

# Ontogenetic integration between force production and force reception: a case study in *Ctenomys* (Rodentia: Caviomorpha)

Aldo I. Vassallo,<sup>1</sup> Federico Becerra,<sup>1</sup> Alejandra I. Echeverría<sup>1</sup> and Adriá Casinos<sup>2</sup>

<sup>1</sup>Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Instituto de investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata – CONICET, Dean Funes 3250, Mar del Plata, 7600, Argentina; <sup>2</sup>Departamento de Biología Animal, Facultat de Biología, Universitat de Barcelona, Avinguda Diagonal 643, Barcelona, Spain

## Keywords:

development, mammals, behaviour, functional morphology, bite force

Accepted for publication:

5 January 2015

## Abstract

Vassallo, A.I., Becerra, F., Echeverría, A.I., Casinos, A. 2015. Ontogenetic integration between force production and force reception: a case study in *Ctenomys* (Rodentia: Caviomorpha) —*Acta Zoologica* (Stockholm) 00: 000–000.

During ontogeny, complex adaptations undergo changes that sometimes entail different functional capabilities. This fact constrains the behaviour of organisms at each developmental stage. Rodents have ever-growing incisors for gnawing, and a powerful jaw musculature. The incisors are long enough, relative to their diameter, to be affected by bending stresses. This is particularly true in the subterranean *Ctenomys* that uses its incisors for digging. We measured bite force (BF) in individuals of different ages using a force transducer. We estimated incisor section modulus  $Z$ , a geometrical parameter proportional to bending strength. A relative strength indicator was calculated as  $S = Z/\text{BF}$  incisor length. We found that ontogenetic BF scales to body mass with positive allometry. However, an ANOVA showed non-significant differences in  $S$ , neither between sexes nor among age classes. This result implies that during growth, incisors might have a rather similar ability to withstand bending stresses from increasing masticatory forces, what may be considered evidence of ontogenetic integration of force production (by muscles) and force reception (by the incisors). This fact well correlates with the observation that pups and juveniles of *C. talarum* incorporate solid foods shortly after birth, and they are able to dig burrows early in life.

Aldo I. Vassallo, IIMyC – Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Dean Funes 3250, (7600) Mar del Plata, Argentina. E-mail: avassall@mdp.edu.ar

## Introduction

When functional morphology is applied to the study of groups of organisms possessing particular specializations, this discipline highlights adaptations to distinctive functional demands or behaviours (Hildebrand 1985; Schwenk 2000; Liem *et al.* 2001; Becerra *et al.* 2012a). Regarding the mandibular apparatus, these studies include, among other aspects, the assessment of dental adaptations related to particular feeding habits (Biknevicius *et al.* 1996; Evans *et al.* 2005), analyses of the temporomandibular joint (Greaves 1980; Verzi and Olivares 2006) and analyses of muscle mechanical advantage in durophagous or digging vertebrates that need to exert significant bite forces to adequately perform in relation to their distinctive lifestyles (Cleuren *et al.* 1995; Herrel *et al.* 2002; Christiansen

2007; Becerra *et al.* 2011). With regard to complex adaptations, defined as those involving multiple characters (e.g. osteological, myological and dental characters), an important issue to address is how development proceeds regarding sets of characters that must act altogether to perform particular behaviours (Carrier 1983; Lowry and Motta 2007). As usually the design of a certain structure or adaptation must be apt (i.e. ‘it should work’) before attaining adulthood, an ontogenetic approach is of particular interest. This capacity does not necessarily imply similar performance between adults and juveniles, but rather an adequate performance according to the functional demands on each stage (see, for example, Galis 1993; Carrier 1996).

The development of complex adaptations was addressed, among others, by Smith (1996) and Herring (2010) when

referring to the concept of ‘ontogenetic integration between form and function’, defined as the genetic, epigenetic or functional association between elements via a set of causal mechanisms, in such a way that any change in one element is reflected by a change in another. Specifically, for the musculo-skeletal system, there are two key issues that Herring (2010) mentioned: the force production and reception (by the muscles and the bones, respectively). It can be expected that both features should be associated during the generation of viable phenotypes.

Within mammals, subterranean rodents have been the focus of numerous researches, mainly because they present many morphological, physiological and behavioural specializations related to the underground niche (Nevo 1999; Begall *et al.* 2007). *Ctenomys* (Rodentia: Caviomorpha; usually called ‘tuco-tucos’ or ‘ocultos’, a name meaning ‘the hidden’) is a widely distributed genus of South American subterranean rodents that uses both the fore-claws and the incisors (both upper and lower) for digging (Vassallo 1998; Becerra *et al.* 2013). This group of animals is characterized by living underground in burrowing systems and conducting the vast majority of their activities in there, that is performing only short excursions on the surface. Hence, a key behavioural trait of these kinds of rodents is the achievement of food sources distant from the burrow openings, only by the extension of their galleries (Lacey *et al.* 2000). Ecological and behavioural traits and comparative digging energetics of the genus were assessed in previous studies such as Malizia *et al.* (1991), Cutrera *et al.* (2010), and Luna *et al.* (2009), among others. The powerful mandibular apparatus of tuco-tucos mainly performs three functions: feeding, digging and aggressive biting, the latter being linked to agonistic intermales encounters, as tuco-tucos are highly territorial and possess a polygynous mating system (Zenuto *et al.* 1999; Becerra *et al.* 2012a). Like other subterranean rodents that use their incisors to assist in loosening soils (chisel-tooth digging; e.g. Van Daele *et al.* 2009), *Ctenomys* exerts forces at the incisor tip that are relatively high when compared to other rodents of similar body mass but different ecology and locomotor behaviours (e.g. *Ctenomys australis* versus *Chinchilla laniger* and *Octodon degus*; see Becerra *et al.* 2014). It is noteworthy that rodent incisors are long enough, relative to their basal diameter, to be significantly affected by bending stresses (Fig. 1), and this is particularly true in *Ctenomys*, which uses its large and procumbent incisors during burrowing (Mora *et al.* 2003).

