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RESEARCH ARTICLE

Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition

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SUMMARY

Feeding conditions during the nestling period may significantly affect whole-life fitness in altricial birds but little is known about the physiological mechanisms responsible for these effects. Permanent changes (irreversible developmental plasticity) in digestive physiology caused by the neonatal diet may form such a mechanism. We previously showed that the lack of starch in the diet of house sparrow (*Passer domesticus*) nestlings between 3 and 12 days post-hatching significantly decreased the activity of intestinal maltase, an enzyme essential for starch digestion. To check whether diet-induced variation in maltase activity in young house sparrows is reversible, we raised them under laboratory conditions from 3 until 30 days of age on diets with either 0% starch or 25% starch, with some individuals experiencing a switch in their assigned diet at 12 days of age. We found evidence for the presence of an internal, presumably genetic, program for changes in the activity of maltase and sucrase, which was, however, significantly affected by diet composition (i.e. environmental factor). Digestive enzyme activity in 30 day old birds was not influenced by diet composition prior to day 12 but instead depended only on diet that was fed between days 12 and 30. We conclude that plasticity in the activity of intestinal disaccharidases in house sparrow nestlings represents completely reversible phenotypic flexibility that can help young sparrows to cope with unpredictable variation in food composition during ontogeny without long-term effects on their digestive system. However, comparison with other species suggests that the magnitude of digestive flexibility in young passerines may be evolutionarily matched to species-specific variation in feeding conditions.

Key words: phenotypic flexibility, digestive enzymes, house sparrow, early nutrition compensation, ecological physiology, ontogeny.

INTRODUCTION

Environmental conditions during the growth period can exert a significant effect on the phenotype of adult individuals (Lindström, 1999; Monaghan, 2008). Altricial birds may be particularly prone to such long-term effects, because of their relatively rapid rate of growth and development. Indeed, several studies have reported that conditions during the nestling period significantly affect the future fitness of adult birds (e.g. Reid et al., 2003; Alonso-Alvarez et al., 2007; Tilgar et al., 2010). In most cases these effects are related to the feeding conditions of nestlings; however, the exact physiological mechanisms (other than changes in body size) whereby early nutrition affects adults are usually more difficult to identify (Monaghan, 2008). Although some studies have found that nestling nutrition may exert long-lasting effects on physiological traits in adulthood (Blount et al., 2003; Verhulst et al., 2006; Criscuolo et al., 2008), others have revealed that after fledging birds can fully compensate for the negative effect(s) of early nutrition on such important parameters as immune function (Birkhead et al., 1999) or stress response (Lendvai et al., 2009).

Phenotypic variability that is expressed by a single genotype under a range of environmental conditions is called phenotypic plasticity (Pigliucci, 2001; Pigliucci, 2005), and can take the form of irreversible developmental plasticity or reversible phenotypic flexibility (Piersma and Drent, 2003). The digestive system is directly exposed to variation in food quantity and/or quality, and exhibits tremendous phenotypic plasticity in response to external factors like diet type, food availability and season, or demands set by reproduction or migration (Starck and Wang, 2005). As the digestive tract is responsible for supporting all functions of the body, permanent effects of early nutrition on the function of the digestive system (i.e. developmental plasticity) might be one of the physiological mechanisms responsible for the long-term effect(s) of experience during the nestling period. However, the persistence of effects of early feeding conditions in altricial birds is almost unknown. Some studies have found that the effects of a short (2–3 day) undernourishment on digestive function in altricial birds can be compensated for relatively quickly (Lepczyk et al., 1998; Brzęk and Konarzewski, 2004) (but see Konarzewski et al., 1996). However, in many organisms the responsiveness of digestive physiology to external factors decreases during ontogeny (Henning and Leeper, 1982; Toloza and Diamond, 1990), which can limit the potential for reversible plasticity after fledging. Similarly, in some organisms even a very short period of diet manipulation may permanently influence digestive physiology (Geurden et al., 2007) (see also Patel and Srinivasan, 2010).

