Evol Biol
DOI 10.1007/s11692-013-9229-4

RESEARCH ARTICLE

Developmental Processes, Evolvability, and Dental Diversification of New World Monkeys

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Received: 3 November 2012 / Accepted: 29 March 2013

Abstract The developmental processes that contribute to variation of morphological traits are the subject of considerable interest when attempting to understand phenotypic evolution. It is well demonstrated that most characteristics of tooth pattern can be modified by tinkering conserved signal pathways involved in dental development. This effect can be evaluated by comparing developmental models with naturally occurring variation within explicit phylogenetic contexts. Here, we assess whether evolutionary changes in lower molar (M) ratios among platyrrhines were channelled by alterations in the balance of activators and inhibitors as predicted by the inhibitory cascade (IC) model (Kavanagh et al. in Nature 449:427–432, 2007). Ordinary linear regression adjusted to M2/M1 versus M3/M1 ratios of 38 species of platyrrhines indicated that the slope and intercept were significantly different from the IC model. Conversely, when the phylogeny was incorporated into the regression analyses (PGLS), variation in molar ratios did not differ from the developmental model. PGLS also showed that changes in molar proportions are not an allometric effect associated with body size. Discrepancies between phylogenetically corrected and non-corrected analyses are mainly due to the departure of Callitrichines from the predicted values. This subfamily displays agenesis of M3 with higher than expected M2/M1 ratios, indicating that M3 fails to develop even when the inhibition by M1 on the subsequent molars is not increased. Our results show that evolution in molar ratios is concordant with slight changes in the proportion of activators and inhibitors that regulate molar development; however, other processes are required to account for variation in the number of teeth.

Keywords Evo-devo · Inhibitory cascade model · Primates · Molar ratios · Phylogenetic generalized least-squares model

Electronic supplementary material The online version of this article (doi:10.1007/s11692-013-9229-4) contains supplementary material, which is available to authorized users.

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Introduction

The role of developmental processes in the evolution of phenotypic traits at the macroevolutionary level has been extensively debated. From formulations that emphasized development as a constraint that imposes limits to an otherwise non-limited variation, the focus has shifted toward understanding the mechanisms that underlie the production of phenotypic variation on which evolutionary processes can work (Gould 1977; Hendrikse et al. 2007; Müller 2007; Oster and Alberch 1982). Nevertheless, it is now widely accepted that in every developmental system, some morphological changes are more likely than others and some are nearly impossible. Consequently, the generation of variation through development is thought to be a

Published online: 07 April 2013

fundamental determinant of evolvability, i.e., the ability of a system to evolve (Wagner and Altenberg 1996).

The mammalian dentition provides an ideal complex system for studying the link between phenotypic variation, development and evolutionary processes. In particular, molar teeth have received great attention over the years; they have been widely used as diagnostic features in taxonomy, in the reconstruction of paleodiets of extinct taxa, and the study of life history traits, among others (Smith 1989; Ungar 2007). More recently, the progress made in elucidation of the mechanisms of tooth development, especially on the basis of experimental studies with rodents, has led to the proposal of different hypotheses that suggest how small changes in a few developmental parameters can cause significant variation in the morphology of molar teeth (Cho et al. 2011; Jernvall 2000; Kavanagh et al. 2007; Plikus et al. 2005; Salazar-Ciudad and Jernvall 2010). Among these, a simple developmental cascade model that accounts for the relative size of lower molars in rodents has been suggested to accurately predict the production of variation in molars among species (Kavanagh et al. 2007).

Kavanagh et al. (2007) showed, by using germ culture of murine teeth, that signaling molecules produced by developing first molars inhibited the development of subsequent molars, while molecules from the surrounding tissues had the opposite effect. The balance between inhibitors and activators (e.g., Ectodin, Follistatin, Bmp3, Bmp4, and Activin βA; see review in Tummers and Thesleff 2009) determines the relative size, time of formation, and presence of second and third molars. The inhibitory cascade model (IC model) further states that the dynamic balance between activators and inhibitors (a/i) could account for the evolvability of teeth (Kavanagh et al. 2007; Polly 2007). Changes in molar proportions could be generated simply by processes acting over the activator versus inhibitor ratio, which favors specific trajectories in the evolution of mammalian dentition (Kavanagh et al. 2007). This model provides a mechanistic explanation for longstanding observed patterns, such as the directionality of size reduction in the molar row and the frequent agenesis of third molars in different lineages (Nieminen 2009). However, murids, from which the developmental models derive, have a particular dental formula, 1.0.0.3/1.0.0.3, with only one incisor, three molars, no canines and no premolars. Whether the same developmental mechanisms also occur in mammals with different dental formulas must be evaluated by testing the expectations derived from these models with the naturally occurring variation in fossils and extant species from other clades. The few studies performed to date, which analyzed either a broad representation of several genera of mammals, or related species from the order Rodentia (Polly 2007; Renvoisé et al. 2009; Wilson et al. 2012), showed that even though the IC model applies to many clades, it cannot be generalized across all mammals. Such findings stress the importance of incorporating clades characterized by high a degree of dental variation, in terms of absolute and relative size as well as number of posterior teeth.

