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Research Article

# The direction of main phenotypic variance as a channel to morphological evolution: case studies in murine rodents

Sabrina Renaud[a,*], Jean-Christophe Auffray[b]

[a] *Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, CNRS, Université Lyon 1, Campus de la Doua, 69622 Villeurbanne, France*
[b] *Institut des Sciences de l'Evolution, UMR 5554, CNRS, Université Montpellier 2, 34095 Montpellier, France*

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**Abstract**

A key issue in evolutionary studies is the means by which evolution can be channeled by intrinsic processes such as genetic and development. Studying the phenotypic variation in a population can shed light on these constraints, because phenotypic variation, being the product of genetic and developmental processes, is the target of both selective screening and random sampling. The main phenotypic variance in populations ("$Pmax$") could thus act as a "line of least resistance to evolution". Based on morphometric analysis of molar evolution in several fossil lineages and modern murine rodents, the role of $Pmax$ as line of least resistance to evolution is investigated: Does evolution along lineages actually occur along $Pmax$? Does this line of least resistance facilitate parallel evolution? What is the relationship of $Pmax$ to developmental processes and functional constraints? Case studies on murine rodent teeth are complemented by examples focusing on mouse mandibles. Compared to teeth, which are mineralized early during development, the mandible, as a bone, is prone to shape changes through remodeling in relation to masticatory muscles and other tissues. Mandible shape may thus vary throughout an animal's life due to allometric growth and, more generally, because of environmental influences. This may lead the mandible's $Pmax$ to align with the direction of plastic and allometric variation. However, the same kind of shape change may also be produced by genetic changes. These examples illustrate how studying patterns of phenotypic variance using geometric morphometrics can help to identify evolutionary processes, bridging several evolutionary levels from intra-group variation to inter-group evolution, and therefore can contribute to an integrated view of phenotypic evolution.

## Introduction

The means by which evolution is constrained and channeled by intrinsic processes such as development is a key issue in evolutionary studies, as these processes might condition the evolvability of traits and their flexibility in response to selection as well as drift (e.g. Beldade et al. 2002; Brakefield 2006). Evolutionary patterns such as parallel evolution may have different interpretations depending on whether or not they have been channeled by intrinsic processes. A similar pattern can be the product of parallel responses to strong comparable and selective pressures, or correspond to similar outputs facilitated by common intrinsic constraints.

The variation existing within a population has the potential to provide clues for deciphering the role of these constraints. The expression of genetic variance is modulated by many genetic, epigenetic, and environmental features which interact with developmental networks to produce the phenotypic variation characteristic of a population. Recognizing the importance of development in conditioning the phenotypic outcome of a given genotype has revolutionized the simplistic view of the genotype-phenotype relationship (e.g. Jernvall 2000; Kavanagh et al. 2007; Salazar-Ciudad and Jernvall 2010; Skinner and Gunz 2010). By integrating both genetic and developmental components, the study of phenotypic variation thus appears fundamental when revisiting morphological evolution with an "evo-devo" perspective.

Furthermore, phenotypic variation is itself a key feature in evolution. Not only is it the phenotypic variation existing in a population on which natural selection operates, but even the output of random processes such as drift depends on this variation, since widespread variants will have a higher chance of being sampled. The evolution of a trait in a given direction may be facilitated when this kind of variation is already present in a population, i.e. as an important component of intra-population variance. Hence, the main direction of intra-population variance has been suggested to constitute a "line of least resistance" to evolution (Schluter, 1996). Evaluating which directions of variance are produced preferentially, their stability over time and space, and their relationship with developmental processes, may thus shed precious light on the role and strength of intrinsic constraints in directing short and long term evolution (e.g. Marroig and Cheverud 2001, 2005; Renaud et al. 2006; Hunt 2007). The aim of the present study is to exemplify how studying the main directions of phenotypic variance, as potential lines of least evolutionary resistance, might help for a better understanding of morphological evolution. Starting from a conceptual background, including methodological issues, case studies of rodent evolution will be used to illustrate the potential of this type of investigation in evolutionary studies.

## Conceptual background

The idea that the main direction of variance may constitute a line of least resistance to evolution was first proposed for genetic variance (Schluter, 1996). The direction of greatest genetic variation (or $Gmax$) corresponds to the major axis of the genetic variance-covariance (VCV) matrix, or $\mathbf{G}$ matrix. This role of $Gmax$ as line of least evolutionary resistance was supported in several studies (e.g. Bégin and Roff 2004; Steppan et al. 2002; McGuigan et al. 2005).

An accurate assessment of the role of the $\mathbf{G}$ matrix and $Gmax$ in evolution requires well known genealogies (Steppan et al., 2002),

*Corresponding author
*Email address:* Sabrina.Renaud@univ-lyon1.fr (Sabrina Renaud)

which are difficult or almost impossible to obtain in wild populations. In contrast, evaluating the phenotypic variance-covariance matrix, or $\mathbf{P}$ matrix, requires measurements of traits in a sample of individuals from a population (Fig. 1A). This type of information may be much easier to obtain. Thus, using the $\mathbf{P}$ matrix as a surrogate to the $\mathbf{G}$ matrix potentially greatly expands the range of evo-devo applications by making it possible to study both wild populations (Cheverud, 1988; Ackermann and Cheverud, 2000; Marroig and Cheverud, 2001), and fossils, for which a direct estimate of the $\mathbf{G}$ matrix is generally impossible (Renaud et al., 2006; Hunt, 2007).

The $\mathbf{P}$ matrix is related to the $\mathbf{G}$ matrix by the equation $\mathbf{P} = \mathbf{H} \cdot \mathbf{G}$, where $\mathbf{H}$ is the heritability matrix (Polly 2004, and references therein). Data about heritability of morphometric characters are scarce, especially in the context of geometric morphometrics. Univariate estimates provide intermediate values (Cheverud, 1988). Multivariate estimates suggest that indeed, $\mathbf{P}$ is significantly correlated to $\mathbf{G}$ and that their main axes of variation ($Pmax$ and $Gmax$) also have similar directions (Siahsarvie, 2012). Such results provide support for the use of the $\mathbf{P}$ matrix as a surrogate of the $\mathbf{G}$ matrix in evolutionary studies. Besides, the $\mathbf{P}$ matrix is interesting in itself, as it contains information not only on the genetic variance but also on non-heritable, environmental and developmental components, which are a central focus of evo-devo studies.

