



Between-population differences in digestive flexibility in the olivaceous field mouse

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ABSTRACT

The flexibility of digestive traits characterizes a standard model of physiological flexibility, demonstrating that animals adjust their digestive attributes in order to maximize overall energy return. Using an intraspecific experimental study, we evaluated the amount of flexibility in digestive tract mass and length in individuals from field mouse populations inhabiting semi-arid and temperate rain forest habitats and acclimated for six months to diets of different qualities. In accordance with the predictions of the theory of digestion, we observed a highly significant relationship between dietary variability and digestive flexibility in both specific digestive chambers and in the total digestive tract mass and length. Specifically, we found higher digestive plasticity in response to diet quality in rodents inhabiting southern temperate ecosystems with higher dietary variability in comparison to individuals from northern semi-arid habitats.

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1. Introduction

Central to our understanding of the physiological responses to different environments is the analysis of the mechanisms that underlie within- and between-species diversity in physiological traits, and the ecological consequences of this variation at different hierarchical levels (Bozinovic et al., 2007). This study attempts to merge an analysis of the mechanistic basis of trait expression with an analysis of the variation of these traits at higher levels of organization over broad geographical scales. The analysis of phenotypic trait variation along a geographical scale is considered a powerful approach for evaluating how physiological traits evolve at both the intra- and inter-specific level (Bozinovic and Rosenmann, 1989; Addo-Bediako et al., 2000; Chown et al., 2004). One of the most important factors causing variations in trait values is phenotypic flexibility. Flexibility is heritable and appears to evolve through natural selection (e.g., Schlichting and Pigliucci, 1998). Ecological and evolutionary physiologists have studied reversible phenotypic flexibility under the paradigm of environmental acclimation and/or acclimatization (Willmer et al., 2000; Garland and Carter, 1994).

The flexibility of digestive traits such as enzymatic and nutrient transport activity, as well as digestive morphology, characterizes a standard model of physiological flexibility (Karasov and Diamond,

1983). Studies have demonstrated that animals adjust their digestive attributes in order to maximize overall energy return (Sibly, 1981). Indeed, recently Naya et al. (2008) applied a meta-analytical approach to literature data of rodent small intestine length flexibility, finding a positive correlation between small intestine length flexibility and latitude, and between the former variable and the number of habitats occupied. This broad statistical analysis is an important piece of evidence on the adaptive value of digestive flexibility in small mammals, and one of the most important conclusions to emerge from this study is that these physiological traits are highly flexible (see also Naya et al., 2007). Nevertheless, because of the nature of that study, the variation between populations was ignored, although this variability is the raw material upon which natural selection acts. By minimizing this variability, interspecific analyses cannot differentiate the proximate factors responsible for variations in a given physiological trait.

In this contribution, we studied the flexibility in digestive tract size in field mice from two contrasting habitats (semi-arid and temperate rain forest) acclimated for six months to diets of different qualities. Testing the effect of diet quality on digestive flexibility is important because numerous studies have observed that digestive traits change by acclimation to environmental temperature and food quality. Indeed, according to digestion theory (Sibly, 1981) animals usually compensate for low quality food by increasing digestive tract dimensions, i.e. by an increase in digestive mass/length (see also Clauss et al., 2008). We aimed to test experimentally at an intraspecific level if there is a positive relation-

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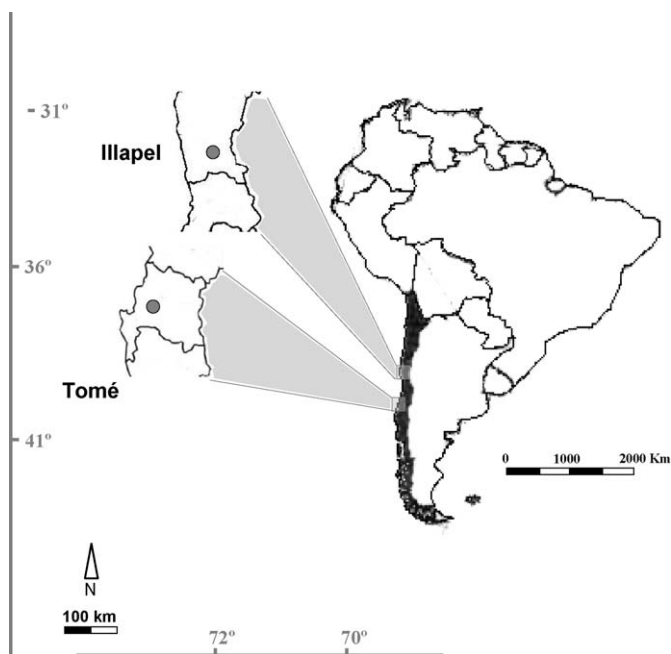


Fig. 1. Geographic localities, latitude and habitats of two populations of the olivaceous field mouse *Abrothrix olivaceus* in Chile.

ship between the amount of flexibility in digestive tract dimension and seasonal variability in the quality of the diet consumed in the wild.

