounts of amino acids, vitamins, and electrolytes (Baker 1977; Baker and Baker 1983a, 1983b), nectar-feeding birds must also conserve protein and salts (Beuchat et al. 1999; Lotz and Martínez del Rio 2004).

The physiological traits that allow nectar-feeding birds to cope with their watery and sugary diets have been studied primarily in hummingbirds. Hummingbirds possess extremely high activity of disaccharidases and high rates of intestinal glucose transport (Beuchat et al. 1979; Karasov et al. 1986; Martínez del Rio 1994). They also have remarkably low protein requirements (McWhorter et al. 2004) and an astounding capacity to retain the scanty electrolytes present in floral nectar (Lotz and Martínez del Rio 2004).

Nectar-feeding flowerpiercers appear to have evolved from a frugivorous ancestor to become specialized nectar robbers of hummingbird pollinated flowers (Burns 1997; Schondube and Martínez del Rio 2003a). Most flowerpiercer species are syntopic with and feed on the same flowers as hummingbirds. They can be described as passerines that feed on the characteristic diet of a hummingbird (Skutch 1954; Schondube and Martínez del Rio 2003a). They also show similar sugar preferences and sucrose assimilation efficiencies to those of hummingbirds (Schondube and Martinez del Rio 2003b). It is natural to predict that their digestive traits have converged with those of hummingbirds. Here, we examine this conjecture. Specifically, we compare some of the physiological traits that presumably underlie the ability of cinnamon-bellied flowerpiercers (*Diglossa baritula*) to assimilate sucrose and protein with those of several hummingbird species. To provide a comparative context, we also compare flowerpiercers and hummingbirds with available data for passerines.

Oligosaccharides (such as sucrose and maltose) and oligopeptides must be hydrolyzed into simple sugars and amino acids by intestinal membrane-bound enzymes before they can be absorbed. Thus, the hydrolytic activity of intestinal enzymes is the basis of the ability of animals to assimilate carbohydrates and protein (Alpers 1987; Martínez del Rio 1990; Martínez del Rio et al. 1995; Witmer and Martínez del Rio 2001). Sucrase-isomaltase catalyzes the hydrolysis of sucrose ($\alpha$ 1–2 $\beta$ glucose, fructose) into its constituent sugars: glucose and fructose. This enzyme also catalyzes the hydrolysis of maltose ($\alpha$ 1–4 glucose, glucose), a disaccharide that results from the digestion of starch and glycogen. Maltose also can be hydrolyzed by maltase-glucoamylase, an enzyme that does not have sucrase activity (Alpers 1987; Martínez del Rio 1990; Martínez del Rio et al. 1995). We measured the activity of the intestinal enzymes sucrase-isomaltase and maltase-glucoamylase. For simplicity we will call these enzymes sucrase and maltase, respectively. As a proxy index for the ability to digest protein, we measured the intestinal enzyme aminopeptidease-N (Kania et al. 1977; Martínez del Rio et al. 1995). Aminopeptidase-N appears to be the primary exopeptidase of the brush-border membrane of mammals and birds. This enzyme hydrolyzes the $NH_2$ terminal residues from oligopeptides to yield dipeptides and free amino acids that can then be absorbed (Maroux et al. 1973; Kania et al. 1977; Martínez del Rio et al. 1995).

We used an allometric approach to compare the total hydrolytic activity of sucrase, maltase and aminopeptidase-N among *D. baritula*, 29 other species of passerines, and 11 species of hummingbirds. This comparison allowed us (1) to explore whether the clades of passerines and hummingbirds differ in the ability to hydrolyze sugars and protein; and (2) to determine whether *D. baritula* exhibits digestive traits similar to those of other passerines, or if it has converged with hummingbirds in digestive function. Because phylogenetic relationships can confound the inferences of allometric analyses (Garland et al. 1992; Garland and Adolph 1994), we corroborated the conclusions of our allometric comparisons using phylogenetically independent contrasts (Felsenstein 1985).

## Materials and methods

### Collection of samples

We collected three species of passerines (*D. baritula*, *Vermivora celata*, and *V. ruficapilla*) and four species of hummingbirds (*Eugenes fulgens*, *Colibri thalassinus*, *Hylocharis leucotis*, and *Selasphorus rufus*) in Nevado de Colima National Park, Jalisco, Mexico. Sample sizes differed for different species (see Table 1). Birds were collected with permission from the National Institute of Ecology (INE), Mexico. Sample sizes reflect permit constraints. Birds were euthanized by thoracic compression and their intestines immediately removed. Intestines were chilled in ice-cold 0.9% saline and divided into three sections of approximately equal length. Each tissue section was slit longitudinally, unfolded flat, and its length and width was measured to obtain an estimate of its "nominal" area. The tissue was then blotted, weighed and stored in liquid $N_2$. Enzymatic activity of three more species of hummingbirds was taken from unpublished data (*Lampornis clemenciae*, *Archilochus alexandri*: T.J. McWhorter, unpublished data; *A. colubris*: C. Martínez del Rio, unpublished data). Measurements of enzymatic activity and gut morphology from other hummingbird and passerine species were collected from the literature (Martínez del Rio and Stevens 1989; Martínez del Rio 1990; Afik et al. 1995; Martínez del Rio et al. 1995; Sabat et al. 1998; Levey et al. 1999; McWilliams et al. 1999; Meynard et al. 1999; Caviedes-Vidal et al. 2000; McWhorther and Martínez del Rio 2000; Sabat 2000). All these authors used the same methodologies, allowing us to compare the data.