The study of the main direction of variance ($Pmax$ or $Gmax$) provides a conceptual and methodological framework to bridge the gap between different evolutionary scales. $Pmax$, estimated at the intra-population level, can be compared to long-term evolutionary trajectories (Fig. 1A, B) to assess the role of genetic/developmental constraints. The main direction of variance and its relation with evolutionary trajectories can further be interpreted in the context of adaptive landscapes (Fig. 1C, D). This representation plots the fitness (z-axis) as a function of two traits (life-history traits, or morphological traits, which may be axes from geometric morphometrics) (Arnold et al., 2001; Polly, 2008). High adaptive regions are represented by peaks, and unfavorable areas, in terms of fitness, by valleys (Fig. 1C). The evolution of populations can be described by trajectories in the adaptive landscape, with the nearest peak attracting populations towards a local optimum of adaptation (e.g. Arnold et al. 2001; Polly 2008). However, the main direction of variance might sometimes constrain and sometimes facilitate evolution towards a one or the other among neighboring peaks (Fig. 1D). The relation between patterns of variance and observed evolutionary trajectories in the adaptive landscape is seldom investigated in real cases because of the difficulties in measuring fitness changes due to subtle multivariate morphometric variations.

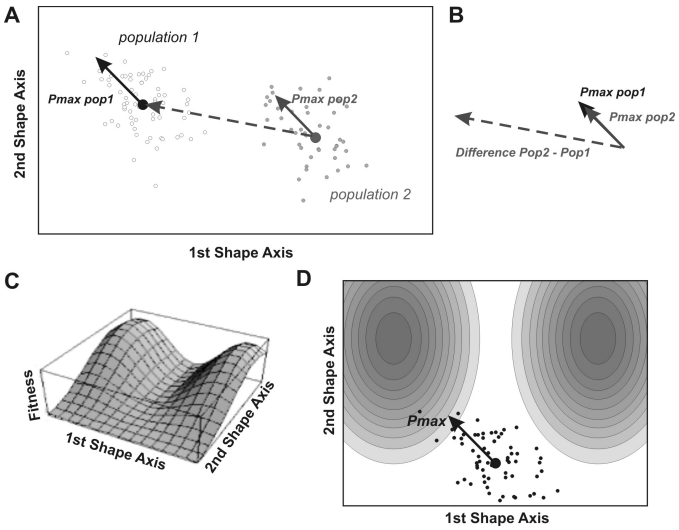


**Figure 1** – . Theoretical illustration of the geometric framework for the study of $Pmax$ and its relationship with the adaptive landscape. A) $Pmax$ and direction of evolution in the morphometric space. A set of morphometric variables can be summarized using independent axes representing directions of variance of decreasing magnitude (1st, 2nd shape axes, etc.). A population can be visualized as a cloud of points in this morphospace. The direction of maximum dispersion of this population is represented by $Pmax$ (first eigenvector of the intra-group VCV matrix). B) The direction of main variance in two populations can be compared using the correlation between their $Pmax$ vectors. $Pmax$ can be further compared with evolutionary directions, which can be evaluated as the difference between group means of two populations, or by the first axis of variation among the means of a set of populations. C) Any morphotype in the morphospace is characterized by its fitness value. An adaptive landscape represents the variations in fitness (values along the vertical z-axis) as a function of traits (for instance along the 1st and 2nd axes of the morphospace) in a population. Two adaptive peaks are shown in the example. D) The variation present in a population (e.g, $Pmax$) can promote evolution towards one or the other adaptive peak.

## Methodological issues

The main direction of phenotypic variance ($Pmax$) can be estimated from quantitative morphological variables by computing the major axes of their variance-covariance (VCV) matrix (the $\mathbf{P}$ matrix). This corresponds to performing a principal component analysis on the variation within the considered sample (e.g. a population, or a fossil assemblage). Successive principal axes describe statistically independent directions of variation. The first one (V1, or $Pmax$) describes the greatest intra-group variance, the second one (V2) describes the second most important direction of intra-group variance, and so forth. Several populations, or species, can be analyzed and represented in a morphospace. Their corresponding $Pmax$ can be projected and compared in this space (Fig. 1A). The direction of $Pmax$ in the different groups can also be quantitatively compared (Fig. 1B) using vector angles (the arccosine of the inner product of the two vector elements). The inner product ranges between -1 (vectors pointing in totally opposite directions) and $+1$ (vectors perfectly pointing in the same direction), similar to simple correlation. Comparing $Pmax$ to other trajectories requires estimating their direction: (1) as difference between two endpoints in evolution (for instance, the difference between an ancestor and its descendent); (2) as the main direction of inter-group variation; or (3) as a direction of morphological change set by its covariation with other factors (e.g., environmental gradients, diet variation, etc.). For instance, the morphological effect of a treatment (e.g., mice bred on standard food vs. mice fed exclusively on soft food) or of a genetic mutation (e.g., a normal strain vs. a genetically manipulated one) can be summarized by a vector connecting the mean of the "control" population to the mean of the "treated" population (e.g. Renaud et al. 2010). The significance of the angle between two vectors is finally estimated using non-parametric models. Among these, a fairly straightforward procedure is to conduct simulations to compute angles between random vectors of the same dimensionality as those being tested (Klingenberg, 1996; Renaud et al., 2006; Marroig and Cheverud, 2010). The corresponding distribution of angles simulates the null hypothesis of no relationship between vectors. If the observed angle is an outlier relative to this distribution, then it can be concluded that it is significantly smaller than expected by chance. A drawback is that random vectors may not accurately represent the distribution of real vectors in the morphospace as these are likely to share some common structure which is not taken into account by the simulation.

An alternative model for the null hypothesis could be using the correlations among a set of real morphometric vectors assumed to randomly explore all directions of the morphometric space (Boell et al., 2011). This approach would take into account the commonalities between vectors describing similar morphological features (say, a rodent mandible). A drawback could be that the distribution of the correlations determining the null hypothesis depends on the set of vectors chosen as a reference. If they are not distributed at random in the morphological space, the vectors will be themselves correlated and will not provide an adequate distribution for assessing the correlation of other vectors of interest. Both of these approaches are designed to compare vectors, such as $Pmax$ of two or more groups. However, it is important to also consider the structure of the entire $\mathbf{P}$ matrix, which can be compared using a Mantel-test. The degree of similarity between matrices can also be evaluated using common principal component analyses (CPCA). Using this method, matrices can be shown to be related in different ways: proportional (when eigenvectors are equal and eigenvalues proportional); characterized by common prin-

cipal components (with equal eigenvectors equal but different eigenvalues); or completely unrelated (with both different eigenvectors and eigenvalues) (e.g. Arnold et al. 2008).

All these methods for comparing vectors and matrices, however, do not take into account the phylogenetic relatedness between populations and species, an issue that will require the development of techniques within the broader context of phylogenetic comparative methods (Klingenberg and Gidaszewski, 2010). Also, these tests assume that vectors or matrices are reliably estimated. Sampling error, however, may severely affect estimates of means, variances, and angles (Polly, 2005; Cardini and Elton, 2007). The uncertainty in the estimates of these parameters can be assessed using bootstrap methods.