As a study model we used the mouse *Abrothrix olivaceus* (Muridae). In Chile, this species is distributed from approximately 20°S in arid and semi-arid habitats to approximately 53°S in temperate rain forest and Patagonian steppe, where individuals consume poor quality diets in which quality markedly changes (Iriarte, 2008). In contrast to previous studies, which usually evaluated the short-term effect (e.g., some weeks) of highly contrasting diets, we decided to evaluate the long-term effect of two diets that were fairly similar in their composition. We did this because we hypothesized that subtle differences in diet composition might also determine adjustments in gut size, i.e., that digestive attributes are really fine-tuned by organisms.

2. Materials and methods

2.1. Animals and experiments

We used adult males and non-reproductive females of the olivaceous field mouse *Abrothrix olivaceus* (Muridae). From January to April 2008, we captured 12 animals (8 males and 4 females), using Sherman live traps, in Illapel, a semi-arid shrubland in northern Chile (31.5°S, 71.1°W, 310 m above sea level) and 12 animals (5 males and 7 females) in Tomé, a rainy temperate coastal forest in southern Chile (36.6°S, 7.9°W, 35 m above sea level). The distance between both localities is 634.03 km (Fig. 1). Accumulated annual rainfalls are 129 mm (mean monthly rainfall \pm SD = 17.5 \pm 19.8 mm; minimum = 0.5 mm; maximum = 48.7 mm) in Illapel and 1200 mm (mean monthly rainfall \pm SD = 106.6 \pm 94.2 mm; minimum = 14.3 mm; maximum = 213.9 mm) in Tomé. Mean annual temperatures are 14.3 \pm 4.2 °C (minimum = 8.3 °C; maximum = 24.7 °C) in Illapel and 12.4 \pm 2.9 °C (minimum = 7.2 °C; maximum = 18.8 °C) in Tomé. Climatic data were obtained from di Castri and Hajek (1976).

Northern populations of this species are mainly omnivorous while southern populations are restricted to a more herbivo-

rous/granivorous diet with a higher intake of low quality food items such as plants, fungi and lichens (Silva, 2005).

All individuals were transported to the laboratory within a week of capture and were individually maintained for six months in rat cages with a bedding of hardwood chips. The animals were randomly assigned to one of two treatments: high food quality (HFQ) and low food quality (LFQ). HFQ chemical composition was: 86.0% dry matter, 5% crude fiber, 4.0% lipids, 20.5% proteins, 46.0% carbohydrates, total energy content = 19.5 \pm 0.3 kJ/g. LFQ chemical composition was: 90.6% dry matter, 6.5% crude fiber, 3.0% lipids, 20.0% proteins, 40.3% carbohydrates, total energy content = 18.4 \pm 0.5 kJ/g (commercial chow provided by Champion S.A., Santiago, Chile).

After random assignment, the Illapel population was divided into an LFQ group consisting of 2 males and 4 females and an HFQ group consisting of 3 males and 3 females. The Tomé population was divided into an LFQ group consisting of 5 males and 1 female and an HFQ group with 2 males and 4 females. Food and water were always given *ad libitum* to all the groups. Animals were kept in a ventilated room exposed to a L:D = 12:12 photoperiod and to a constant ambient temperature of 21 °C. Animals were sacrificed after six months of dietary acclimation, between 09:00 and 10:00 h, and the complete digestive tract was quickly removed and dissected free of mesenteric attachments, without stretching the tissue. Digestive organs (stomach, small and large intestines and caecum) were washed with saline solution, and their length was measured with a plastic ruler (\pm 1 mm) and a digital caliper (\pm 0.01 mm; Mitutoyo Corp., Kanagawa, Japan). Digestive organs were then dried to constant mass in an oven at 60 °C for 15 days, after which they were weighed with an analytical balance (\pm 0.0001 g; Chyo JK-180, Chyo Balance Corp., Kyoto, Japan) (see Bozinovic et al., 1990).