### Intestinal enzymatic activity

Intestinal tissues were thawed at 5°C and homogenized (30 s, OMNI 5000 homogenizer at setting 6) in nine volumes of 350 mmol $l^{-1}$ mannitol in 1 mmol $l^{-1}$ HEPES/KOH, pH 7.5. Disaccharidase activities were measured following Martinez del Rio et al. (1995) and Schondube et al. (2001). In brief, tissue homogenates (100 µl) diluted with 350 mmol $l^{-1}$ mannitol in 1 mmol $l^{-1}$ HEPES/KOH were incubated at 40°C with 100 µl of 56 mmol $l^{-1}$ sugar (sucrose or maltose) solutions in 0.1 mol $l^{-1}$ maleate/NaOH buffer, pH 6.5. After a 10- to 20-min incubation, reactions were arrested by adding 3 ml of a stop/developing Glucose-Trinder (one bottle of Glucose-Trinder 500 reagent [Sigma, St. Louis, Mo., USA] in 250 ml 1.0 mol $l^{-1}$ TRIS/HCL, pH 7, plus 250 ml of 0.5 mol $l^{-1}$ $NaH_2PO_4/Na_2HPO_4$, pH 7). After 15 min at 20°C, absorbance of the resulting solution was measured at 505 nm with a Beckman DU-64 spectrophotometer. Aminopeptidase-N assays were done using L-alanine-*p*-nitroanilide as a substrate. Briefly, 10 µl of tissue homogenate diluted with mannitol/KOH

**Table 1** Body mass, gut nominal area and total enzymatic activity for the species used in this study; values are means ± SE

| Species | *n* | Body mass (g) | Gut nominal area ($cm^2$) | Total sucrase activity (μmol/min) | Total maltase activity (μmol/min) | Total aminopeptidase-N activity (μmol/min) | Source |
|---|---|---|---|---|---|---|---|
| Trochilidae | | | | | | | |
| *Selasphorus rufus* | 3 | 3.3 ± 0.1 | 2.1 ± 0.2 | 16.6 ± 3.1 | 7.7 ± 0.9 | 0.4 ± 0.1 | This study |
| *Selasphorus platycercus* | 2 | 3.3 ± 0.1 | 1.5 ± 0.07 | 12.7 ± 2.2 | 6.1 ± 0.9 | 0.1 ± 0.01 | McWhorter and Martínez del Rio (2000) |
| *Archilochus alexandri* | 2 | 3.2 ± 0.1 | 2.2 ± 0.01 | 9.8 ± 1.9 | 7.7 ± 1.4 | 0.2 ± 0.02 | McWhorter (unpublished data) |
| *Archilochus colubris* | 5 | 3.68 ± 0.2 | 1.6 | 9.1 ± 0.9 | 18.7 ± 1.3 | | Martínez del Rio (unpublished data) |
| *Lampornis clemenciae* | 2 | 8.4 ± 0.3 | 3.7 ± 0.02 | 28.5 ± 0.2 | 28.2 ± 1.3 | 0.6 ± 0.07 | McWhorter (unpublished data) |
| *Eugenes fulgens* | 3 | 7.1 ± 0.1 | 3.5 ± 0.3 | 21.4 ± 2.4 | 17.0 ± 1.9 | 0.7 ± 0.2 | This study |
| *Amazilia rutila* | 4 | 4.5 ± 0.6 | 2.2 | 8.3 ± 0.9 | 20.7 ± 3.5 | | Martínez del Rio (1990) |
| *Hylocharis leucotis* | 3 | 3.6 ± 0.1 | 2.52 ± 0.13 | 16.0 ± 2.1 | 7.6 ± 0.9 | 0.6 ± 0.1 | This study |
| *Cynanthus latirostris* | 3 | 2.9 ± 0.1 | 1.7 | 5.6 ± 0.5 | 14.0 ± 1.3 | | Martínez del Rio (1990) |
| *Chlorostilbon canivetii* | 4 | 2.1 ± 0.1 | 1.2 | 4.5 ± 0.4 | 8.5 ± 0.3 | | Martínez del Rio (1990) |
| *Colibri thalassinus* | 3 | 4.8 ± 0.2 | 3 ± 0.02 | 26.4 ± 3.3 | 18.9 ± 1.8 | 0.7 ± 0.1 | This study |
| Tyrannidae | | | | | | | |
| *Pitangus sulfuratus* | 3 | 73.3 ± 5.7 | 21.7 | 6.9 ± 1.0 | 39.9 ± 6.2 | | Martínez del Rio (1990) |
| *Myiozetetes similis* | 3 | 28.9 ± 2.6 | 11.3 | 3.1 ± 0.5 | 29.7 ± 4.1 | | Martínez del Rio (1990) |
| *Empidonax difficilis* | 2 | 10.9 ± 1.3 | 4.7 | 0.3 ± 0.1 | 7.1 ± 2.5 | | Martínez del Rio (1990) |
| Phytotomidae | | | | | | | |
| *Phytotoma rara* | 5 | 45.6 ± 0.9 | 18.2 | 119 | 419.9 | 21.8 | Meynard et al. (1999) |
| Muscicapidae | | | | | | | |
| *Catharus guttatus* | 1 | 33 | 11.2 | 0.2 | 26.9 | 15.3 | Witmer and Martínez del Rio (2001) |
| *Catharus ustulatus* | 1 | 37 | 12.6 | 0.05 | 31.1 | 11.2 | Witmer and Martínez del Rio (2001) |
| *Catharus minimus* | 1 | 43.5 | 14.8 | 0.2 | 23.6 | 12.7 | Witmer and Martínez del Rio (2001) |
| *Catharus aurantiirostris* | 2 | 25.2 ± 2 | 9.1 | 0 | 5.5 ± 1.1 | | Martínez del Rio (1990) |
| *Hylocichla mustelina* | 3 | 61.8 ± 2.1 | 22.2 | 0.3 ± 0.02 | 29.9 ± 2.65 | 25.2 ± 1.1 | Witmer and Martínez del Rio (2001) |
| *Turdus migratorius* | 3 | 67.5 ± 1.1 | 22.9 | 0.4 ± 0.1 | 60 ± 8.7 | 29 ± 3.5 | Martínez del Rio (1990) |
| *Turdus rufopalliatus* | 3 | 70.4 ± 5.8 | 24.2 | 0 | 9.4 ± 2.1 | | Martínez del Rio (1990) |
| Sturnidae | | | | | | | |
| *Sturnus vulgaris* | 8 | 75 ± 1.2 | 25.7 | 0 | 67.7 ± 4.9 | 29.4 ± 1.8 | Martínez del Rio et al. (1995) |
| Bombycillidae | | | | | | | |
| *Bombycilla cedrorum* | 4 | 36.3 ± 1.5 | 22.3 | 20.2 ± 1.1 | 145.3 ± 13.4 | 11.4 ± 2.1 | Witmer and Martínez del Rio (2001) |
| Passeridae | | | | | | | |
| *Passer domesticus* | 7 | 26.7 ± 0.9 | 10.7 | 9.9 ± 0.5 | 110 ± 8.09 | 7.9 ± 1.3 | Caviedes-Vidal et al. (2000) |
| Parulidae | | | | | | | |
| *Dendroica pinus* | 7 | 11.6 ± 0.4 | 6 | 2.2 ± 0.1 | 17.3 ± 2.7 | 3.7 ± 0.6 | Levey et al. (1999) |
| *Dendroica coronata* | 7 | 12.5 ± 0.3 | 5.8 | 4.2 ± 0.1 | 43.3 ± 1.6 | 7.7 ± 0.1 | Afik et al. (1995) |
| *Vermivora ruficapilla* | 3 | 8 ± 0.2 | 6 ± 0.1 | 3.2 ± 0.8 | 20.5 ± 2.6 | 2.4 ± 0.8 | This study |
| *Vermivora celata* | 3 | 8.2 ± 0.3 | 5.4 ± 0.1 | 2.1 ± 0.9 | 17.2 ± 3.1 | 1.5 ± 0.04 | This study |
| *Icteria virens* | 3 | 21.9 ± 2 | 10.8 | 3.3 ± 0.2 | 58.7 ± 0.8 | | Martínez del Rio (1990) |
| Icteridae | | | | | | | |
| *Icterus pustulatus* | 3 | 28.2 ± 4.1 | 7.3 | 8.5 ± 0.9 | 60.8 ± 6.3 | | Martínez del Rio (1990) |
| *Icterius spurius* | 3 | 18.5 ± 2.6 | 6.6 | 13.6 ± 0.5 | 104.4 ± 3.3 | | Martínez del Rio (1990) |
| *Agelaius phoeniceus* | 8 | 57.5 ± 2.0 | 15.5 ± 0.5 | 4.6 ± 0.07 | 106.6 ± 1.3 | | Martínez del Rio et al. (1995) |
| *Molothrus ater* | 8 | 40.8 ± 1.7 | 12.9 ± 0.6 | 4.12 ± 0.1 | 83.3 ± 6.3 | | Martínez del Rio (unpublished data) |
| *Quiscalus mexicanus* | 3 | 95.9 ± 5.0 | 30.4 ± 1.8 | 10 ± 0.9 | 195.4 ± 3.9 | | Martínez del Rio (unpublished data) |
| *Cacicus melanicterus* | 3 | 84.9 ± 18 | 23.4 | 18.5 ± 5 | 223.8 ± 4.5 | | Martínez del Rio (1990) |
| Fringillidae | | | | | | | |
| *Diuca diuca* | 13 | 32.7 ± 0.8 | | 5.9 ± 0.2 | 66.3 ± 2.7 | 12.08 ± 0.3 | Sabat et al. (1998) |
| *Zonotrichia capensis* | 13 | 20.6 ± 0.3 | | 3.6 ± 0.3 | 37.4 ± 2.9 | 8.4 ± 0.5 | Sabat et al. (1998) |
| Cardinalidae | | | | | | | |
| *Saltator coerulescens* | 3 | 45.6 ± 0.3 | 16.4 | 4 ± 0.4 | 67.4 ± 0.5 | | Martínez del Rio (1990) |
| *Passerina leclancherii* | 2 | 15 ± 2.1 | 5.5 | 3.8 ± 0.2 | 47.7 ± 0.2 | | Martínez del Rio (1990) |
| Thraupidae | | | | | | | |
| *Diglossa baritula* | 4 | 8.1 ± 0.1 | 3.7 ± 0.1 | 3.25 ± 0.31 | 30.1 ± 2.0 | 0.7 ± 0.05 | This study |