## Case studies: the molar tooth and the mandible of murine rodents

Rodents are the most diverse order of mammals, with ca. 2000 species including nearly half of all mammalian species. Among them, the subfamily of murine rodents (Murinae, or Old World mice and rats) includes today ca. 120 genera and 550 species (Wilson and Reeder, 2005). Their radiation involved numerous morphological and life-history traits, among which the diversification in diet caused considerable variation in the selective pressure on morphological traits related to food processing, such as teeth and mandibles (e.g. Misonne 1969; Michaux 1971; Michaux et al. 2007) (Fig. 2). The house mouse (*Mus musculus*) belongs to the Murinae, and, as an emblematic laboratory model, a rich background on its genetics and development is available from experimental studies (e.g. Klingenberg et al. 2001, 2003; Workman et al. 2002; Shimizu et al. 2004; Kassai et al. 2005; Kavanagh et al. 2007; Boell et al. 2011).

The first model investigates the evolution of molar tooth shape in fossil and modern representatives of murine rodents. The following issues will be addressed: (1) Is the main direction of variance conserved across lineages? This is a prerequisite for a potential role as a line of least evolutionary resistance. (2) Does the main direction of variance actually parallel the evolutionary trajectory along a lineage? This provides correlational evidences for $Pmax$ being a line of least resistance to evolution. (3) If the main direction of variance is shared across lineages, and serves as a line of least evolutionary resistance, can it contribute to facilitate parallel evolution in different lineages? (4) Both selection and random processes can "surf" on these lines of least resistance. Are there means to disentangle their signature on morphological evolution? (5) How does the main direction of phenotypic variance relate to developmental processes and function? These questions will be answered using molar teeth, which mineralize early in development and are not prone to change with late growth except for wear. Whether plasticity in bones affects the main direction of variance and its role as line of least resistance to evolution will be, in contrast, investigated using the house mouse mandibles, which are subject to remodeling throughout life.

The material investigated therefore includes a set of fossil and modern first upper molars (UM1) of murine rodents, and mandibles of modern house mice (Fig. 2). Molar samples include specimens of murine rodents from Western Europe from the Miocene to present day (Tab. 1). They document the molar shape evolution along three fossil lineages which, starting with an ancestral form, *Progonomys*, lead independently to *Stephanomys* (Renaud et al., 1996, 2005, 2006), to *Paraethomys* (Renaud et al., 1999a) and to the wood mouse *Apodemus sylvaticus* (Renaud et al., 2005). The paleontological record was completed by two modern populations of wood mice, as well as two populations of the house mouse *Mus musculus domesticus*, which are used to exemplify evolution on islands (Renaud et al., 2011). Mandible data, in contrast, are from a sample of laboratory mice from an outbred strain (OF1), bred in controlled conditions at the PBES (Ecole Normale Supérieure, Lyon, France) and sacrificed at various ages from weaning (22 days) up to six months of age. This cross-sectional ontogenetic series was compared to variation in a sample from a natural population (Gardouch, France).

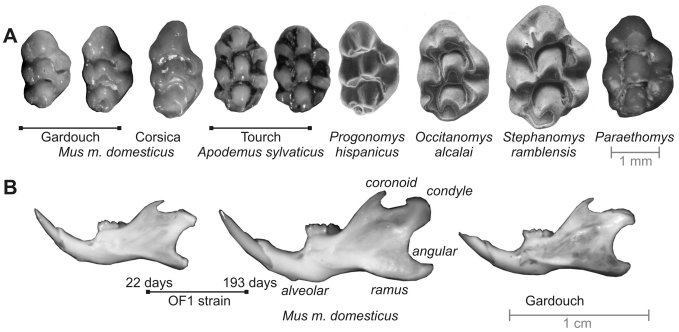


**Figure 2** – Examples of first upper molars (A) and mandibles (B) of murine rodents considered in this study. A) First upper molars (lingual side to the right) in various modern and fossil murine rodents. From left to right, teeth exemplify variation in a house mouse continental population (Gardouch, France); the insular population of Corsica; variation in a wood mouse population (Tourch, France); evolution along the *Stephanomys* lineage, and *Paraethomys*. B) House mouse mandibles. The two mandibles on the left visualize ontogenetic variation in the OF1 laboratory strain (left, a specimen sacrificed at weaning; right, a six-months old specimen). The mandible to the right corresponds to a wild population (Gardouch, France).

Shape was quantified using a Fourier analysis of the 2D outline (basis of the crown for the molar and labial view of the bone for the mandible). Using this approach, each outline was described by successive trigonometric functions of decreasing wavelength, the harmonics. Each was weighted by Fourier coefficients constituting shape variables after size standardization. Consideration of the first seven harmonics appeared as a satisfactory compromise between information content and number of variables for both characters (e.g. Renaud and Michaux 2007). The 14 resulting Fourier coefficients (2 Fourier coefficients per 7 harmonics) were used as shape variables.

The main direction of phenotypic variance, $Pmax$, was calculated based on the variance-covariance (VCV) matrix of the 14 shape variables. It was evaluated at the intra-group level, with a group corresponding to a population of modern specimens, an assemblage of fossil teeth, or a taxon including several fossil deposits or modern populations. Directions of evolution were calculated for each lineage as the first axis of the inter-group VCV matrix, calculated on the group means.

$\mathbf{P}$ matrices were compared using Mantel t-tests. Similarity between vectors ($Pmax$ and evolutionary directions) was assessed by comparing their observed correlation R to the distribution of R from fifty thousand simulated random vectors. For vectors of 14 elements, this provided the following significance threshold values for the absolute value of $R$ (a significant probability meaning that the observed $R$ is larger than expected based on the distribution of $R$ between random vectors): $p < 0.01$ , $R = 0.651$ (*); $p < 0.001$, $R = 0.770$ (**); $p < 0.0001$, $R = 0.860$ (***). Note that the absolute value of R was considered, because the +/- direction of $Pmax$ (and of any eigenvector) is arbitrary.

### Impact of sampling on Pmax estimate

As a preliminary analysis, in order to investigate the effect of sampling on the estimate of the $\mathbf{P}$ matrix and $Pmax$, the structure of morphological variance-covariance was computed in two samples of house mice (Fig. 3) and its variation assessed by bootstrapping. The samples were molars from the Fango population in Corsica ($N = 53$) and mandibles from the French Gardouch population ($N = 68$). Each sample was bootstrapped 100 times. The bootstrap procedure was repeated in random subsamples with $N = 50$ (for the Gardouch population starting from $N = 68$), $N = 25$ and $N = 10$. This demonstrated that $Pmax$ from bootstrapped samples were in a vast majority of cases significantly correlated to the observed $Pmax$ (Tab. 2). However, when $N$ decreased, some of the estimates of $Pmax$ show large differences and become inaccurate. The percentage of variance explained by $Pmax$ varied considerably even in bootstrapped samples with the original sample size (Tab. 2). It tended to be slightly overestimated when sample size decreased. Thus, overall, bootstrap analyses confirm previous findings suggesting that reliably assessing $Pmax$ and $\mathbf{P}$ matrices does require large number of specimens per population (Prôa et al., 2013).