Platyrrhini represents an interesting group for examining the link between evolutionary changes in molar proportions and the activator–inhibitor ratios that control tooth development for several reasons. First, Platyrrhines are a monophyletic group which probably colonized South America during the Oligocene period (around 23–33 million years ago) and evolved in isolation from the Old World primates. Second, within the continent they experienced a radiation with divergence in several lineages, occupation of a large range of ecological niches, and great morphological diversification (Fleagle 1999; Rosenberger et al. 2009; Tejedor 2008). Third, they greatly vary in molar size and some lineages present the agenesis of third molars. This is particularly relevant to test the predictions of the IC model regarding the mechanisms that result in molar agenesis, which has not being addressed yet.

Thus, the main objective of this paper is evaluating whether the IC model accounts for variation in relative size of lower molars among New World monkeys. If the a/i ratio in molar development favored a direction of evolutionary phenotypic changes, we expect that variation in relative molar size among species conforms the axis of variation predicted by the IC model. Additionally, we analyzed molar proportions within species to evaluate whether population level variation is related to changes in the parameters of the IC model. Previous studies have found that changes in single parameters regulating dental development may explain variation among individuals in different aspects of dental morphology (Salazar-Ciudad and Jernvall 2010). Thus, although the original formulation of the IC model had implications for macroevolutionary scales only, it was of interest to test this developmental model at both intra and inter-specific scales.

Variation in body size has been suggested to be a primary factor underlying the phenotypic diversification among Platyrrhines (Marroig and Cheverud 2005; Rosenberger 1992; Rosenberger et al. 2009), so we also evaluated whether the relative molar size was associated with variation in the overall growth shown by this clade. Mechanisms that regulate the size of particular organs (e.g. teeth) are intimately involved with those that regulate overall organismal growth (Shingleton 2011). Therefore, the final size of individual organs is controlled not only by organ-intrinsic regulators of growth, including conserved extracellular signaling molecules such as the activators and inhibitors of Kavanagh's model, but also by systemic factors that stimulate the growth of most organs and ensure

appropriate scaling with body size (Parker 2011). Consequently, studying dental development jointly with changes in overall size may contribute to understanding the interactions between common and local factors that drive the diversification of molar morphologies. We expect that if an association among developmental processes regulating relative molar size and those controlling overall patterns of growth exists, a significant correlation between among-species variation in relative molar size and body size should be observed (Hartwig 1996; Rosenberger 1984; Smith and Jungers 1997).

We used ordinary least squares (OLS), reduced major axis (RMA), and phylogenetic regression methods (PGLS; Harvey and Pagel 1991; Rohlf 2001) to determine whether the relative size of mandibular molars in platyrrhines fits into the IC model. The phylogenetic relationships were studied using multiple coding and non-coding nuclear DNA sequences and Bayesian methods (Drummond et al. 2006; Lemey et al. 2009). Then, we tested the among-species variation in relative molar size against body size variation in an explicit phylogenetic context.

Materials and Methods

Morphometric Measurements, Phylogenetic Relationships, and Body Size Data

We studied 38 species of 15 extant genera of platyrrhines from America (Table 1 Supp. Mat.; Wilson and Reeder 2005). These genera represent all spectra of morphological variation in the Parvorder of New World monkeys (Fleagle 1999). The mesiodistal length and buccolingual breadth of the lower molars were obtained from Rosenberger (1992) and Plavcan (1990). Dental measurements were obtained to the nearest 1/10 mm at the crown surface as the largest diameters along the 2 axes. In order to evaluate the probable influence of inter-observer error, we first estimated the amount of difference between mean values obtained for the same species by both authors. We concluded that the variation introduced by inter-observer error was negligible. Based on these measurements an overall molar size for each species was estimated as the product of the mean mesiodistal length and buccolingual breadth. In the following analyses, we used the sex-pooled mean of these measurements for each species. All samples were sex-balanced to avoid possible bias due to sexual dimorphism in dental size.

To estimate the phylogenetic relationships for the 38 platyrrhine species, we analyzed DNA sequences using Bayesian methods. For each species we obtained a total of 11,450 bp, including a mitochondrial sequence (CytB) download from Genbank, and 16 nuclear sequences (AFF2, AXN1, BCOR, CNR1, ERC2, MAPKAP1, NEGR1, NPAS3, RAG2, RPGRIP1, SIM1, SMCX, SMGS1, TYR, USH2A, ZIC3) obtained from Perelman et al. (2011) (Table 1 Suppl. Mat.). We estimated phylogenetic relationships and relative divergence times under a relaxed molecular clock model for 38 platyrrhine species, with *Homo* as an outgroup, using BEAST v1.6.1 (Drummond et al. 2006; Drummond and Rambaut 2007). The phylogenetic tree obtained is presented in Fig. 1. This phylogenetic tree is in general agreement with other recent estimations (Fig. 1; Opazo et al. 2006; Perelman et al. 2011; Perez et al. 2012; Wildman et al. 2009), being identical to the relationships estimated by Opazo et al. (2006) and Wildman et al. (2009).