buffer were mixed with 1 ml of a pre-warmed (40°C) assay mix (2.04 mmol $l^{-1}$ L-alanine-*p*-nitroanilide in 0.2 mol $l^{-1}$ $NaH_2PO_4$/$Na_2HPO_4$, pH 7). The reaction was incubated at 40°C and arrested after 10 min with 3 ml of ice-cold 2 N acetic acid, and absorbance was measured at 384 nm.

To determine pH optima, we used a 0.1 mol $l^{-1}$ maleate/NaOH buffer system (for sucrose and maltose), and a 0.2 mol $l^{-1}$ $NaH_2PO_4$/$Na_2HPO_4$ buffer system (for aminopeptidase-N) with pH ranging from 5 to 8.5. Disaccharide (56 mmol $l^{-1}$) and L-alanine-*p*-nitroanilide (2.04 mmol $l^{-1}$) concentrations were held constant. Measurements reported in the results were conducted at optimal pH (to the nearest 0.5; Table 1). Kinetics parameters were measured at concentrations ranging from 0.78 to 200 mmol $l^{-1}$ for sucrose and maltose, and concentrations ranging from 1.5 to 40 mM for L-alanine-*p*-nitroanilide. We used a non-linear Gauss-Newton routine to obtain the maximal hydrolysis rates for each of the different substrates ($V_{max}$) and their apparent binding constants ($K_m$, the concentration at which the rate of hydrolysis equals

$V_{max}/2$; Table 1). See Martínez del Rio (1990) and Martínez del Rio et al. (1995) for justification of the use of intestine homogenates and reaction rates relative to $V_{max}$ under assay conditions.

## Data analysis

All data were log-transformed, with the exception of enzyme activities standardized by intestinal area. We fitted allometric lines relating intestinal area and enzyme hydrolytic capacities to body mass without including the value of *D. baritula*. We used a linear model to compare between the slopes and intercepts of the regression lines for hummingbirds and passerines:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_3$$

where $y$ is log(intestinal area) or log(hydrolytic activity), $x_1$ is log(body mass), and $x_2$ is a dummy variable that equals 0 or 1 depending on whether the data point belongs to a hummingbird or a passerine, and $\beta$s are regression coefficients (see Ramsey and Shafer 1996). If $\beta_2$ was significantly different from zero, we inferred that the two allometric lines had a different intercept. If $\beta_3$ was significantly different from zero, we inferred that the lines had a different slope. If any of these parameters was statistically indistinguishable from zero, we removed it from the model. We considered the value of *D. baritula* different from that of either hummingbirds or passerines if the 95% confidence interval of the flowerpiercer's values did not overlap with the 95% confidence interval of the corresponding regression lines. Because species in the sturnid-muscicapid lineage lack intestinal sucrase activity, we removed species in this clade from all analyses involving this enzyme.