**Table 1** – Sampling of the fossil deposits and modern localities that delivered the first upper molars (UM1) considered in this study. Age in million years is provided for the fossil localities, together with the number of first upper molars measured (UM1). Data from Renaud et al. 1996, 1999a,b, 2005, 2006, 2011.

| Group/Lineage | Locality | Abbreviation | Genus | Species | Age (myr) | UM1 |
|---|---|---|---|---|---|---|
| *Stephanomys* | La Roma 4B | ROM4B | *Progonomys* | *hispanicus* | 9.6 | 4 |
| | La Roma 4C | ROM4C | *Progonomys* | *hispanicus* | 9.5 | 8 |
| | Masia Del Barbo 2B | MBB | *Progonomys* | *hispanicus* | 9.2 | 20 |
| | Peralejos D | PERD | *Progonomys* | *hispanicus* | 8.7 | 16 |
| | Dionay | DIO-PH | *Progonomys* | *hispanicus* | 8.6 | 15 |
| | Puente Minero | PM | *Occitanomys* | *sondaari* | 8.3 | 20 |
| | Tortajada A | TOA | *Occitanomys* | *sondaari* | 8.1 | 20 |
| | Masada Del Valle 2 | MDV2 | *Occitanomys* | *adroveri* | 7.3 | 20 |
| | Concud 3 | CC3 | *Occitanomys* | *adroveri* | 7.0 | 20 |
| | Los Mansuetos | LM | *Occitanomys* | *adroveri* | 6.9 | 20 |
| | Valdecebro 3 | VDC3 | *Stephanomys* | *ramblensis* | 6.3 | 21 |
| | Las Casiones | KS | *Stephanomys* | *ramblensis* | 6.1 | 20 |
| | La Gloria 4 | GLO4 | *Stephanomys* | *dubari* | 5.9 | 12 |
| | Castelnou 3 | C3 | *Stephanomys* | *dubari* | 5.6 | 15 |
| | La Tour | LT | *Stephanomys* | *dubari* | 5.6 | 5 |
| | Sète | STE-SD | *Stephanomys* | *donnezani* | 3.1 | 79 |
| | Lo Fournas 13 | LF13 | *Stephanomys* | *donnezani* | 3.0 | 30 |
| | Balaruc 2 | BAL2-SC | *Stephanomys* | *calveti* | 2.7 | 44 |
| | Pla De La Ville | PLV-SC | *Stephanomys* | *calveti* | 2.5 | 101 |
| | Seyne | SEY-ST | *Stephanomys* | *thaleri* | 2.5 | 30 |
| | Moreda 1B | MOR | *Stephanomys* | *minor* | 2.4 | 60 |
| | Balaruc 6 | BAL6 | *Stephanomys* | *thaleri* | 2.3 | 30 |
| | Lo Fournas 4 | LF4 | *Stephanomys* | *thaleri* | 2.0 | 30 |
| | Iles Medas | ILM | *Stephanomys* | *balcellsi* | 1.9 | 44 |
| | Casablanca 1 | CAS | *Stephanomys* | *progressus* | 1.8 | 30 |
| *Apodemus* | Dionay | DIO-PL | *Parapodemus* | *lugdunensis* | 8.6 | 18 |
| | Sète | STE-AD | *Apodemus* | *dominans* | 3.1 | 39 |
| | Balaruc 2 | BAL2-AD | *Apodemus* | *dominans* | 2.7 | 43 |
| | Pla De La Ville | PLV-AD | *Apodemus* | *dominans* | 2.5 | 10 |
| | Seynes | SEY-AD | *Apodemus* | *dominans* | 2.5 | 20 |
| | Vergranne | VER | *Apodemus* | *sylvaticus* | 0.45 | 13 |
| | Orgnac 3 | OR3 | *Apodemus* | *sylvaticus* | 0.35 | 8 |
| | Montpellier | MTP | *Apodemus* | *sylvaticus* | modern | 14 |
| | Tourch | TOU | *Apodemus* | *sylvaticus* | modern | 88 |
| *Paraethomys* | Oued Tabia | OTAB | *Progonomys* | *cathalai* | 9.5 | 3 |
| | Afoud8 | AF8 | *Paraethomys* | sp. | 5.2 | 2 |
| | Wanou | WAN | *Paraethomys* | *miocaenicus* | 7.8 | 2 |
| | Khendek El Ouaich | KEO | *Paraethomys* | *miocaenicus* | 7.7 | 2 |
| | Amama 2 | AMA2 | *Paraethomys* | *miocaenicus* | 7.6 | 1 |
| | Azib | AZB | *Paraethomys* | *pusillus* | 5.3 | 4 |
| | Amama 3 | AMA3 | *Paraethomys* | *anomalus* | 2.9 | 7 |
| | Irhoud DV | IDV | *Paraethomys* | *darelbeidae* | 1.0 | 30 |
| | Sidi Abdallah 1 | SABH1 | *Paraethomys* | *rbiae* | 1.5 | 1 |
| | Irhoud Neand. | IRHN | *Paraethomys* | *filfilae* | 0.6 | 13 |
| *Mus musculus* | Gardouch | GARD | *Mus* | *musc. dom.* | modern | 68 |
| | Corsica | CO | *Mus* | *musc. dom.* | modern | 62 |

## Molar shape: Stability of Pmax across time and phylogeny

The phenotypic signature of conserved genetic/developmental constraints should be indicated by a relative invariance of $Pmax$ in different lineages (Badyaev and Foresman, 2000; Marroig and Cheverud, 2001). $Pmax$ and $\mathbf{P}$ matrix of the first upper molar (UM1) were estimated and shown to be conserved in two lineages spanning over 10 million years of evolution (Renaud et al., 2006) (Tabs. 3 and 4). A second study (Renaud et al., 2009) demonstrated that the same pattern of variance is conserved also when the wood mouse (*Apodemus sylvaticus*) is compared to the house mouse (*Mus musculus domesticus*), two species that diverged some 10 million years ago (Lecompte et al., 2008). Whatever the basic tooth shape characteristic of the species, the main direction of intra-population variance corresponds to a trend from narrow to broad teeth (Figs. 3 and 4).