In interspecific and ontogenetic scaling studies, the two hypotheses currently accepted for predicting scaling changes among individuals are based on the assumption that shape is similar (‘geometrical similarity’) or, inversely, that the increasing body mass should generate a differentiated growth in diameters and lengths (‘elastic similarity’) and, hence, in proportions and shape (see Schmidt-Nielsen 1991 for review; see also Alexander 1985). However, an important open question is whether animals from different ontogenetic stages can be considered geometrically similar (Valverde *et al.* 2005). With

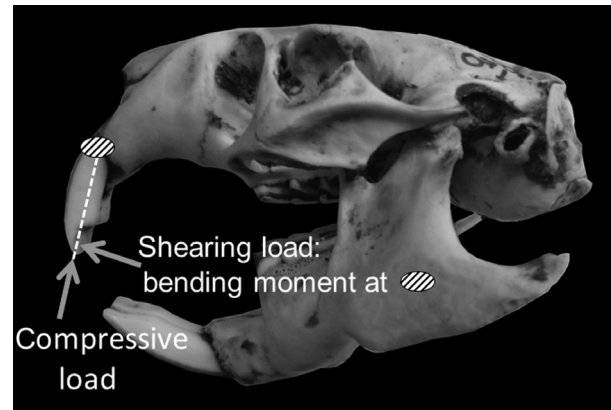


Fig. 1—Skull of *Ctenomys talarum* showing compressive and shearing loads upon incisors.

respect to the production and reception of forces by the jaw apparatus, some predictions are of relevance. If an animal is supposed to maintain geometric similarity throughout its ontogeny, muscle cross-sectional area should scale to (body mass)<sup>0.67</sup> and, hence, bite force should scale to (body mass)<sup>0.67</sup>. On the other hand, canine or incisor section modulus ( $Z$ , a geometrical parameter proportional to bending strength) should scale to (body mass)<sup>1.00</sup> and to (bite force)<sup>1.50</sup> (Becerra *et al.* 2011, 2012b). The specific scaling of body parts or features probably underlies the emergence of complex adaptations.

Previous researches have shown that the digging behaviour in different species of the genus *Ctenomys* is already present shortly after birth (Echeverría 2011; Echeverría and Vassallo 2011). Yet, in those taxa, this particular conduct involves both force production and the corresponding force reception. Therefore, to test the existence of a complex adaptation between the structures that make possible both activities, namely mandibular muscles and teeth, the analyses must be carried out in terms of ontogenetic trajectory. This study focuses on the force produced by the mandibular adductor muscles, but it cannot be excluded the possibility of the chisel-tooth digging being accomplished by the recruitment of the neck muscles too. As previous studies on complex adaptation have been focused on adults (e.g. Lessa *et al.* 2008), the linkage between force production and reception in tuco-tucos would be a clear example of ‘ontogenetic integration between form and function’, as suggested by Herring (2010) (see above). Briefly, we were handling a case study in evolutionary morphology. The test of this possible ontogenetic integration was the aim of this research. For that goal, the *in vivo* bite forces, as the outcome of the effective muscle force production (e.g. Becerra *et al.* 2013), were compared to the incisor strength, estimated by their section modulus (see, for example, Irgens 2008). We assumed the hypothesis of geometrical similarity between structures, along different ontogenetic stages, to predict scaling changes. Finally, as bite force values

are available from specimens of different ages, we hypothesized that similar relative values of incisor bending strength throughout ontogeny indicate the existence of integration between force production and reception.

## Materials and Methods

### Specimens

The study was based on 51 live specimens of *C. talarum* (24 females; 27 males) of different ages: pups (<40 g;  $n = 9$ ), juveniles (40–100 g;  $n = 13$ ) and adult individuals (>100 g;  $n = 29$ ). Individuals were collected from August to November 2008 from a population occupying natural grasslands and dune habitats near the coastal village of Mar de Cobo, Buenos Aires Province, Argentina (37°45'S, 57°56'W). Animals were captured using tubular mesh live traps located at burrow entrances. They were housed individually in plastic cages (42 cm × 34 cm × 26 cm) with wood shavings as bedding. The animal room was maintained within the thermoneutrality range of the study species (24 ± 1 °C). All animals were fed *ad libitum* with a diet composed of a variety of vegetables (see Zenuto *et al.* 2001), and they were regularly weighed using an electronic scale (0.01 g). All procedures followed ABS/ASAB Guidelines for the Treatment of Animals in Behavioral Research, and Argentinean laws. The use of *C. talarum* was approved by CONICET (National Council of Scientific and Technical Researches, Argentina) and University of Mar del Plata, Argentina. After all the *in vivo* measurements were registered, individuals were released at good physical condition at the site where they were captured.

### In vivo bite force measurement

Individual bite force (BF) at incisor tip was measured with a strain gauge load cell force transducer [by Necco Technologies, Mar del Plata, Argentina, 0–20,000 g (range), error 1 g; for a more detailed description, see Becerra *et al.* (2011)] during sessions in which animals were induced to bite the transducer plates. These plates were covered with a thin protective coating made of leather, to prevent animals from damaging their incisors. The temporal sequence of the experiments was as follows: (i) animals were induced to bite defensively by taking them out of their cages; (ii) each session lasted ~1 min and consisted of biting trials that included several bites; (iii) trials ended when the animals refused to bite the transducer, which in some instances occurred after 30–40 s of recording; and (iv) each session was repeated 4–6 times per individual. Bite force measurements were recorded on a PC using the software Terminal (Windows XP, Microsoft), and the highest recording from all sessions was assumed to represent the maximum bite performance for each individual, following Becerra *et al.* (2011). Incisor width ( $Iw$ ) and depth ( $Id$ ) were measured in the same ontogenetic series using a digital calliper (to the nearest 0.01 mm). Other body measurements such as

body mass, body length, naso-occipital length, and tail length were also recorded.

