RESEARCH ARTICLE

The Evolution of Modularity in the Mammalian Skull II: Evolutionary Consequences

Gabriel Marroig · Leila T. Shirai · Arthur Porto · Felipe B. de Oliveira · Valderes De Conto

Abstract Changes in patterns and magnitudes of integration may influence the ability of a species to respond to selection. Consequently, modularity has often been linked to the concept of evolvability, but their relationship has rarely been tested empirically. One possible explanation is the lack of analytical tools to compare patterns and magnitudes of integration among diverse groups that explicitly relate these aspects to the quantitative genetics framework. We apply such framework here using the multivariate response to selection equation to simulate the evolutionary behavior of several mammalian orders in terms of their flexibility, evolvability and constraints in the skull. We interpreted these simulation results in light of the integration patterns and magnitudes of the same mammalian groups, described in a companion paper. We found that larger magnitudes of integration were associated with a blur of the modules in the skull and to larger portions of the total variation explained by size variation, which in turn can exert a strong evolutionary constraint, thus decreasing the evolutionary flexibility. Conversely, lower overall magnitudes of integration were associated with distinct modules in the skull, to smaller fraction of the total variation associated with size and, consequently, to weaker constraints and more evolutionary flexibility. Flexibility and constraints are, therefore, two sides of the same coin and we found them to be quite variable among mammals. Neither the overall magnitude of morphological integration, the modularity itself, nor its consequences in terms of constraints and flexibility, were associated with absolute size of the organisms, but were strongly associated with the proportion of the total variation in skull morphology captured by size. Therefore, the history of the mammalian skull is marked by a trade-off between modularity and evolvability. Our data provide evidence that, despite the stasis in integration patterns, the plasticity in the magnitude of integration in the skull had important consequences in terms of evolutionary flexibility of the mammalian lineages.

Keywords Morphological integration · Constraints · Evolvability · Selection · Evolutionary flexibility

Introduction

Evolvability can be defined as the ability of a population or species to respond to selection (Hansen 2003) or, more precisely, it is the ability of a given species to evolve in the direction of selection (Hansen and Houle 2008). Selection and genetic architecture interact to produce evolutionary change, a relationship explicit in the multivariate response to selection equation $\Delta z = G\beta$, where Δz is the evolutionary response to selection, G is the additive genetic variance/covariance (V/CV) matrix and β is the directional selection gradient (Lande 1979). Embodied in G is the pattern of variation and covariation of genetic traits, a very important aspect of the genetic architecture because even when selection operates on single traits, other traits respond accordingly. This correlated evolution occurs due to the integration of traits within modules, as well as due to the integration among different modules, a feature that characterizes complex hierarchical systems and organisms (Wagner et al. 2008). Modularity relates directly to

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evolvability because a modular architecture may favor evolvability by allowing changes in one module without much interference in others. At the same time, modules interact among themselves. In other words, modules present a certain degree of integration and independence, which promotes coordinated or quasi-independent responses to selection. Neither extreme integration nor extreme independence are necessarily favorable in terms of the evolutionary potential of any population (Wagner and Altenberg 1996; Ancel and Fontana 2000; Hansen 2003; Schlosser and Wagner 2004). There is always a trade-off between the two and the evolvability of a population.

Modularity is empirically recognized by the presence of correlation between parts of organisms and the absence of correlations between these and other parts of the same organisms ("correlation pleiades" *sensu* Berg 1960), being a manifestation of the morphological integration principle (Olson and Miller 1958). According to this principle, a modular organization is considered to be the outcome of functional and/or developmental relationships between traits; in other words, traits related by development or function have greater influence on each other than on those without shared function or developmental origin/interaction. Furthermore, traits in the same module usually share a common genetic basis through pleiotropy, epistasis and linkage disequilibrium (Cheverud 1982, 1984; Chernoff and Magwene 1999) and, therefore, they are expected to evolve as an integrated unit.

When studying morphological integration, two aspects are important to be considered together: patterns and magnitudes of integration on one side; their evolutionary consequences on the other. However, although considerable effort has been made in comparisons and analyses of integration patterns, both the magnitude of integration and their evolutionary consequences have often been neglected in the literature. The magnitude of integration has received particular attention in our companion work (Porto et al. 2009), in which we compared integration patterns and magnitudes in several mammalian taxa. The general result was that, while integration patterns remained surprisingly similar across these taxa, there was considerable variation in the overall magnitude of integration, even in closely related groups. Furthermore, smaller magnitudes of integration were associated with increased modularity (see Fig. 3 in Porto et al. 2009). While the general trends were described, the evolutionary consequences of these changes were still not addressed. Therefore, our aim here is to explore how differences in the magnitude of integration affect the direction and magnitude of evolution or, in other words, how strong is the influence of the magnitude of integration over evolvability and flexibility.

Morphological integration studies are usually performed on correlation matrices, for which several well established analytical tools are available (Cheverud et al. 1989). Although the morphological integration framework has explicit expectations about the relationship between modularity and the evolvability of any population (e.g., Wagner and Altenberg 1996), those expectations have rarely been tested empirically. We think one possible reason for this present state of the art is the lack of analytical tools to compare morphological integration patterns and magnitudes between groups that explicitly relate these aspects to the Quantitative Genetics framework. One possible step to accomplish this fusion of morphological integration studies with evolutionary quantitative genetics is to use analytical tools suited for V/CV matrices, like the random skewers (RS) approach which is based on the multivariate response to selection equation (Lande 1979; Cheverud and Marroig 2007). Under this approach, random selection gradients are simulated and multiplied by the V/CV matrices in order to obtain simulated evolutionary responses that can then be compared between populations (Cheverud 1996; Marroig and Cheverud 2001; Cheverud and Marroig 2007). We believe that Lande's (1979) multivariate response to selection equation can be a meaningful way to achieve the union between morphological integration studies and evolutionary quantitative genetics in a practical way. In fact, recently another group converged to the same approach independently (Hansen and Houle 2008).

