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On the relationship between sugar digestion and diet preference in two Chilean avian species belonging to the Muscicapoidea superfamily

Relación entre digestión de azúcar y preferencia en la dieta en dos especies
de aves chilenas de la superfamilia Muscicapoidea

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ABSTRACT

It has been hypothesized that species belonging to the Sturnidae-Muscicapidae lineage, despite having generalist diets comprising fruits with sugars of diverse kinds, do not express intestinal sucrase. In order to increase the taxonomical range of species for which sucrase intestinal activity has been investigated, we analyzed the relationship between enzymatic activity (sugar digestion) and feeding preference for native fruits containing sucrose, in two South American members of the superfamily Muscicapoidea, the Austral thrush (*Turdus falcklandii*) and the Chilean mockingbird (*Mimus thenca*). We hypothesized that these birds would lack intestinal sucrase activity and that in preference tests they would reject sucrose solutions. Both thrushes and mockingbirds lacked significant intestinal sucrase activity. Considering the phylogenetic constraint hypothesis for sucrose digestion in the Muscicapoidea superfamily, our results support the notion that lack of sucrase activity is a shared derived-character only for the Cinclidae-Sturnidae-Turdinae lineage, and suggests that the selective pressure that these birds can exert on the plants whose seeds they disperse and whose flowers they visit are consistent across world hemispheres. Food preference by thrushes was significantly biased toward glucose and fructose, showing scant to nil consumption of sucrose, thus corroborating a positive relationship between digestion capabilities and food preference for different sugar types.

Key words: intestinal hydrolases, *Mimus thenca*, physiological constraint, sucrase activity, sugar preferences, *Turdus falcklandii*.

RESUMEN

Se ha planteado que el linaje Sturnidae-Muscicapidae no expresa sacarasa intestinal. Con el fin de aumentar el rango taxonómico para el cual se ha investigado la actividad de sacarasa intestinal, analizamos la relación entre la actividad enzimática (digestión de azúcar) y preferencias dietarias por ítemes que contienen sacarosa, en dos miembros Sudamericanos de la superfamilia Muscicapoidea, el zorzal (*Turdus falcklandii*) y la tenca (*Mimus thenca*). Hipotetizamos que estas aves no debieran presentar sacarasa intestinal y que debieran rechazar soluciones de sacarosa en pruebas de preferencia. Ninguna de las especies presentó una actividad significativa de sacarasa intestinal. A la luz de los nuevos antecedentes de la relación filogenética del grupo Muscicapoidea, nuestros resultados apoyan la hipótesis respecto que la pérdida de la sacarasa intestinal es un carácter derivado y compartido sólo por el linaje Cinclidae-Sturnidae-Turdinae y sugiere que las presiones selectivas que esas aves ejercen sobre las plantas cuyas semillas dispersan y cuyas flores visitan, son consistentes entre ambos hemisferios. La preferencia de alimentación de zorzales fue significativamente sesgada hacia el consumo de glucosa y fructosa, mostrando un escaso a nulo consumo de sacarosa, corroborando así, una relación positiva entre la capacidad de digestión y preferencias de alimentación por diferentes tipos de azúcares.

Palabras clave: hidrolasas, *Mimus thenca*, preferencia por azúcares, restricción fisiológica, sacarasa, *Turdus falcklandii*.

