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SHORT COMMUNICATION

Intestinal α-glycosidase transcriptional responses during development and diet adjustment in altricial birds

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ABSTRACT

We describe developmental changes in maltasic activity and its mRNA until adulthood, and in response to an increase in dietary starch. We studied house sparrows (*Passer domesticus*), which undergo a natural switch from insects to a starch-containing seed diet during development, and zebra finches (*Taeniopygia guttata*), which have a relatively fixed starchy seed diet during development. In zebra finches, in which maltasic activity increased with age but not with dietary starch, α-glycosidase (AG) mRNA was not affected by either age or dietary starch level. In house sparrow nestlings, in which maltasic activity increased with age and with added starch, AG mRNA was higher when birds were fed a diet with added starch but did not increase with age. These results are consistent with the idea that the apparent programmed developmental increase in maltasic activity is not mainly under transcriptional control of AG mRNA, whereas induction of maltasic activity by increased dietary starch is.

KEY WORDS: Digestive enzymes, Transcriptional control, Phenotypic flexibility

INTRODUCTION

Major changes in intestinal enzymes occur during development in vertebrates, but only in mammals are the associated transcriptional responses well described, based on studies in about a dozen species (Karasov and Douglas, 2013). A fundamental question is whether the patterns in mammals are general for vertebrates. In developing eutherian mammals, activity increases markedly for starch-digesting α-glycosidases [sucrase-isomaltase (SI) and maltase-glucoamylase (MG)], and decreases after weaning for milk disaccharide-digesting lactase-phlorizin hydrolase, in most cases accompanied by parallel changes in the expression of their genes (Galand, 1989; Karasov and Douglas, 2013). A variety of studies have shown that these changes often occur in the absence of specific signals from either the gastrointestinal (GI) tract lumen or circulation, although levels of some hormones and growth factors are involved in maturation and growth. Earlier or later inclusion of specific substrates in the diet may advance or delay changes in an enzyme's activity and expression of its mRNA (Karasov and Douglas, 2013). The picture that emerges is of a putative genetic program that controls developmental changes in expression of intestinal α-glycosidases (Galand, 1989) that can be modified by environmental factors such as a change in diet composition. In humans, maltasic activity derives from two α-glycosidases in the intestinal apical membrane (brush-border membrane): maltase glucoamylase [MG, Enzyme Commission number (EC) 3.2.1.20 and 3.2.1.3, encoded by the *MGAM* gene] and sucrase isomaltase (SI, EC 3.2.148 and 3.2.1.10 encoded by the *SI* gene) (Nichols et al., 2003). In rats, addition of carbohydrate to the diet induces increases in both maltasic and sucrasic activity, and these are accompanied by increases in the expression of *MGAM* and *SI* (Karasov and Douglas, 2013).

Changes in intestinal α-glycosidases and their mRNA during post-hatch development in birds has barely been studied. Changes in α-glycosidase mRNA in chickens (*Gallus gallus domesticus*; Galliformes) were described only for the first 7 days post-hatch (Sklan et al., 2003; Uni et al., 1999) and without any changes in diet composition. In this paper, we describe developmental changes in maltasic activity and its mRNA until adulthood, and in response to modification of dietary starch, in two altricial species in a different avian order, Passeriformes, from chickens. We studied two models of altricial development: house sparrows, *Passer domesticus* (Linnaeus 1758) (Brzek et al., 2009; Caviedes-Vidal and Karasov, 2001), which undergo a natural switch from a low- to a high-starch diet (i.e. from >80% insect diet to 80% seed diet; Anderson, 2006) during development, and zebra finches, *Taeniopygia guttata* (Vieillot 1817) (Brzek et al., 2010), which have a relatively fixed starchy seed diet during development (Zann, 1996). Using mainly samples of tissue in which maltasic activity had previously been characterized in nestling and adult zebra finches (Brzek et al., 2010) and in nestling house sparrows (Brzek et al., 2009, 2011), as well as those characterized in a new experiment with adult house sparrows, we tested several predictions about gene expression that arose from the general patterns seen during mammalian development and from the more limited information available on birds.

In chickens, changes in sucrasic activity immediately before and after hatching seemed to correlate with changes in α-glycosidase mRNA (AG mRNA) levels (Karasov and Douglas, 2013; Sklan et al., 2003; Uni et al., 1999, 2003), suggesting that developmental variation in sucrasic activity is transcriptionally controlled. Note that for birds, we refer to the mRNA as AG mRNA because, to our knowledge, none of the cloned avian α-glycosidases has been expressed and had its expression product characterized for activity. Based on patterns in mammals and the chicken, we predicted (1) that post-hatch increases in maltasic activity, which occur in both house sparrows (Brzek et al., 2009; Caviedes-Vidal and Karasov, 2001) and zebra finches (Brzek et al., 2010), would be matched by increases in AG mRNA.