### Incisor strength

The section modulus ( $Z$ ) is a geometric parameter corresponding to a given cross section used in engineering to design beams and other structures subjected to bending stress. In biomechanics, it is used to study the resistance of long bones and teeth. The section modulus to anteroposterior bending was estimated considering the incisor cross-sectional area as an ellipse (being the major axis  $Iw$ , and minor axis  $Id$  represented by its mediolateral and anteroposterior diameters, respectively; see Verzi *et al.* 2010) by the following equation:

$$Z = \pi \cdot (Iw) \cdot (Id)^2 / 32$$

Then, following Alexander (1983, 1985), we defined a relative bending strength indicator for the incisors ( $S$ ) as:

$$S = Z / BF \cdot l$$

where the incisor length ( $l$ ) is the distance from the distal end of the incisor to its base where it enters the premaxilla (labial side). The strength indicator (i.e. a ratio of section modulus to bending moment) includes the *in vivo* bite force values (BF) and the incisor length, which is needed to consider the bending moment at the basal section of the incisor (Fig. 1, dashed area). Due to the difficulty of measuring the incisor length in live specimens given the presence of soft tissues, this variable was estimated from regressions of incisor length versus naso-occipital length measured in preserved skulls of both juvenile and adult individuals matching the size range of live individuals. This estimation was possible as the occipital region remains well marked in live specimens and allows the comparison with preserved skulls. The regression equations were  $y = 0.25 \times -3.50$ ,  $R^2 = 0.93$  (females,  $n = 14$ ), and  $y = 0.30 \times -4.42$ ,  $R^2 = 0.94$  (males,  $n = 15$ ). Naso-occipital length measured in live specimens allowed to estimate incisor length using these equations.

### Statistics

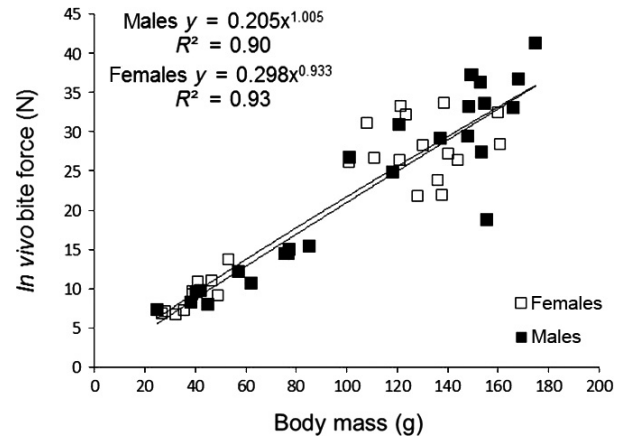
Allometric analyses were carried out assuming Model II (standardized major axis regression) (Sokal and Rohlf 1995; Warton *et al.* 2006). Confidence intervals at 95% were calculated for both the  $y$ -intercept ( $a$ ) and the exponent ( $b$ ) of the allometric equation  $y = a \times b$  (Huxley 1932) using the software SMATR (Falster *et al.* 2006). ANCOVAs on bite force and incisor section modulus were performed, with body mass as the continuous variable and sex as categorical predictor. Changes during postnatal ontogeny in the incisor relative strength indicator ( $S$ ) were analysed using a two-way analysis of variance (two-way ANOVA). Factors (independent variables) used in the two-way ANOVA were age category (pups, juveniles and adults), sex (female, male) and the age category by sex interaction. We included the sex as a factor to determine whether sexual

dimorphism needs to be accounted for in the analyses. ANOVA was performed using STATISTICA v10.0 (StatSoft, Milton Keynes, UK).

**Results**

In both sexes, the bite force at incisor tip scaled to body mass with positive allometry and the allometric coefficient was significantly higher ( $F_{1,24} = 47.80$  and  $F_{1,23} = 40.40$ ;  $P < 0.001$ , males and females) than the predicted one (0.67) under geometric similarity (Table 1, Fig. 2). No significant differences were observed between sexes, neither in the slope nor in the y-intercept, as it is shown by confidence intervals (Table 1) and the ANCOVA results ( $F_{1,47} = 0.02$ ,  $P = 0.89$ ).

In both sexes, the section modulus ( $Z$ ) of upper and lower incisors, which was calculated on the basis of incisor measurements taken from individuals of the same ontogenetic series, scaled to body mass with positive allometry; that is, exponents were significantly higher than 1 (upper incisors  $F_{1,23} = 62.0$  and  $41.9$ ; lower incisors  $F_{1,23} = 66.40$  and  $72.50$ ; males and females;  $P < 0.001$ ) (Table 1, Fig. 3). Again, no significant differences between sexes were observed neither in the slope nor in the y-intercept, according to confidence intervals (Table 1) and to ANCOVA results ( $F_{1,47} = 1.01$ ,  $P = 0.32$ , and  $F_{1,47} = 2.22$ ,  $P = 0.14$ , for upper and lower incisors, respectively). On the other hand, when incisor section modulus was regressed against bite force, no allometric coefficient was significantly different from the isometric value of 1.5 in either sex (upper incisors  $F_{1,23} = 0.34$  and  $1.57$ ,  $P = 0.56$  and  $0.22$ , males and females; lower incisors  $F_{1,23} = 1.83$  and  $0.68$ ,  $P = 0.19$  and  $0.42$ , males and females) (Table 1). Once again, considering both confidence intervals (Table 1) and ANCOVA results, no significant differences between sexes were



**Fig. 2**—Regression of *in vivo* bite force versus body mass in an ontogenetic series of *Ctenomys talarum*.

observed, neither in the slope nor in the y-intercept (ANCOVA:  $F_{1,47} = 1.15$ ,  $P = 0.29$ , and  $F_{1,47} = 3.24$ ,  $P = 0.08$ , for upper and lower incisors, respectively). After the body size effect was controlled for the incisor section modulus ( $Z$ ) and the bite force (BF), residuals for both variables were regressed against each other and significant results were obtained for both upper ( $F_{1,48} = 7.16$ ;  $P = 0.01$ ) and lower incisors ( $F_{1,48} = 20.32$ ;  $P < 0.01$ ). These residuals were positively correlated (Fig. 4). Finally, it was found that incisor relative strength indicator ( $S$ ) was not predicted by the body mass. For both sexes, the regressions of  $S$  (upper and lower incisors) against body mass did not depart significantly from the null (zero) slope (Fig. 5). Mean values of  $S$  were  $0.0076 \text{ mm}^2/\text{N}$  (CV 45.6%) and  $0.0047 \text{ mm}^2/\text{N}$  (CV 34.6%), for upper and lower incisors, respectively. When considering the three age

**Table 1** Allometric equations ( $y = ax^b$ ) relating *in vivo* bite force (BF), incisor section modulus ( $Z$ ) and body mass in an ontogenetic series of Talas’ tuco-tuco (*Ctenomys talarum*)