The general framework used here was extensively developed by Hansen and Houle (2008) which defined several useful statistics to explore the evolutionary consequences of modularity and integration as captured in V/CV matrices. Respondability ($\bar{r}$) is the norm of the evolutionary response vector, which means that it is a measure of the magnitude of the evolutionary change. Evolvability ($\bar{e}$) is the projection of the respondability on the selection gradient and therefore corresponds to the ability of a population to evolve in the direction to which selection is pushing. Due to correlation among traits, another informative statistic is one that captures the evolvabilty of a trait or dimension whiles the others traits or dimensions are not allowed to change (Hansen and Houle 2008). This is equivalent to the expected response in one trait or dimension while the others are under stabilizing selection, and was called conditional ($\bar{c}$) evolvability by Hansen and Houle (2008). Autonomy ($\bar{a}$) is the proportion of evolvability that remains after conditioning on other traits under stabilizing selection and can be estimated by the ratio of conditional evolvability by evolvability. Finally, Hansen and Houle (2008) acknowledged that the ratio between evolvability and respondability is equivalent to the cosine of the angle formed between the evolutionary response and the selection gradient, even though no name was provided for this latter statistic. We here follow the suggestion (T. Hansen, personal communication) that the designation

"evolutionary flexibility" could be used for the correlation between these two vectors (the evolutionary response and the selection gradient). In terms of the biological interpretation, evolutionary flexibility captures the ability of a population to track closely the direction selection is acting, irrespectively of the magnitude of evolutionary response. Another way to envisage the same point is that evolvability captures the magnitude of the evolutionary response under selection in some direction of the morphospace while evolutionary flexibility captures the alignment of the response with the selection gradient. Therefore, evolvability and flexibility capture different aspects of the ability of a given species to evolve in the direction of selection.

The orientation and magnitude of the evolutionary responses, as well as their degree of similarity with the selection gradient applied, can therefore be very informative about the evolutionary properties of the matrices/populations being compared. In this study, we applied the RS method to analyze the consequences of changes in the magnitude of integration on the morphological evolution of the skull, exploring the relationship between evolvability, evolutionary flexibility, autonomy, constraints and morphological integration magnitudes in 15 mammalian orders. As far as we know, this is the first large scale study clearly linking modularity and integration with their evolutionary consequences in a large group such as mammals.

Materials and Methods

Samples, Landmarks, Measurements and Matrices

The analyzed sample, definition of landmarks and measurements, as well as procedures to obtain V/CV matrices, were described in detail in the companion paper (Porto et al. 2009). However, we think one important point is worth emphasizing here. When dealing with modularity, as well as with its evolutionary consequences, it is important to obtain the best possible representation of phenotypic and genetic patterns and magnitudes of integration. This can only be accomplished with proper sample sizes in order to reduce the effects of sampling error on matrices estimation. Additionally, to us, the proper representation of modularity for any biological group is one that removes sources of variation and covariation that are not directly related to the genotype–phenotype map per se. For example, if one was to estimate a P-matrix without regard to sexual differences within a population, a substantial part of the correlation observed between traits would be due to extant differences between sexes, and not directly to the underlying genetic architecture. The same rationale applies to others sources of variation like geographic variation or between-species differences. Therefore, all matrices used here and in the

companion paper (including G-matrices) are pooled within-groups correlation or V/CV matrices properly controlled for these other sources of variation whenever appropriate (see Table 1 in Porto et al. 2009).

Magnitude of Integration

Besides the V/CV matrices, we also calculated the pooled within-groups correlation matrices for each taxon in our dataset. These matrices were used to calculate the average coefficient of determination (r^2), which is an index frequently employed in morphological integration studies (Cheverud et al. 1989; Marroig and Cheverud 2001; Porto et al. 2009). This index is simply the average of squared correlation coefficients and measures the overall level of integration among all traits. The r^2 is a scale-independent index and is particularly suitable to compare taxa with very different body sizes, such as the mammal groups studied here. The relationship of this index with particular modules within the cranium, as well as with the phylogeny of the mammal taxa under investigation, has been explored in detail in the companion paper (Porto et al. 2009).

Evolutionary Simulations

In order to explore the impact of the overall level of integration on the evolutionary properties of each taxon, the r^2 values were correlated with the results of a simulation of evolutionary responses to selection involving the respective V/CV matrices (Cheverud and Marroig 2007). This approach is based on the multivariate response to selection equation: $\Delta z = G\beta$ (Lande 1979). This equation explicitly relates evolutionary response (Δz) under directional selection to the force of selection operating individually upon each trait (β) and to the patterns of heritable variation (G-matrix). G-matrices can be substituted by their phenotypic counterparts, the P-matrices, if they are sufficiently similar (Arnold 1981; Arnold and Phillips 1999; Lovsfold 1986; Cheverud 1988, 1996; Marroig and Cheverud 2001; Roff 1995; Reusch and Blanckenhorn 1998; Waitt and Levin 1998; Reale and Festa-Bianchet 2000; House and Simmons 2005; Akesson et al. 2007; Oliveira et al. 2009). Considering that this similarity was demonstrated for the mammal groups studied here (Porto et al. 2009), we used P-matrices as a surrogate for G-matrices.

We subjected each phenotypic V/CV matrix to 1,000 random selection vectors (β) and obtained the respective response vectors. Each random selection vector was drawn from a uniform distribution with individual values for the elements ranging from −1 to +1; therefore, these vectors were uncorrelated with each other (average $r = 0.136 \pm 0.101$) and spanned a wide range of possibilities in the morphological space (morphospace).

We normalized all simulated selection vectors to a length of one and multiplied them by each taxon matrix. The 1,000 response vectors obtained (Δz) were then correlated to the first principal component of each matrix and we counted the cases in which correlation coefficients were equal or higher than 0.7. The first principal component of a V/CV matrix is what Schluter (1996) called a "line of least evolutionary resistance" because it is the axis holding the largest portion of genetic (or phenotypic) variation (represented by the symbol p_{max} here). In theory, evolutionary changes along p_{max} will be facilitated even though selection may not be aligned with it. The number of evolutionary responses aligned with p_{max} is a measure of how much constraints embodied in the pattern and magnitude of integration of the phenotypic matrices would bias the cranial evolution of the mammalian lineages compared here. In fact, if we divide the absolute number of simulated responses aligned with p_{max} by the total number of random selection vectors applied (1,000), we can think of this ratio as a measure of constraints (referred hereon as constraints only, for simplicity). While any vector correlation with 35-elements above 0.45 is highly significant ($P < 0.001$), counting the absolute number of evolutionary responses aligned with p_{max} (or $\boldsymbol{\beta}$, see below) still involves the choice of a threshold. Therefore, another way to present the results of these simulations is to use the average vector correlation between the evolutionary responses and p_{max}. We present here both the absolute number and the average vector correlation because the former is a more intuitive number, readily interpretable in terms of the proportions of responses aligned with p_{max} while the latter is a "decision-free" statistic; only the averages were used in the following illustrations.