Inclusion of extra carbohydrate in the diet induces disaccharidase activity in house sparrow nestlings and fledglings (Brzek et al., 2009, 2011), but not in house sparrow adults (Caviedes-Vidal et al., 2000) or zebra finches (Brzek et al., 2010). Considering that in rats,

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diet-induced increases in activity of SI and MG are accompanied by increases in expression of their genes (see above), we predicted (2) that inclusion of extra carbohydrate in diets would increase levels of AG mRNA in young house sparrows but not in zebra finches or adult house sparrows.

MATERIALS AND METHODS

Collection, feeding and maintenance of birds

We used adult and nestling house sparrows collected in the wild and then maintained in captivity, and captive, colony-raised adult and nestling zebra finches. Because all the methods on collection, feeding and maintenance were developed and described in previous studies with nestling house sparrows (Brzek et al., 2009, 2011), adult house sparrows (Caviedes-Vidal et al., 2000) and nestling and adult zebra finches (Brzek et al., 2010), we do not repeat them here in detail. All experimental procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

Experimental diets and feeding

Synthetic diets used in these studies were fed in liquefied form to nestlings and in powdered form to post-fledge birds. The diets were composed of the same ingredients but differed in the amount of corn-starch (carbohydrate), casein (protein) and corn oil (lipid) (Table 1). For each species, there were at least two diets, nearly isocaloric, that were named according to their relative amounts of starch; for clarity we have retained the names of the diets that were used in the respective source studies. Although there is a '0 starch' diet for house sparrows, zebra finch nestlings and adults require some starch in their diets (Brzek et al., 2010), but for both species the higher starch diet contained an additional 0.21–0.25 g starch g^{-1} diet dry mass. Nestlings from the same clutch were randomly assigned to different diets. Nestlings were syringe-fed hourly 15 times per day with meal sizes calculated using age-specific energy requirements for each species. Post-fledging birds were provided with powdered diet and drinking water *ad libitum*. In general, food intake of young birds and adults of both species did not differ by diet, and neither did body mass differ by diet at the time tissues were harvested (Brzek et al., 2009, 2010, 2011; Caviedes-Vidal et al., 2000).

Table 1. Composition (% of dry mass) of diets used in experiments

	Zebra finch		House sparrow			
			Nestlings and fledglings		Adults	
	MS*	HS*	0 starch‡	+starch‡	0 starch	+starch
Corn starch	25.4	46.2	0	25.4	0	25.4
Casein (protein)	46.2	25.4	59.6	46.2	59.6	34.2
Corn oil	8.0	8.0	20	8.0	20	20
Alphacel non-nutritive bulk	4.9	4.9	4.9	4.9	4.9	4.9
Silica sand	5.0	5.0	5.0	5.0	5.0	5.0
Amino acids, vitamins, mineral salts, etc.§	10.5	10.5	10.5	10.5	10.5	10.5
Energy (kJ g^{-1})¶	16.1	16.0	19.0	16.1	19.0	18.8

Zebra finch diets applied to adults and nestlings; house sparrow diets differed between nestlings and fledglings (30 days old), and adults.
*Diet as named and described in Brzek et al. (2010). MS, medium starch; HS, high starch.
‡Diet as named and described in Brzek et al. (2009).
§Content as described by Brzek et al., Lepczyk et al. (1998).
¶Energy content, approximate value calculated.

Experimental schedules

Nestling zebra finches

The performance of zebra finch nestlings was tested at three time points (days post-hatch, dph; hatch day=0) during their development: (i) the phase of rapid development of the GI tract, which occurs at 5 dph, corresponding to about 28% of the days to fledging (18 days) (Brzek et al., 2010); (ii) the day of the peak in intestine mass, which occurs at 8 dph, corresponding to about 44% of the days to fledging; and (iii) the time of acquisition of adult body mass, which occurs at 15 dph, corresponding to about 83% of the days to fledging. Previously unpublished data supporting these designations are given in Fig. S1.

Nestling house sparrows

The performance of house sparrow nestlings, which fledge at about 14 dph (faster than zebra finches) was tested at three time points during their development, corresponding to the same three developmental phases used in zebra finches (Brzek et al., 2009; Caviedes-Vidal and Karasov, 2001): (i) the phase of rapid development of the GI tract, which occurs at 4 dph, corresponding to about 28% of the days to fledging; (ii) the day of the peak in intestine mass, which occurs at 6 dph, corresponding to about 43% of the days to fledging; and (iii) the time of acquisition of adult body mass, which occurs at 12 dph, corresponding to about 85% of the days to fledging.

Post-fledging zebra finches and house sparrows

Adult zebra finches were raised on the diets for 15–21 days. Fledgling house sparrows were raised on their respective diets from 3 to 30 dph. Adult house sparrows were fed for 1 day on natural seeds mixed 50:50 with their respective powdered synthetic diet, and then 14–15 days on pure synthetic diet.