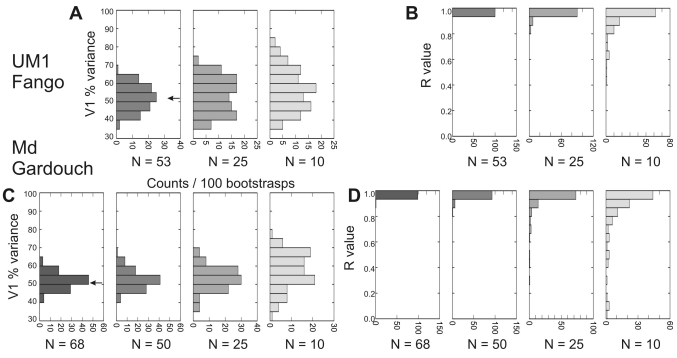


**Figure 3** – Impact of sampling on the estimate of $Pmax$. Two populations with fairly large sample size were considered: Corsica for first upper molars ($N$ = 53; A and B) and Gardouch for mandibles ($N$ = 68; C and D). The initial samples were bootstrapped 100 times. The number of specimens in the bootstrapped samples was then progressively decreased to 50 (starting from 68 for the mandible set), 25 and 10 specimens, and bootstraps repeated. The first eigenvector was extracted in all corresponding VCV matrix, providing estimates of $Pmax$ that were compared to the initial $Pmax$. The distribution of the percentage of variance explained by $Pmax$ (A, C) and the correlation $R$ with the original vector (B, D) are shown. Initial percentage of variance is represented by an arrow (UM1: 51.3%; mandible: 50.6%).

## Pmax as a line to least resistance to molar shape evolution

Murine rodents diversified in Europe around 10 million years ago from the primitive, generalist *Progonomys* (Michaux, 1971; Renaud et al., 1999b). One lineage developed a peculiar dental specialization termed stephanodonty (Schaub, 1938), characterized by longitudinal crests connecting the transverse rows of cusps on the upper molars (Fig. 2) which slide in corresponding gutters on the occluding lower molars. Teeth also became larger and higher-crowned along the lineage. Supposed to increase masticatory efficiency, these morphological changes have been interpreted as adaptations to a more abrasive diet, probably grass. This interpretation is supported by comparative studies in extant murines with similar teeth and diets (Renaud and Michaux, 2004;

**Table 2** – Effect of sampling on estimate of $Pmax$. $Pmax$ was computed in two samples with large sample size of house mice, for first upper molar shape variation (Fango, Corsica) and for mandible shape variation (Gardouch, mainland France). A bootstrap procedure (100 replications) was repeated in random subsamples decreasing from initial sample size to $N$ = 50, $N$ = 25, and $N$ = 10. Mean, standard deviation (SD), maximum and minimum of the distribution in bootstrapped samples are provided for the percentage of variance explained by $Pmax$ (%$V1$) and the correlation (absolute value of $R$, in order to take into account the arbitrary +/- direction of the eigenvector) between bootstrapped and initial estimates of $Pmax$ (including the percentage of significant correlations [%*], with a threshold of $R$ = 0.651 corresponding to $p < 0.01$.

| | | % V1 | | | | R | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Initial | | $N$ = 53 | $N$ = 25 | $N$ = 10 | | $N$ = 53 | $N$ = 25 | $N$ = 10 |
| UM1 Fango | Mean | 51.3 | | 52.4 | 53.6 | 56.6 | | 0.988 | 0.972 | 0.910 |
| | SD | | | 6.8 | 9.5 | 11.1 | | 0.012 | 0.034 | 0.111 |
| | Max | | | 67.0 | 71.4 | 82.7 | | 0.999 | 0.998 | 0.997 |
| | Min | | | 37.6 | 36.5 | 36.0 | | 0.943 | 0.839 | 0.463 |
| | % * | | | | | | | 100 | 100 | 95 |
| Md Gardouch | | | $N$ = 68 | $N$ = 50 | $N$ = 25 | $N$ = 10 | $N$ = 68 | $N$ = 50 | $N$ = 25 | $N$ = 10 |
| | Mean | 50.6 | 52.0 | 52.5 | 53.3 | 57.1 | 0.981 | 0.974 | 0.923 | 0.829 |
| | SD | | 4.2 | 4.9 | 6.4 | 9.6 | 0.017 | 0.024 | 0.113 | 0.220 |
| | Max | | 63.3 | 66.6 | 69.4 | 76.0 | 0.997 | 0.998 | 0.996 | 0.989 |
| | Min | | 41.6 | 40.6 | 38.6 | 34.9 | 0.909 | 0.863 | 0.274 | 0.057 |
| | % * | | | | | | 100 | 100 | 97 | 86 |

Renaud et al., 2005). The origin of these specialized phenotypes has been related to climatic changes, which caused a shift from dominantly closed landscapes towards more open environments (Fox and Koch, 2003; deMenocal, 2004).

The possible role of $Pmax$ in constraining morphological evolution in response to these environmental changes was tested by comparing $Pmax$ with the directions of molar shape changes in the lineage (Renaud et al., 2006). Indeed, the lineage leading from *Progonomys* to *Stephanomys dubari* (from around 10 to 5 million years ago) evolved along a direction of shape change parallel to $Pmax$ in the ancestor population (Fig. 4) (correlation between inter-group $V1$ and $Pmax$ of *Progonomys hispanicus*, $R$ = 0.896***). Yet, the same lineage also showed one case of departure from a model of evolution along lines of least resistance: the transition from the Miocene *Stephanomys* to the Pliocene representatives of the genus (between 7 and 3.5 million years) implied a drastic change of direction along an evolutionary trajectory statistically independent from the ancestral $Pmax$ (Fig. 4A). As a consequence, the direction of evolution along the whole lineage (*Stephanomys*-total on Fig. 4B) was only marginally correlated with the $Pmax$ in the ancestral population (correlation between inter-group $V1$ and $Pmax$ *Progonomys hispanicus*, $R$ = 0.635) (Fig. 4B). An alternation of evolutionary modes, either along lines of least resistance or not, seems to suggest changes in the selection regime. Responses to weak or even intermediate selective pressures might be facilitated along the lines of least resistance, since they represent the major pattern of covariation among phenotypic traits. Yet, in the case of strong selection in favor of phenotypes expressing rare covariation among traits, constraints can be overriden (Beldade et al., 2002) and evolution might follow directions unrelated to the lines of least resistance.

Based on paleoenvironmental proxies (Zachos et al., 2001; Fox and Koch, 2003), we may infer that the environmental trend driving morphological evolution along the *Stephanomys* lineage was regular and of limited magnitude from 10 to 6 million years. The climatic trend accelerated afterwards leading to extremes of variation in the Pleistocene. An initial response, occurring along the lines of least resistance and mainly corresponding to a broadening of the teeth, might have been sufficient to deal with small environmental changes. In contrast, the evolution of the stephanodont pattern away from the direction of least resistance set by the main structure of variance and covariance in teeth morphology may have been crucial for adaptation to the much more pronounced change in the environment which occurred later in the history of the lineage. This extreme specialization was an evolutionary dead-end, and *Stephanomys* did not survive the extreme Pleistocene climatic fluctuations and went extinct about 1.2 million years ago. That specialists might be more prone to extinction in times of environmental change seems to be a general occurrence in the evolution of life on Earth (e.g. Leonard et al. 2007) and one which is likely important to understand how living species are and will be affected by global changes in our climate and environment (Clavel et al., 2011).