Phylogenetically independent contrasts (Felsenstein 1985; Garland and Adolph 1994) were calculated using PDAP (Phenotypic Diversity Analysis Programs, v. 5; Garland et al. 1993, 1999) on log-transformed values (area-specific enzyme activity data were not log-transformed). Evolutionary relationships among passerine species, and bird families were determined using Sibley and Ahlquist's (1990) phylogenetic hypothesis. Relationships among hummingbird species were obtained from J.F. Ornelas et al. (unpublished observations; see Fig. 1). In order to standardize the values of the phylogenetic independent contrasts, we calculated arbitrary branch lengths using the automatic routines of PDAP. Branch lengths were corrected to ensure that the standardized independent contrasts fulfilled analysis assumptions (Garland et al. 1992).

A phylogenetic contrast measures the evolutionary change in a trait between two branches. Thus, the relationship between the phylogenetic contrasts of two traits (say hydrolytic capacity and body mass) measures their correlated evolution. Two clades can differ because a trait changes faster with changes in another, or

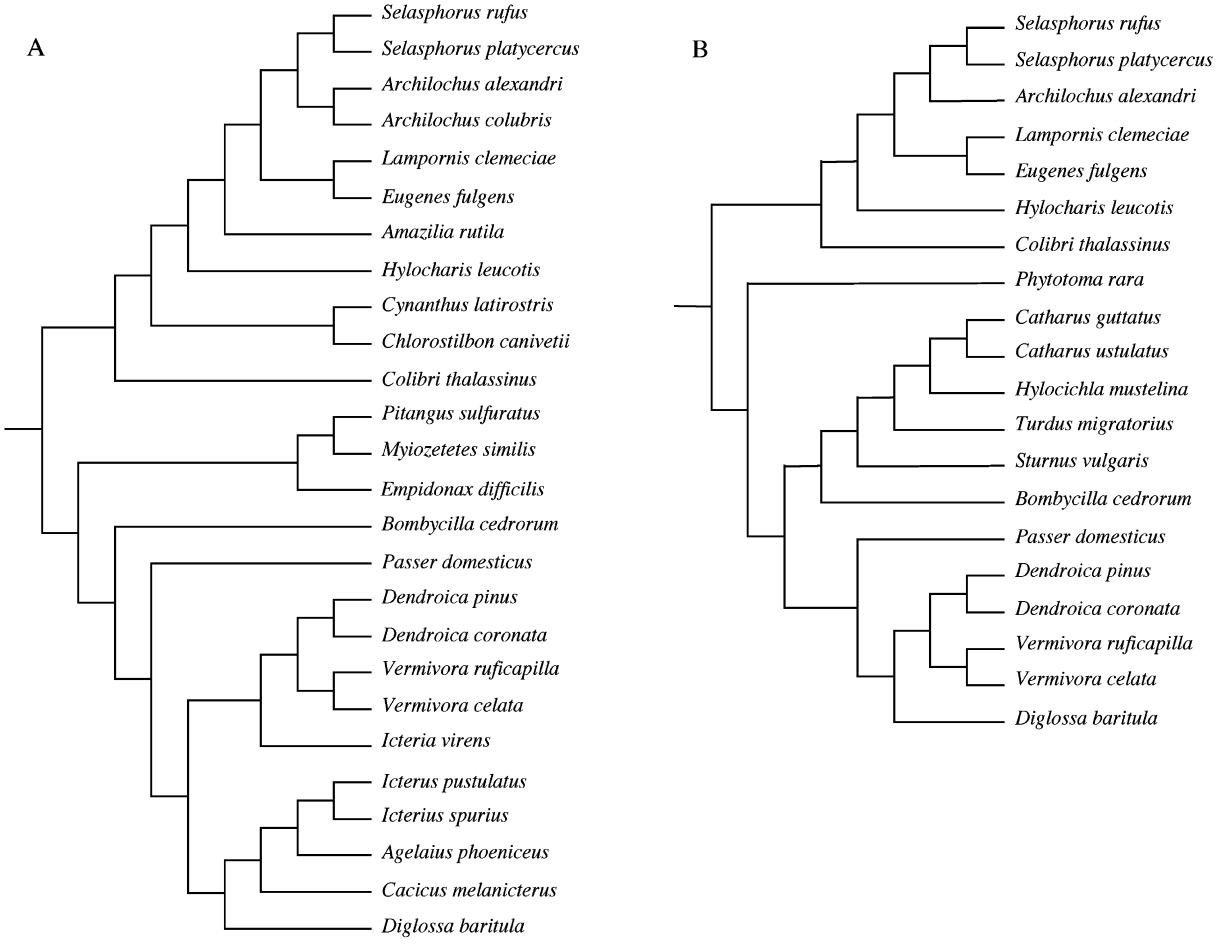


**Fig. 1A, B** Phylogenetic hypotheses used to obtain the phylogentically independent contrasts (PIC) of enzyme activity and gut nominal area. Evolutionary relationships among passerine species, and bird families were determined using Sibley and Ahlquist's (1990) phylogenetic hypothesis. Relationships among hummingbird species were obtained from J.F. Ornelas et al. (unpublished observations). Phylogeny (**A**) was used to obtain PIC values for sucrase, maltase and gut nominal area. Because the sturnid-muscicapid clade lacks intestinal sucrase activity, it was not included in analyses involving this enzyme. Because aminopeptidase-N activity has been measured in fewer species, we used a reduced tree in analyses involving this enzyme (**B**)

because a clade has a consistently higher (or lower) value in one trait for the same value in the other trait. Two clades can differ in the slope of the allometric relationship ($b$ in the equation $y = ax^b$) or in its intercept ($a$). These two situations translate into different relationships among phylogenetic contrasts. If the clades differ only in the intercept of their allometric relationships, then the relationship between the contrasts of both clades falls along a single line, with the exception of the contrast of the node that separates the clades. The value of this contrast estimates the magnitude of the evolutionary change in the intercept of the allometric relationships between the clades. If there is a difference between the clades, this contrast falls outside of the common relationship. When the allometric relationships differ in slope, then the relationships for the contrasts in each clade fall along separate lines. The interpretation of this result is that the rate of evolutionary change in one trait relative to the other differs between clades.

We separated contrast data into two groups: contrasts within hummingbirds ("hummingbird contrasts") and contrasts within passerines ("passerine contrasts"). We fitted regressions on standardized phylogenetic contrasts through the origin without using the values for the contrast calculated in the node separating hummingbirds from passerines (the "basal" node) or the node separating *D. baritula* from its sister clade. We tested for differences between hummingbirds and passerines in two ways: (1) if the slopes of the relationships differed, we inferred that the clades were different, and (2) if the slopes of the relationship did not differ between clades, we calculated a common relationship and assessed whether the value of the basal contrast fell within the 95% confidence interval of this common relationship.