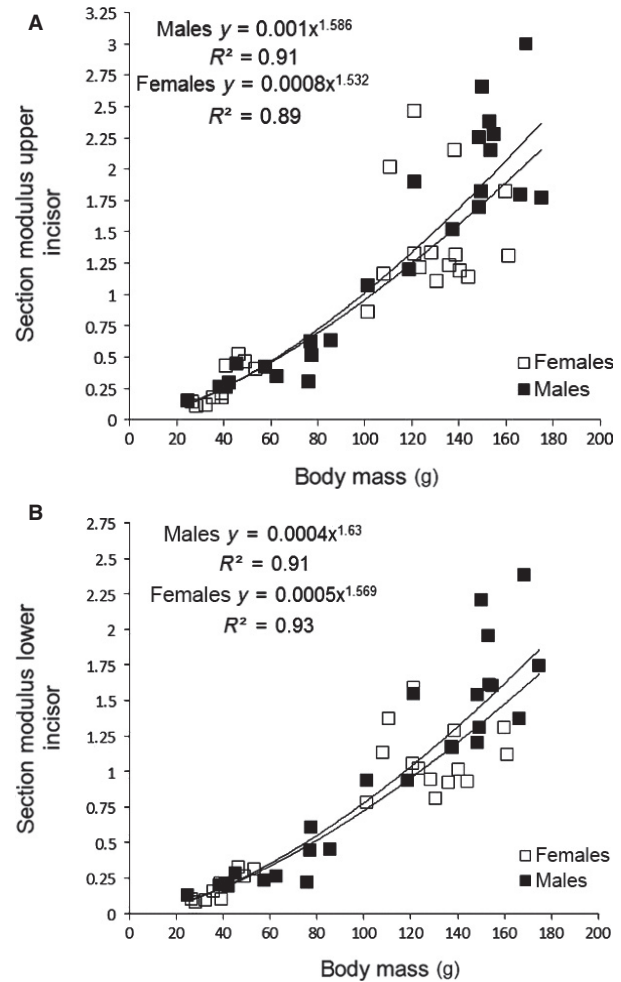
Equation	$n$	$R^2$	95% confidence interval	
			$b$	$a$
<b>Bite force versus body mass</b>				
Males $y = 0.205 \times^{1.005}$	26	0.90	0.884–1.144	0.1129–0.3726
Females $y = 0.298 \times^{0.933}$	25	0.93	0.832–1.046	0.1856–0.4783
<b>Z upper incisors versus body mass</b>				
Males $y = 0.001 \times^{1.586}$	25	0.91	1.399–1.797	0.0003–0.0017
Females $y = 0.0008 \times^{1.532}$	25	0.89	1.332–1.761	0.0003–0.0022
<b>Z lower incisors versus body mass</b>				
Males $y = 0.0004 \times^{1.630}$	25	0.91	1.435–1.857	0.00016–0.0011
Females $y = 0.0005 \times^{1.569}$	25	0.93	1.402–1.757	0.00024–0.0012
<b>Z upper incisors versus bite force</b>				
Males $y = 0.0087 \times^{1.553}$	25	0.92	1.375–1.754	0.0049–0.0154
Females $y = 0.0061 \times^{1.643}$	25	0.88	1.415–1.907	0.0030–0.0126
<b>Z lower incisors versus bite force</b>				
Males $y = 0.0004 \times^{1.632}$	25	0.91	1.435–1.857	0.00016–0.0011
Females $y = 0.0005 \times^{1.569}$	25	0.93	1.402–1.757	0.00024–0.0012

classes in the analysis (pups, juveniles and adults), non-significant differences were observed in  $S$  neither between sexes (two-way ANOVA;  $F_{1,44} = 0.19$ ,  $P = 0.66$ ) nor between ages ( $F_{2,44} = 0.70$ ,  $P = 0.50$ ).

## Discussion

The main finding of the present study was that, even though ontogenetic bite force (BF) in the rodent *Ctenomys talarum* scales against body mass with a positive allometry, there were non-significant differences in the relative strength indicator for the incisors ( $S$ ) between either sexes or age classes. This result implies that the incisors have a rather similar ability to withstand bending stresses from increasing masticatory forces throughout postnatal ontogeny, suggesting an integration of force production (by the jaw adductor muscles) and force reception (by the incisors). This assertion is further supported by the fact that in the studied ontogenetic series, the residuals of BF were significantly related to those of incisor section modulus.

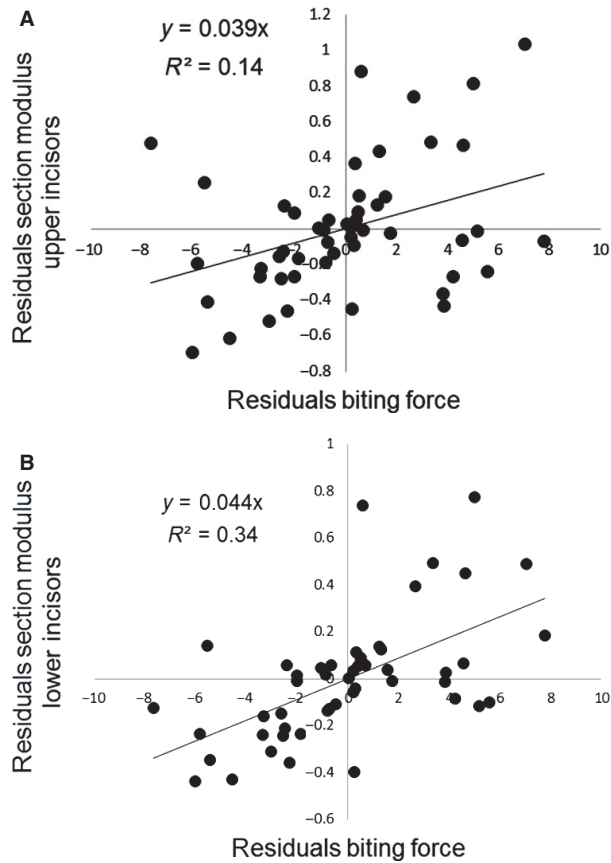
Relatively elongated dental pieces such as carnivore canines and rodent incisors are not supportive structures – in the same way than bones are – although they are subjected to loads imposed by masticatory muscles and food reaction forces (Feldhamer *et al.* 2007; Ungar 2010). Interspecific analyses have shown that a significant proportion of the adaptations exhibited by different teeth morphologies is dictated by loading regimes related to particular trophic habits and the action of the jaw adductor musculature. For example, by studying the upper incisor design in living felids, canids and hyaenids, Biknevicius *et al.* (1996) and Christiansen and Adolfsson (2005) showed that incisor strength to anteroposterior bending was explained by their differences in killing and feeding behaviours. Within the order Rodentia, Vieytes *et al.* (2007) studied the incisor enamel microstructure in species of octodontoid rodents having different digging adaptations, pointing out that the primary functional requirements of dental enamel are resistance to abrasive wear and prevention of the propagation of internal fractures. These authors found relatively higher values of the external index ( $EI$ , calculated as the ratio between the thickness of radial enamel + zone of prismless enamel and the total enamel thickness) in species specialized to chisel-tooth digging such as the extinct genus *Eucelophorus*. In addition,  $EI$  values of other studied taxa showed a gradient from lowest values in the arboreal *Dactylomyia* and the fossorial *Octodontomys* to highest in the subterranean *Ctenomys talarum* – the species in our study – which digs in relatively hard soils (Vassallo 1998). Vieytes *et al.* (2007) suggested that, beyond the phylogenetic constraints, dental structure and microstructure may display particular adaptations according to certain functional requirements. Taking into account that chisel-tooth digging in subterranean rodent species implies much stronger reaction forces upon the incisors than ground-dwelling or arboreal behaviours, Becerra *et al.* (2012b) found that some genera previously identified as