INTRODUCTION

Omnivorous species often encounter different types of food and require diverse digestive adjustments for absorbing different types and concentrations of substrates (Karasov & McWilliams 2005). For example, many studies have shown a positive correlation between the concentration of dietary substrates and enzyme levels (Karasov & Hume 1997) or retention time of food in the gut (López-Calleja et al. 1997). To digest sucrose birds must first hydrolyze it into its components, glucose and fructose, which are then transported into intestinal cells (Karasov 1996). The hydrolysis of sucrose takes place as a result of the action of sucrase-isomaltase, a membrane bound intestinal enzyme (Vonk & Wenster 1984, and references therein). It is expected that birds with a co-evolutionary history with food items containing a particular type of sugar should have proper digestive enzymes to handle that sugar, as observed for example, in hummingbirds (Martínez del Río 1990). However, Martínez del Río et al. (1995) found that species of birds belonging to the large monophyletic sturnid-muscicapidae lineage, which includes starlings (Sturnidae), mockingbirds (Mimidae), and thrushes (Muscicapidae), with a generalist diet that include fruits among other food items, do not express intestinal sucrase (Martínez del Río et al. 1995). Birds without this enzyme suffer from osmotic diarrhoea if they ingest food items containing sucrose (Martínez del Río & Stevens 1990). Martínez del Río et al. (1992) hypothesized that the sugar preferences of those birds lacking a particular digestive enzyme represent a significant selective pressure which maintains the absence of sucrose from the fruit pulp and nectar of the plants whose seeds they disperse and whose flowers they pollinate (Lotz & Nicholson 1996). Based in these results Martínez del Río et al. (1995) proposed the existence of a phylogenetic constraint for sucrose digestion in the complete Sturnidae-Muscicapidae lineage (currently included in the monophyletic superfamily Muscicapidae; see Voelker & Spellman 2004).

To our knowledge, the study of biochemical digestive capacities for sucrose digestion has been conducted only in eight

species of the Muscicapidae group: three genus belonging to the Turdidae family (genus *Catharus*, four species; *Hylochila*, one species and *Turdus*, one species) and only one member of the Sturnidae, *Sturnus vulgaris* (Schondube & Martínez del Río 2004). Besides, with the exception of the migratory species *Catharus ustulatus*, all studied birds are restricted to the northern hemisphere. In order to evaluate the generality of the phylogenetic constraint hypothesis for sucrose digestion and its consequences for feeding preferences, we analyzed the biochemical and digestive capacity to digest sugars in two southern South American members of the Muscicapidae lineage, the Austral thrush (*Turdus falcklandii*) and the Chilean mockingbird (*Mimus thenca*). These species are abundant members of Chilean bird assemblages (De la Peña & Rumboll 1998). Although there is no detailed study describing their diets, preliminary studies suggest that both species are omnivores, and that they include significant amounts of fruits, in addition to animal preys, in their diets (Sabag 1993). The endemic Chilean mockingbird is the primary disperser of several mistletoes (Martínez del Río et al. 1995, Soto-Gamboa & Bozinovic 2002) and is the main pollinator of *Puya berteroniana*, an abundant plant of Chilean matorral (González-Gómez et al. 2004). The Austral thrush is an important seed disperser of some tree species in southern Chile (Johnson 1967, Willson et al. 1996). In accordance with the phylogenetic constraint hypothesis, we expected that these two bird species would lack intestinal sucrase activity and that in preference tests they would reject sucrose solutions as other members of the Muscicapidae lineage do.

MATERIAL AND METHODS

Eight *T. falcklandii* and seven *M. thenca* individuals were captured with mist nets or with air guns in Santiago, Chile (33°24' S, 71°42' W) in September of 2002. Birds were transported to the laboratory and maintained at 22 ± 2 °C, L:D 12:12, individually in 50 x 50 x 50 cm cages, and they were fed with larvae of *Tenebrio molitor*, cat chow and tap water *ad libitum*. Birds were acclimated to laboratory

conditions for one week before the experiments. Behavioral experiments were done only with *T. flacklandii* because the individuals of *M. thenca* did not acclimate well to laboratory conditions. Experiments comprised choice tests where we offered a sugar solution against water, following the procedures developed by Martínez del Río et al. (1988). We offered thrushes a choice of two paired graduated tubes containing either tap water or a glucose plus fructose mixture (1:1, 1.66 M) for 4 h starting at 07:00 h, in three consecutive days. Then, we switched the solution to an iso-caloric solution of sucrose (0.87 M), and the entire procedure was repeated. After three days, the solution was changed again to a 1:1 glucose and fructose mixture. Consumed volumes were determined and corrected for evaporative losses using control tubes located outside the experimental cage.