## Results

Sucrase, maltase, and aminopeptidase-N activity in the flowerpiercer *D. baritula* followed classical Michaelis-Menten kinetics. Table 2 shows details on the enzymatic activity of *D. baritula*. Sucrase and maltase activities standardized by intestinal nominal area ($cm^2$) and intestinal mass (mg) were positively correlated both within ($0.83 < r < 0.96$ for all species collected in this study) and among species ($r = 0.93$, $P < 0.001$ and $r = 0.86$, $P = 0.001$ for sucrase and maltase respectively, $n = 7$). In the results presented here we will standardize enzyme activities by intestine nominal area (μmol $\cdot$ $min^{-1}$ $cm^{-2}$ of nominal area), or use the total summed activity (μmol/min). Gut nominal area, body mass, and summed enzymatic activities for *D. baritula* and other species are listed in Table 1.

Sucrase and maltase activities standardized by gut nominal area were positively correlated in both passerines and hummingbirds (see Fig. 2). The regression lines for passerines and hummingbirds had both different slopes ($F_{(\text{slope})1,26} = 23.2$, $P < 0.001$) and different intercepts ($F_{(\text{intercept})1,26} = 69.0$, $P < 0.001$). Per unit of sucrase activity, passerines had more than twice as much maltase activity as hummingbirds. The point for *D. baritula* fell well within the passerine relationship. A phylogenetically corrected analysis revealed the same pattern. The contrast in sucrase activity was positively correlated with the contrast in maltase activity, but the slope between these two contrasts differed between the clades ($F_{(\text{slope})1,21} = 13.86$, $P = 0.001$). The value for the independent contrast comparing passerines with hummingbirds fell between the regression lines for these two groups and outside their respective 95% confidence intervals (Fig. 2).

Intestinal nominal area of hummingbirds and passerines increased allometrically with body mass (Fig. 3), following the same trend in both clades ($F_{(\text{slope})1,35} = 0.05$, $P = 0.82$, $F_{(\text{intercept})1,\ 35} = 0.23$, $P = 0.23$). The scaling relationships between both maltase and aminopeptidase-N activity and body mass were indistinguishable between passerines and hummingbirds (Fig. 3). Aminopeptidase-N activity in *Diglossa baritula* fell below the 95% confidence interval of the common allometric line. The intercept of the scaling relationship between sucrase activity and body mass differed between these two groups ($F_{(\text{intercept})1,24} = 22.5$, $P < 0.001$), but the slopes were not different ($F_{(\text{slope})1,24} = 2.3$, $P = 0.14$). Hummingbirds had on average ten times more sucrase activity than did passerines. Contrary to expectations, *Diglossa baritula*'s sucrase activity was within the passerine allometric line's 95% confidence interval and below the 95% confidence interval for the hummingbird line.

Phylogenetic analyses were largely concordant with the standard allometric results (Fig. 4). There was no statistical difference between hummingbirds and passerines in the slopes of the relationships between the contrast in body mass and the contrast in all other variables (intestinal area: $F_{(\text{slope})1,20} = 2.35$, $P = 0.14$; sucrase: $F_{(\text{slope})1,20} = 0.0002$, $P = 0.99$; maltase: $F_{(\text{slope})1,20} = 1.161$, $P = 0.29$; aminopeptidase-N: $F_{(\text{slope})1,15} = 0.04$, $P = 0.83$). The contrast for the basal node fell within the 95% confidence intervals of the regression lines of body mass against maltase, aminopeptidase-N and intestinal nominal area. However, the contrast of the basal node was different from the regression line relating the sucrase

**Table 2** *Diglossa baritula*'s sucrase, maltase and aminopeptidase-N activities

| | Sucrase | Maltase | Aminopeptidase-N |
|---|---|---|---|
| Activity per section of the gut (μmol/min $cm^2$) | | | |
| Proximal | 2.21 ± 0.12 | 20.91 ± 0.71 | 0.38 ± 0.05 |
| Medial | 0.88 ± 0.19 | 7.88 ± 1.58 | 0.17 ± 0.01 |
| Distal | 0.15 ± 0.03 | 11.49 ± 0.45 | 0.15 ± 0.03 |
| Total activity (μmol/min) | 3.25 ± 0.31 | 30.14 ± 2.01 | 0.7 ± 0.05 |
| Maximal digestive capacity (Vmax total in μmol/min) | 10.16 ± 0.94 | 33.17 ± 2.21 | 0.81 ± 0.06 |
| Km (μmol) | 59.5 | 2.81 | 3.92 |
| pH optima | 6 | 5.5 | 7 |