Data on body size for each genus and species was collected from the literature in order to characterize the allometric effect on the dental variables of the groups included in this study (for more details on how body mass data were calculated see Smith and Jungers 1997).

Statistical Methods

We first fit the IC model proposed by Kavanagh et al. (2007) to the relative size of lower molars. The IC model examines how molar initiation and size are regulated along the dental row during individual development. The model assumes an anterior–posterior sequence of formation, where the formation of M1 precedes that of the other 2 molars, as in the case of the lower molar teeth in platyrrhines (Henderson 2007). Relative lower molar size results from an inhibitory cascade throughout molars following the equation:

$$Y = 1 + [(a - i)/i\,](X - 1),$$

where Y is molar area relative to its position, X is molar position (i.e., 1, 2, or 3), a is the activator, and i is the inhibitor. The $(a - i)/i$ represents the relative strengths of the activators versus the inhibitors. Molar areas are derived from the above equation as, $M1 = 1$, $M2 = a/i$, and $M3 = 2a/i - 1$. The relative molar size (M2/M1 vs. M3/M1) can be predicted using the following formula: $M3/M1 = 2\,(M2/M1) - 1$. According to the IC model, variations in a/i can account for changes in proportional molar size: high values of a/i lead to similar size, whereas a decrease in a/i results in larger first molars.

To compare the IC model defined by Kavanagh et al. (2007) with the model adjusted to the relative molar size exhibited by platyrrhines, we also fit the simple linear regression model $\boldsymbol{y = a + bx + e}$ to the data using 3 different algorithms: RMA, OLS and PGLS. In this model, $\boldsymbol{y}$ is the m3/m1 proportion, $\boldsymbol{x}$ is the M2/M1 proportion, $\boldsymbol{b}$ is the regression coefficient, and $\boldsymbol{e}$ is the error term. In conventional RMA and OLS analyses, $\boldsymbol{e}$ is assumed to be