We also correlated simulated random selection vectors ($\boldsymbol{\beta}$) and their correspondent response vectors (Δz), counting the number of responses significantly aligned with each $\boldsymbol{\beta}$ applied. Here we used a more relaxed criteria, counting the cases in which correlations coefficients were higher than 0.45 (or lower than -0.45), which is the expectation for correlations between 35-element vectors occurring by chance alone. This counting can be thought as a measure of the capacity of the population to respond in the same direction imposed by selection. Dividing this absolute number by the total number of responses (1,000) resulted in an index which can be seen as a measure of morphological evolutionary flexibility. Alternatively, the average correlation between the 1,000 random selection gradients and the corresponding evolutionary responses can also be used as a measure of evolutionary flexibility. Again, we present both here since the latter statistic is a decision-free number (the average), while the former involve a somewhat arbitrary decision (the correlation threshold adopted to count a vector of response aligned or not with $\boldsymbol{\beta}$, in our case $r \geq 0.45$), but it is more intuitive than the average correlation.

Variation related to size may be an important factor influencing phenotypic evolution of any quantitative trait, and can be expressed by the total variation within a taxon explained by p_{max}. To study this influence, we correlated this factor to r^2 values and to the absolute numbers of responses to selection aligned with p_{max} (constraints).

Similarly, integration patterns in specific regions/modules of the skull could also affect changes in the overall phenotype. To account for this aspect, we used data derived from theoretical matrices of functional/developmental relationships among characters (Cheverud 1995; Marroig and Cheverud 2001; Porto et al. 2009). Details regarding the constructions of these matrices were described in the companion paper (Porto et al. 2009). We calculated the ratio between the average correlation of integrated (within modules) and non-integrated (between modules) traits (avg+/avg−) for nine developmental/functional hypotheses. We called these "modularity ratios", and studied their correlation to the constraints and flexibility indexes, as well as other statistics, as described below.

Respondability, Autonomy and Evolvability

Hansen and Houle (2008) formally defined some indexes which could help to understand the evolutionary consequences of modularity: respondability ($\bar{r}$), autonomy ($\bar{a}$) and evolvability (conditional and unconditional—Table 1). These indexes are also based on the multivariate response to selection equation, and can be computed through an approach similar to the one outlined above, using random selection vectors, or alternatively through approximation formulae; details of the calculations can be found in Hansen and Houle's paper (2008). We present only the results based on random selection vectors. Similarly, calculations of those indexes can be carried on raw and mean standardized matrices and although the absolute values of the statistics varied, both resulted in very similar patterns. Therefore, we chose to present only the indexes calculated on mean standardized matrices. To further explore the consequences of morphological integration, we correlated the indexes proposed by Hansen and Houle (2008) to the r^2 values of the mammal groups studied. Table 1 summarizes all abbreviations and indexes used in this paper, as well as their associated biological meaning.

Results

Magnitude of Integration

The magnitude of integration, measured by the average of squared correlation coefficients between traits (r^2), varied considerably across taxa; the lowest value (*Homo*,

Table 1 Abbreviations and symbols used for each variable investigated in this study, how they were measured, and their biological meaning

Symbol	Measure	Simulation equations	Biological interpretation
P or P-matrix	Pooled within-group phenotypic V/CV matrix		The amount and pattern of variation and covariation within a group
p_{max}	First principal component of the P-matrix		The linear combination of traits accounting for the largest portion of phenotypic variance within a group; the phenotypic line of least resistance to evolutionary change (LLER)
Δz	Vector of differences in the averages before and after selection		Direction and magnitude of the evolution
β	Selection gradient (in this paper being simulated and all of them with a norm of one)	Uniform distribution random generator number	A vector describing the directional selection operating individually upon each trait independently from the genetic covariance with other traits in the system
Size	First principal component score of any group; or cubic root of the body size		Measures the absolute size of any species
r^2	Average of the squared correlation coefficients of a given correlation matrix (coefficient of determination)		Measures the overall magnitude of integration among traits in a given correlation matrix; theoretical measurement of the constraints embodied in a correlation matrix
Flexibility ($\bar{f}$)	Cosine of the angle between the selection vector (β) and the response vector (Δz); or the ratio between evolvability and respondability		Measures how close to the direction of selection is the evolutionary response vector
Respondability ($\bar{r}$)	Norm of the response vector; or the length of the predicted response to selection (Δz)	$\mathbf{E}\left[\left(\beta'\mathbf{P}^2\beta\right)^{1/2}\right]$	Measures how rapidly a population will respond under directional selection
Evolvability ($\bar{e}$)	Projection of the response vector over the selection vector; or the length of response in the direction of β	$\mathbf{E}[\beta'\mathbf{P}\beta]$	Ability of a given population to evolve in the direction of selection
Conditional evolvability ($\bar{c}$)	Length of the response vector assuming the presence of stabilizing selection; or the evolvability multiplied by the autonomy	$\mathbf{E}\left[\left(\beta'\mathbf{P}\beta\beta'\mathbf{P}^{-1}\beta\right)^{-1}\right]$	Ability of a given species to evolve in the direction of selection when under stabilizing selection
Autonomy ($\bar{a}$)	Measures the proportion of the genetic variation in a given trait (or linear combination of traits) that is independent of other traits, given the action of stabilizing selection; or the conditional evolvability divided by the evolvability	$\mathbf{E}\left[\left(\beta'\mathbf{P}^{-1}\beta\right)^{-1}\right]$	Proportion of the evolvability that remains after conditioning on other traits
Constraints	Average vector correlation between the response vector and p_{max}, alternatively, the absolute number of significant vector correlations		Measures the relative influence of p_{max} on the direction of the evolutionary responses

$r^2 = 0.05$) was around nine times lower than the highest value (Peramelimorphia, $r^2 = 0.44$). This is equivalent to an average value of correlation among traits three times higher in Peramelimorphia than in humans. Higher r^2 values, indicating higher overall level of integration, were mainly associated with four of the metatherian orders (Peramelimorphia, Diprotodontia, Didelphimorphia and Dasyuromorphia). Besides methaterians, other highly integrated taxa were hyracoids, carnivores, cingulates and *Papio* (Table 2). Lower r^2 values included rodents, scandents, macroscelids and most of the primates; those results were detailed in our companion paper (Porto et al. 2009).

The r^2 values (in logarithmic scale) and absolute skull size were not correlated ($r = 0.059$, $P = 0.799$; Fig. 1a). This was found when using, as a measure of size, the scores of each taxon on the first principal component extracted from the pooled-within groups V/CV matrix of all mammals studied. The same result was found using the cubic root of the body weight as a measure of size.