Young house sparrows (*Passer domesticus* L.) show a gradual increase in the consumption of high-starch dietary items (e.g. seeds) during ontogeny (Anderson, 2006), which is accompanied by a simultaneous increase in the activity of maltase, an intestinal enzyme crucial to the digestion of starch (Caviedes-Vidal and Karasov, 2001). Recently, we showed that maltase activity in 12 day old nestlings, fed on a starch-free diet imitating the insect-rich food of very young nestlings, was only half that observed in birds fed on a starch-containing diet mimicking a mix of insects and seeds, typical of older nestlings (Brzęk et al., 2009). Thus, an apparent genetic program responsible for ontogenetic changes in expression of intestinal disaccharidases can be modified by diet composition. However, our previous study did not reveal whether these changes are permanent (irreversible) or not. Adults of house sparrows and other passerines studied so far either do not change the mass-specific activity of their intestinal disaccharidases in response to dietary starch (Afik et al., 1995; Martinez del Rio et al., 1995; Caviedes-Vidal et al., 2000) or show non-specific and presumably non-adaptive increases in the activity of all intestinal enzymes (including disaccharidases) in response to lowered dietary starch content (Sabat et al., 1998; Brzęk et al., 2010). Therefore, fledged house sparrows might possess a low capacity for further adaptive modulation of maltase activity and the diet-induced changes observed in nestlings may be permanent. Moreover, diet switching in house sparrows is accompanied by innate behavioral changes in food preference (Mueller, 1986), which may indicate that the whole process is controlled by genes and therefore involves irreversible changes in digestive physiology.

Here, we report the results of an experiment in which we raised house sparrow nestlings under laboratory conditions using the same diets as in our previous study (Brzęk et al., 2009). However, young birds around the typical age of fledging were switched to alternative diets and the activity of their intestinal enzymes was subsequently measured 17–18 days later. If phenotypic plasticity of maltase expression in fledgling-age house sparrows represents an irreversible developmental plasticity, we predicted that the activity of enzymes observed 18 days after fledging should be at least partly affected by diet during the nestling period. In contrast, if these changes represent reversible phenotypic flexibility, enzyme activity in older fledglings should depend exclusively on diet consumed after the diet switch and should be independent of food during the nestling period.

MATERIALS AND METHODS

Animal maintenance and experimental treatments

Most methods used in nestling maintenance were identical to those in our previous experiment and are described in detail elsewhere (Brzęk et al., 2009). Briefly, 3 day old house sparrow nestlings (day 0 is the day of hatching) were collected in May–July 2008 from natural nests located on the campus of the University of Wisconsin, Madison, and housed individually in our laboratory. Nestlings were hand-fed two synthetic diets, as previously (Brzęk et al., 2009): (a) diet '0', intended to mimic insects consumed by very young house sparrows and which contained no carbohydrates, 20% corn oil, and 59.63% casein by dry mass; (b) diet '+', intended to mimic the mixture of insects and seeds typical of older nestlings, which contained 25.4% corn starch, 8% corn oil and 46.23% casein. In comparison to previously described feeding techniques (Brzęk et al., 2009), the size of the hourly meal offered to nestlings on days 3–5 was increased to 0.4, 0.55 and 0.65 g, respectively. When nestlings reached day 12 (close to the typical fledging time of house sparrows; 14–15 days) (Anderson, 2006), some birds were switched to the alternative diet. On days 13 and 14 these birds were fed a mixture of the two diets in a 50:50 mass proportion, and during subsequent days were fed the alternative diet to that offered on days 3–12. Thus, we created groups '+/0' (i.e. switched from diet + to diet 0) and '0/+' (switched in the opposite direction). Another two groups, '+/+' and '0/0' were fed all the time on the same diet (sample size, N=8 for each group). To control for nest effect, when more than one nestling was collected from the same natural nest, individuals were randomly assigned to different experimental groups.

Beginning on day 12, hourly meal size was adjusted in relation to the begging effort of individual nestlings (they were fed until they stopped begging; mean meal size 0.9–1 g). Sixteen day old birds were transferred to individual cages and hand-fed using the same technique until day 29–30. Food intake did not differ between the four treatment groups over the entire experimental period or over sub-periods before and after nestlings were moved to cages (ANOVA, P>0.2 for all comparisons).

When fledglings reached day 29–30, they were killed with CO_2, and dissected to remove and weigh the intestines, gizzard, liver, pancreas and pectoral muscles. Intestines were flushed with ice-cold avian Ringer solution to clear them and cut into three sections, corresponding to the proximal, middle and distal regions of the intestine, and immediately preserved in liquid nitrogen. For the sake of brevity, we refer to all these birds as 30 day old birds.