**Fig. 3**—Regression of incisors' section modulus ( $Z$ ) versus body mass in an ontogenetic series of *Ctenomys talarum*. —**A.** upper incisors —**B.** lower incisors.

subterranean or fossorial (tuco-tucos, *Ctenomys*; coruros, *Spalacopus*; and viscachas, *Lagostomus*) presented both a powerful jaw adductor musculature and a relatively high incisor bending strength. Therefore, the remaining question is as follows: Are both features functionally associated during ontogeny?

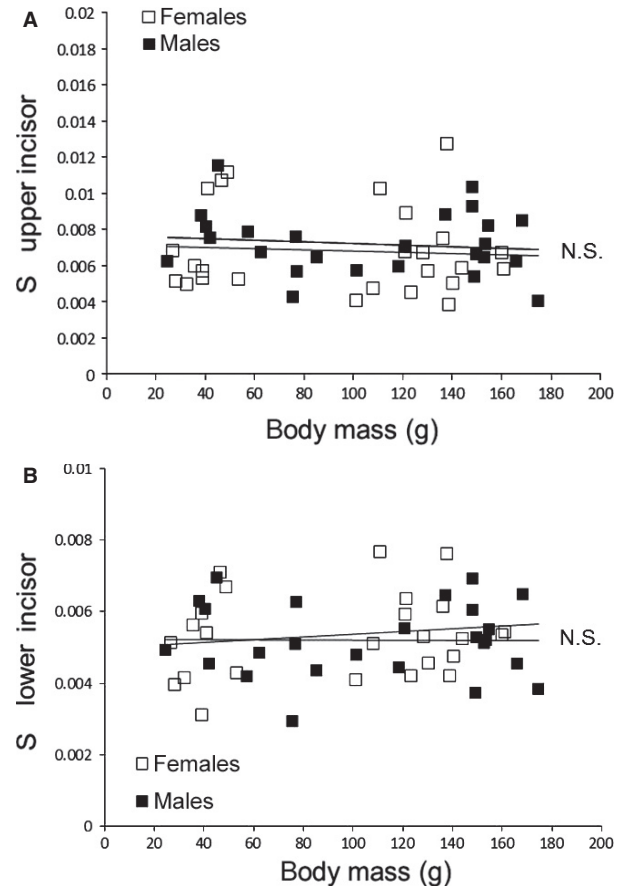
As it was previously stated, ontogenetic integration refers to the genetic, epigenetic or functional association among elements via a set of causal mechanisms, in such a way that any change in one element is reflected by a change in another (Smith 1996). An ontogenetic integration between force production (by muscles) and force reception (by bones or dental pieces) is one of the ways how this phenomenon may occur. Herring (2010) suggested that integration is presumably the result of the selection of a mechanism matching body systems that work together, which is crucial for the emergence of particular types of adaptations. In the ontogenetic series of *Ctenomys talarum*, we found that the regression analyses of the



**Fig. 4**—Residuals of incisors' section modulus and bite force, both versus body mass, regressed now against each other (both sexes pooled), in an ontogenetic series of *Ctenomys talarum*. —**A.** upper incisors —**B.** lower incisors.

relative strength indicator  $S$  against body mass do not significantly depart from the zero slope, and non-significant differences were observed between sexes (Fig. 5 and two-way ANOVA). Accordingly, throughout postnatal development, the incisors have a similar ability to withstand loading stresses in spite of increasing masticatory forces. We suggest that this similarity occurs precisely because of a growth pattern consisting of both bite forces and incisor section modulus scaling to body mass in a similar way with positive allometry (Figs 2 and 3; Table 1). However, when bite force and incisor section modulus were regressed against each other, it was found to be an isometric relationship (Table 1).