Response to Simulated Selection, Integration and Evolvability

The number of response vectors aligned with p_{max} (constraints) followed the same basic pattern observed for r^2, in which metatherians, hyracoids, cingulates, lagomorphs, carnivores and *Papio* exhibited higher values than all other taxa (Table 2). The variation in the V/CV matrices explained by p_{max} was also considerably higher in these taxa. The number of response vectors aligned with the

Table 2 All variables measured in the P-matrices and simulations of the mammalian lineages: the morphological integration index (r^2), natural logarithm of the skull size (ln size), responsability ($\bar{r}$), evolvability ($\bar{e}$), conditional evolvability ($\bar{e}$), autonomy ($\bar{a}$), flexibility measured as the average correlation between the evolutionary responses and the selection gradients ($\bar{f}$), flexibility measured as the absolute number of evolutionary responses significantly correlated with the selection gradients ($\bar{f}$ abs.), constraints measured as the average vector correlation between evolutionary responses and p_{max} (Cons.), constraints measured as the number of evolutionary responses significantly correlated with p_{max} (Cons. Abs.), the percentage of variation explained by p_{max} (% p_{max}), oral, neurocranium (Neural), Neuroface and Total integration modularity ratios

Group	r^2	Ln Size	$\bar{r}$	$\bar{e}$	$\bar{c}$	$\bar{a}$	$\bar{f}$	$\bar{f}$ abs.	Cons.	Cons. (abs.)	p_{max} %	Oral	Neural	Neuro-face	Total
Didelphimorphia	0.35	4.99	0.097	0.028	0.0006	0.039	0.30	96	0.89	872	68.1	1.20	0.82	1.01	1.04
Paucituberculata	0.15	3.80	0.016	0.005	0.0001	0.032	0.33	151	0.85	830	61.2	1.97	0.48	1.15	1.08
Dasyuromorphia	0.28	3.86	0.017	0.005	0.0001	0.046	0.32	154	0.87	865	63.8	1.25	0.70	1.04	0.99
Diprotodontia	0.39	5.55	0.223	0.059	0.0006	0.020	0.27	62	0.92	909	75.6	1.43	0.67	1.01	1.00
Peramelimorphia	0.44	4.74	0.095	0.024	0.0001	0.014	0.26	32	0.93	920	80.4	1.32	0.79	1.03	1.00
Hyracoidea	0.26	4.99	0.085	0.024	0.0001	0.005	0.30	89	0.89	884	67.2	1.71	0.55	1.11	1.10
Macroscelidea	0.07	3.96	0.008	0.004	0.0001	0.042	0.49	708	0.65	554	30.4	2.63	0.66	1.27	1.24
Cingulata	0.17	5.01	0.074	0.022	0.0003	0.024	0.31	102	0.88	871	64.0	1.70	0.98	1.06	1.15
Scandentia	0.09	4.29	0.011	0.005	0.0002	0.051	0.46	507	0.58	425	29.3	1.68	1.01	1.29	1.31
Lagomorpha	0.19	4.64	0.052	0.018	0.0007	0.052	0.36	201	0.84	822	54.8	1.75	0.71	1.03	1.02
Carnivora	0.21	5.29	0.077	0.027	0.0011	0.059	0.36	175	0.82	787	53.7	1.34	0.83	1.05	1.08
Perissodactyla	0.10	6.30	0.186	0.082	0.0016	0.024	0.46	546	0.69	614	35.5	2.09	0.84	1.13	1.38
Artiodactyla	0.11	5.52	0.093	0.038	0.0011	0.036	0.42	360	0.74	674	41.1	1.97	0.96	1.08	1.35
Rodentia	0.09	3.86	0.004	0.002	0.0001	0.073	0.49	685	0.68	580	33.1	1.72	0.94	1.29	1.33
Gorilla	0.07	5.92	0.171	0.080	0.0120	0.167	0.49	720	0.68	596	32.9	1.67	1.00	1.20	1.40
Homo	0.05	5.73	0.081	0.044	0.0061	0.147	0.58	963	0.58	425	23.9	1.80	1.38	1.35	1.85
Pan	0.06	5.57	0.083	0.045	0.0097	0.234	0.57	960	0.59	443	24.8	2.55	0.63	1.37	1.57
Papio	0.23	5.66	0.238	0.072	0.0060	0.130	0.31	145	0.89	882	65.2	1.68	0.62	1.19	1.12
Allouatta	0.15	5.09	0.066	0.028	0.0032	0.139	0.43	437	0.77	731	41.7	1.82	0.76	1.17	1.24
Cebus	0.12	4.95	0.063	0.027	0.0020	0.090	0.44	388	0.61	456	32.2	2.05	0.80	1.27	1.44
Callithrix	0.07	4.22	0.017	0.009	0.0013	0.162	0.54	924	0.55	362	23.0	2.20	0.99	1.15	1.50