Harvesting and storage of tissues

Birds were killed by exposure to CO_2, weighed (±0.1 g), and dissected to remove the small intestine. We do not report here on other tissues harvested, which is reported elsewhere (Brzek et al., 2009, 2011, 2010; Caviedes-Vidal et al., 2000). The small intestine (between the pyloric sphincter and vestigial cecae) was flushed with ice-cold Ringer solution, weighed (±0.1 mg), cut into three sections corresponding to proximal, middle and distal regions, and immediately preserved in liquid N_2 and stored at −80°C (for enzyme assays) or in RNALater. Although maltasic activity was originally measured and reported for all three regions in both house sparrows (Brzek et al., 2009, 2011) and zebra finches (Brzek et al., 2010), gene expression was measured and reported, along with maltasic activity, only in the middle section because this is a region where maltasic activity is routinely high and where a previous study of both house sparrows (Gatica-Sosa et al., 2016) and zebra finches (C.G.-S. and P.B., unpublished data – see Fig. S2) showed that gene expression was highest. The single exception was that in adult house sparrows, we used proximal intestine, where activity was highest, because of loss of some midgut samples.

Measurement of RNA

Total RNA from intestinal samples was extracted using PureLink Micro-to-Midi Total RNA Purification System (Invitrogen, Carlsbad, CA, USA), and was quantified using microspectrophotometry (Nano-Drop Technologies, Wilmington, DE, USA). RNA integrity was measured in all samples using the Experion System (Bio-Rad

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Laboratories, Hercules, CA, USA), except in adult house sparrows fed on starch and starch-free diets, in which RNA integrity was assessed in agarose gel stained with GelRed (Biotium Inc., Hayward, CA, USA). Purified RNA was converted to cDNA immediately, or after storage at −80°C, using the iScript cDNA synthesis kit according to the manufacturer's instructions (Bio-Rad Laboratories). For AG mRNA, GenBank accession numbers are EU855810.1 for zebra finches and GQ919053.1 for house sparrows (Gatica-Sosa et al., 2016), and for the reference genes *ß-actin* and *GAPDH* they are AY045726 and AF255390, respectively. Suitability of GAPDH and *ß-actin* as reference genes was evaluated using BestKeeper software (Pfaffl et al., 2004). Real-time PCR to quantify mRNA levels relative to the two reference genes was performed using iQ SYBRGreen Supermix (Bio-Rad Laboratories) as described previously (Gatica-Sosa et al., 2016).

Data management

Results are given as means±1 s.e.m. (*n*=number of birds per treatment, with each treatment defined according to age and diet). Maltasic activity and AG mRNA levels as a function of age and formulated diet (Table 1) were compared using two-way ANOVA with interaction followed by *post hoc* Student's least significant difference comparisons. Data from hatchling house sparrows, which had unspecified wild diets, were compared with data from house sparrows at 4 dph, eating formulated diet using a *t*-test. Correlations between maltasic activity and AG mRNA levels were tested using least-squares linear regression. For all these analyses, ANOVA assumptions regarding normality and homoscedasticity were tested using Levene and Shapiro–Wilk tests, respectively. When normality was not met, a $\log_{10}$ transformation was used. All tests were carried out using JMP PRO, version 13 (SAS Institute Inc., Cary, NC, USA: 1989-2007). In all cases, the significance level was set at $P<0.05$.