To test whether thrushes could digest sucrose we used Malcarney et al.'s (1994) method. Birds were fasted overnight and the concentration of blood plasma glucose was measured. Then birds fed with either sucrose (3 g per kg, administered in 0.87 M solutions) or a 1:1 mixture of glucose and fructose (3 g per kg, administered in 1.66 M solutions) with a rubber tube. Blood was obtained after puncturing the brachial vein and plasma glucose was measured from plasma colorimetrically using the Glucose Trinder kit (Sigma). We analyzed changes in plasma glucose as a function of time and sugar ingestion using repeated measures ANOVA with sugar type as a factor.

The activity of intestinal enzymes was measured in both *T. flacklandii* and *M. thenca*. Birds were euthanized with CO₂ (following University of Chile bio-ethical guidelines for animal experimentation), their digestive tracts were excised and washed with a 0.9 % NaCl solution. Because previous reports indicated that digestive enzyme activities may vary along the intestine (Martínez del Río et al 1988), we measured the disaccharidase activities along the intestinal axis. Intestines were divided into five sections of similar length and immediately frozen in liquid nitrogen. Prior to enzymatic analyses, tissues were thawed, and homogenized (30 s in a ULTRA TURRAX T25 homogenizer) in 20 volumes of 0.9 % NaCl

solution. Disaccharide activity was determined according to the method of Dahlqvist (1964) as modified by Martínez del Río (1990). In order to validate the colorimetric method of enzyme determination, together with sucrase assays, we determined maltase activity, which has been found in all passerines studied to date (Schondube & Martínez del Río 2004). Briefly, tissue homogenates (100 mL), were incubated at 40 °C with 100 mL of 56 mmol L⁻¹ sugar solutions in 0.1 M Maleate/NaOH buffer, pH 6.5. After 10 min, reactions were stopped by adding 3 mL of a stop developing solution (one bottle of Glucose Trinder 500 reagent (Sigma) in 250 mL 0.1 mol L⁻¹ TRIS/HCl, pH 7 plus 250 mL of 0.5 NaH₂PO₄, pH 7). Absorbance was measured at 505 nm with a Sequoia Turner 390 spectrophotometer after 18 min at 20 °C. The activities of enzymes are presented as standardized hydrolytic activity (UI per g wet tissue, UI = $\mu\text{mol hydrolyzed min}^{-1}$, see Martínez del Río et al. 1995 for a detailed explanation). We evaluated enzyme activity as a function of intestinal position using a repeated measures ANOVA. To satisfy the assumptions of the repeated measures ANOVA model, we tested for sphericity using the Mauchly test, which in all cases was nonsignificant. All data is reported as mean \pm SE.

RESULTS

Thrushes increased their consumption of the glucose:fructose solution from day one to day three by 54 % (Tukey HSD test, $P = 0.03$). In contrast, when animals were offered a sucrose solution, their consumption decreased from day four to day six (Tukey HSD test, $P < 0.05$). Consumption of sucrose solutions on day six was only 10 % of the maximal consumption of monosaccharide mix solution ingested on day three (Fig. 1), and three out of eight birds refused to consume the sucrose solution. In order to test if birds developed conditioned aversion for all sugar solutions or aversion only for the sucrose solution, we offered birds a monosaccharide solution on day seven. Birds rapidly increased consumption to similar values to those observed on day three, when we recorded maximal consumption of sugar solutions (Fig. 1).