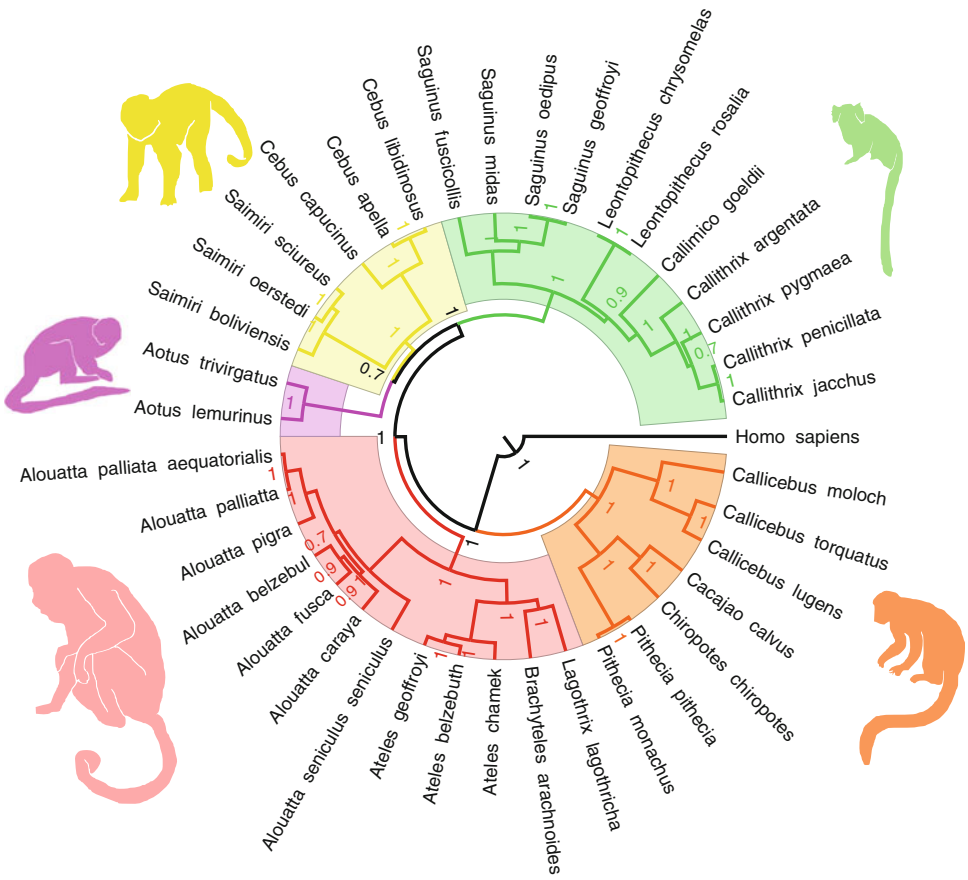

Fig. 1 Phylogenetic relationships for the 38 platyrrhine species analyzed based on DNA sequences. Most of the clades are strongly supported with high values (>0.8), and only the relationship between *Aotus* and Cebinaes has a lower value (54). The traditional division of Platyrrhine into 5 main clades is also shown. These clades correspond to the families Pitheciidae and Atelidae, and the subfamilies Aotinae, Cebinae, and Callitrichinae, with Atelidae as the closest relative to these 3 subfamilies (Opazo et al. 2006; Wildman et al. 2009)

independent. However, the use of linear methods, such as RMA and OLS, that do not take into account the phylogenetic signal (i.e., tendency for related species to resemble each other) of the variables might bias the estimation of parameters in the model. To account for phylogenetic non-independence, PGLS assumes that $\boldsymbol{e}$ has a covariance matrix that is derived from the phylogenetic tree (Rohlf 2001). We used a phylogenetic covariance matrix estimated from the phylogenetic tree obtained with BEAST, which uses the maximum likelihood estimate of lambda parameter (Orme et al. 2012). The significance of the regression model was assessed using 95 % confidence intervals (CI) and *F* tests (Orme et al. 2012).

Finally, we tested the association of molar proportions (M3/M1 and M2/M1) with body size by using the PGLS model where molar proportions (M3/M1 and M2/M1) were the dependent variables and log body size was the independent variable. This analysis allows us to evaluate the effect of body size independently from the phylogenetic structure in our dataset.

As a complementary analysis, we calculated the phylogenetic signal to evaluate the concordance of the phylogenetic structure (BEAST tree) with dental measurements and body size. In particular, we calculated the *K* statistic proposed by Blomberg et al. (2003) that provides a univariate measure of the strength of phylogenetic signal in the data relative to the expected under Brownian motion character evolution along the specified phylogenetic tree. *K* values near 0 indicate a lack of signal, while values near 1 are expected if the character evolved under a BM model (Blomberg et al. 2003). Higher values are interpreted as evidence of strong phylogenetic signal. The significance of the *K* statistic was assessed via permutation tests with 10,000 replications.

Statistical analyses were performed using the ape, caper and picante packages for R 2.13.0 (R-Development Core Team 2012) and PAST 2.11 (Hammer et al. 2001) software.

Results

Intraspecific Variation in Molar Proportions

We analyzed the intraspecific variation in molar proportions in a set of 8 species for which more than 25 specimens were available (with sample size ranging between 25 and 48). Most species did not display a linear relationship

between M2/M1 and M3/M1 areas (Fig. 2). The adjustment to a linear model was poor, with coefficients of determination R^2 ranging between 0.01 and 0.6 (Table 1). These results suggest that variation in M3/M1 ratio among individuals from the same species cannot be predicted by M2/M1, and thus changes in relative molar size at this scale do not seem to be controlled by changes in the a/i ratio. The coefficients of variation (CV) within these 8 species showed that M3 was the most variable molar (a well known phenomenon), having in some species twice the variation observed in M1 and M2. Conversely, the CVs for these two molars were similar.

Interspecific Variation in Molar Proportions

The RMA model obtained for the 38 platyrrhine species showed that molar proportions in this group did not follow the expectations of the IC model (Fig. 3). The 95 % CI of the parameters of platyrrhine data confirmed the significant differences found between the observed data and the predicted model (Table 2). Similar results were obtained using OLS (Table 2). Moreover, the members of Callitrichinae have lost the M3, but the relative size of M2 is larger than the threshold value predicted by the IC model for the loss of M3, indicating that when inhibition increases, its effect is greater on M3 than on M2. Likewise, some species of the Pitheciidae and Atelidae fall in the region where $M1 < M2 > M3$, a pattern that requires not only a decrease in inhibition but also an early arrest of M3 development.

The test of phylogenetic signal for the dental variables and body size indicated *K* values significantly higher than expected under Brownian motion character evolution along the specified phylogenetic tree (Table 3). When the phylogenetic structure of the data was incorporated into the analysis, the parameters for the observed model were not significantly different from those for the IC model (Fig. 3; Table 2). As dental proportions have a strong phylogenetic structure (Table 2), the species that belong to the same phylogenetic clade are not independent. The PGLS analysis revealed that the evolutionary changes in the relative size of lower molars in this group are closer to the predicted values based on developmental parameters. Moreover, the adjusted R^2 obtained for the PGLS model was 0.648. Thus, alterations in the inhibition/activation ratio can explain the variation in relative molar size observed among New World monkeys, with the exception of the morphology exhibited by Callitrichinae. Within this clade, other developmental changes are required for explaining the severe reduction in M3, given their M2/M1 size ratio. Note that given PGLS incorporates information about the evolutionary relationships of organisms in the analysis, the loss of M3 among callitrichines does not bias the estimation of the model, as did happen when RMA and OLS were used.