Another two groups of nestlings (N=9 for each group) were raised with identical methods on + and 0 diets and dissected on day 12, to examine nestling condition immediately before the diet switch.

Intestinal enzyme assays

Assays of intestinal digestive enzymes were performed as described in detail elsewhere (Brzęk et al., 2009), with two modifications: (a) intestinal samples analyzed in the previous study represented only part of each intestinal section (proximal, medial and distal) – in the present experiment we cut intestines from each section longitudinally and used one-half of each section for enzyme assays (the other was preserved for other assays), resulting in better analysis of enzyme activity over the whole intestine length; (b) in addition to maltase and aminopeptidase-N, we also analyzed sucrase activity. The sucrase assay was identical to the maltase assay described in (Brzęk et al., 2009), except sucrose was substituted for maltose as the substrate.

We expressed mass-specific activity of enzymes as micromoles of substrate processed per minute per gram wet tissue. We calculated the summed hydrolysis activity of the entire small intestine, which is an index of the total hydrolytic capacity, by multiplying mass-specific activity in the proximal, medial and distal intestinal regions by their respective masses, and summed over the three regions. We also calculated the ratio of values found for maltase to that of aminopeptidase-N (hereafter M/A). This ratio represents the relative investment in carbohydrate- and protein-digesting enzymes and is not affected by potential differences in intestinal morphology or villus area between groups (see Ramirez-Otarola et al., 2011).

Data analysis

Data were analyzed by means of one-way ANOVA/ANCOVA, with diet treatment as the main factor. For organ size, initial and/or final body mass were included in the model as covariates when they were significant. All tests were carried out separately for 12 day old birds (two experimental groups) and 30 day old birds (four experimental groups). We also used ANOVA to compare summed enzyme activities in 30 day old birds with those of 12 day old nestlings fed

on the same diet as fledglings before the diet switch (e.g. 30 day old group +/0 with 12 day old group +). Here, we checked for changes in enzymatic activity after the diet switch. All tests were carried out using SAS software. In all tests, the significance level was set at $\alpha<0.05$.

RESULTS

Diet treatment had no significant effect on body mass or on the mass of most internal organs of nestlings and fledglings (including intestine mass; Tables 1–3). The gizzard was the only organ whose mass was significantly affected by experimental treatment but there was no consistent pattern of diet effect (Tables 1 and 3).

Twelve day old house sparrow nestlings raised on diet + had more than twice the summed activity of both disaccharidases (maltase and sucrase) than birds raised on diet 0 (maltase: $F_{1,16}=33.40$, $P<0.0001$, Fig. 1A; sucrase: $F_{1,16}=51.20$, $P<0.0001$, Fig. 1B). In contrast, the activity of aminopeptidase-N in the same 12 day old nestlings was not significantly affected by diet treatment ($F_{1,16}<0.01$, $P=0.97$, Fig. 1C), resulting in significantly higher values of M/A ratio in the + group ($F_{1,16}=29.66$, $P<0.0001$, Fig. 1D).

Diet treatment also had a highly significant effect on summed activity of both disaccharidases in 30 day old fledglings (maltase: $F_{3,28}=11.37$, $P<0.0001$, Fig. 1A; sucrase: $F_{3,28}=10.76$, $P<0.0001$, Fig. 1B). Birds fed on diet + between days 12 and 30 (groups +/+ and 0/+) showed *ca*. 40–80% higher activity of maltase and sucrase than birds fed on diet 0 during the same interval (groups 0/0 and +/0; Fig. 1A,B). In contrast, the diet offered to nestlings before day 12 had no significant effect on the activity of disaccharidases on day 30 (Fig. 1A,B). Similarly, the summed activity of aminopeptidase-N on day 30 was not significantly affected by diet treatment ($F_{3,28}=1.32$, $P=0.29$, Fig. 1C). M/A was affected by dietary treatment in the same way as maltase activity ($F_{3,28}=9.26$, $P=0.0002$); strikingly, it was significantly higher in the switched group 0/+ than in birds fed on starch-containing diet all the time (group +/+; Fig. 1D).