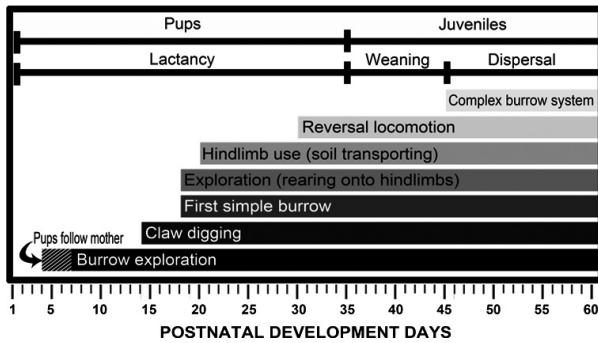
As it was pointed out by Ford and Corruccini (1985), ontogenetic scaling greatly varies among different species. The scaling of mechanical stress on bones or other structures, such as teeth, could be very different. In the case of dental pieces, stresses might vary according to loading or trophic habits, as it was already quoted above. However, specific predictions have been made for the scaling of ontogenetic series. For example, Miller and Burton (2001) postulated positive



**Fig. 5**—Plot of incisors' relative bending strength indicator ( $S$ ,  $\text{mm}^2/\text{N}$ ) versus body mass in an ontogenetic series of *Ctenomys talarum*. —**A.** upper incisors —**B.** lower incisors.

intraspecific allometry for sexually selected traits (SS), while non-SS traits might rather display isometry or negative allometry, although this prediction shows important exceptions, as it was stated by Valverde *et al.* (2005). In *C. talarum*, both the section modulus and the bite force scale to body mass faster than what was predicted for geometrically similar animals (see Introduction). However, as non-significant differences are observed between sexes, there is no reason to consider any of those traits as sexually selected. On the other hand, other dental traits, such as the incisor procumbency, show sexual dimorphism in this species even after controlling for body size (Becerra *et al.* 2012a).

The functional association between force production and reception is further supported by the positive relationship between the residuals of the incisor section modulus and the residuals of bite force (Fig. 4). This significant association can be considered evidence of integration because it suggests that the individuals that were able to exert higher bite forces also had strengthened incisors, and *vice versa*. It should be noted that although we took into account the highest recorded value



**Fig. 6**—Postnatal ontogeny of salient behaviours related to digging in *Ctenomys talarum* (modified from Echeverría 2011).

from all sessions in our *in vivo* bite force measurements, the variability introduced by their behaviour (i.e. willingness to bite) might be the cause of the low  $R^2$ . Our analysis was based upon a geometrical parameter, the incisor section modulus, which is proportional to the bending strength (see, for example, Alexander 1983; Bacigalupe *et al.* 2002). As in the ever-growing rodent incisors, dentin and enamel continue being deposited after tooth eruption and, therefore, their mechanical properties might change (Plikus *et al.* 2005), future studies should consider ontogenetic variation in the strength of incisor materials.

The results have a further implication regarding some aspects of the behavioural ontogeny of this species. For example, as it was observed by Echeverría (2011); Fig. 6, *C. talarum* pups engage in digging activities shortly after birth (~14 days old). When they are 20 days old (shortly after thermoregulatory independence age, which occurs near postnatal day 15), well before natal dispersion (~60 days), pups are able to construct simple burrows to shelter, extending in some cases the natal burrow system. Ending the weaning age, juveniles have the ability to excavate complex burrows with nest chamber and feeding tunnels. Due to the considerable reaction forces that result from soil breaking, digging produces significant loads on the claws, bones and incisors (i.e. the ‘digging tools’). Therefore, the rather similar ability to withstand loading stresses (i.e. similar  $S$  values) by the incisors during postnatal ontogeny may be related to the early manifestation of digging behaviour in this species.

Another example of developmental integration between force production and force reception comes from experimental studies using foods of different hardness. Ravosa *et al.* (2010) showed that rabbits which grew up being fed on an overuse diet (i.e. fracture resistant), developed larger cross-sectional areas in jaw muscle fibres and relatively increase the amount of type II fibres, which confers an elevated force-generating capability. As a counterpart, when compared to animals that were fed on a soft diet, the former exhibited higher levels of jaw-joint biomineralization;

that is, their force reception structures became strengthened. Current hypotheses propose that the developmental integration between force production and force reception is probably driven by the fact that bones and dental pieces respond to mechanical stimuli arising from muscle loading, most likely through cell signalling involving mechano-transduction (see, for example, Farnum 2007; and references therein), which has been early regarded as an epigenetic process (Carter *et al.* 1998). For example, it was found that during bone formation, skeletal unloading induces resistance to hormone–insulin growth factor I (IGF-I) and, thus, inhibits the activation of IGF-I signalling pathways, probably partially by downregulation of integrin cell signalling (Sakata *et al.* 2004). As a consequence of this unloading, it was observed a decrease in the proliferation of osteoblasts and their osteoprogenitor cells. Similar regulatory effects may be expected from occlusal loading acting upon teeth and mandibular morphogenesis (Brin *et al.* 1990). As it was summarized by Kablar (2010), the key feature that connects the vertebrate skeleton and the musculature appears to be their ability to react to mechanical stimuli, which has important implications for both adaptation and morphogenesis.

The different species within the genus *Ctenomys* show a considerable variability in their incisor shape, mainly in relation to the relevance of chisel-tooth digging (Vassallo 1998; Mora *et al.* 2003; Vassallo and Mora 2007). Previous analyses focusing on functional cranial traits, including the incisors, have shown that the ontogenetic trajectories of different *Ctenomys* species can differ in both the slope and the y-intercept, partially contributing to the substantial morphological variability in incisor bending strength (Verzi *et al.* 2010). Further investigation on other *Ctenomys* species with varying incidence of chisel-tooth digging, bite force and incisor characteristics will be of great interest to extend the present ontogenetic analysis.

### Acknowledgements

We thank Philip Cox (Hull York Medical School) and an anonymous reviewer for their comments on an earlier version of this manuscript. This work was presented at the symposium ‘Evolution of the Rodents: Anatomy, Palaeontology and Functional Morphology’, ICVM 10, Barcelona 2013. Grant sponsors PIP 1380 (CONICET); PICT 2121 (ANPCyT); CGL2011-23919/BOS (Ministerio de Economía y Competitividad – Secretaría de Estado de Investigación, Desarrollo e Innovación, España; 2012–2014).