We further evaluated whether variation in molar proportions was associated with differences in body size among the 38 species studied here. The PGLS model including molar proportions as dependent variables and log body size as the independent variable showed that this variable explained only 11 % of variation in molar proportions independently from phylogeny (Adjusted R^2: 0.116).

Discussion

The results obtained for the intraspecific analysis indicated that proportions of lower molars do not follow a linear relationship, and hence M3/M1 ratio cannot be predicted based on M2/M1 values as is expected by the IC model (Fig. 2). This pattern contrasts with changes in relative molar size observed at a macroevolutionary level, where the position of individual species in morphospace supports a strong relationship between M3/M1 versus M2/M1 proportions (Fig. 3). Consequently, the main direction of phenotypic variance among the species studied here cannot be derived based on the morphologies produced within them. Two general scenarios can be envisioned to account for such discrepancies. On one hand, variation at intra and inter-specific scales may actually depend on modulation of the same underlying developmental processes -e.g. the proportion of activators and inhibitors in the IC model- but the magnitude of variation in such processes may be different. On the other hand, different developmental mechanisms may underlie the production of variation in phenotypic traits at both scales. The current literature suggests that a highly conserved set of pathways is involved in dental development (Tummers and Thesleff 2009), and thus the first alternative seems to be more likely. Under this hypothesis, the lower levels of variation observed within species (Fig. 2) might reflect the lack of variation in the processes responsible for the development of the traits under study. However, for other molar traits it has been proposed that different mechanism may account for similar phenotypic variation, depending on the evolutionary scale considered (Ledevin et al. 2010). The correspondence of intra and inter-specific variation with the expectations derived from developmental models has not been extensively addressed yet, making difficult to assess the relation between the processes that generate phenotypic variation at both scales. Experimental studies that introduce changes in the magnitude of developmental processes by modifying genetic and environmental factors and test