There was a highly significant increase in the summed activity of maltase and sucrase between days 12 and 30 in birds fed on diet 0 before day 12 (for all comparisons: $F_{1,15}>35$, $P<0.0001$; Fig. 1A,B). Birds fed on diet + throughout the entire experiment showed a significant increase in the summed activity of sucrase between days 12 and 30 ($F_{1,15}=7.77$, $P=0.014$) and no change in the summed activity of maltase ($F_{1,15}=0.31$, $P=0.59$). Birds switched from diet + to diet 0 showed no change in the activity of sucrase ($F_{1,15}=0.00$, $P=0.98$) and tended to show a decrease in the activity of maltase ($F_{1,15}=3.98$, $P=0.065$). The summed activity of aminopeptidase-N was significantly lower on day 30 than on day 12 in all groups (for all comparisons: $F_{1,15}>20$, $P<0.0005$; Fig. 1C). In contrast, M/A ratio increased significantly between days 12 and 30 in all groups (for all comparisons: $F_{1,15}>18$, $P<0.005$; Fig. 1D).

DISCUSSION

Effect of experimental diet switch on activity of intestinal enzymes

The results of our experiment clearly indicate that the phenotypic plasticity of digestive enzymes in young house sparrows represents fully reversible phenotypic flexibility. As in our previous study (Brzęk et al., 2009), we found that house sparrow nestlings fed on starch-free diet 0 between days 3 and 12 had a significantly lower activity of disaccharidases than birds raised on diet +, which includes starch (Fig. 1A,B). However, disaccharidase activity measured in 30 day old fledglings was completely independent of the diet fed to nestlings before day 12, and corresponded only to the diet offered to fledglings after day 13. At the same time, we observed a significant increase in