Values area means ± SE

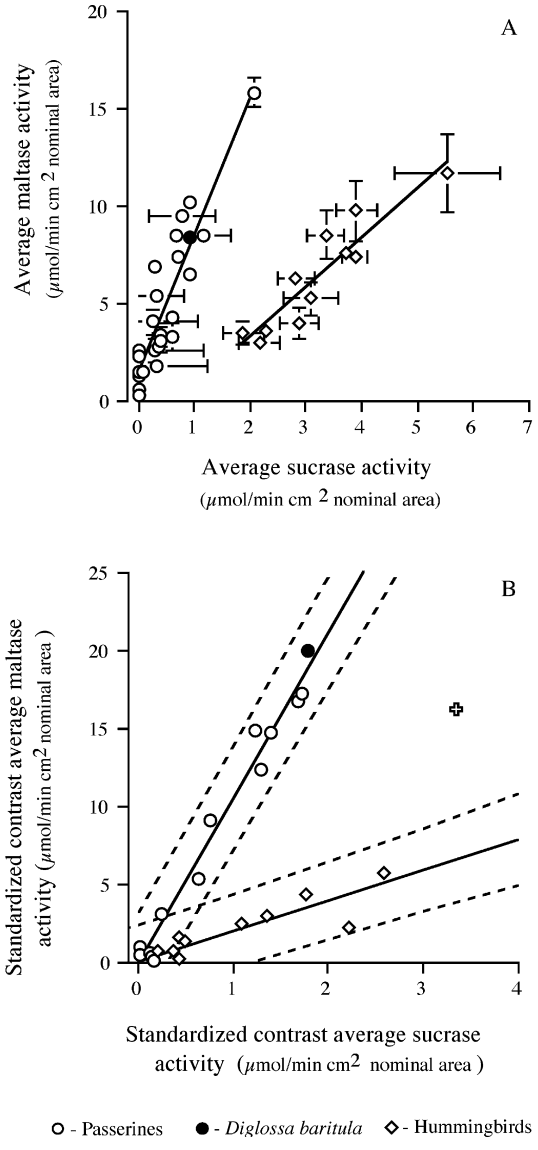


**Fig. 2A, B** Sucrase and maltase activities standardized by gut nominal area were positively correlated in both passerines ($y = 1.63 + 7.01x$, $r^2 = 0.86$) and hummingbirds ($y = -1.7 + 2.55x$, $r^2 = 0.85$). The regression lines for passerines and hummingbirds had different slopes and intercepts (**A**). Passerines had more than twice as much maltase activity per unit of sucrase activity as hummingbirds. *Diglossa baritula* fell within the passerine relationship. An analysis on phylogenetically independent contrasts analysis revealed the same pattern (**B**). *Diamonds* are non transformed values (**A**) or contrasts within the hummingbird clade (**B**), *open circles* are non transformed values (**A**) or contrast among passerines (**B**), and the *filled circle* represents *D. baritula*. The *cross* (in **B**), represents the contrast of the basal node between hummingbirds and passerines. Values in **A** represent species mean ± SE. *Dashed lines* (in **B**) represent 95% confidence belts for the regression lines

and the body mass contrasts, indicating a significant difference in sucrase activity between the two clades (Fig. 4). Although *D. baritula*'s values for sucrase, maltase and gut nominal area were unremarkable in standard allometric analyses, the contrast including *D. baritula* behaved unexpectedly in one of the phylogenetic comparisons. Its contrast for sucrase activity was outside of the 95% confidence interval of the corresponding phylogenetic regression.

## Discussion

Our results show that with the exception of sucrase activity, several digestive traits of hummingbirds are indistinguishable from those of passerines. Sucrase activity was on average ten times higher in hummingbirds than in passerines. Hummingbirds and passerines also differed in the relationship between intestinal maltase and sucrase activities. Passerines had two times more maltase activity per unit of sucrase activity than did hummingbirds. Although we expected sucrase activity of *D. baritula* to be similar to that of hummingbirds, it was much lower. Surprisingly, with the exception of aminopeptidase-N activity, the intestinal area and intestinal enzyme activities of *D. baritula* were very similar to those of other passerines.

### Sucrase activity in hummingbirds and passerines

Sucrase activity was the only digestive trait that differed between hummingbirds and passerines. Hummingbirds had much more sucrase activity than passerines. Given that hummingbirds are probably the most specialized nectar-feeding birds (Stiles 1981, 1985; Suarez and Gass 2002) and that they include large amounts of sucrose in their diets, this difference is not surprising and can be safely assumed to be the result of the adaptation of hummingbirds to a sucrose-rich diet. However, this is likely to be only part of the explanation. In addition to feeding on sucrose, hummingbirds have extremely high metabolic rates (Wolf et al. 1975; Weathers and Stiles 1989; Tiebout 1991; Suarez and Gass 2002). To fuel these, they require a digestive system that delivers a high flux of sugars (Karasov et al. 1986; McWhorter and López-Calleja 2000; Suarez and Gass 2002). We hypothesize that explaining interspecific variation in the activity of digestive enzymes requires accounting for differences in both diet and metabolic demands.

Our adaptive explanation for the remarkable sucrase activity of hummingbirds is based on the assumption that the observed variation in enzyme activity between clades was the result of interspecific differences, rather than simply to differences in sucrose activity associated with the diet of the individuals at the time of capture. Two lines of evidence suggest that variation in sucrase activity between the clades is not the result of dietary acclimatization. First, available evidence suggests that in birds disaccharidases such as sucrase and maltase are remarkably unresponsive to changes in diet (Afik et al. 1995; Sabat et al.1998; Levey et al. 1999; Caviedes-Vidal et al. 2000). Second, the *D. baritula* individuals that we