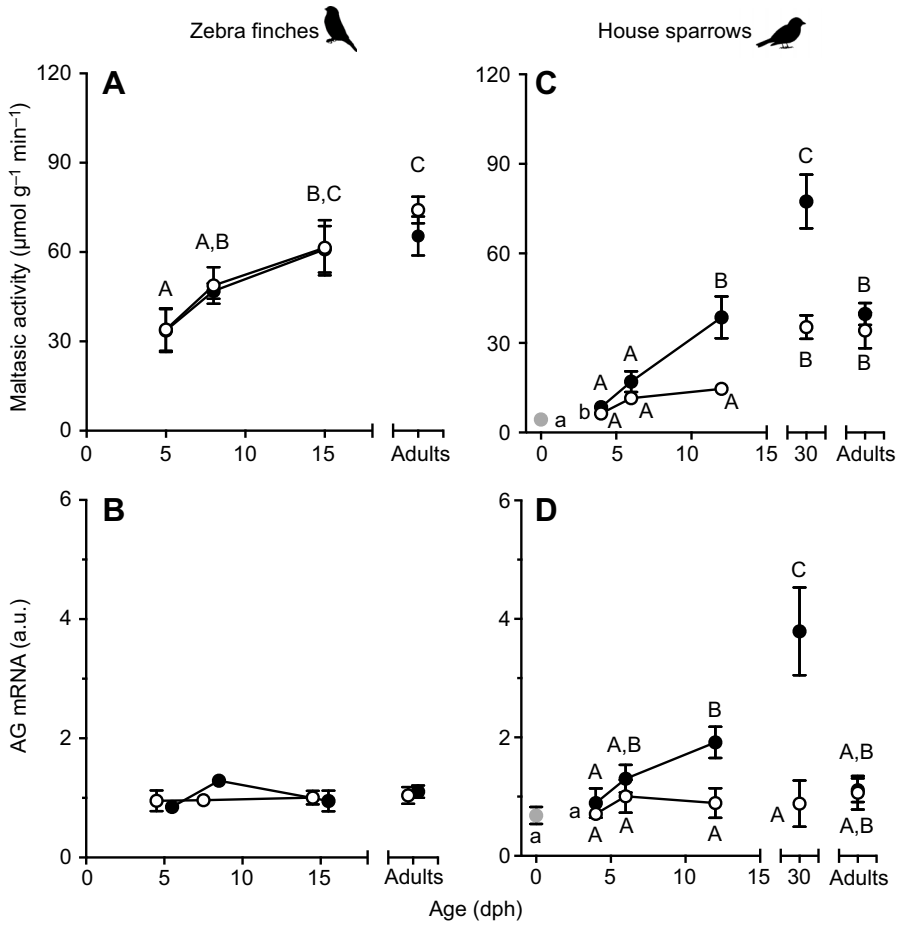

Fig. 1. Intestinal maltasic activity and α-glycosidase (AG) mRNA levels as a function of age in zebra finches and house sparrows raised on diets with differing starch content. (A) Maltasic activity of zebra finches raised on a medium-starch (MS) diet (open circles) and a high-starch (HS) diet (filled circles). (B) AG mRNA levels of zebra finches raised on the two diets (symbols as in A). (C) Maltasic activity of house sparrows raised on a zero starch (0 starch) diet (open circles) and a starch-containing (+starch) diet (filled circles). (D) AG mRNA levels of house sparrows raised on the two diets (symbols as in C). Values are means±s.e.m. Within each plot, different letters represent significant differences ($P<0.05$) for the age and diet effect. In the house sparrow plots, the left-most circle colored in gray represents values for nestlings removed from nests in the wild on the day of hatching. These house sparrow hatchlings differed in maltasic activity from 4 days post-hatch (dph) nestlings fed formulated diet ($t_{7.89}=2.62$, $P=0.031$; signified by different lowercase letters) but they did not differ in AG mRNA levels ($t_{7.22}=0.63$, $P>0.5$; signified by same lowercase letter). All other values within a plot that share the same uppercase letters do not differ significantly by Student's least significant difference tests. The number of individuals used in each group by species, assay, diet and age was as follows: zebra finches: 4 individuals of 5, 8 and 15 dph for both diets (i.e. HS and MS) for mRNA and maltasic activity, but 3 were measured for maltase in the 5 dph MS group, and 5 and 6 adults were assayed for both mRNA level and maltase activity for MS and HS, respectively; house sparrows: 4 individuals of 4, 6, 12 and 30 dph and adults for both diets (i.e. 0 starch and +starch diets) were assayed for mRNA and maltase activity, but 8 0 starch-fed 12 dph nestlings for maltase, and 4 and 10 hatchlings for mRNA and maltase were assayed, respectively. a.u., arbitrary units.

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During initial planning of experiments, we sought a power (i.e. 1−β) of 0.8. Considering the anticipated variance and differences by age and diet in our measurements of enzyme activity and mRNA (Gatica-Sosa et al., 2016), we planned for a minimum of at least 4 individuals for each combination of age and diet and exceeded this minimum for both house sparrows and zebra finches.

RESULTS AND DISCUSSION

In zebra finch nestlings, maltasic activity increased with age ($F_{3,26}$=10.9, P<0.001) but was not affected by dietary starch level ($F_{1,26}$=0.4, P>0.5; Fig. 1A; Table S1). As predicted, AG mRNA levels were not affected by dietary starch level ($F_{1,27}$=0.3, P>0.6) but, contrary to our prediction, they did not increase with age ($F_{3,27}$=1.8, P=0.18; Fig. 1B). In house sparrow nestlings, maltasic activity increased with age up to 30 dph ($F_{4,42}$=26.9, P<0.001), and was higher in birds raised continuously on the diet with added starch ($F_{1,42}$=22.7, P<0.001; Fig. 1C). As predicted, AG mRNA levels in house sparrows were higher in those raised continuously on the diet with added starch ($F_{1,38}$=17.0, P=0.002; Fig. 1D). However, contrary to our prediction, AG mRNA levels in house sparrows did not increase with age on both diets (diet×age interaction, $F_{4,38}$=5.4, P=0.0016; Fig. 1D) as maltasic activity had (cf. 0 starch diet in Fig. 1C,D).