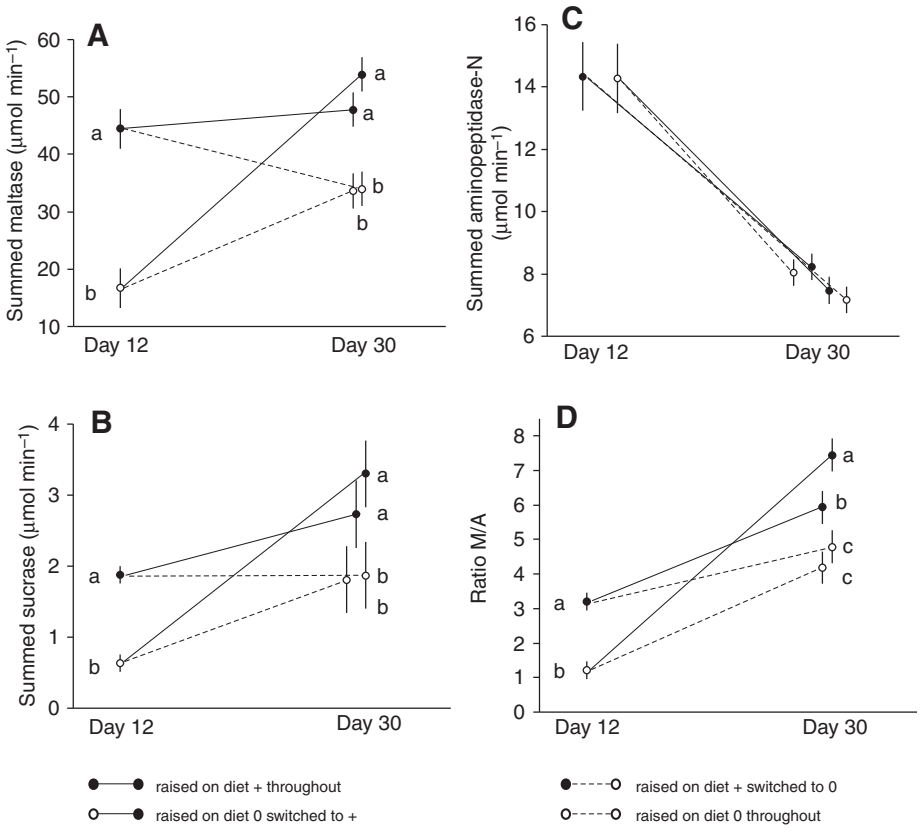

Fig. 1. Summed activity of maltase (A), sucrase (B) and aminopeptidase-N (C), and ratio of summed activity of maltase to that of aminopeptidase-N (M/A, D) in the present experiment. Means ± s.e.m. are shown. Filled circles indicate groups fed on diet + (25% starch), and unfilled circles those fed on diet 0 (0% starch), between days 3 and 12 (means for day 12) and between days 12 and 30 (means for day 30). Lines indicate the course of changes between days 12 and 30 in birds fed on diet + (solid lines) and 0 (dashed lines) during that time. Different letters indicate significant differences between groups on days 12 and 30 (they do not refer to changes between days 12 and 30). In C, groups for day 30 are (from left to right): 0/0, +/+, 0/+ and +/0.

the summed activity of maltase between days 12 and 30 in birds fed throughout the experiment on diet 0 (Fig. 1A). A similar increase was also found in nestlings fed on the same diet between days 6 and 12 (Brzęk et al., 2009). This pattern indicates the presence of an intrinsic, genetic program responsible for an increase in the disaccharidases activity that occurs even when carbohydrates are completely absent in food. However, this genetic program is significantly modified by diet composition, as group 0/+ showed much higher activity of maltase on day 30 than group 0/0, in spite of the birds being fed on the same diet before day 12. In contrast, the summed activity of maltase did not change between days 12 and 30 in group +/+, and, most astonishingly, we found that a diet switch opposite to that faced by wild birds (in group +/0) produced a near-significant decrease in the summed activity of maltase (Fig. 1A). Thus, environmental factors (diet composition) exert the prevailing effects on ontogeny of the capacity for carbohydrate digestion in house sparrows and can probably even reverse its normal course.

The direction and magnitude of changes in the activity of maltase and sucrase were very similar (compare Fig. 1A and 1B). This is not surprising because the sucrase–isomaltase complex contributes to maltase activity (Semenza and Auricchio, 1989). However, experimental treatment had no significant effect on the activity of aminopeptidase-N (Fig. 1C). Therefore, the diet effects observed for disaccharidases cannot be explained by non-specific mechanisms such as changes in intestinal morphology or properties of cell membranes that might affect all membrane-bound enzymes in a similar fashion. Moreover, because diet treatment had no significant effect on the size of the intestines (Tables 1–3), all changes in the capacity for digestion of carbohydrates reflect adjustments in the mass-specific activity of enzymes. Presumably, diet composition had a direct effect on the expression of disaccharidases. Potentially, the diet effect observed in our experiment could reflect either the induction of disaccharidase activity by starch in diet + or its suppression by

Table 1. Summary of results of ANOVA or ANCOVA on the effects of diet on body mass and size of internal organs in nestlings analyzed on day 12

	F	d.f.	*P*	Diet + (g)	Diet 0 (g)
Initial body mass (day 3)	0.52	1,16	0.48	10.57±0.48	10.07±0.48
Body mass on day 12	0.15	1,16	0.70	26.81±0.59	27.13±0.59
Intestine mass[a]	0.66	1,15	0.43	1.348±0.070	1.429±0.070
Pancreas mass[b]	1.57	1,15	0.23	0.244±0.015	0.217±0.015
Gizzard mass[b]	5.77	1,15	0.03	0.740±0.015	0.691±0.015
Liver mass[a]	2.09	1,15	0.17	1.071±0.049	0.969±0.049
Pectoral muscle mass	0.00	1,16	0.98	2.768±0.187	2.774±0.187

Mean values (±s.e.m.) are shown.
Diet +, 25% starch; diet 0, 0% starch.
[a]Initial body mass was significant as covariate (intestines: $F_{1,15}$=6.64, P=0.021; liver: $F_{1,15}$=12.39, P=0.0031). [b]Final body mass was significant as covariate (pancreas: $F_{1,15}$=6.11, P=0.026; gizzard: $F_{1,15}$=11.64, P=0.0039).