### References

- Alexander, R. M. C. N. 1983. On the massive legs of a Moa (*Pachyornis elephantopus*, Dinornithes). *Journal of Zoology* **201**: 363–376.
- Alexander, R. M. C. N. 1985. Mechanics of posture and gait of some large dinosaurs. *Zoological Journal of the Linnean Society* **83**: 1–25.
- Bacigalupe, L. D., Iriarte-Díaz, J. and Bozinovic, F. 2002. Functional morphology and geographic variation in the digging apparatus of

- cururos (Octodontidae: *Spalacopus cyanus*). *Journal of Mammalogy* **83**: 145–152.
- Becerra, F., Echeverría, A. I., Vassallo, A. I. and Casinos, A. 2011. Bite force and jaw biomechanics in the subterranean rodent Talas tuco-tuco (*Ctenomys talarum*) (Caviomorpha: Octodontoidea). *Canadian Journal of Zoology* **89**: 334–342.
- Becerra, F., Echeverría, A. I., Marcos, A., Casinos, A. and Vassallo, A. I. 2012a. Sexual selection in a polygynous rodent (*Ctenomys talarum*): an analysis of fighting capacity. *Zoology* **115**: 405–410.
- Becerra, F., Vassallo, A. I., Echeverría, A. I. and Casinos, A. 2012b. Scaling and adaptations of incisors and cheek teeth in caviomorph rodents (Rodentia, Hystricognathi). *Journal of Morphology* **273**: 1150–1162.
- Becerra, F., Casinos, A. and Vassallo, A. I. 2013. Biting performance and skull biomechanics of a chisel tooth digging rodent (*Ctenomys tuconax*; Caviomorpha; Octodontoidea). *Journal of Experimental Zoology A* **319**: 74–85.
- Becerra, F., Echeverría, A. I., Casinos, A. and Vassallo, A. I. 2014. Another one bites the dust: bite force and ecology in three caviomorph rodents (Rodentia, Hystricognathi). *Journal of Experimental Zoology A* **321**: 220–232.
- Begall, S., Burda, H. and Schleich, C. E. 2007. Subterranean Rodents: News from Underground. Springer Verlag, Berlin.
- Biknevicius, A. R., Van Valkenburgh, B. and Walker, J. 1996. Incisor size and shape: implications for feeding behaviors in saber-toothed 'cats'. *Journal of Vertebrate Paleontology* **16**: 510–521.
- Brin, I., Steigman, S. and Michaeli, Y. 1990. Effect of occlusal functional force on incisor socket morphology and location in the rat mandible. *Anatomical Record* **226**: 36–72.
- Carrier, D. R. 1983. Postnatal ontogeny of the musculoskeletal system in the black-tailed jack rabbit (*Lepus californicus*). *Journal of Zoology* **201**: 27–55.
- Carrier, D. R. 1996. Ontogenetic limits on locomotor performance. *Physiological Zoology* **69**: 467–488.
- Carter, D., Mikic, B. and Padian, K. 1998. Epigenetic mechanical factors in the evolution of long bone epiphyses. *Zoological Journal of the Linnean Society* **123**: 163–178.
- Christiansen, P. 2007. Evolutionary implications of bite mechanics and feeding ecology in bears. *Journal of Zoology (London)* **272**: 3–443.
- Christiansen, P. Y. and Adolfsson, J. S. 2005. Bite forces, canine strength and skull allometry in carnivores (Mammalia, Carnivora). *Journal of Zoology (London)* **266**: 133–151.
- Cleuren, J., Aerts, P. and De Vree, Y. F. 1995. Bite and joint force analysis in *Caiman crocodylus*. *Belgian Journal of Zoology* **125**: 79–94.
- Cutrera, A. P., Mora, M. S., Antenucci, C. D. and Vassallo, A. I. 2010. Intra- and interspecific variation in home-range size in sympatric tuco-tucos, *Ctenomys australis* and *C. talarum*. *Journal of Mammalogy* **91**: 1425–1434.
- Echeverría, A. I. 2011. Ontogenia del comportamiento en el roedor subterráneo *Ctenomys talarum* (Rodentia: Ctenomyidae). Ph.D. dissertation. Mar del Plata, Argentina: University of Mar del Plata. 208 pp.
- Echeverría, A. I. and Vassallo, A. I. 2011. Role of maternal odors on foraging behavior during postnatal development in a solitary subterranean rodent *Ctenomys talarum*. *Acta Ethologica* **15**: 91–99.
- Evans, A. R., Hunter, J., Fortelius, M. and Sanson, G. D. 2005. The scaling of tooth sharpness in mammals. *Annales Zoologici Fennici* **42**: 603–613.
- Falster, D. S., Warton, D. I. and Wright, I. J. 2006. SMATR: Standardised major axis tests and routines, ver 2.0. <http://www.bio.mq.edu.au/ecology/SMATR/>.
- Farnum, C. E. 2007. Postnatal growth of fins and limbs through endochondral ossification. In: Hall, B. K. (Ed.): *Fins into Limbs: Evolution, Development, and Transformation*, pp. 118–151. The University of Chicago Press, Chicago.
- Feldhamer, G. A., Drickamer, L. C., Vessey, S. H., Merritt, J. F. and Krajewski, C. 2007. *Mammalogy: Adaptation, Diversity, and Ecology*. Johns Hopkins University Press, Baltimore.
- Ford, S. M. and Corruccini, R. S. 1985. Intraspecific, interspecific, metabolic, and phylogenetic scaling in platyrrhines primates. In: Jungers, W. L. (Ed.): *Size and Scaling in Primate Biology*, pp. 401–435. Plenum Press, New York.
- Galis, F. 1993. Interactions between the pharyngeal jaw apparatus, feeding behavior, and ontogeny in the cichlid fish, *Haplochromis piceatus*: a study of morphological constraints in evolutionary ecology. *Journal of Experimental Zoology* **267**: 137–154.
- Greaves, W. S. 1980. The mammalian jaw mechanism – the high glenoid cavity. *American Naturalist* **116**: 432–440.
- Herrel, A., O'Reilly, J. C. and Richmond, A. M. 2002. Evolution of bite performance in turtles. *Journal of Evolutionary Biology* **15**: 1083–1094.
- Herring, S. 2010. Muscle-bone interactions and the development of skeletal phenotype. In: Hallgrímsson, B. and Hall, B. K. (Eds): *Epigenetics*, pp. 221–237. University of California Press, Berkeley.
- Hildebrand, M. 1985. Digging in quadrupeds. In: Hildebrand, M., Bramble, D. M., Liem, K. F. and Wake, D. B. (Eds): *Functional Vertebrate Morphology*, pp. 89–109. Belknap Press, Cambridge.
- Huxley, J. 1932. *Problems of Relative Growth*. Methuen, London.
- Irgens, F. 2008. *Continuum Mechanics*. Springer-Verlag, Berlin.
- Kablar, B. 2010. Role of skeletal muscle in the epigenetic shaping of organs, tissues, and cell fate choices. In: Hallgrímsson, B. and Hall, B. K. (Eds): *Epigenetics*, pp. 256–270. University of California Press, Berkeley.
- Lacey, E., Patton, J. L. and Cameron, G. N. 2000. *Life Underground: The Biology of Subterranean Rodents*. Chicago University Press, Chicago, Illinois.
- Lessa, E. P., Vassallo, A. I., Verzi, D. H. and Mora, M. S. 2008. Evolution of the morphological adaptation for digging in living and extinct ctenomyid and octodontid rodents (Rodentia: Caviomorpha: Octodontoidea). *Biological Journal of the Linnean Society* **95**: 267–283.
- Liem, K. F., Bemis, W. E., Walker, W. F. and Grande, L. 2001. *Functional Anatomy of the Vertebrates: An Evolutionary Perspective*, 3rd edn. Thomson Learning-Brooks/Cole, Belmont.
- Lowry, D. and Motta, P. J. 2007. Ontogeny of feeding behavior and cranial morphology in the white spotted bamboo shark *Chiloscyllium plagiosum*. *Marine Biology* **151**: 2013–2023.
- Luna, F., Antenucci, C. D. and Bozinovic, F. 2009. Comparative energetics of the subterranean *Ctenomys* rodents: breaking patterns. *Physiological and Biochemical Zoology* **82**: 226–235.
- Malizia, A. I., Vassallo, A. I. and Busch, C. 1991. Population and habitat characteristics of two sympatric species of *Ctenomys* (Rodentia: Octodontidae). *Acta Theriologica* **36**: 87–94.
- Miller, E. H. and Burton, L. E. 2001. It's all relative: allometry and variation in the baculum (os penis) of the harp seal, *Pagophilus groenlandicus* (Carnivora: Phocidae). *Biological Journal of the Linnean Society* **72**: 345–355.
- Mora, M., Olivares, A. I. and Vassallo, A. I. 2003. Size, shape and structural versatility of the skull of the subterranean rodent *Ctenomys* (Rodentia, Caviomorpha): functional and morphological analysis. *Biological Journal of the Linnean Society* **78**: 85–96.
- Nevo, E. 1999. *Mosaic Evolution of Subterranean Mammals. Regression, Progression, and Global Convergence*. Oxford University Press, New York.