## Lines of least resistance and parallel evolution

Related species, especially if under similar ecological and/or environmental pressures, are expected to show a variable extent of parallel evolution. Parallelism in evolution might become especially pronounced if genetic and developmental constraints, common to different lineages, channel evolution towards a similar preferential direction. Thus, we compared molar shape evolution along several loosely related lineages to estimate whether they occurred in a similar direction which is consistent with our assessment of $Pmax$. Evolving in the same time period as *Stephanomys* from another representative of the primitive genus *Progonomys*, a lineage developed, mainly in North Africa from the Miocene to the Pleistocene, which led to an independent acquisition of stephanodont crests (Jaeger et al., 1975) in *Paraethomys* (Fig. 2). This genus underwent an evolutionary acceleration between 3 and 1 million years ago, probably in relation to climate change, and went extinct soon after, in the Late Pleistocene (Renaud et al., 1999a). Beyond the acquisition of stephanodont crests, which suggests a common adaptive response to grass eating, it is still an open question whether the evolution of molar shape in *Paraethomys* occurred along lines of least resistance, which might have been the same as the one leading to the unusual morphology of *Stephanomys*.

To answer this question, we compared directions of evolution in the two lineages (Fig. 4). Although characterized by idiosyncratic molar shape and independent evolution, the *Paraethomys* lineage displayed a direction of evolution highly correlated with that of the *Stephanomys* lineage (directions of evolution estimated by inter-group $V1$; *Paraethomys* vs. total *Stephanomys* lineage: $R$ = 0.815**; vs. early *Stephanomys* lineage: $R$ = 0.926***). This direction of evolution is similar to that of $Pmax$ estimated in various murine species: in the ancestor population of the *Stephanomys* lineage (*Progonomys hispanicus*: $R$ = 0.875***), the wood mouse (*Apodemus sylvaticus* Tourch, $R$ = 0.877***), and even the house mouse (Gardouch population: $R$ = 0.844**). Thus, overall, these results are consistent with the idea of commonalities in processes (genetic and/or developmental ones), which might facilitate parallel evolution in related linages.

## Selection and random processes "surfing" along lines of least resistance

Phenotypic evolution is in essence multivariate. Focusing exclusively on $Pmax$ (= $V1$ of the $\mathbf{P}$ matrix) neglects subsequent components of the variation ($V2$, $V3$, etc.), which may also represent significant directions of evolutionary changes. Selection might tend to favor evolution mostly along one specific direction, which seems often to coincide, to a large extent, with $Pmax$. Random processes, however, should not occur along preferential trajectories and changes should simply be proportional to the variance-covariance structure in the population. Thus, one can try to disentangle the effects of random processes from those of selection by comparing the proportions of variance on successive axes between intra- and inter-group VCV matrices (Roff, 2000; Ackermann and Cheverud, 2004; Arnold et al., 2008). With this aim, we compared

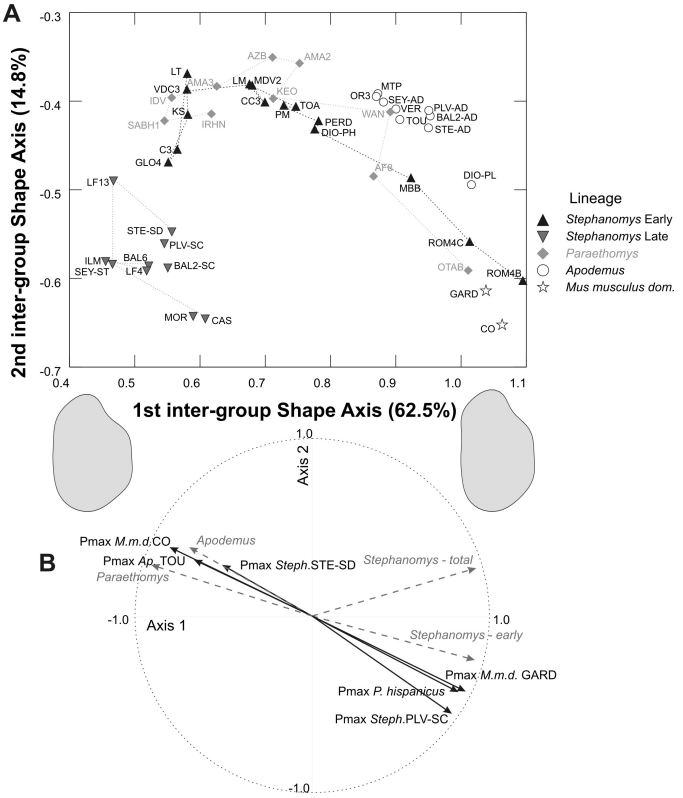


**Figure 4** – $Pmax$ and evolution of the first upper molar of murine rodents. A) Temporal and phylogenetic differentiation of the first upper molar along three lineages of murine rodents: *Stephanomys*, *Paraethomys*, and *Apodemus*, and two house mouse populations. Each symbol corresponds to the mean shape of a population, plotted in a morphospace defined by the first two principal axes of the total inter-group variation. Along the first axis, molar outlines visualize the shape changes from a PC1 score of 0.0 to one of 2.0 (0.5 for PC2). B) Relationships between $Pmax$ (first vector of intra-group variance, full black arrows) and directions of evolution (first vector of inter-group variance, dotted grey arrows). All vectors are projected on the axes represented on (A). Vectors pointing in a similar directions (+ and - arbitrary) suggest that molar shape changes share common components. Note that the morphospace was constructed using 14 shape variables; the correlation of the vectors is thus expressed on a 14-dimensional space. All vectors are here scaled to unity (shown using a circle of radius 1): vectors shorter than unity point into a multivariate direction out of the plane (e.g. $Pmax$ of *Stephanomys* in STE-SD).

(Tab. 5): (1) intra-group variance in several populations/species; (2) inter-group variance in lineages, where selection is assumed to have played a major role; (3) inter-group variance in lineages, where random processes are more likely to have occurred.

The first axis describing the inter-group variance along lineages evolving under directional selection (e.g. *Stephanomys* or *Paraethomys*) is expected to represent significantly more variance than its counterpart at the intra-group level. Indeed, $Pmax$ represented between 30% and 55% of variance in all groups considered (Tab. 5), an appreciably smaller percentage than the first axis of inter-group variance in *Paraethomys* (66%) and *Stephanomys* (77% in the early part of the lineage).