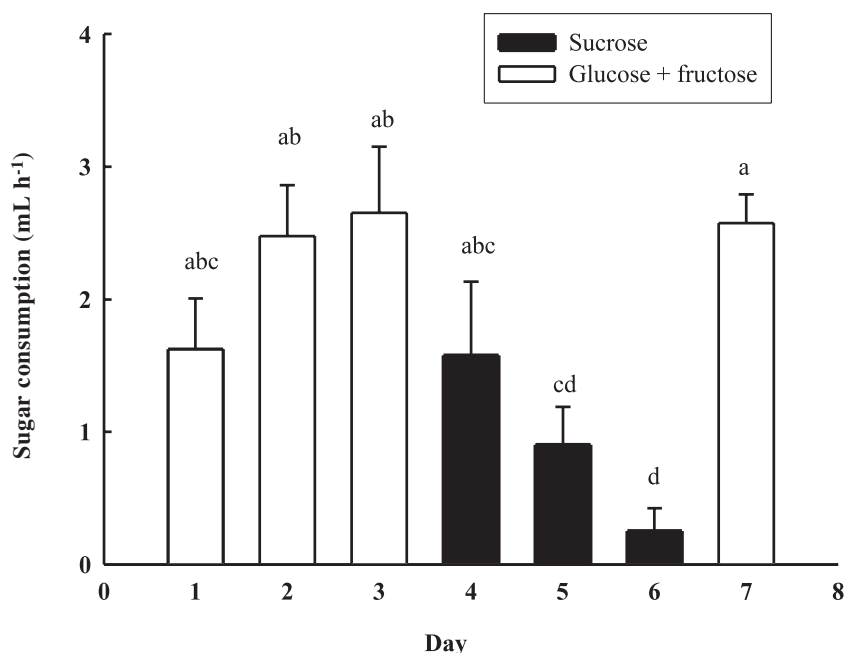


Fig. 1: Consumption of a mixture 1:1 of glucose plus fructose (1.66 M, at days 1, 2, 3, and 7) and iso-caloric sucrose solution (0.87 M, days 4, 5, and 6) in *Turdus falcklandii*. Ingestion is expressed as mL h⁻¹ (mean \pm SE). Similar letters indicate non-significant differences among days.

Consumo de una mezcla 1:1 de glucosa mas fructosa (1,66 M, a los días 1, 2, 3 y 7) y una solución isocalórica de sacarosa (0,87 M, días 4, 5 y 6) en *Turdus falcklandii*. La ingesta es expresada como mL h⁻¹ (promedio \pm EE). Letras similares indican diferencias no significativas entre días.

We found significant maltase activity in all individuals of both species. Maltase activity decreased distally along the length of the small intestine in the Austral Thrush (Fig. 2, repeated-measures ANOVA, $F_{4,16} = 2.89$, $P = 0.05$) and the Mockingbird (repeated-measures ANOVA, $F_{4,24} = 8.77$, $P = 0.0002$). We found no evidence of significant sucrase activity in any individual of both species (Fig. 2). Average level of plasma glucose in fasted birds was 567.2 ± 36.8 mg per 100 mL (mean \pm SE). A repeated measures ANOVA revealed no significant effect of sugar type on plasma glucose (ANOVA $F_{1,10} = 0.54$, $P < 0.48$), but a strong effect of time ($F_{3,30} = 15.47$, $P = 0.001$) and a highly significant interaction between time and sugar type ($F_{3,30} = 4.69$, $P < 0.001$). The a-posteriori Dunnett-test revealed that after thrushes were fed on sucrose, blood glucose levels did not change significantly (all comparisons, $P > 0.10$). However, when they were fed on glucose and fructose, blood glucose levels increased at 15 and 30 min and then decreased 60 min after ingestion (Dunnett-test $P < 0.01$, Fig. 3).