- Plikus, M. V., Zeichner-David, M., Mayer, J. A., Reyna, J., Bringas, P., Thewissen, J. G. M., Snead, M. L., Chai, Y. and Chuong, C. H. M. 2005. Morphoregulation of teeth: modulating the number, size, shape and differentiation by tuning Bmp activity. *Evolution and Development* 7: 440–457.
- Ravosa, M. J., Ning, J., Costley, D. B., Daniel, A. N., Stock, S. R. and Stack, M. S. 2010. Masticatory biomechanics and masseter fiber-type plasticity. *Journal of Musculoskeletal and Neuronal Interaction* 10: 46–55.
- Sakata, T., Wang, Y., Halloran, B. P., Elalieh, H. Z., Cao, J. and Bickle, D. D. 2004. Skeletal unloading induces resistance to insulin-like growth factor-I (IGF-I) by inhibiting activation of the IGF-I signaling pathways. *Journal of Bone and Mineral Research* 19: 436–446.
- Schmidt-Nielsen, K. 1991. Scaling. Why is Animal Size so Important? Cambridge University Press, Cambridge.
- Schwenk, K. 2000. Feeding: Form, Function and Evolution in Tetrapod Vertebrates. Academic Press, New York.
- Smith, K. K. 1996. Integration of craniofacial structures during development in mammals. *American Zoologist* 26: 70–79.
- Sokal, R. R. and Rohlf, F. J. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., San Francisco, California.
- Ungar, P. 2010. Mammal Teeth: Origin, Evolution, and Diversity. The Johns Hopkins University Press, Baltimore.
- Valverde, E., Casinos, A., Alba-Fernández, C. and Del Río, L. 2005. Lumbar ontogenetic allometry and dimorphism in humans. A case for comparison between interspecific and intraspecific scaling. *European Journal of Morphology* 42: 185–192.
- Van Daele, P. A. A. G., Herrel, A. and Adriaens, D. 2009. Biting performance in teeth-digging African mole-rats (*Fukomys*, Bathyergidae, Rodentia). *Physiological and Biochemical Zoology* 82: 40–50.
- Vassallo, A. I. 1998. Functional morphology, comparative behavior, and adaptation in two sympatric subterranean rodents genus *Ctenomys* (Caviomorpha: Octodontidae). *Journal of Zoology* 244: 415–427.
- Vassallo, A. I. and Mora, M. S. 2007. Interspecific scaling and ontogenetic growth patterns of the skull in living and fossil ctenomyid and octodontid rodents (Caviomorpha: Octodontoidea). In: Kelt, D. A., Lessa, E. P., Salazar-Bravo, J. A. and Patton, J. L. (Eds): The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson, pp. 945–968. University of California Publications in Zoology, Berkeley.
- Verzi, D. H. and Olivares, A. I. 2006. Craniomandibular joint in South American burrowing rodents (Ctenomyidae): adaptations and constraints related to a specialized mandibular position in digging. *Journal of Zoology* 270: 488–501.
- Verzi, D. H., Alvarez, A., Olivares, A. I., Morgan, C. and Vassallo, A. I. 2010. Ontogenetic trajectories of key morphofunctional cranial traits in South American subterranean ctenomyid rodents. *Journal of Mammalogy* 91: 1508–1516.
- Vieytes, E. C., Morgan, C. C. and Verzi, D. H. 2007. Adaptive diversity of incisor enamel microstructure in the South American burrowing rodents of family Ctenomyidae (Caviomorpha). *Journal of Anatomy* 211: 296–302.
- Warton, D. I., Wright, I. J., Falster, D. S. and Westoby, M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* 81: 259–291.
- Zenuto, R. R., Lacey, E. A. and Busch, C. 1999. DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. *Molecular Ecology* 8: 1529–1532.
- Zenuto, R. R., Vassallo, A. I. and Busch, C. 2001. A method to study social and reproductive behavior of subterranean rodents in captivity. *Acta Theriologica* 46: 161–170.