In contrast, the lineage of the wood mouse does not seem to have evolved under strong directional selection. After originating from the primitive *Progonomys*, it did not undergo much morphological change and evolved into a group of taxa related to the modern wood mouse (*Apodemus*), which is still living in Europe today (Michaux et al., 1997). Presumably, while *Stephanomys* colonized niches in the new open habitats, *Apodemus*, a generalist, survived the climatic fluctuations by tracking its forest habitat in a mosaic landscape (Renaud et al., 2005). If this hypothetical reconstruction is correct, it is reasonable to assume that stabilizing selection maintained a fairly constant pattern of tooth morphology in this lineage. Consistent with this expectation, the first axis of inter-group variance in *Apodemus* explained a comparable amount of variation (47%) to those within groups (Tab. 5).

In conclusion, the approach exemplified in this study seems promising and provides clues on the selection regime which might have been the main driver of evolution in these groups (Ackermann and Cheverud, 2004; Marroig and Cheverud, 2010). It is important to bear in mind, however, that this approach requires a large number of groups to reliably estimate the matrix of inter-group variances and its structure, and an extensive sampling of specimens for estimating the intra-group variance-covariance matrix, which will be used to compute the percentages of variance explained by different components (Fig. 3).

## Beyond lines of least resistance: genetics and function

Some phenotypes seem more widespread than others in a population. This might be because the corresponding genotype is more common or because developmental processes are channelling phenotypic variation in a specific direction. Indeed, using quantitative trait loci (QTL) analyses, it has been shown that the mouse mandible is characterized by some recurrent patterns of shape change which are associated with specific genetic traits (Klingenberg et al., 2001). In the murine first upper molar, the pattern of associated with $Pmax$ corresponds to a trend from slender to broad molars (Renaud et al., 2006, 2009) (Figs. 2, 4). The position of the cusps is determined early during embryogenesis by the position of signaling centers (enamel knots), and the size of their surrounding inhibitory field (Jernvall, 2000). A broadening of the molar can be triggered by a concerted increase in breadth of the developmental field, which will later become a tooth, together with an increase in the lateral spacing of the primary enamel knots and their inhibitory field. This might be mediated by changes in the regulation of genes controlling tooth development (e.g. Mustonen et al. 2003). Such effects should be global and concern all molars and all cusps of a tooth. Indeed, strong integration was found in all six molars (upper and lower

**Table 3** – Correlation among $\mathbf{P}$ matrices, estimated using Mantel tests. Above the diagonal, $p$ values; below the diagonal, $R$ values. In bold significant probabilities.

| *R/p* | *Prog.hisp.* | *Steph.* STE | *Steph.* PLV | *Apod.* TOU | *M.m.d.* GARD | *M.m.d.* CO |
|---|---|---|---|---|---|---|
| *Prog.hisp.* | - | **0.005** | **< 0.001** | **0.004** | **< 0.001** | 0.088 |
| *Steph.* STE | **0.272** | - | **< 0.001** | **< 0.001** | **< 0.001** | **< 0.001** |
| *Steph.* PLV | **0.512** | **0.610** | - | **< 0.001** | **< 0.001** | **< 0.001** |
| *Apod.* TOU | **0.285** | **0.400** | **0.392** | - | **0.001** | **< 0.001** |
| *M.m.d.* GARD | **0.570** | **0.547** | **0.513** | **0.339** | - | **< 0.001** |
| *M.m.d.* CO | 0.142 | **0.556** | **0.475** | **0.361** | **0.566** | - |

**Table 4** – Correlation between $Pmax$ in various groups. Below the diagonal, correlation of the vectors $R$ = inner product of the two vector elements. Above the diagonal, significance of the correlation, obtained by comparing the observed $R$ to the distribution of $R$ between random vectors. In bold significant correlations ($p$ < 0.01, $R$ = 0.651).

| *R/p* | *Prog.hisp.* | *Steph.* STE | *Steph.* PLV | *Apod.* TOU | *M.m.d.* GARD | *M.m.d.* CO |
|---|---|---|---|---|---|---|
| *Prog.hisp.* | - | | *** | *** | *** | * |
| *Steph.* STE | 0.461 | - | | * | | *** |
| *Steph.* PLV | **0.960** | 0.545 | - | *** | *** | ** |
| *Apod.* TOU | **0.860** | **0.749** | **0.891** | - | *** | *** |
| *M.m.d.* GARD | **0.939** | 0.629 | **0.963** | **0.917** | - | ** |
| *M.m.d.* CO | 0.721 | **0.876** | **0.779** | **0.898** | **0.848** | - |

**Table 5** – Structure of the inter-group and intra-group variance in different cases of evolution of the first upper molar in murine rodents. Upper panel, inter-group variance (VCV matrix) estimated on group means of a set of fossil and/or modern populations documenting the evolution along the lineages of *Stephanomys* (total: from *Progonomys* to *Stephanomys progressus*; early: from *Progonomys hispanicus* to *Stephanomys dubari*), *Paraethomys*, and *Apodemus*. Lower panel, intra-group variance ($\mathbf{P}$ matrix) in a set of fossil populations from the *Stephanomys* lineage, and in modern populations of the wood mouse (*Apodemus sylvaticus*, Tourch, France) and the house mouse (*Mus musculus domesticus*, Gardouch and Corsica, France). $N$, number of items (specimens for intra-group and group means for inter-group) used for the calculation of the VCV matrix. $V1$ (= $Pmax$), $V2$, $V3$: % of variance explained by the first three eigenvectors.

| | **Model** | | **N** | **V1** | **V2** | **V3** |
|---|---|---|---|---|---|---|
| Inter-group | *Stephanomys* | total | 24 | 59.9 | 23.0 | 7.9 |
| | *Stephanomys* | early | 14 | 77.4 | 15.2 | 2.3 |
| | *Paraethomys* | total | 10 | 66.2 | 14.5 | 6.7 |
| | *Apodemus* | total | 9 | 47.4 | 28.0 | 11.7 |
| Intra-group | *Prog. hisp.* | total | 63 | 54.3 | 15.6 | 8.5 |
| | *Stephanomys* | STE | 79 | 35.5 | 25.8 | 9.6 |
| | *Stephanomys* | PLV | 101 | 34.1 | 26.8 | 11.3 |
| | *Apodemus* | Tourch | 88 | 36.8 | 14.6 | 11.1 |
| | *Mus musc. dom.* | Gardouch | 68 | 38.9 | 19.9 | 12.7 |
| | *Mus musc. dom.* | Corsica | 62 | 49.2 | 17.0 | 10.5 |

ones) in mice Renaud et al. (2009), such that when upper teeth become broader, lower molars do the same.