DISCUSSION

Based in the phylogeny proposed by Sibley and Ahlquist (1990) for the Muscicapoidae relationships, Martínez del Río et al. (1995) proposed that the lack of sucrase activity is a derived character shared by all members of the former Muscicapidae-Sturnidae lineage. That proposition was based in the study of five members of the Turdinae and two of the Sturnidae groups, which were assumed to belong to a monophyletic group, following the phylogenetic study of Sibley & Ahlquist (1990) where Sturnidae, Turdinae, and Muscicapinae (which includes Saxicolini and Muscicapini) were found to share a common ancestor. Recently, Voelker & Spellman (2004), reanalyzing the phylogenetic relationship of the Muscicapidae using molecular markers, proposed that Turdinae does not belong to the Muscicapidae, and that Turdinae-Saxicolini-Muscicapini is not a monophyletic group. Since our results indicate that both thrushes and mockingbirds lacked significant intestinal sucrase activity, our findings support the notion

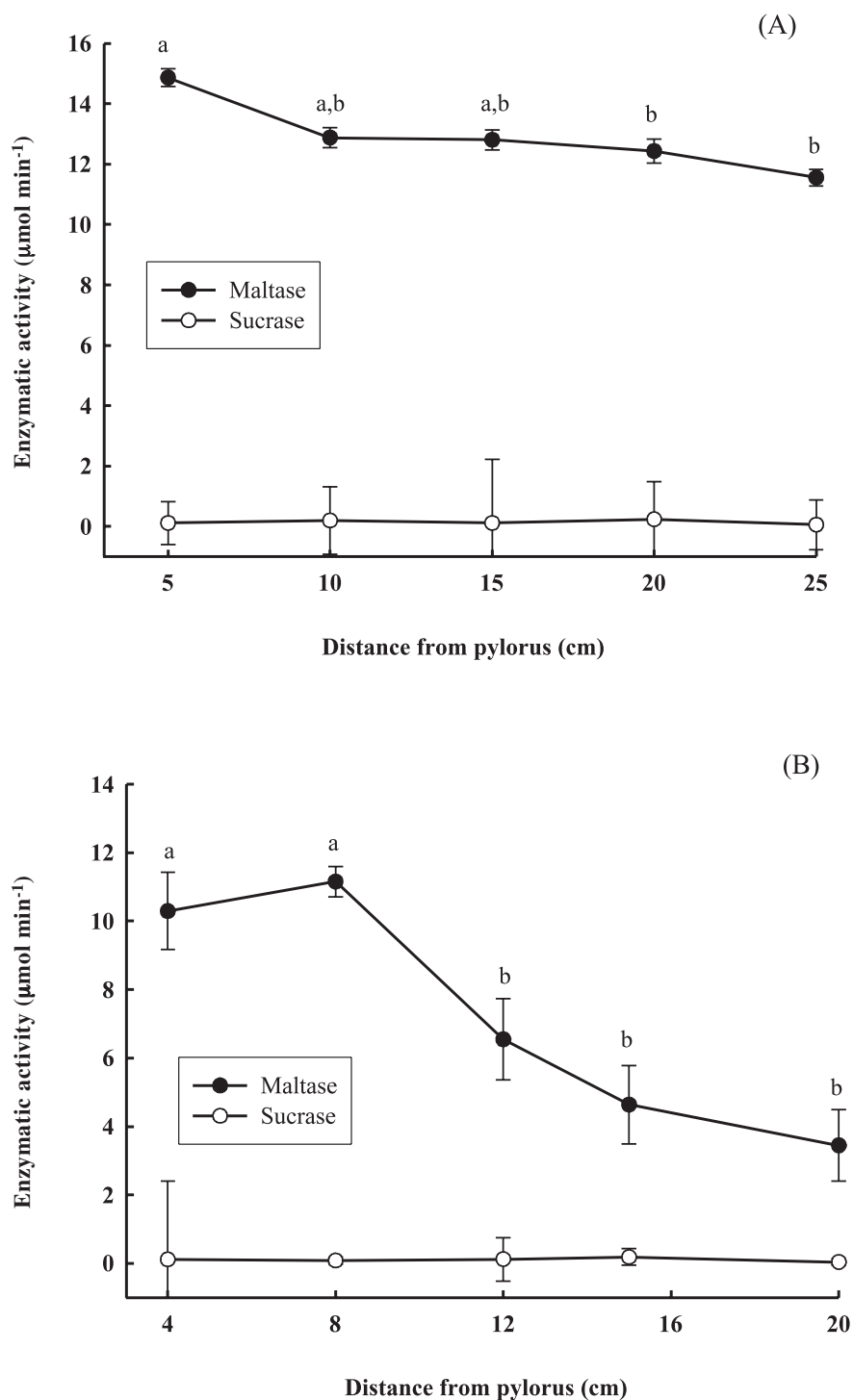