2.2. Statistical analysis

Differences in body mass (m_b) after the acclimation period were evaluated by two-way ANOVA, with populations and diet treatments as fixed factors. Digestive tract variables by population were evaluated using one-way ANOVA (with diet treatments as the main factor) or one-way ANCOVA if a significant relationship between the dependent variable and body mass was detected. For each comparison, we estimated the standardized size effect (SSE) using Hedges' unbiased estimator (d) and its corresponding asymptotic standard error (Kline, 2004). SSE differences between populations were considered to reflect differing digestive plasticity. The overlap level between asymptotic standard errors within populations is a measure of the magnitude of digestive plasticity. In short, a factor or a value of plasticity is considered statistically significant if the 95% confidence around its effect excludes zero. Prior to the analysis, we tested the test assumptions. Statistical analyses were performed using the statistical package Statistica version 6.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

No significant differences in m_b due to effects of population and dietary treatment were observed, but a time effect could be detected (see Table 1; Tomé initial m_b = 29.8 \pm 0.60 g; Illapel initial m_b = 27.0 \pm 1.4 g; Tomé final m_b = 34.9 \pm 0.90 g; Illapel final m_b = 32.5 \pm 1.8 g).

In agreement with digestive theory, which predicts that animals eating poor quality food will have larger digestive tracts, we did find differences in digestive tract length due to the effect of food quality (Table 2). However, the digestive morphology of individuals from the semi-arid habitat was more constrained than that

Table 1Results of repeated measures ANOVA for body mass. Fisher *F*-test value and associated probabilities are indicated.

	SS	Df	MS	<i>F</i>	<i>P</i> -value
Locality	81.90	1	81.90	2.62	0.12
Diet	33.84	1	33.84	1.08	0.31
Locality × diet	28.99	1	28.99	0.93	0.35
Error	625.72	20	31.29		
Time	341.87	1	341.87	54.98	<0.0001
Time × locality	0.26	1	0.26	0.04	0.84
Time × diet	15.53	1	15.53	2.50	0.13
Time × locality × diet	8.09	1	8.09	1.30	0.27
Error	124.36	20	6.22		

of those from the temperate habitat. Indeed, only caecum length increased significantly in individuals from the semi-arid habitat acclimated to LFQ (Table 2; Figs. 2 and 3). Digestive tracts of individuals from the southern temperate forest were more flexible, and those acclimated to LFQ exhibited higher values for stomach and large intestine masses, and also for stomach, caecum, small and large intestines lengths (Table 2; Figs. 2 and 3).

4. Discussion

Variation in distribution can be explained by data on physiological traits of species and populations (Pither, 2003). Indeed, the limits of geographic ranges call for – among other ecological and evolutionary data – the intrinsic physiological properties to predict their responses to environmental biotic and abiotic variables and how they may affect the range of species and populations (Stevens, 1989; Ispolatov and Doebeli, 2009). Studies of phenotypic variation expressed by the same species in different habitats have been central in evolutionary ecology, but have not yet been used very much in evolutionary physiology (Piersma and Drent, 2003).

In this study, we found greater digestive plasticity in populations from a higher latitude, which appears to be correlated with seasonal changes in diet quality. Specifically, we found that digestive flexibility is more noticeable in the temperate forest population, where strong seasonal changes in diet quality occur. In northern Chile, *A.*

olivaceus is omnivorous throughout all seasons, with an ingestion of high quality food items such as arthropods and insect larvae ranging from 17 to 31%. The southern populations of this species exhibited a more herbivorous diet with a higher intake of low quality food items such as plants, fungi and lichens (Silva, 2005).

Our experimental design was conservative regarding population comparisons. Indeed we used, as this kind of study usually does (e.g., Naya et al., 2008), relatively high diet qualities. Thus, we were working at the edge of the reaction norm. Since we found differences for a narrow range of experimental diets, our results strongly suggest that both populations in fact differ in digestive tract flexibility.

These data seem to support our predictions since digestive traits increase by acclimation (Gross et al., 1985; Green and Millar, 1987; Bozinovic et al., 1988), acclimatization (Gebczynska and Gebczynski, 1971; Derting and Noakes, 1995), or local adaptation to poor food quality (Corp et al., 1997; Sassi et al., 2007). Indeed, digestion theory predicts that animals will compensate for low quality food by increasing tract dimensions, i.e., increasing digestive mass and/or length.

Although the study of different populations – in this case two populations – is a first approach to understanding whether differences in reversible phenotypic plasticity have a genetic basis (Garland and Adolph, 1991; Kawecki and Ebert, 2004), it is necessary to be cautious in interpreting the differences as genetic.

Table 2Arithmetic means (±SE) for digestive organs (dry mass in g and length in cm) of individuals of *Abrothrix olivaceus* from two contrasting geographic localities and acclimated to high and low food quality. Fisher *F*-test value and associated probabilities are indicated.