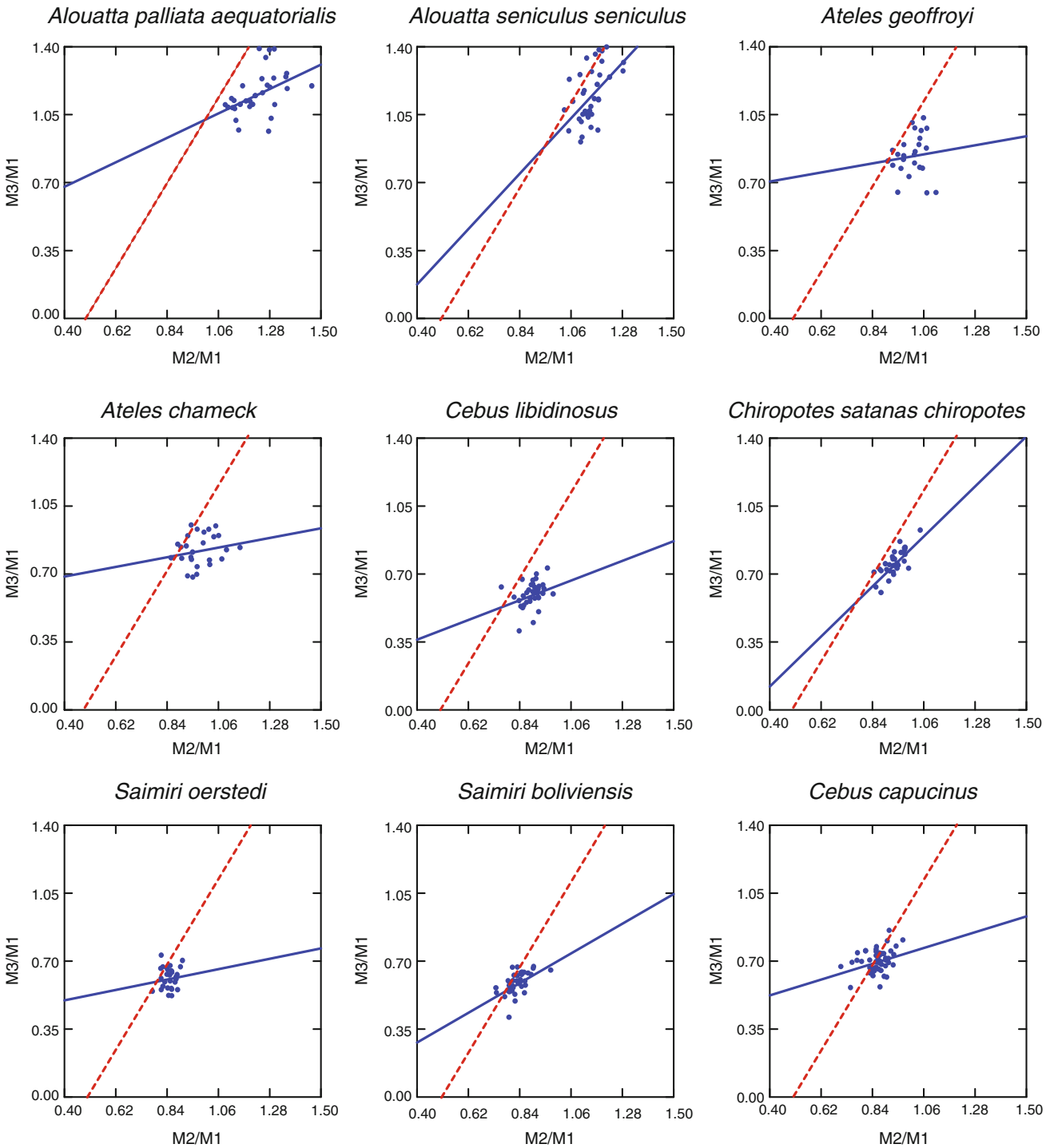

Fig. 2 Intraspecific variation in molar proportions. *Lines* represent the linear adjustment to the data (*solid lines*) and the predictions of the IC model (*dotted lines*)

for their effects on specific traits can shed light on this matter.

We show that the fit of the lower molar proportions of New World monkeys to the IC model proposed by Kavanagh et al. (2007) depends on the regression model used. When the data were fitted to the RMA and OLS regression models, the relative molar size did not follow the expectations of the IC model. These two regression models have been previously used to contrast the predicted and observed molar ratios in different organisms (Kavanagh et al. 2007; Polly 2007; Renvoisé et al. 2009). However, one of their main limitations is that they fail to account for shared

Table 1 Ordinary least-squares (OLS) analysis for intraspecific variation in molar proportions

Species	N	r	R^2	Slope	Intercept	M1 CV	M2 CV	M3 CV
Alouatta palliata aequatorialis	32	0.456	0.208	1.252 (0.727/1.668)	−0.389 (−0.899/0.263)	0.106	0.103	0.121
Alouatta seniculus seniculus	35	0.527	0.278	2.463 (1.494/3082)	−1.684 (−2.406/−0.549)	0.110	0.107	0.139
Ateles geoffroyi vellerosus	25	0.106	0.011	1.989 (1.341/6.397)	−1.184 (−5.69/−0.516)	0.072	0.067	0.141
Ateles paniscus chameck	26	0.199	0.040	1.135 (0.556/3.412)	−0.299 (−2.568/0.267)	0.107	0.119	0.145
Cebus apella libidinosus	35	0.308	0.095	1.499 (−0.742/3.825)	−0.753 (−2.833/−0.059)	0.073	0.091	0.123
Cebus capucinus capucinus	48	0.330	0.109	1.123 (0.705/1.453)	−0.276 (−0.564/0.093)	0.061	0.080	0.098
Chiropotes satanas chiropotes	30	0.765	0.585	1.532 (1.151/1.86)	−0.687 (−1.001/−0.323)	0.096	0.093	0.106
Saimiri oerstedi oerstedi	29	0.128	0.016	1.903 (1.465/6.354)	−1.018 (−4.822/−0.634)	0.048	0.048	0.089
Saimiri sciureus boliviensis	40	0.561	0.315	1.24 (0.61/1.642)	−0.452 (−0.788/0.081)	0.09	0.09	0.1

The coefficients of variation (CV) for molar areas are also included

95 % CI are shown within brackets. *N,* sample size. M1, M2 and M3: lower first second and third molars