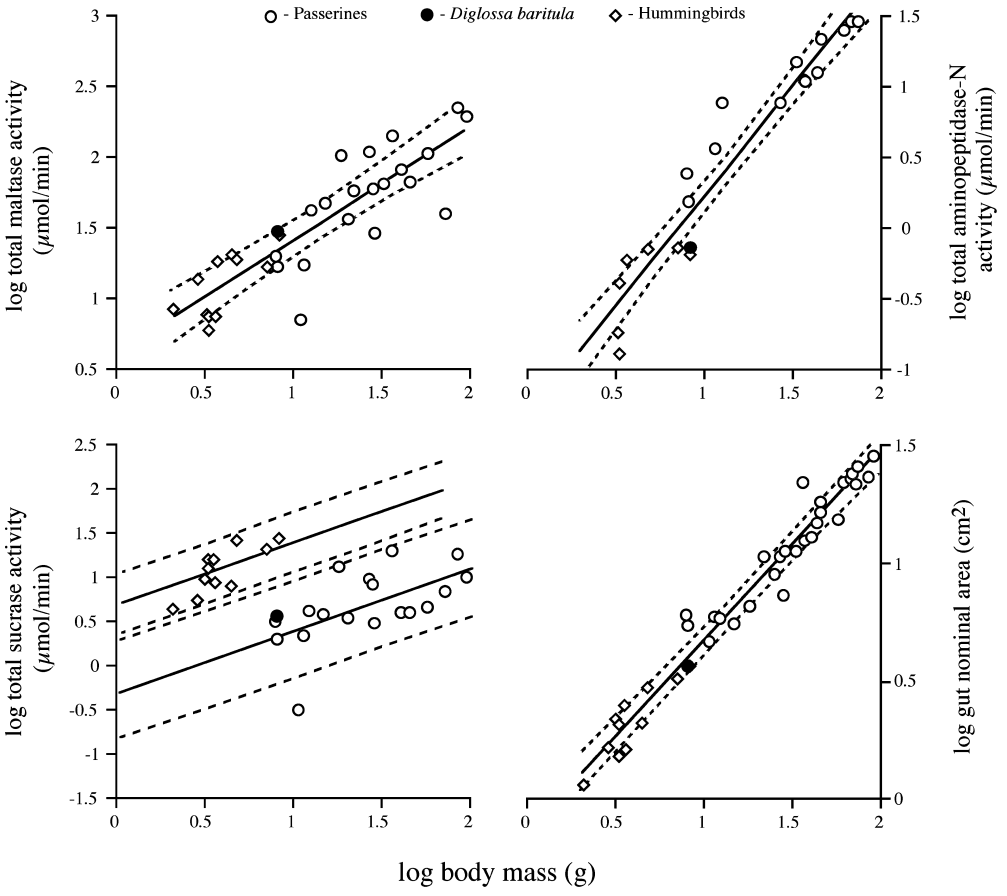


**Fig. 3** Intestinal nominal area of hummingbirds and passerines increased allometrically with body mass ($\log y = -0.13 + 0.79 \log x$). There were no statistically significant differences between the two clades. The scaling relationships between both maltase ($\log y = 0.6 + 0.82 \log x$) and aminopeptidase-N ($\log y = -1.29 + 1.53 \log x$) activity and body mass were indistinguishable between passerines and hummingbirds. The intercept of the scaling relationship between sucrase activity and body mass in passerines ($\log y = -0.38 + 0.77 \log x$) and hummingbirds ($\log y = 0.62 + 0.77 \log x$) differed, but the slopes did not. Hummingbirds had on average more than ten times more sucrase activity than passerines (i.e., $10^{0.62}/10^{-0.38} = 11.5$). With the exception of aminopeptidase-N activity, the values of *D. baritula* were within the 95% confidence interval (*dashed lines*) of the common allometric line of passerines or of the common passerine-hummingbird line

studied fed largely on the same diet as hummingbirds, but exhibited lower sucrase activity.

## The association between maltase and sucrase activities

The relationship between intestinal sucrase and maltase activity differed notably between hummingbirds and passerines. Because sucrase-isomaltase has maltase activity (Alpers 1987; Martínez del Rio 1990), the relationship between the activity of these two enzymes reveals that (1) the intercept estimates the intestinal maltase activity that occurs in the absence of the enzyme sucrase, and (2) the slope indicates the amount of maltase activity per unit of sucrase activity (Sørensen et al. 1982; Martínez del Rio 1990). The intercept for passerines (1.63 μmol $\text{min}^{-1}$ $\text{cm}^{-2}$) was different from zero ($t_{26} = 4.12$, $P = 0.0004$). Thus, in passerines some maltase activity appears to be independent of sucrase activity. In contrast, in hummingbirds, the intercept of the relationship between the activity of the two enzymes was not significantly different from zero ($t_{10} = -1.55$, $P = 0.15$), suggesting that all maltose digestion in hummingbirds was the result of sucrase-isomaltase activity. Passerines exhibited 2.8 times more maltase activity per unit of sucrase activity than hummingbirds. The ratio of maltase to sucrase in hummingbirds is similar to that observed in other non-passerines such as wild and domestic chickens (Hu et al. 1987; Biviano et al. 1993; Jackson and Diamond 1995; Uni et al. 1998), turkeys (Sell et al. 1988; Albatshan et al. 1992), and monk parakeets (M.C. Witmer and C. Martínez del Rio, unpublished data). We interpret this result to be a consequence of a molecular difference in the enzyme that hydrolyzes sucrose between hummingbirds and other non-passerines, and passerines.

## Aminopeptidase-N activity

The allometric relationships between sucrase, maltase, and gut nominal area with body mass had similar exponents (0.77, 0.82, and 0.79, respectively). Note that these exponents are not far from 3/4, the value expected for traits that deliver energy to fuel metabolic rate (Calder 1984; West et al. 1997, 1999), or from 0.67, the scaling exponent of metabolic rate in birds (Bennett and Harvey 1986; Reynolds and Lee 1996; Frappell et al. 2001). In contrast, the scaling exponent of aminopeptidase-N was 1.53. Why was the allometric exponent of aminopeptidase-N regression so much higher from those of other digestive traits? The aminopeptidase-N activities of hummingbirds were not statistically different from those of passerines. However, because the number of hummingbird species in our sample was small, this comparison may have had low statistical power. Note that *D. baritula* and three hummingbird species