Fig. 1 a Plot of skull size
against the morphological
integration index (r^2) (both in
natural log scale), **b** Plot of
flexibility against skull size, **c**
Plot of constraints (as measured
by the average vector
correlation between p_{max} and
Δz) against skull size, **d** Plot of
flexibility against constraints, **e**
Plot of the percentage of the
total variation associated with
the first principal component
(p_{max}) against constraints, **f** Plot
of the modularity ratio
(measured as the ratio between
the within modules × between
modules correlations) for the
total integration against the
percentage of the total variation
associated with PC1 (p_{max})
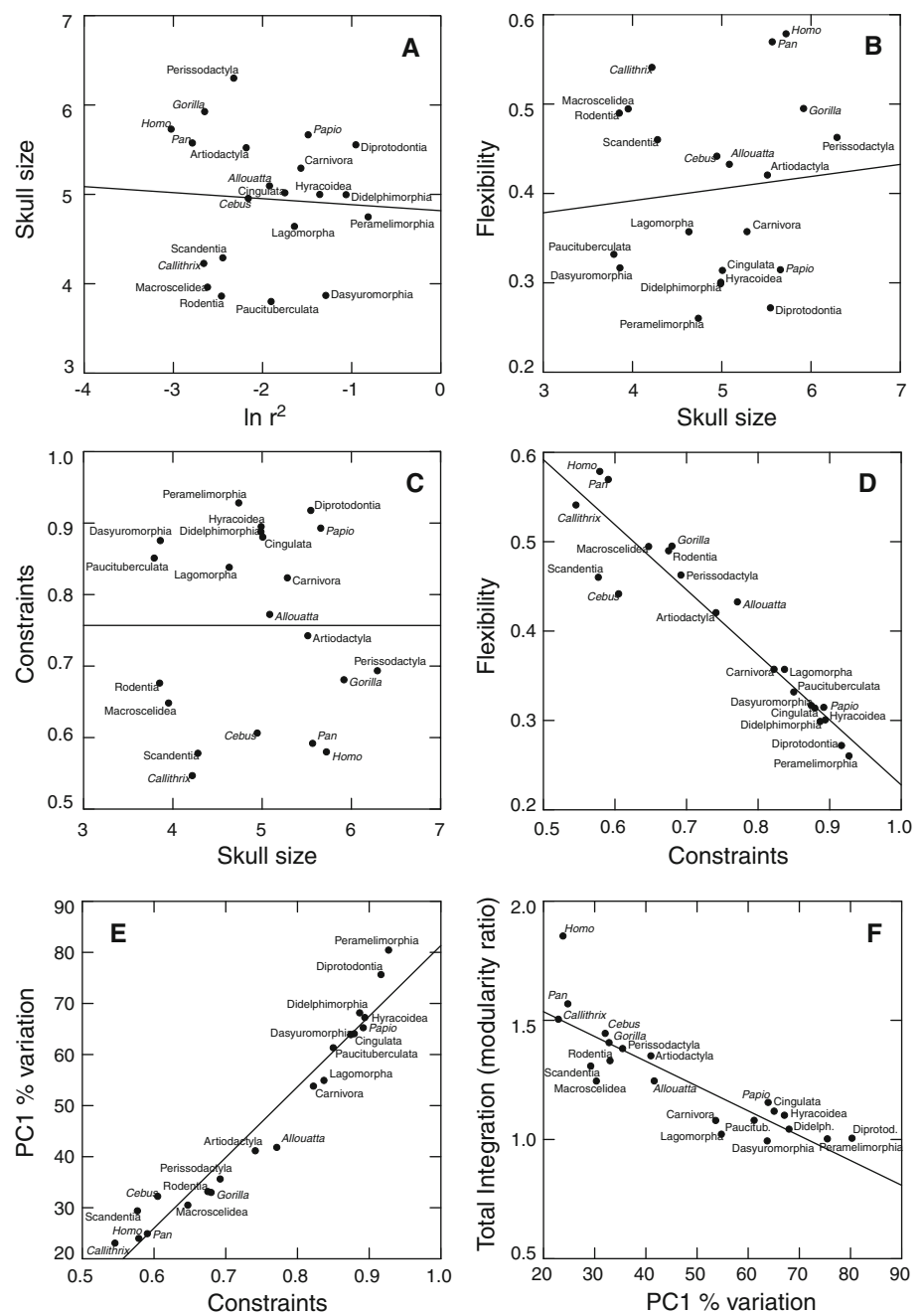
selection gradients (flexibility) exhibited the opposite pattern: higher among many Eutheria and lower among most metatherians and eutherians with high r^2 values. Although some of the primate genera displayed the highest values (*Homo*, *Pan* and *Callithrix*), it should be noted that *Papio* yielded a value as low as paucituberculate marsupials; other genera, like *Cebus* and *Alouatta*, fell in between these extremes (Table 2). Conditional evolvabilities are higher among all primates and low (close to only 1% of the highest values) among Hyracoidea, Paucituberculata, Rodentia, Peramelimorpha, Macroscelidae and Dasyurimorpha. Autonomy follows the same basic pattern

observed for flexibility with low values among Hyracoidea, Peramelimorpha, Diprotodontia, Perissodactyla, Cingulata, Paucituberculata and the highest values again among primates.

The morphological integration index (r^2) was strongly correlated with the absolute number of responses aligned with p_{max} ($r = 0.902$, $P < 0.0001$) as well as with the average correlation between the response vectors and p_{max} ($r = 0.92$, $P < 0.0001$; Fig. 2). Additionally, r^2 exhibited a high positive correlation with the percentage of variation explained by p_{max} ($r = 0.957$, $P < 0.0001$; see Fig. 3 in Porto et al. 2009) and strong negative correlation with the

Fig. 2 Plots of the flexibility and constraints against the morphological integration index (r^2)
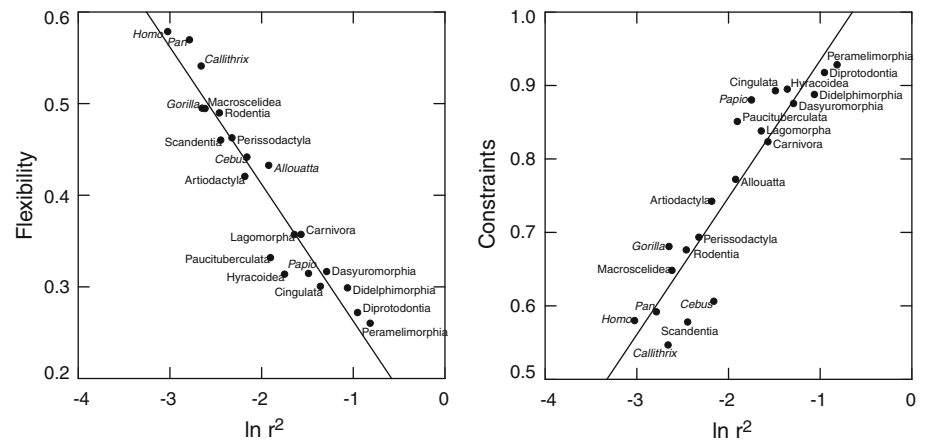
Table 3 Correlation between the natural logarithm of the morphological integration index (r^2), absolute size (ln of the skull size), respondability, evolvability, conditional evolvability, autonomy, evolutionary flexibility (ratio evolvability/respondability), constraints, the percentage of variation explained by p_{max} and the modularity ratios (Oral, Neurocranium, Neuroface and Total Integration) are all found below the diagonal with P values above the diagonal. Bold values significant at $P < 0.05$

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	MI index	–	0.799	0.209	0.871	**0.034**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.022**	**0.000**	**0.000**
2	Skull Size	−0.059	–	**0.000**	**0.000**	**0.006**	0.249	0.669	1.000	0.841	0.939	0.301	0.986	0.176
3	Respondability	0.286	**0.821**	–	**0.000**	0.058	0.803	0.247	0.138	0.160	0.349	0.739	0.327	0.704
4	Evolvability	−0.038	**0.921**	**0.922**	–	**0.002**	0.225	0.801	0.839	0.992	0.941	0.670	0.976	0.350
5	Conditional Evol.	**−0.465**	**0.580**	0.420	0.645	–	**0.000**	**0.022**	0.118	0.073	0.297	0.371	**0.023**	**0.012**
6	Autonomy	**−0.603**	0.263	0.058	0.277	**0.822**	–	**0.001**	**0.005**	**0.003**	0.069	0.289	**0.003**	**0.001**
7	Flexibility	**−0.961**	0.099	−0.264	0.058	0.496	0.692	–	**0.000**	**0.000**	**0.001**	**0.018**	**0.000**	**0.000**
8	Constraints	**0.920**	0.000	0.335	0.047	−0.351	**−0.585**	**−0.950**	–	**0.000**	**0.001**	**0.018**	**0.000**	**0.000**
9	$p_{max}\%$	**0.957**	−0.047	0.318	0.002	−0.399	**−0.612**	**−0.975**	**0.973**	–	**0.001**	**0.023**	**0.000**	**0.000**
10	Oral	**−0.726**	−0.018	−0.215	−0.017	0.239	0.404	0.686	**−0.660**	**−0.687**	–	0.698	**0.002**	**0.007**
11	Neurocranium	**−0.496**	0.237	−0.077	0.099	0.206	0.243	**0.511**	**−0.511**	−0.492	−0.090	–	0.249	**0.002**
12	Neuroface	**−0.820**	−0.004	−0.225	0.007	0.493	**0.624**	**0.810**	**−0.797**	**−0.786**	**0.635**	0.263	–	**0.000**
13	Total Integration	**−0.881**	0.307	−0.088	0.215	**0.539**	0.663	0.907	**−0.870**	**−0.861**	0.568	0.646	0.778	–