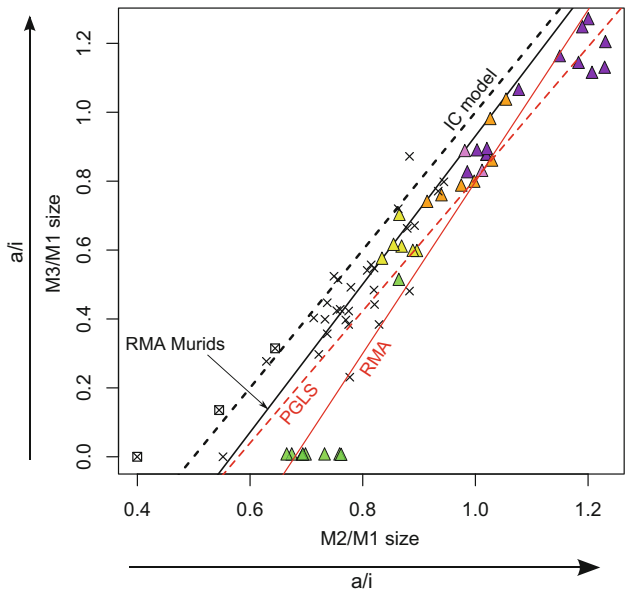

Fig. 3 Platyrrhines molar proportions adjusted to reduced major axis (RMA) and phylogenetic generalized least-squares (PGLS) models compared to the inhibitory cascade (IC) prediction model. Data obtained by Kavanagh et al. (2007) on murine species (*crosses*) as well as on the experiments with *Mus musculus* (*squares*) were added for comparisons. *Violet triangles*: Atelidae; *orange*: Pitheciidae; *pink*: Aotinae; *green*: Callithricinae; *yellow*: Cebinae. Note that for RMA and OLS phylogenetically uncorrected data were used (Color figure online)

Table 2 Reduced major axis regression (RMA), phylogenetic generalized least-squares (PGLS), and ordinary least-squares (OLS) analyses for interspecific variation in molar proportions

Method	a	95 % CI a	b	95 % CI b	R^2
IC model	−1	–	2	–	–
RMA	−1.693	−1.919/−1.465	2.492	2.225–2.734	0.93
OLS	−1.605	−1.852/−1.377	2.397	2.146–2.637	0.92
PGLS	−1.11	−1.336/−0.884	1.916	1.686–2.147	0.83

The values of the intercept (a) and slope (b) expected under the inhibitory cascade model (IC) are also shown. Note that for RMA and OLS phylogenetically uncorrected data were used

95 % *CI* confidence interval for intercept and slope for each model

Table 3 Phylogenetic signal (K-statistic) for molar size and proportions, and body size

Variable	K	P
Body mass	2.83	0.0001
Log body mass	3.09	0.0001
M1 area	3.08	0.0001
M2 area	4.02	0.0001
M3 area	4.62	0.0001
M2/M1	2.57	0.0001
M3/M1	4.18	0.0001

M1, M2 and M3: lower first second and third molars

evolutionary history, which results in closely related species being more similar to each other than expected by chance alone. Consequently, the RMA and OLS models might lead to a poor estimation of the intercept and slope, resulting in a misunderstanding of the relationship between the variables of interest (Felsenstein 1985; Ives and Zhu 2006; Rohlf 2001). Controlling for phylogeny, the parameters for the observed regression model were not significantly different from those predicted by the IC model. If we examine Fig. 3, we find that species from the 5 clades tend to be clustered in morphological space. Differences between the ordinary and phylogenetic regression models could be particularly related to the effect of the cluster conformed by 10 species from the Callitrichinae clade, which are treated as independent observations by the RMA and OLS models. Clearly, our results reinforce the importance of using more realistic models that incorporate the phylogenetic structure instead of the standard

regression techniques (also see Felsenstein 1985; Freckleton et al. 2011; Garland et al. 2005; Ives and Zhu 2006; Rohlf 2001).

The parameters of the PGLS model fitted to the relative size of lower molars of New World monkeys agreed with the values predicted by the IC model. Thus, the main evolutionary trend in molar proportions among platyrrhines might have resulted from an alteration in the inhibition/activation ratio among species. These results confirm our expectation that the mechanisms of dental development facilitated the evolution of lower molars along a specific trajectory that was characterized by an antero-posterior increase or decrease in molar size. Some clades, such as Atelidae and Pitheciidae, have a weak inhibitory cascade that results in molars of similar size or even larger distal molars (Fig. 3). A similar trend has been observed among Old World primates (Polly 2007). Conversely, Cebinae and Callimico species are characterized by a strong inhibitory cascade that results in small distal molars. Our results also showed areas of the morphospace that are hardly occupied, such as the region where morphologies carachterized by M1 > M2 < M3 are expected. Similar findings have been made in other clades, suggesting that certain morphologies result from developmental mechanisms that seldom occur in the evolution of mammals (Polly 2007; Renvoisé et al. 2009). The comparison of morphospaces built upon known developmental mechanisms and the variation among species or higher taxa allows to evaluate the relative importance of underlying intrinsic factors in structuring the evolution of adult forms. In this particular case, the empty areas represent phenotypes that cannot be originated by changes in the proportion of activators and inhibitors. The production of those morphologies would require either major changes of already existing mechanisms of dental developmental or the introduction of new mechanisms. Experimental studies, both in vivo and in silico, are the most promissory alternative for determining the type of modifications needed to produce variation in different directions of the developmental morphospace.