Table 2. Summary of results of ANOVA or ANCOVA on the effects of diet on body mass and size of internal organs in nestlings analyzed on day 30

	Diet			Initial body mass			Final body mass		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Initial body mass (day 3)	0.08	3,28	0.97	x	x	x	x	x	x
Body mass on day 12	0.85	3,28	0.48	n.s.	n.s.	n.s.	x	x	x
Body mass on day 30	1.68	3,26	0.20	n.s.	n.s.	n.s.	x	x	x
Intestine mass	0.99	3,27	0.41	n.s.	n.s.	n.s.	4.27	1,27	0.048
Pancreas mass	1.25	3,27	0.31	n.s.	n.s.	n.s.	7.78	1,27	0.0096
Gizzard mass	4.22	3,28	0.014	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Liver mass	0.65	3,26	0.59	9.92	1,26	0.004	5.13	1,26	0.032
Pectoral muscle mass	0.2	3,26	0.89	13.65	1,26	0.001	16.52	1,26	0.0004

The lower degree of freedom for final body mass on day 30 is caused by missing data for two birds (from groups 0/0 and +/+). n.s., covariate is non-significant; x, covariate is not relevant for that particular trait.

Table 3. Mean values (±s.e.m.) of body mass and size of internal organs in nestlings analyzed on day 30

	Diet 0/0	Diet +/+	Diet 0/+	Diet +/0
Initial body mass (day 3)	10.10±0.43	10.14±0.43	10.29±0.43	10.00±0.43
Body mass on day 12	27.80±0.52	27.62±0.52	27.61±0.52	28.62±0.52
Body mass on day 30	25.05±0.64	24.57±0.64	24.17±0.64	26.11±0.64
Intestine mass	0.864±0.027	0.842±0.027	0.869±0.028	0.909±0.028
Pancreas mass	0.152±0.007	0.136±0.007	0.154±0.007	0.148±0.007
Gizzard mass	0.480±0.034[a,b]	0.398±0.034[a]	0.440±0.034[a]	0.561±0.034[b]
Liver mass	0.690±0.023	0.689±0.023	0.724±0.024	0.720±0.024
Pectoral muscle mass	3.910±0.145	3.759±0.149	3.807±0.152	3.783±0.157

Diet 0/0, 0% starch diet throughout; diet +/+, 25% starch diet throughout; diet 0/+, 0% starch diet followed by a switch to 25% starch diet after day 12; diet +/0, 25% starch diet followed by a switch to 0% starch diet after day 12.
Different letters indicate significant differences between experimental treatments.

the higher lipid content in diet 0 (see Caviedes-Vidal et al., 2000), mediated by transcriptional or post-transcriptional mechanisms (see Honma et al., 2007). We plan to investigate these questions in future experiments.

The direction of the observed diet-induced modulation of carbohydrases follows predictions of the adaptive modulation hypothesis (Karasov and Diamond, 1988) that the activity of a digestive enzyme should be matched to the relative level of its dietary substrate so that energy/nutrients are not wasted on enzymes in excess of need. Although the prevalence of consumed animal material generally decreases during ontogeny in the house sparrow, the animal content in the nestling diet just before fledging (days 12–13) varies widely among populations [40–90%; cf. fig. 6.5 in Anderson (Anderson, 2006)], presumably reflecting the seasonal or spatial variation in food availability. Therefore, high developmental flexibility of the digestive system may help young house sparrows deal with those changes without apparent long-term effects on digestive function. We did not find any detectable short-term effect of diet manipulation on body mass or mass of pectoral muscles (Tables 1–3). However, all birds in our study were fed *ad libitum*, and both applied diets were supplemented with vitamins and microelements. We hypothesize that, under natural conditions, unusual diet composition is related to periods of food shortage, and it might then be more difficult for nestlings to compensate quickly for a period of retarded body growth.