Fig. 2: Enzyme activities along the intestine of *Turdus falcklandii* (A) and *Mimus thenca* (B). A significant decrease of maltase activity was observed toward the distal section in both species. Similar letters indicated non-significant differences among intestine segments. Note the absence of sucrase activity along the intestinal axis.

Actividad enzimática a lo largo del intestino delgado de *Turdus falcklandii* (A) y *Mimus thenca* (B). Una disminución significativa de la actividad de maltasa fue observada hacia la porción distal del intestino en ambas especies. Letras similares indican diferencias no significativas entre los segmentos del intestino. Nótese la ausencia de actividad de sacarasa a lo largo del eje intestinal.

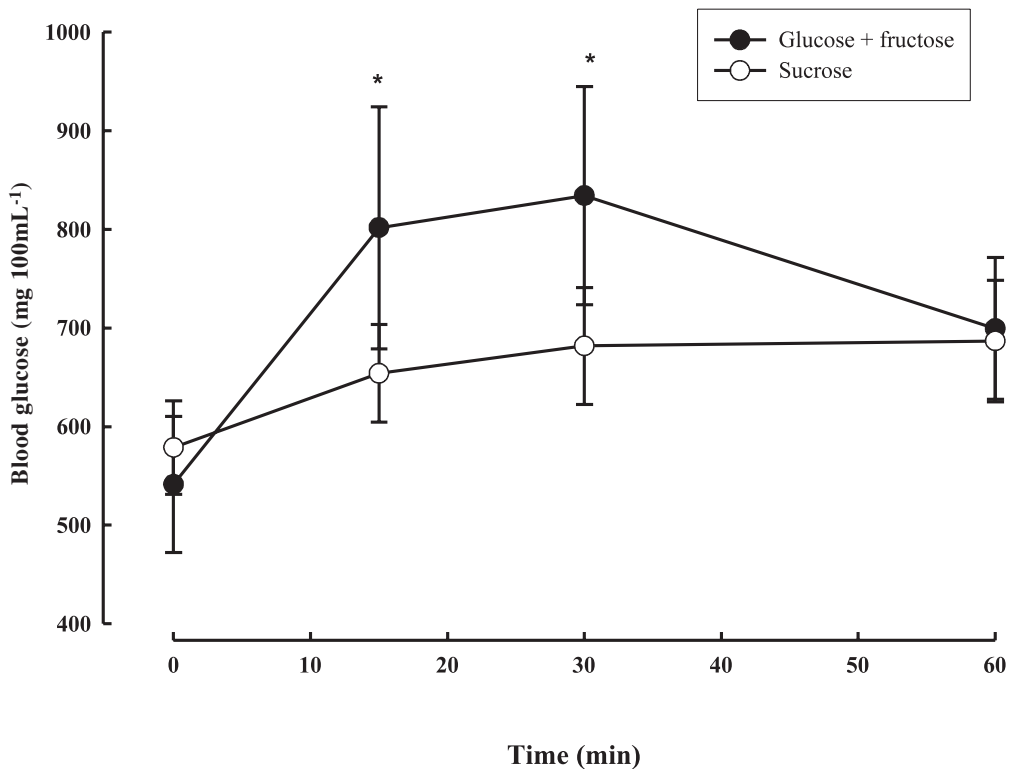


Fig. 3: Sugar tolerance test in *Turdus falcklandii*. Blood glucose changes were measured before (time 0) and after birds were fed on sucrose (0.87 M, open circles) or an iso-caloric fructose-glucose mixture (1:1; 1.66 M, filled circles). An * indicates significant differences after a repeated-measures ANOVA and Dunnett test, $P < 0.05$.