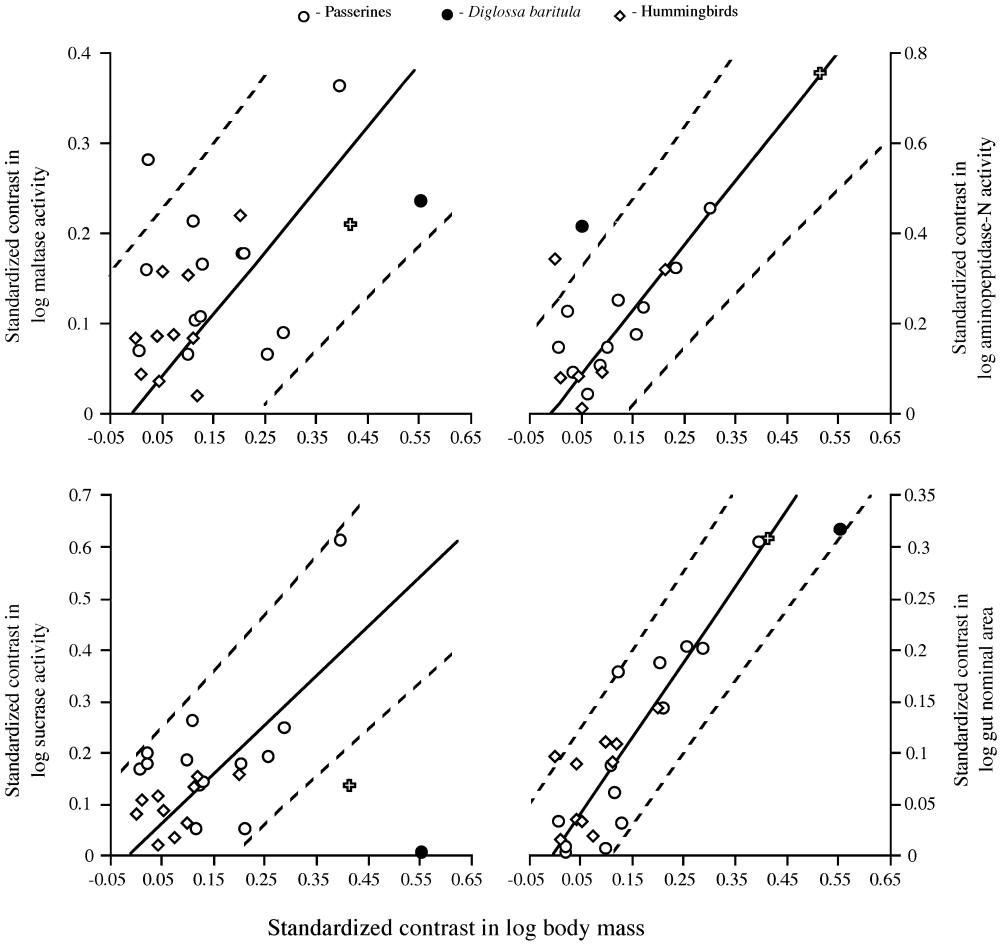


**Fig. 4** There was no differences between hummingbirds and passerines in the slopes of the relationships between the phylogenetically independent contrast in body mass and the contrast in all other variables. The contrast for the basal node (*cross*) fell within the 95% confidence intervals (*dashed lines*) of the regression lines of maltase, aminopeptidase-N and intestinal nominal area vs. body mass. However, the contrast of the basal node was different from the regression line relating the sucrase and the body mass contrasts, indicating a significant difference in sucrase activity between passerines and hummingbirds

exhibited aminopeptidase-N values that fell under the 95% confidence interval of the regression line (Fig. 3).

If aminopeptidase-N activity of hummingbirds is indeed lower than that of passerines, placing both data sets on a common allometric line would make the slope of this line steeper. Indeed, the slope of the allometric regression of passerines alone was shallower (mean ± SE, 1.13 ± 0.107) than that of hummingbirds and passerines together. Both standard allometric analyses and phylogenetic independent contrasts suggested that *D. baritula* had lower aminopeptdase-N activity than expected. This difference can be attributed to the low protein, high sugar diet that these animals ingest. However, because of our small sample size, this inference must be treated with caution. More data on hummingbirds and nectar-feeding passerines are needed to examine the conjecture that the habit of feeding on nectar is associated with low expression levels of intestinal aminopeptidase-N.

## Is the sucrase activity of *D. baritula* similar to or different from that of other passerines?

A comparison between Figs. 3 and 4 revealed an apparently contradictory result. Although the standard allometric analysis indicated that sucrase activity in *D. baritula* was no different from that expected of a passerine of its size (Fig. 3), the standardized contrast that includes *D. baritula* fell outside the 95% confidence interval for the phylogenetic regression line (Fig. 4). This result highlights the sometimes peculiar results that can be obtained when calculating phylogenetic contrasts from data sets that lack some taxa or from phylogenies with unknown branch lengths. We interpret this apparent contradiction as the result of using arbitrary branch lengths to calculate our phylogenetic independent contrast (PIC). Because sucrase activity in *D. baritula* does not seem to be unusually high (Fig. 3; Table 2), the standard allometric analysis probably provides a more realistic assessment of *D. baritula*'s sucrase activity relative to that of other passerines. Phylogenetic contrasts have statistical advantages over standard analyses that do not account for phylogenetic relationships (Felsenstein 1985; Garland et al. 1992, 1999). However, conclusions inferred from phylogenetically explicit analyses must be tempered by recognition of their methodological limitations.

## How is *D. baritula* able to survive on a sucrose-rich diet?

*Diglossa baritula* is a nectar-robber that feeds largely on the same diet as hummingbirds. Yet, we unexpectedly found lower sucrase activity in this species compared to

hummingbirds. How is *D. baritula* able to survive on the sucrose-rich diet characteristic of hummingbirds? The ecologically relevant question is not how much sucrose flowerpiercers can assimilate, but whether the amount of sucrose that they are able to digest is enough to satisfy their daily energy requirements (Schondube 2003). As mentioned above, hummingbirds have extraordinarily high metabolic rates (Weathers and Stiles 1989; Powers and Conley 1994; Nagy et al. 1999; Suarez and Gass 2002). We suggest that a comparison of digestive capacities should account for differences in metabolic demands to provide information on how digestive processes can affect the feeding behavior and ecology of nectar-feeding birds. Although the total sucrase activity of *D. baritula* was approximately eight times lower than the one exhibited by a hummingbird of the same body mass (*Lampornis clemenciae*, $8.4 \pm 0.3$ g), its maximal sucrose digestive capacity (expressed in kJ of energy hydrolyzed per unit time) standardized by its predicted field metabolic rate (FMR; Nagy et al. 1999) was only two times lower (sucrose hydrolysis/expected FMR = 0.96 and 1.92 for *D. baritula* and *L. clemenciae*, respectively). Although this comparison narrows the gap, the notable difference between hummingbirds and flowerpierecers remained. A mathematical model, based on sucrase enzymatic activity and intestinal volume, predicted that the maximal amount of sucrose that *D. baritula* can process in a day is close to 2 g/12 $h^{-1}$ (Schondube and Martínez del Rio 2003b; see McWhorter and Martínez del Rio 2000 and Martínez del Rio et al. 2001 for the method used to predict maximum hydrolytic capacity). This amount of sugar provides *D. baritula* with enough energy to satisfy its predicted FMR ($\approx$1.5 kJ $day^{-1}$; Nagy et al. 1999; Schondube 2003). Although total sucrase activity in *D. baritula* is low when compared with that of hummingbirds, it seems sufficient, albeit barely, to allow it to live on a sucrose-rich diet (Schondube and Martínez del Rio 2003b). Hummingbirds seem to maintain much larger spare capacity to assimilate sucrose than flowerpiercers (Schondube 2003).

In conclusion, our comparisons suggest that hummingbirds are unique among birds in their capacity to hydrolyze sucrose. This remarkable capacity is probably a result of both their sucrose-rich diet and their high metabolic rate. In spite of their obviously specialized nectar-robbing bill morphology (Schondube and Martínez del Rio 2003a), flowerpiercers do not seem to have converged with hummingbirds in their ability to hydrolyze sucrose. Their capacity to hydrolyze sucrose is lower than that of hummingbirds even when it is standardized by metabolic rate. Although flowerpiercers probably represent the most specialized nectar-feeding lineage of passerines in the New World, their digestive traits do not match those of the legitimate pollinators of the flowers that they rob.

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