The interaction between activators and inhibitors involved in dental development favored certain evolutionary changes in molar proportions, but clearly, they did not represent a constraint for phenotypic diversification among platyrrhines. Callitrichinae clade members, with the exception of Callimico, do not fit into the IC model. According to this model, agenesis of third molars is expected when the M2/M1 ratio attains a value lower than 0.5 (Kavanagh et al. 2007); however, Callithricinae is characterized by the absence of M3 with a M2/M1 ratio between 0.66 and 0.76. This finding contrasts with the changes in molar proportions exhibited by species of murid rodents, in which the loss of M3 is associated with a stronger reduction of M2 (Fig. 3). The departure from the developmental model tested of those species that have lost the M3 raises the question of whether mechanisms other than changes in the a/i ratio might be involved in regulating variation in molar relative size and the number of teeth. A visual inspection of Fig. 3 suggests that when the species without M3 are included, a polynomial model or two lineal models with different slopes might adjust better than a simple linear regression. Thus, additional parameters should be considered to account for the pattern of variation in lower molars within the group under study. In this sense, a mechanism that has been shown to affect the number of teeth that form is the alteration of the size of the molar field. Experimental studies demonstrate that a small molar field, as generated by recombination or in an Eda mutant, leads to the formation of a reduced number of teeth while the increase in the number of mesenquimatic cells results in an increase in the number of teeth (Cai et al. 2007; Catón et al. 2005; Hu et al. 2006). Moreover, Cai et al. (2007) proposed that tooth size and number might be regulated independently and thus changes in the number of molars can occur without modifying the molar area.

Studies on extant human populations and other non-human primates might also contribute to our understanding of the mechanisms of tooth agenesis. The failure to develop normal teeth is a common phenomenon in modern humans, which typically affects the teeth that develop latest in each tooth class of the secondary dentition, with the agenesis of M3 being the most frequent (Nieminen 2009). The greater susceptibility shown by the last developing teeth suggest an overall reduction of odontogenic potential that could be produced by quantitative changes during dental development (Nieminen 2009). To date, mutations in many genes have been identified in human families with tooth agenesis, and some of them, such as mutations in *MSX1* and *PAX9*, appear to be commonly associated with reduced dimensions, shortened roots, and simplified forms (Jumlongras et al. 2004; Klein et al. 2005; Lidral and Reising 2002; Nieminen et al. 2001). Whether or not the same genes are involved in the loss of M3 in platyrrhines is still a subject of research. Only one study has evaluated the *PAX9* gene in 3 platyrrhine genera (*Callithrix*, *Saimiri*, and *Aotus*) of the Cebidae family, and the results obtained show that New World monkeys, with exception of *Aotus*, have the same mutations in this gene (Pereira et al. 2006). Consequently, molar agenesis in *Callithrix* does not seem to be associated with *PAX9* gene polymorphisms. Further studies evaluating a larger number of genes, which are known to regulate molar development, are required in order to determine the specific molecular mechanisms responsible for dental variation within this clade.

Under the hypothesis that processes controlling dental development and overall patterns of growth are integrated, we expected to find a significant association between

among-species variation in relative molar size and variables describing the species-specific life histories. Previous studies have suggested that size reduction of later developing teeth, with the occurrence of M3 agenesis, among callitrichines might be a correlated effect of noticeable reduction in body size (Kanazawa and Rosenberger 1988). Alternatively, Plavcan and Gomez (1993) proposed that the loss of the third molar in callitrichines might be a continuation of a platyrrhine trend that was unrelated to body size reduction because smaller third molars were observed in other platyrrhines that did not show reduction in body size. Our results suggest that although there is a slightly significant association between molar proportions and body size, the latter variable explains only 12 % of molar variation when phylogenetic relationships are taken into account.

In summary, we compared evolutionary changes in phenotypic traits with the variation predicted by a model of dental development that was built upon experimental work on mice. Overall, our results show that the interspecific variation in relative molar size can be interpreted to result from slight changes in the parameters that control the activation and inhibition of molar tooth development. However, the same mechanism fails to explain the M3 agenesis within platyrrhines and thus other processes need to be incorporated into the model and tested on different data-sets. These sorts of developmental models are of great interest because they provide clues regarding the mechanisms of the observed evolutionary changes, although the actual genetic basis still remains unknown. Evidence gathered over the last few years has shown that modulation of tooth patterns is usually achieved by modifying existing and highly conserved genetic pathways (Tummers and Thesleff 2009). Consequently, our understanding of the factors that modulate dental development at the organismal-level can be a powerful tool in evaluating the mechanisms that are responsible for phenotypic changes at macroevolutionary scales. In this way, further studies that evaluate, in a phylogenetic context, hypotheses about the association of phenotypic traits and candidate genes with known effects on specific development processes will contribute to identifying the developmental determinants of molar variation in different clades.

Acknowledgments The authors would like to acknowledge the contribution of Michael Plavcan for providing dental measurements for several of the species analyzed here and for reading and commenting on the manuscript. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata and Grants from the FONCyT, PICT-2011-0307 (V. B and S. I. P). P. N. G was supported *by a fellowship from Alberta Innovates Health Solutions and the* CIHR Training Program in Genetics, Child Development and Health (Alberta Children's Hospital).

Conflict of interest The authors have no conflict of interest to declare.

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