The evolution of flexibility of digestive physiology in altricial birds

Fully reversible, diet-induced phenotypic flexibility of some intestinal enzymes, similar to that found in the present study, has also been described for maltase and sucrase in precocial chickens (although down-regulation of enzyme activity after the diet switch was slower than up-regulation) (Biviano et al., 1993), and for aminopeptidase-N in the rodent *Phyllotis darwini* (Sabat et al., 1999). However, altricial birds show probably the fastest rate of growth in the animal world (Case, 1978), which presumably exerts strong selection pressure for an optimal genetic program for development of digestion rather than for its plasticity. Phenotypic flexibility can evolve (Pigliucci, 2001; Pigliucci, 2005), and between-species comparisons of the magnitude of phenotypic flexibility can be a valuable although indirect tool in evaluating its adaptive value (Doughty and Reznick, 2004). The capacity for flexibility of the digestive system may involve costs (Pigliucci, 2005), and therefore natural selection should favor its loss when the expected variation in food type is low. Indeed, adult house sparrows have a much less variable diet than young birds (Anderson, 2006) and do not show diet-induced modulation of their disaccharidases (Caviedes-Vidal et al., 2000). This loss of phenotypic plasticity with age is perhaps not surprising, as external factors can presumably exert larger effects on physiological traits during their development (by affecting mechanisms controlling ontogenetic changes) than after they reach maturity in adults. However, it seems there is also a large inter-specific variation in the level of flexibility of digestive physiology present in young passerine birds. For example, zebra finch nestlings (*Taeniopygia guttata*), a granivorous specialist that does not change diet during ontogeny, exhibit much smaller and less flexible ontogenetic changes in mass-specific activity of disaccharidases than young house sparrows (Brzęk et al., 2010). Moreover, zebra finches cannot thrive on a starch-free diet. This low phenotypic flexibility of digestive physiology might reflect the low diet flexibility observed in free-living zebra finches (Zann, 1996). A similar low level of flexibility of digestive physiology was observed in young song thrushes (*Turdus philomelos*) (Konarzewski et al., 1996) and European starlings (*Sturnus vulgaris*) (Schew and Ricklefs, 1998). On the other hand, nestlings of the sand martin (*Riparia riparia*), an aerial insectivore that faces variable food availability, show quite large flexibility of their digestive system (Brzęk and Konarzewski, 2001; Brzęk and Konarzewski, 2004).

We hypothesize that the level of flexibility of digestive physiology in passerine nestlings is positively correlated with species-specific variation in feeding conditions, analogous to an evolutionary match between diet composition and the activity of carbohydrate-digesting enzymes observed in adult passerines (Kohl et al., 2011; Ramirez-Otarola et al., 2011). Indeed, some recent studies showed that flexibility in the size of the gastrointestinal tract is related to variability of environmental factors at both the intra- and inter-specific level (Cavieres and Sabat, 2008; Naya et al., 2008). We predict that a high variation in food quantity/quality faced by altricial nestlings should select for a higher flexibility of digestive physiology, whereas in other species factors like predator pressure and sibling competition select for the fastest possible pace of development (including development of digestive physiology), even at the cost of its lower flexibility. In turn, high levels of phenotypic flexibility of digestive physiology may facilitate evolutionary adaptation to new food niches (Ghalambor et al., 2007). We hypothesize that this capacity may be part of a suite of physiological adaptations that increase the potential for colonization in the house sparrow, a very successful invasive species (see Lee et al., 2005). However, the presence of significant inter-specific variation in the level of phenotypic flexibility of digestive physiology suggests that even though young house sparrows are capable of complete compensation for a period of abnormal development of digestive function, we cannot exclude the possibility that such effects are permanent in other species. Strikingly, Ramirez-Otarola and colleagues recently suggested that physiological modification caused by diet composition in the early stages of ontogeny can explain differences between adult passerines in the activity of digestive enzymes (Ramirez-Otarola et al., 2011). The results of our present experiment show that this is not the case at least for house sparrow (and, generally, is unlikely for species that switch diet during ontogeny), but the question is open as to whether it holds for other species. However, more studies are needed to check the generality of our predictions.

In summary, we have shown that diet composition (i.e. an environmental factor) significantly affects the capacity for carbohydrate digestion in house sparrow nestlings, but this effect can be fully compensated for soon after fledging. We conclude that even large, phenotypically plastic adjustments of the gastrointestinal tract to diet composition in house sparrow nestlings may have no lasting effect on the digestive physiology of adult birds. In fact, nestling and fledgling house sparrows can continue normal growth and development on a diet whose composition is very different from that consumed by wild birds, even to the extreme of a complete lack of carbohydrates, or following a diet switch directly opposite to that occurring in nature. However, the results of this and earlier studies suggest the presence of a continuum in the magnitude of developmental flexibility of digestive physiology in passerine birds, presumably related to variation in food quantity and quality. Finally, our results indicate that the ontogeny of digestive physiology is not limited to the nestling period and fledglings can still undergo significant changes in their digestive tract (see Stein and Williams, 2006).

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