Habitat/locality	High food quality	Low food quality	<i>F</i> _{1,22}	<i>P</i> -value
<i>Northern semi-arid (Illapel)</i>				
Stomach				
Mass	0.083 (0.012)	0.085 (0.016)	0.03	0.861
Length	24.33 (3.38)	26.83 (3.81)	1.44	0.258
Small intestine ^a				
Mass	0.163 (0.016)	0.145 (0.012)	1.58	0.240
Length	368.33 (41.52)	349 (29.77)	1.23	0.293
Ceacum				
Mass	0.074 (0.019)	0.063 (0.020)	0.763	0.403
Length	30.5 (4.13)	40.16 (6.49)	9.460	0.012
Large intestine				
Mass	0.044 (0.014)	0.039 (0.017)	0.383	0.550
Length	100.33 (7.26)	102.66 (10.68)	0.033	0.860
<i>Southern temperate forest (Tomé)</i>				
Stomach				
Mass	0.098 (0.026)	0.141 (0.022)	9.48	0.012
Length	23.16 (2.13)	25.33 (0.516)	5.83	0.036
Small intestine				
Mass	0.279 (0.059)	0.306 (0.042)	0.827	0.385
Length	335.83 (35.69)	382.16 (43.0)	4.12	0.070
Ceacum				
Mass	0.106 (0.100)	0.111 (0.027)	0.013	0.910
Length	27.833 (7.16)	36.833 (5.56)	5.90	0.035
Large intestine				
Mass	0.037 (0.016)	0.070 (0.020)	9.80	0.011
Length	52.83 (17.29)	89.66 (8.68)	21.74	0.001

^a Analysis of co-variance was applied when a significant relationship between body mass and the dependent variable was detected.

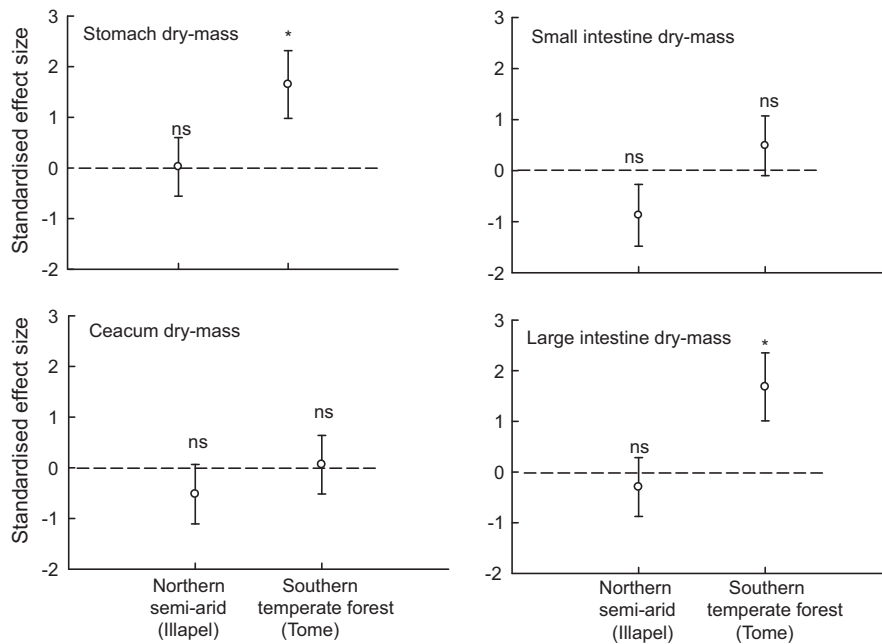


Fig. 2. Effect of dietary acclimation on the dry mass of digestive chambers and total digestive tract for two populations of the olivaceous field mouse. The standardized size effect (magnitude of digestive plasticity) was estimated as well as its corresponding asymptotic standard error. The overlap level between asymptotic standard errors within populations was used to contrast the magnitude of digestive plasticity. *Significant, ns = non-significant standardized size effect.

In fact, without an explicit approach, our results only show that changes in the size of the digestive chambers and the whole tract (size effect) are adaptive in the temperate climate. Also, since digestive adjustments in small mammals typically occur after 1–2 weeks of acclimation (Bozinovic et al., 1988, 1990), the length of our experiment allows us to be confident regarding the presence of plasticity.