average correlation of the response vectors with the selection gradients ($r = -0.961$, $P < 0.0001$; Fig. 2). Table 3 presents all correlations and associated probabilities for the following variables: morphological integration index (r^2 in logarithmic scale), skull size (in logarithmic scale), respondability, evolvability, Conditional Evolvability, Autonomy, Evolutionary Flexibility (the ratio evolvability/respondability or the average correlation between the response vectors with the selection gradients), Constraints (the average correlation between the responses and p_{max}), the percentage of variation explained by p_{max}, and the modularity ratios (Oral, Neurocranium, Neuroface and Total Integration). Autonomy and flexibility were negatively correlated with r^2 (Fig. 2) as well as with the constraints (Fig. 1d) captured in p_{max} (average correlation between the evolutionary responses and p_{max} and the % of variation explained by p_{max}). Furthermore, the modularity ratios were negatively correlated also with both measures

of constraints (r^2 and the number of responses aligned with p_{max}—Fig. 5) and positively correlated with both autonomy and flexibility (Table 3, Fig. 5). Additionally, the modularity ratios (Table 3, Fig. 1f) are also negatively and significantly correlated with the proportion of variation explained by size (p_{max}). Both our measures of flexibility and constraints present no correlation with the skull size (Fig. 1b, c, respectively).

Results can be summarized as follows: (1) there were no significant relationships of respondability and evolvability with other variables, except for the positive and high association with skull size and moderate correlation with conditional evolvability; (2) Conditional evolvability was positively and highly correlated with autonomy and moderately correlated with flexibility, skull size, neuroface and total integration modularity ratios, and negatively correlated with the morphological integration index (r^2); (3) Flexibility and autonomy were positively correlated

between them and additionally presented the same pattern of relationship with all remaining variables. Particularly interesting were the positive and significant correlations of flexibility and autonomy with the modularity ratios, as well as the negative correlation of flexibility and autonomy with the morphological integration index (r^2), constraints, and % of variation associated with p_{max}; (4) Constraints and % of variation associated with p_{max} were also highly correlated (Fig. 1e) and additionally presented the same pattern of relationship with all remaining traits, with a strong correlation with morphological integration index and a negative correlation with modularity ratios.

Discussion

Both respondability (the total amount of evolutionary change) and evolvability were strongly correlated with absolute size. The positive relationship between absolute size and evolvability and respondability would be a trivial result, if we were dealing with non-standardized matrices, because if a fixed selection vector with the same magnitude and direction is applied upon two populations of organisms of different sizes, the larger one will have a larger response to selection and therefore a larger projection of that response upon the selection vector (evolvability). In other words, both are measures related to the magnitude of evolutionary change and therefore are scale-dependent. The same holds for conditional evolvability, that measures the population ability to respond to selection in one direction after conditioning on the stabilizing selection exerted by the correlation among traits. However, we presented results based on average standardized matrices that, in principle, were adjusted for differences in scale (every V/CV matrix is divided, element-by-element, by the result of the cross-product of the vector of averages: $\mathbf{V}\mathbf{V}^{\mathbf{T}}$, where $\mathbf{V}$ is the vector of averages and $\mathbf{V}^{\mathbf{T}}$ is the transposed vector). Standardized and non-standardized results were basically the same and we presented in Tables 2 and 3 the standardized results. Notice that despite this scale-correction, respondability and evolvability still show a strong correlation with absolute size of the organisms. This result is intriguing, but we can only speculate at this point that for some reason the magnitude of evolution was larger on larger organisms even after correcting for differences in scale. Moreover, this is not an artifact of the scaling standardization applied here since we tested different scaling factors (like using the skull size to scale the matrices) with the same results. Notice also that evolvability and respondability have no correlation with any measure of constraints or modularity. These results are probably due to the macroevolutionary scale of this study. We believe that infrageneric studies relating modularity and integration to

measures of evolvability and respondability will be more successful due to, usually but not necessarily, the smaller differences in the scale (size) of the organisms involved.

Conversely, evolutionary flexibility as well as autonomy were independent of absolute size (scale), because both are defined as a ratios: between evolvability and respondability in the first case, and between conditional evolvability and evolvability in the second. We will focus on these two measures (flexibility and autonomy), because we think they are the most informative for the discussion on the evolutionary consequences of morphological integration and modularity on a macroevolutionary scale. However, it is important to note that evolvability and respondability are essentially measures of the magnitude of the evolutionary change, while autonomy and flexibility are measures more related to the pattern (direction) of evolutionary change. Depending on the questions pursued and the scale of the study (micro or macroevolutionary), some of these statistics may be more useful than others.

Evolutionary flexibility is defined here as the capacity of a given species or morphological complex structure (represented by the V/CV matrix) to respond in the direction that selection is pushing. Accordingly, evolutionary flexibility can be measured by the angle (or the cosine of the angle) formed between the evolutionary response and the selection gradient. If the evolutionary response is closely aligned with the selection gradient, then the angle is small and the correlation between the vectors is high. Accordingly, we can say that the structure in question is evolutionarily flexible in that direction of the morphospace. Conversely, if the evolutionary response is not closely aligned with the selection gradient, then the angle is large and the correlation between the vectors is low. Consequently, we can say that such structure is not flexible in that direction of the morphospace, which means that V/CV patterns heavily deflected the evolutionary response from the path through which selection has pushed the population. Autonomy ($\overline{a}$) is the proportion of evolvability that remains after conditioning on others traits under stabilizing selection and can be estimated by the ratio between conditional evolvability and evolvability. Notice that both autonomy and flexibility were highly correlated in our sample (Fig. 3) and also presented the same pattern of correlation with all remaining variables analyzed (Table 3).