The most distinctive feature in house sparrows and zebra finches regarding development of intestinal maltasic capacity is that mass-specific maltasic activity continued to increase with age (Fig. 1A,C). In house sparrows, the mass-specific activity continued to rise to a value at 30 dph of more than 10 times the value at hatching (Fig. 1C; see also Caviedes-Vidal and Karasov, 2001). In contrast, in chickens and in poultry, mass-specific maltasic activity rises to about four times the value at hatching very quickly, and then remains relatively constant thereafter (Karasov et al., 2002). The intestines of avian species generally increase in size until about half of the adult body mass is reached (Karasov et al., 2002). The overall maltasic capacity over the full intestine length, which is the product of intestinal mass and mass-specific maltasic activity, increases with age mainly as a result of the continued increase in intestinal size but, in house sparrows, also as a result of the increase in mass-specific activity. Avian altricial species have faster postnatal growth rates than similar-sized precocial species. Altricial species' continually increasing enzymatic capacities, achieved through higher mass-specific activity and/or intestine mass, may be a prerequisite for supporting their higher growth rate.

Contrary to our prediction, increases in mass-specific maltasic activity with age were not associated with increases in mRNA levels in house sparrows or zebra finches (Fig. 1B,D). The explanation is not as simple as a lack of transcriptional flexibility, in light of the obvious flexibility in response to the level of dietary starch (Fig. 1D). Other possible explanations include changes in small intestine structure such as (i) increases with age in microvillous surface area per mg or per cm^2 nominal intestinal area and (ii) increase in the proportion of cells on the villi that are mature and hence exhibiting high expression, and/or changes in enzyme dynamics such as (iii) changes in the relationship of enzyme synthesis rate in relation to degradation rate. Relatively large amounts of the enzyme per unit apical membrane, measured by western blot, for example, would be apparent in the case of the last two explanations but not the first.

As we predicted, increased mass-specific maltasic activity was associated with increases in mRNA level in nestling and fledgling house sparrows fed a diet with increased starch (Fig. 1C,D). Although the diets also had different lipid content (Table 1), which has been shown to alter disaccharidase activity in house sparrows (Brzek et al., 2013), other studies with house sparrows have confirmed that induction of maltasic activity by increased dietary starch occurs even when dietary lipid is held constant, and as quickly as 24 h after a diet switch (Brzek et al., 2013; Rott et al., 2017). The correlation of induced maltasic activity with increases in its mRNA level by the starch diet ($F_{1,22}$=47.7, P<0.0001; Fig. 2A) was in contrast to the lack of correlation between these values for birds fed the starch-free diet ($F_{1,22}$=0.399, P=0.53; Fig. 2B). These patterns are consistent with the hypothesis that dietary induction of this enzyme is under transcriptional control, which concurs with similar findings in mammals (Karasov and Douglas, 2013). In young house sparrows, the inducing effect of dietary starch on maltasic activity and its mRNA is entirely reversible (Brzek et al., 2011; Karasov, 2011), reflecting flexibility in both transcription and activity. But, the inducing effect of increased dietary starch on activity and mRNA was not apparent in either adult house sparrows (Fig. 1D; see also Caviedes-Vidal et al., 2000) or nestling or adult zebra finches (Fig. 1B). A possible explanation is a total lack of transcriptional flexibility, perhaps due to the loss of some key