Several studies have attempted to link the plasticity of digestive mechanisms to dietary flexibility and vice versa (Karasov and Diamond, 1988). The ability to modulate digestive physiology according to diet quality may be advantageous in environments

with unpredictable resources. From this perspective, if these populations have inhabited their habitats long enough to permit adaptation, the population inhabiting temperate rain forests would be expected to present greater phenotypic flexibility than the population dwelling in a semi-arid habitat. This is because a higher phenotypic flexibility seems to be more important than the maintenance of high, constant capacities and a large digestive tract size, since the latter would involve higher maintenance costs and comparatively lower energy savings. It is generally accepted that organism performance must be the result of the integrated phe-

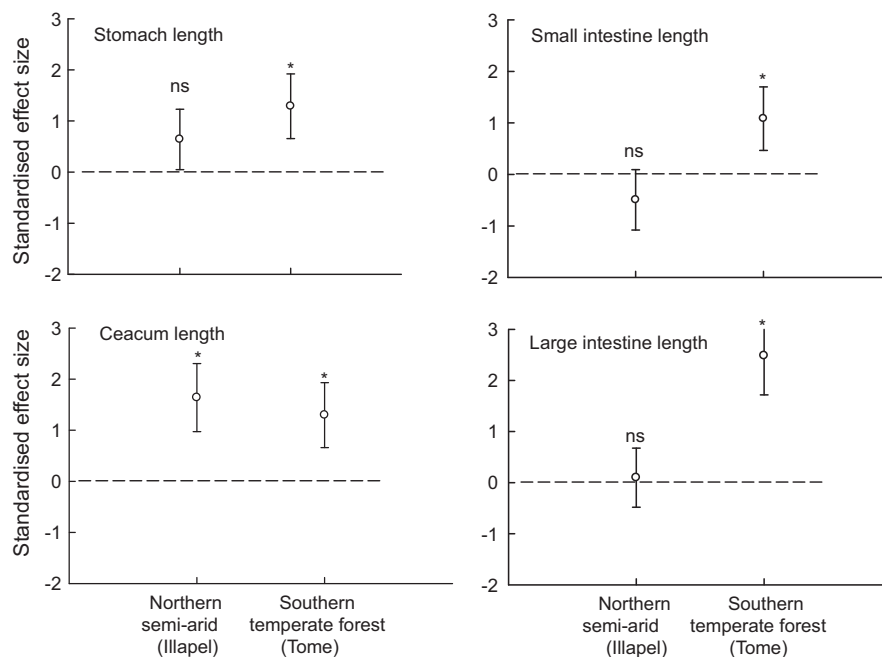


Fig. 3. Effect of dietary acclimation on the length of digestive chambers and total digestive tract for two populations of the olivaceous field mouse. The standardized size effect (magnitude of digestive plasticity) was estimated as well as its corresponding asymptotic standard error. The overlap level between asymptotic standard errors within populations was used to contrast the magnitude of digestive plasticity. *Significant, ns = non-significant standardized size effect.

notypic functioning of different levels of biological organization. This fact implies that any underlying structure/organ can set limits to the performance, even if the performance is below its intrinsic physiological maximum. Several experimental studies as well as statistical evidence have suggested that rates of energy expenditure are centrally limited. That is, regardless of the particular mode of energy expenditure, shared features of energy acquisition and utilization limit energy expenditure to a common value (Drent and Daan, 1980). Also, digestive features are able to change in response to environmental cues, indicating that if there is a digestive limit to expenditure it is not rigid but rather flexible. The match between function and induced plasticity in morphology, together with the correspondence between empirical data and theoretical models, supports the hypothesis that digestive tract size flexibility is an adaptation. This idea stems from the fact that animals with higher metabolic expenditures should support their demand by increasing food intake which needs to match rates of digest flow and nutrient absorption, which also requires an increase in the size of digestive chambers.

This kind of within-species comparative study appears to be of paramount importance to test the adaptive value of phenotypic flexibility (Doughty and Reznick, 2004), which in this paper was digestive flexibility embedded within a dietary variability hypothesis.

Finally, we observed that individuals from temperate ecosystems not only exhibited a steeper slope in their reaction norm but also a higher intercept, i.e. digestive organ masses were noticeably higher (by nearly 50%) in individuals from the southern temperate forest. This result is probably correlated with the higher consumption of plant material in the latter ecosystem. It is well known that individuals from populations of *Clethrionomys glareolus* and *Microtus agrestis* that consume greater amounts of plant material have larger digestive tracts than animals from populations that mainly live on seeds (Hansson, 1985; Hansson and Jaarola, 1989), and that specimens of *Apodemus sylvaticus* from a seed-eating population have larger small intestines than specimens from a population that largely predares on invertebrates (Corp et al., 1997).

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