Evolutionary constraints are defined here as any limitation on the course or outcome of evolution (Arnold 1992), which is usually translated as bias on the direction, ratio or magnitude of evolutionary change. On the following discussion, it is important to keep in mind that the morphological integration index is a measure of constraints related directly to the matrix itself (see Cheverud et al. 1989; Marroig and Cheverud 2001), while the average vector correlation of the responses with p_{max} is a measure
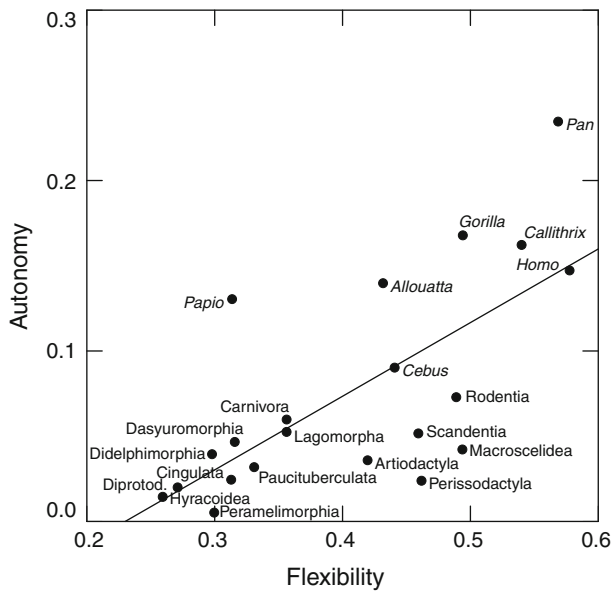
**Fig. 3** Plot of the autonomy against flexibility

related to the interaction between selection and constraints embodied in the P-matrix (or G-matrix). Additionally, considering that the morphological integration index r^2 captures the overall magnitude of correlation among traits, the average vector correlation between response and p_{max} (constraints) is a metric that evaluates the evolutionary consequence of this magnitude.

Our results revealed a clear association among the overall morphological integration, the degree of modularity and the relative capacity of each mammalian taxa to respond to selection. In general, the higher the overall integration in the skull (i.e., tighter association between traits), the lower the ability to respond in the same direction to which selection is pushing (i.e., lower evolutionary flexibility); marsupials were clear examples of this behavior. In contrast, taxa with low overall integration respond more often in the direction of the simulated selection vectors, i.e., had higher evolutionary flexibility,

like most primates in our sample. Moreover, the response to random selection vectors of taxa with low flexibilities most of the time followed the main axis of variation in the population (p_{max}), which is a size-related axis. Less integrated taxa, in turn, presented only a fraction of the responses aligned with p_{max}. This means that highly integrated taxa, like marsupials, tended to exhibit size-related responses regardless of the selection vector applied, while loosely integrated mammals could generally follow the evolutionary path pushed by natural selection. Furthermore, mammals with larger modularity ratios were exactly those with lower overall magnitudes of integration and had higher evolutionary flexibility and autonomy, and less influence of the constraints; the reverse was true for those taxa with lower modularity ratios (had larger magnitude of integration, lower flexibility and autonomy, and higher constraints values, Porto et al. 2009).

In our companion work, we provided evidence that changes in modularity in mammals are not related to pattern evolution, but are instead related to changes in the magnitude of integration within and between modules (Porto et al. 2009). Our results on the responses to random selection vectors suggest that this has interesting evolutionary implications. First, the number of selection responses aligned with p_{max} presented a high positive correlation with the morphological integration magnitude (r^2), evidencing that more integrated skulls responded more often along the line of p_{max}; metatherians and *Papio* (baboons) are good instances of this norm. Another important finding in these lineages was that, despite selection was uniformly distributed in the morphospace (excluding size selection vectors), around 90% of all responses were aligned with size. This illustrates that larger overall magnitude of integration strongly bias the direction in which evolutionary change can happen. Additionally, in groups with high overall integration, the distribution of vector correlations between responses to selection and p_{max} was highly skewed towards higher correlations when compared to lineages with low overall integration (Fig. 4).