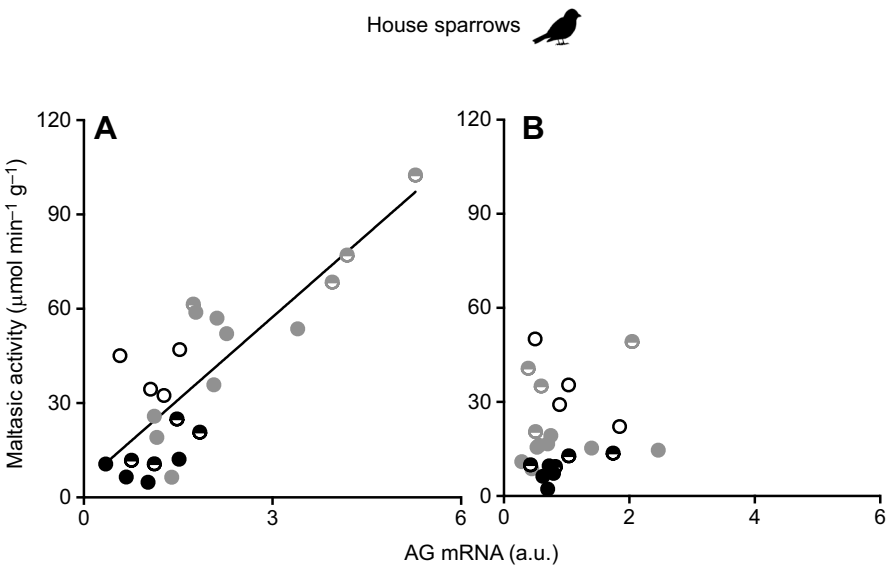

Fig. 2. Correlation of maltasic activity and AG mRNA levels. Maltasic activity in house sparrow nestlings and adults fed starch-containing (+starch) diet was correlated with an increase in AG mRNA levels [$F_{1,22}$=47.7, P<0.0001; y=17.52(±2.54)x+4.77(±5.53), r^2=0.68; A], while this correlation was not apparent for birds fed a starch-free diet ($F_{1,22}$=0.399, P=0.53, r^2=0.02; B). Black-filled, black half-filled, gray-filled, gray half-filled and open circles represent 4, 6, 12 and 30 dph house sparrow nestlings and adults, respectively.

components of the transcription activation signals associated with increased dietary starch. Resolution of this awaits the characterization of the transcriptional promoter(s) of the AG genes in birds.

Differences among species or even cohorts within species in transcriptional and enzymatic flexibility in response to increased dietary starch are expected from an ecological and evolutionary perspective. In nature, house sparrow nestlings transition from a diet of mainly arthropods, which are high in protein and low in carbohydrates, to a diet of mainly seeds, many of which are high in carbohydrates and low in protein (Rott et al., 2017). Flexibility in maltasic activity and AG mRNA levels maximizes the digestibility of starch when it is at high dietary levels and minimizes the cost of synthesizing excess enzyme when starch is at low levels (Rott et al., 2017). In contrast, the regulatory capacity for reversible digestive flexibility is arguably wasteful in specialist feeders. This may explain why house sparrow adults, which essentially are specialists on non-animal foods, did not modulate maltasic activity or AG mRNA when fed diets with different amounts of starch (Fig. 1). Likewise, zebra finches are diet specialists that consume only carbohydrate-rich seeds beginning at hatching (Brzek et al., 2010), and neither nestlings nor adults modulated maltasic activity or AG mRNA levels when fed diets with different amounts of starch. Hence, intestinal α-glycosidase and its mRNA are stimulated by dietary carbohydrate in nestling house sparrows but not in adult house sparrows or zebra finches.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: C.G.-S., P.B., W.H.K., E.C.-V.; Methodology: C.G.-S., P.B., M.M., W.H.K., E.C.-V.; Validation: C.G.-S., M.M., E.C.-V.; Formal analysis: C.G.-S., W.H.K., E.C.-V.; Investigation: C.G.-S., P.B., W.H.K., E.C.-V.; Resources: W.H.K., E.C.-V.; Data curation: C.G.-S., P.B., W.H.K., E.C.-V.; Writing – original draft: C.G.-S., W.H.K., E.C.-V.; Writing – review and editing: C.G.-S., P.B., M.M., W.H.K., E.C.-V.; Visualization: W.H.K., E.C.-V.; Supervision: W.H.K., E.C.-V.; Project administration: W.H.K., E.C.-V.; Funding acquisition: W.H.K., E.C.-V.