Pruebas de tolerancia al azúcar en *Turdus falcklandii*. Los cambios de la glucosa sanguínea fueron medidos antes (tiempo 0) y después de que las aves fueran alimentadas con sacarosa (0.87M, círculos abiertos) o con una mezcla isocalórica de fructosa-glucosa (1:1; 1.66 M, círculos llenos). El asterisco indica diferencias significativas después de una prueba de ANOVA para medidas repetidas y un test de Dunnett, $P < 0.05$.

that the lack of sucrase activity is a shared derived-character of the monophyletic Cinclidae-Sturnidae-Turdinae lineage (Martínez del Río et al. 1988, Martínez del Río 1990). We note that there is no evidence of a lack of sucrase activity for members of the actual Muscicapidae family. However, further studies are needed to elucidate if the lack of sucrase is a derived character shared by the entire superfamily Muscipoidea or if sucrase is only lacking in the Cinclidae-Sturnidae-Turdinae group.

The behavioural responses of thrushes to sucrose were remarkably similar to those exhibited by other species in this lineage (Martínez del Río et al. 1988, Malcarney et al. 1994). The plasma glucose response tests also suggest that *T. falcklandii* is a sucrose-intolerant species. Accordingly, *T. falcklandii*

exhibited marked sugar preferences: subjects chose the glucose-fructose solution and rejected the sucrose solution, probably developing a conditioned aversion to this sugar. This behavior is correlated with a nearly constant concentration of glucose in the plasma after austral thrushes were fed a sucrose solution (Martínez del Río 1990, Malcarney et al. 1994). What are the ecological correlates of these physiological traits in these two Chilean species? Our data suggest that the selective pressure that these birds can exert on the plants whose seeds they disperse and whose flowers they visit are consistent across continents, since species belonging to the same genus (*Turdus*) or family (Mimidae) but inhabiting different hemispheres, specifically north-America (see Karasov & Levey 1990 for *Turdus* and Mimidae species) and south-America (this

study), possess similar sugar digestive capabilities and broad food preferences. Given that different bird species have very diverse digestive physiologies, with some species, such as hummingbirds, specialized on sucrose-rich nectars, and others, such as passerine nectarivores, with a more generalist diet that might include glucose and fructose (Martínez del Río 1990), it could be expected to observe evolutionary diversification of nectar compounds in plants. In this vein, Baker & Baker (1983) and Martínez del Río et al. (1992) have pointed out the contrasting sugar composition of bird pollinated *Puya* species. For example, the flowers of blue puya, *Puya berteroniana*, in central Chile are mostly visited by the Chilean Mockingbird *M. thenca* (González-Gómez et al. 2004), and this *Puya* species secretes dilute nectars that contain primarily glucose and fructose. In contrast, *Puya* species pollinated by hummingbirds secrete concentrated nectars that contain high sucrose contents (Nicolson & Fleming 2002, González-Gómez et al. 2004), suggesting some level of coevolutionary coupling between nectar types and nectarivore species. The super family Muscicapoidae is diverse (608 spp) and cosmopolitan (IBC 2005), and it includes many species that feed on nectar and fruit (Bockheim & Congdom 2001, Nicolson & Fleming 2002, Schondube & Martínez del Río 2003). Their inability to digest sucrose seems to have worldwide influence on the evolution of the sugar composition of nectar and fruit pulp of diverse plant species.

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