Fig. 4 The vector correlation distribution observed between p_{max} and the Δz for two groups: *Homo* and Didelphimorphia. Notice that *Homo* yields a much more uniform distribution, meaning that many evolutionary responses are not aligned with p_{max}, while in Didelphimorphia the bulk of responses presented high correlations with p_{max}
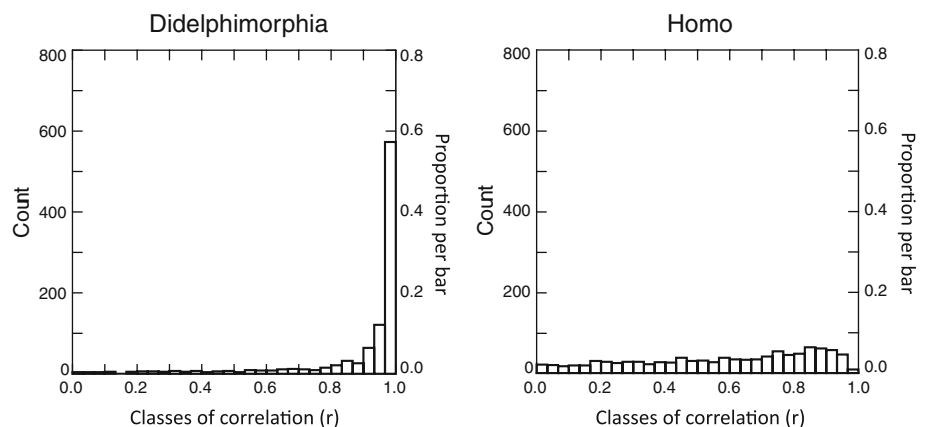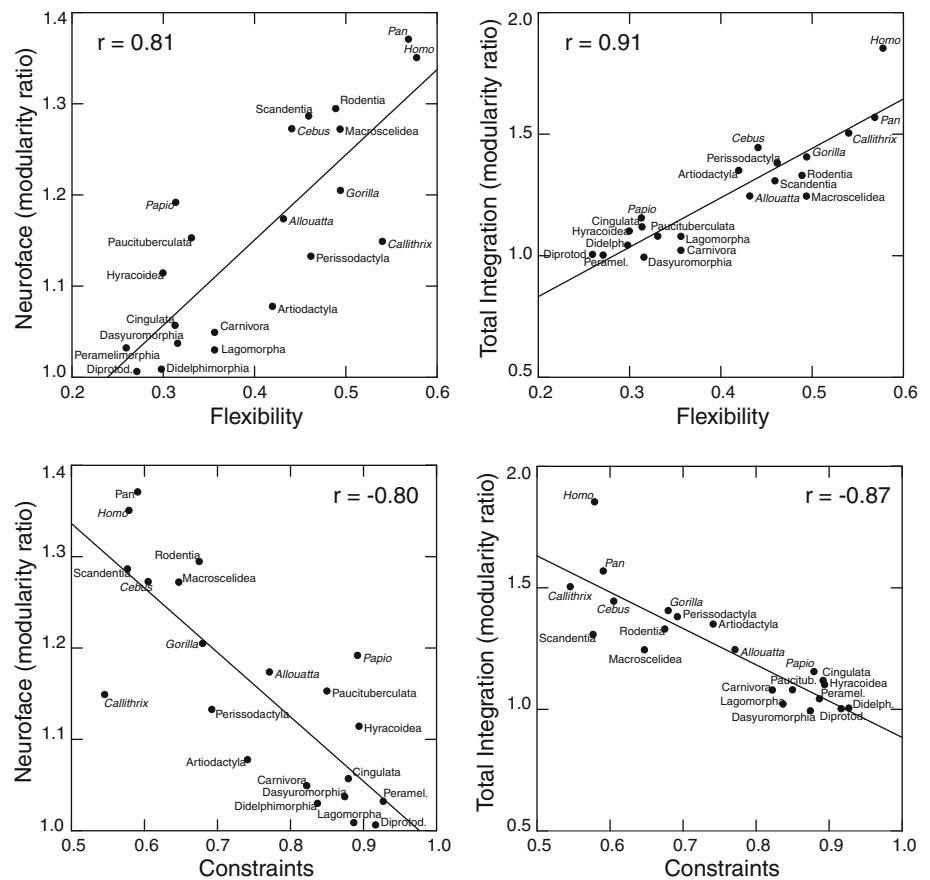
Conversely, higher overall integration (r^2) was strongly and negatively associated with the capacity of a taxon to respond in the direction of selection (Fig. 2). Taxa with high overall integration generally have poorly modularized skulls (as measured by the modularity ratios—Porto et al. 2009), and, therefore, low modularization was associated to lower evolutionary flexibility (Fig. 5), as expected by the theory of modularity (Berg 1960; Cheverud 1984; Wagner and Altenberg 1996). Moreover, these groups with the highest integration also presented the highest percentages of within-group variation explained by p_{max} ($r = 0.957$). Taken together, these results show that an overall tight integration of traits constrains evolutionary change to occur along the axis defined by p_{max}, implying in low evolutionary flexibility and autonomy. In other words, in those groups where the overall magnitude of integration was larger, the amount of the total variation attributed to size was also larger (see Fig. 3 in Porto et al. 2009); in these groups, p_{max} (variation related to size) acts as a strong constraint on the potential evolutionary change. Two good examples are Peramelimorphia and Diprotodontia, which presented around 90% of all responses to random selection vectors aligned with p_{max} and, at the same time, almost 80% of the total variation explained by p_{max} alone. In fact, this result is even more impressive if we consider that the

size dimension is only a tiny portion of the total morphospace available (can be calculated as $(1/2)^{n-1}$ where n corresponds to the number of dimensions, in our case 35). For example, with only two axis, the total space corresponding to size variation is 50%, with three traits would be 25%, with four traits 12.5%, and with 35 traits is only 0.0000000058% of the total space available.

It is important to note that although our dataset is large and representative, some mammalian groups have not been sampled, and with the exception of primates, we have little resolution of the variation within orders. Nevertheless, given the diversity of skulls analyzed, it is reasonable to conclude that morphological integration patterns are conserved among mammals, while magnitudes are variable (see our companion paper Porto et al. 2009). This has already been demonstrated for New World monkeys (Marroig and Cheverud 2001) and preliminary analyses of other primates (Oliveira et al. 2009) and Didelphimorphia (Shirai and Marroig, submitted), indicate that, within mammalian orders, the magnitude of integration is certainly much more plastic than the patterns.

Our results demonstrate that this has important evolutionary consequences showing that the studies of morphological integration magnitudes deserve more attention than they had in the past. Given that integration

patterns among populations is basically similar in all mammals sampled, evolutionary changes that decrease the overall magnitude of integration will impact evolutionary flexibility and autonomy, especially if between-module correlation is reduced more that within modules correlation, as we found for mammals (Porto et al. 2009). Therefore, enhanced modularity will allow a population to track more closely adaptive environmental changes because the constraints imposed by the genetic variance/covariance among traits will not be strong and generally the evolutionary response will follow the direction of natural selection. Our results also allow us to predict that mammalian groups with high magnitudes of integration in the skull will have their evolutionary changes strongly biased by the line of least evolutionary resistance (p_{max}) which in our samples is basically attributable to size (allometric variation). The overall magnitude of morphological integration is not associated with the absolute size of organisms but it is strongly associated with the proportion of the total variation in skull morphology captured by size.

At this point we can only speculate about the mechanisms causing stasis of pattern and evolution of magnitudes in the integration of the mammalian skull. However, it seems likely that pattern conservation is due to internal stabilizing selection acting on largely shared developmental/functional processes and relationships among traits (Porto et al. 2009; Estes and Arnold 2007). Changes in magnitudes of integration could be a result of genetic drift, natural selection or both (Jones et al. 2003); nevertheless, considering the potential adaptive significance of those changes, we suspect that they were driven by selection. Following this rationale, although patterns of integration have been strongly refractory to change due to constrains imposed by stabilizing selection on basic developmental processes, directional selection could promote significant changes by acting on magnitudes of integration in the mammalian skull. Changes in magnitude, therefore, are the key to understand cranial diversity in mammals.

Conclusions

The evolution of modularity in the mammalian skull was dominated by a stasis of integration patterns associated with changes in overall integration magnitudes. Larger magnitudes were associated with less distinct modules, larger variation associated with p_{max}, less evolutionary flexibility, and stronger constraints. In contrast, lower overall magnitudes of integration were associated with more noticeable modules in the skull, smaller fraction of the total variation associated with p_{max} and, consequently, more evolutionary flexibility and weaker constraints.

Therefore, the apparent plasticity in the integration magnitude observed in mammals probably had important consequences for the evolutionary potential of these lineages, with stronger integration associated with a smaller capacity to respond in the same direction of selection, and with weaker integration associated to responses more aligned to selection. We suspect that pattern stasis is due to stabilizing selection acting through shared developmental and functional processes, while magnitude changes are due to directional selection.

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