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Supplementary information
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References
Anderson, T. R. (2006). *Biology of the Ubiquitous House Sparrow*. Oxford: Oxford University Press.
Brzek, P., Kohl, K., Caviedes-Vidal, E. and Karasov, W. H. (2009). Developmental adjustments of house sparrow (Passer domesticus) nestlings to diet composition. *J. Exp. Biol.* **212**, 1284-1293.
Brzek, P., Lessner, K. M., Caviedes-Vidal, E. and Karasov, W. H. (2010). Low plasticity in digestive physiology constrains feeding ecology in diet specialist, Zebra finch (*Taeniopygia guttata*). *J. Exp. Biol.* **213**, 798-807.
Brzek, P., Kohl, K. D., Caviedes-Vidal, E. and Karasov, W. H. (2011). Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition. *J. Exp. Biol.* **214**, 2755-2760.
Brzek, P., Kohl, K. D., Karasov, W. H. and Caviedes-Vidal, E. (2013). Activity of intestinal carbohydrases responds to multiple dietary signals in nestling House sparrows. *J. Exp. Biol.* **216**, 3981-3987.
Caviedes-Vidal, E. and Karasov, W. H. (2001). Developmental changes in digestive physiology of nestling house sparrows, Passer domesticus. *Physiol. Biochem. Zool.* **74**, 769-782.
Caviedes-Vidal, E., Afik, D., Martinez del Rio, C. and Karasov, W. H. (2000). Dietary modulation of intestinal enzymes of the house sparrow (Passer domesticus): testing an adaptive hypothesis. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **125**:11-24.
Galand, G. (1989). Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. Comparative development, effects of glucocorticoids, molecular mechanisms, and phylogenetic implications. *Comp. Biochem. Physiol. B* **94**, 1-11.
Gatica-Sosa, C., Brzęk, P., Chediack, J. G., Cid, F. D., Karasov, W. H. and Caviedes-Vidal, E. (2016). Differential transcriptional responses underlie dietary induction of intestinal carbohydrase activities in house sparrow nestlings. *J. Anim. Physiol. Anim. Nutr.* **100**, 236-242.
Karasov, W. H. (2011). Digestive physiology: a view from molecules to ecosystem. *Am. J. Physiol.Regul. Integr. Comp. Physiol.* **301**, R276-R284.
Karasov, W. H. and Douglas, A. E. (2013). Comparative digestive physiology. *Compr. Physiol.* **3**, 741-783.
Karasov, W. H., Wright, J. and Leonard, M. L. (2002). Nestling digestive physiology and begging. In *The Evolution of Begging: Competition, Cooperation, and Communication* (ed. J. Wright and M. L. Leonard), pp. 199-219. Dordrecht: Kluwer Academic Publishers.
Lepczyk, C. A., Karasov, W. H. and Caviedes-Vidal, E. (1998). Digestive responses during food restriction and realimentation in nestling house sparrows. *(Passer domesticus). Physiol. Biochem. Zool.* **71**, 561-573.
Nichols, B. L., Avery, S., Sen P., Swallow, D. M., Hahn, D. and Sterchi, E. (2003). The maltase-glucoamylase gene: common ancestry to sucrase-isomaltase with complementary starch digestion activities. *Proc. Natl. Acad. Sci. USA* **100**, 1432-1437.
Pfaffl, M. W., Tichopad, A., Prgomet, C. and Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper–Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* **26**, 509-515.
Rott, K. H., Caviedes-Vidal, E. and Karasov, W. H. (2017). Intestinal digestive enzyme modulation in house sparrow nestlings occurs within 24 h of a change in diet composition. *J. Exp. Biol.* **220**, 2733-2742.
Sklan, D., Geyra, A., Tako, E., Gal-Gerber, O. and Uni, Z. (2003). Ontogeny of brush border carbohydrate digestion and uptake in the chick. *Br. J. Nutr.* **89**, 747-753.
Uni, Z., Noy, Y. and Sklan, D. (1999). Posthatch development of small intestinal function in the poult. *Poult. Sci.* **78**, 215-222.
Uni, Z., Tako, E., Gal-Garber, O. and Sklan, D. (2003). Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. *Poult. Sci.* **82**, 1747-1754.
Zann, R. A. (1996). *The Zebra Finch. A Synthesis of Field and Laboratory Studies*. Oxford, United Kingdom: Oxford University Press.

Table S1. Results of two-way ANOVAs to evaluate the effect of diet, age and their interaction on maltasic activity and AG mRNA of ZEBF and HOSP, respectively.

Measurement					
Maltase activity					
	Species				
	ZEBF (n=34)	Source of Variation	*df*	*F*	*P-value*
		Age	3	10.88	0.0001
		Diet	1	0.36	0.5564
		Interaction	3	0.21	0.8923
		Error	26		
		Total	33		
	HOSP (n=52)	Source of Variation	*df*	*F*	*P-value*
		Age	4	26.91	0.0001
		Diet	1	22.74	0.0001
		Interaction	4	5.38	0.0014
		Error	42		
		Total	51		
AG mRNA level					
	Species				
	ZEBF (n=35)	Source of Variation	*df*	*F*	*P-value*
		Age	3	1.75	0.18
		Diet	1	0.25	0.62
		Interaction	3	1.37	0.27
		Error	27		
		Total	34		
	HOSP (n=48)	Source of Variation	*df*	*F*	*P-value*
		Age	4	5.36	0.0016
		Diet	1	17.01	0.0002
		Interaction	4	5.48	0.0014
		Error	38		
		Total	47		