A broadly similar pattern of variation was also found in insular populations of the house mouse. Compared with continental populations, the first upper molar of Corsican mice is slender. This elongation is nevertheless not related to a narrowing of the latitudinal rows of cusps on the tooth, but to a local, anterior elongation of the tooth, to the point of the appearance of an additional cusplet (Renaud et al., 2011). This anterior elongation was a recurrent pattern in several insular populations. It was speculated that it might involve a differential incorporation of a vestigial bud, anterior to the developmental field of the first molar, which usually aborts as the first molar forms (Prochazka et al., 2010; Renaud et al., 2011).

The morphological signature of the two patterns of variance (narrowing of the tooth vs. anterior elongation) suggests a discrepancy. One corresponds to a generalized effect on all molars (narrowing/broadening; Renaud et al. 2009), the other one to a localized change in the first upper molar (anterior elongation; Renaud et al. 2011). They were attributed to different candidate developmental mechanisms. Yet, they also seem to share some common features: they both affect the shape of the tooth without changing the longitudinal alignment of the cusps. Indeed, $Pmax$ in Corsican populations (related to the anterior elongation) is similar to $Pmax$ in continental populations of house mice and to that found in several other murines, that are not characterized by an anterior elongation (Tabs. 3 and 4). This might be explained by a functional constraint. Murine rodents are characterized by a longitudinal chewing movement (propalinal direction), which is achieved by arranging cusps in longitudinal rows that slide into gutters on the occluding tooth (Lazzari et al., 2008). This mechanism inevitably constrains the arrangement of the cusps because any change of the longitudinal arrangement would disrupt function and will therefore be strongly counter-selected. However, a global narrowing/broadening of the tooth, achieved by changing the spacing of the longitudinal rows in a concerted way between occluding teeth, does not perturb this arrangement. In a different way, the anterior elongation of the first upper molar is also consistent with functional requirements. In conclusion, the congruence between functional expectations, inter-group evolutionary trajectories, and $Pmax$ suggests that genetic and developmental systems coevolved in order to match the requirements of the propalinal masticatory movement (Butler, 1985; Lazzari et al., 2008).

## Pmax and plasticity of the mouse mandible

In murine rodents, the molar tooth shape is determined during prenatal development and this is particularly evident for the first upper molar, which is the first to develop and the one which influences all the others in a cascade of spatial interactions along the molar row (Kavanagh et al., 2007). Once erupted, murine molar teeth remain unchanged throughout life except that they wear with use. Mandibles, in contrast, are bony structures and they are actively remodeled by their constant interactions with the muscles and other tissues during and after prenatal development (Katsaros et al., 2001; Mavropoulos et al., 2004, 2005). Mice for instance have only reached about 80% of their adult skull size at weaning (Zelditch et al., 2003), which leaves room for further growth and remodeling. The importance of shape change late during growth is illustrated using a sample of laboratory mice bred in controlled conditions (Fig. 5). All these animals had the third molars fully erupted and would have been considered as adult in a wild population. Their $Pmax$ (Fig. 5C) was largely in the direction of allometry (correlation of $Pmax$ with direction of allometric variation, estimated by regressing shape onto mandibular size, $R$ = 0.997***) and similar to $Pmax$ of wild animals, with fully erupted teeth and hence considered as subadults and adults (Gardouch, France: $R$ = 0.713*). Thus, for mandibles, which are highly plastic, $Pmax$ can strongly be influenced by growth and environmental factors. Shape changes among populations of a same species could occur following $Pmax$ by mere differences in their age structure, which could be enough to generate shape differences because of allometry. Size differences are likely to produce shape variation in mandibles even simply because of physics and the non-linear changes in the forces required during mastication to move mandibles of different sizes (Satoh, 1997; Cardini and Tongiorgi, 2003; Michaux et al., 2007). Size is seen as a highly labile evolutionary character (Bünger and Hill, 1999; Dupont and Holzenberger, 2003), and accordingly it might display rapid divergence among populations or related species (Nevo, 1989; Ganem et al., 1995; Dayan and Simberloff, 1998; Kingsolver and Pfennig, 2004; Cardini et al., 2007). The importance of size-related shape changes can thus makes $Pmax$ collinear with allometry within populations, so that size and allometric shape changes become a line of least evolutionary resistance (Marroig and Cheverud, 2005, 2010).

Because of interactions with muscles and surrounding tissues, mandible shape may also vary in response to environmental factors such as food consistency (e.g. Katsaros et al. 2001; Mavropoulos et al. 2004; Renaud et al. 2010). This plastic effect was demonstrated in laboratory mice and it was also shown to be collinear to allometry and $Pmax$ estimated in wild populations (Renaud and Auffray, 2010). The same areas of the mandible, especially the zone of insertion of the masticatory muscles, seem to recurrently emerge as very variable. It is thus

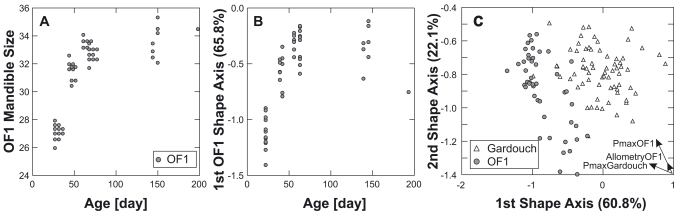


**Figure 5** – Variation in mandible size and shape in the house mouse (*Mus musculus domesticus*). A) Mandible size increase with age of the animal in a laboratory strain (OFI). B) Mandible shape changes along growth of the same OFI mice. Shape is estimated by scores on $Pmax$. C) Morphospace including OFI mice and a wild-trapped population (Gardouch, France). $Pmax$ and allometry of OFI mice, and $Pmax$ of a wild population, were projected into a common morphospace (length of the vectors arbitrary).

strongly contributing to $Pmax$. However, this zone is also strongly involved in allometric shape changes, plastic response to food consistency, genetic (Klingenberg et al., 2001), and biogeographic variation (Renaud and Michaux, 2003; Siahsarvie et al., 2012). Such areas might be under strong functional requirements, making them prone to both genetic and plastic variation, and preferential targets of adaptive evolution. This illustrates that $Pmax$, even when including a component of plastic variation, might be relevant to infer complex evolutionary processes beyond patterns of morphological differences.

## Concluding remarks

The geometric morphometric framework we used in this study has a great potential for investigating the role of intrinsic constraints in channeling evolution. Thus, it can help to bridge evolutionary studies at different scales, from micro- (intra- and inter-population variance) to macro-evolution (species and supra-specific differences). It can also be instrumental in identifying the mechanisms involved in evolutionary divergence (e.g. mutational effect, developmental mechanism, effect of a treatment). However, the mechanisms behind morphological change in evolution are diverse and complex, and might involve genetics, plasticity, developmental and environmental factors. Experimental studies will be needed together with descriptive approaches to fully understand the relative roles of these components and better disentangle processes from patterns.

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