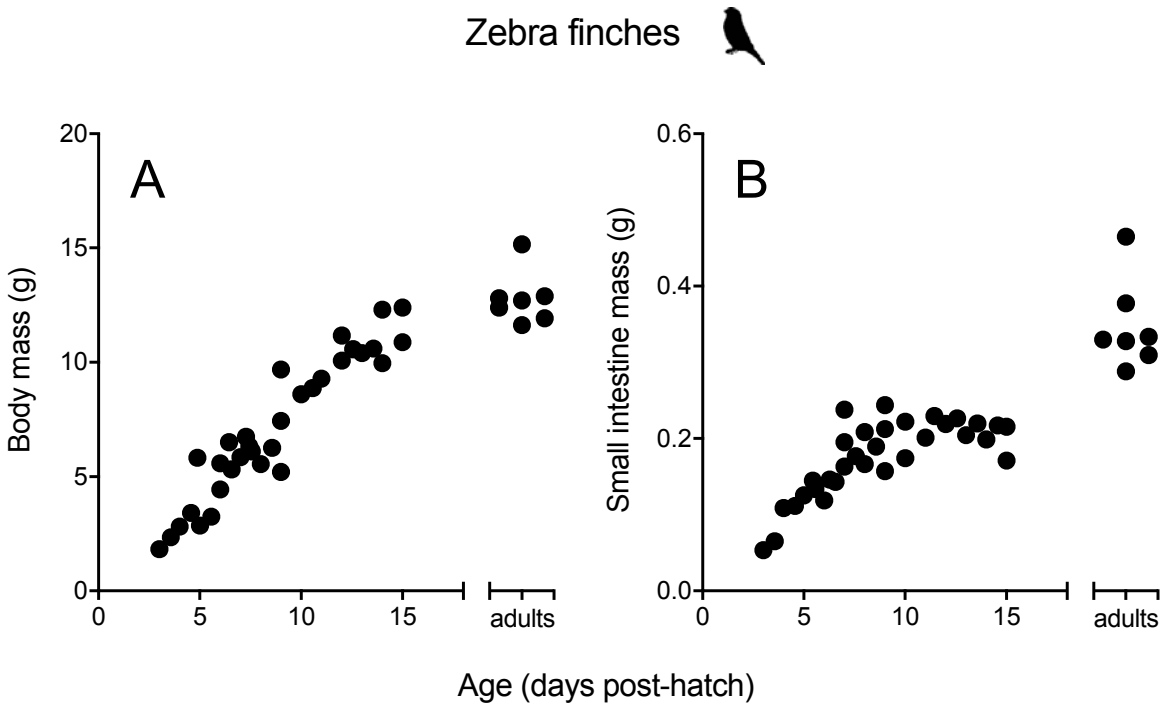

Fig. S1. Body mass (A) and small intestine mass (B) in zebra finch nestlings as a function of age (days post hatch) and as compared to adults. In this preliminary study, the birds received daily a mixture of seeds and bird food and fresh water *ad libitum*, and every other day a specially prepared egg food to support growth and reproduction. Each point is an individual bird.

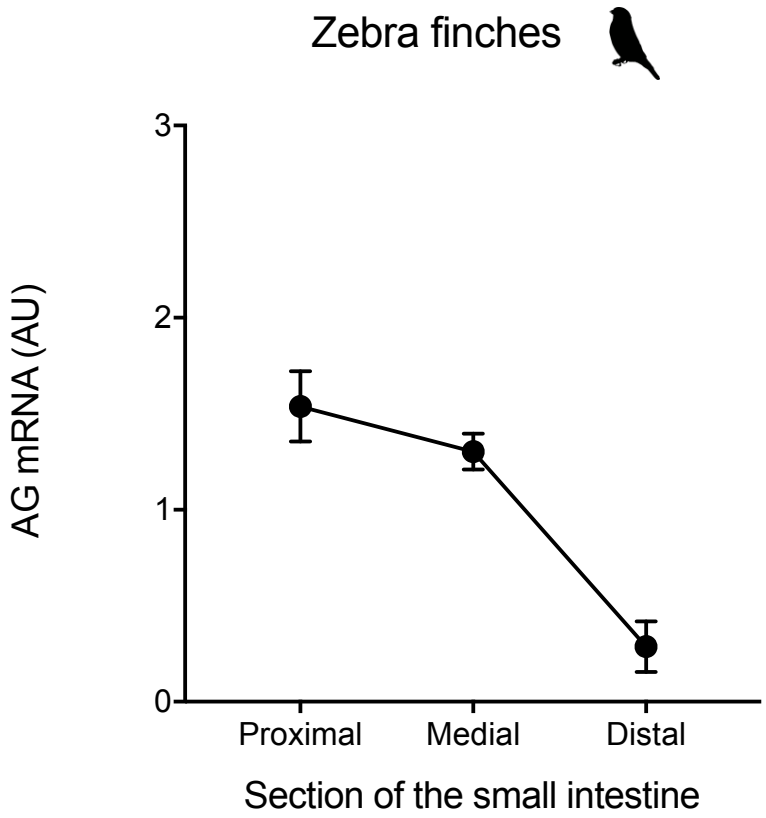

Fig. S2. Amount of intestinal AG mRNA as a function of intestinal position in adult Zebra finches. AG mRNA differed with intestinal position ($F_{2,13.9}$=22.49, P<0.0001) and was significantly lower in distal intestine (P<0.05) than in either of the more proximal regions, which did not differ from each other (P=0.18). Data for 11 birds eating either diets MS or HS were pooled because AG mRNA did not differ as a function of diet (P=0.17).