

## MACROEVOLUTIONARY DYNAMICS AND COMPARATIVE METHODS OF MORPHOLOGICAL DIVERSIFICATION

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### ABSTRACT

Among the comparative approaches that have been used to understand the patterns of morphological diversification, those related to the detection and evaluation of large-scale evolutionary trends have recently been highlighted. A new method known as the analysis of skewness (ANSKEW) allows partitioning between the passive and driven trends associated with the random occupation of a bounded morphological space and a single morphological attractor, respectively. This partitioning provides a better understanding of the relative role of processes that occur at distinct hierarchical levels associated with the macroevolutionary trends of morphological diversification. In this paper, we used this new approach to understand the patterns of morphological diversification in Erodiscini (Coleoptera, Curculionidae, Otidocéphalinae) beetles. When genera were used as subclades, ANSKEW revealed that 19.9% of the body size variation in the Erodiscini was attributable to driven trends, i.e., a morphological attractor, whereas 80.1% of the variation was attributable to the occupation of different adaptive zones by distinct subclades (a passive process), with the passive components being significant (based on 5,000 bootstrap samples). This simple approach to partitioning provided insights into the intrinsic dynamics of body size evolution in this group without the need to consider explicit phylogenetic structures. Such analyses could provide a starting point for further evaluation of adaptive variation at multiple hierarchical levels and of the processes underlying the relationship between variation in body size and other ecological, physiological and behavioral aspects.

**Key words:** Analysis of skewness, macroevolution, morphological diversity

### INTRODUCTION

The comparative method has long been the most common approach for identifying morphological adaptations, i.e., the relationship between biological and environmental variation [2]. However, it was only in the 1980s that the theoretical and methodological aspects of the comparative approach were formalized to allow a better understanding of adaptive variation. This change was triggered by many factors, including a general improvement in our understanding of evolutionary biology [8], an increasing interest in the formal reconstruction of the phylogenetic history of organisms (and, more recently, the possibility of using molecular variation – see Felsenstein [3]), and the recognition that species (or any other taxa) do not provide independent observations for correlative

statistical analyses (see Harvey and Pagel [10] and Diniz-Filho [2] for a brief history of these methods).

Among the many possible comparative approaches for understanding biological diversity and the process of diversification, those related to the detection and evaluation of evolutionary trends have recently been highlighted [12,14-16]. Indeed, there are numerous examples of trends in time and space, e.g., species richness increases from temperate to tropical zones, the number of cell types increases with increasing DNA content, and species become large-bodied through evolutionary time. The evolution of this complexity has been extensively discussed elsewhere [1,4,5,13,15,16]. In particular, trends in the evolution of body size have been recognized since the 19<sup>th</sup> century and formed the basis of neolamarckian models of evolution, especially the orthogenesis theory developed by paleontologists [9,13].

Evolutionary biologists have been concerned with the detection of these simple patterns in time and space and with the processes underlying

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these broad-scale trends. Of course, teleological, metaphysical and neo-lamarckian models for the existence of evolutionary trends, such as orthogenesis, are no longer accepted, although the mechanisms underlying evolutionary trends are still being debated. For example, if adaptation occurs only at individual level, as orthodox Darwinism states, then it would be necessary to advocate that the directional selective pressures that generate these trends have been acting continuously over an enormous number of generations. Alternatively, macroevolutionary models of discontinuous evolution, including punctuated equilibrium models, state that these changes can be concentrated in a few moments of evolution [7].

McShea [12,15,16] revisited the analytical methods and evolutionary interpretations of broad scale trends, with emphasis on morphological diversification, and discussed the theoretical and methodological advances in this area. In this paper, we revisit the main aspects of modern methods for analyzing evolutionary trends and their interpretation, and apply a new approach specifically developed to evaluate these methods. We also use this new approach to examine the patterns of morphological diversification in Erodiscini (Coleoptera: Curculionidae: Otdocephalinae) beetles [20].

### Evolutionary Trends

Initially, it is necessary to understand how overall patterns of morphological diversity can help us understand evolutionary trends. A “morphological space” may be defined by plotting species (or any other taxa) in a Euclidian space in which each dimension is a morphological trait [12]. For example, species vary in body size (a complex trait that is usually associated with many ecological, physiological and behavioral aspects of organisms) [1] and have a spatial distribution defined by this trait. A general pattern in this space is that most species are small-bodied (although the smallest possible species do not form the most diversified taxa) and a few species are large-bodied [1,5,13]. The resulting aggregation of species at a given position in space generates an asymmetrical distribution, the explanation for which requires a consideration of the macroevolutionary dynamics of these species throughout this morphological space [6].

If ancestral species started at the center of the space and diversified randomly with respect to a given trait, then a normal distribution of values for a parameter, e.g., body size, would be expected.

Random diversification implies that the chance of evolving towards larger or smaller values in this space, e.g. a larger or smaller body size, is equal [12]. This normal distribution of phenotypes could be interpreted as if a new adaptive peak had appeared in the morphological space and further diversification would occur randomly around it. Alternatively, a skewed distribution in morphological space suggests non-random diversification. A right skewed distribution of body sizes, for example, might suggest that large-bodied species somehow have more adaptive advantages in which body size evolves at higher rates so that the distribution of species along the morphological axis will be right skewed. Such a situation suggests that there would be an ‘attractor’ in morphological space. This attractor may be characterized as a region in which there are selective advantages for individuals of the species [12], and would appear independently in different clades or subclades (Fig. 1A).

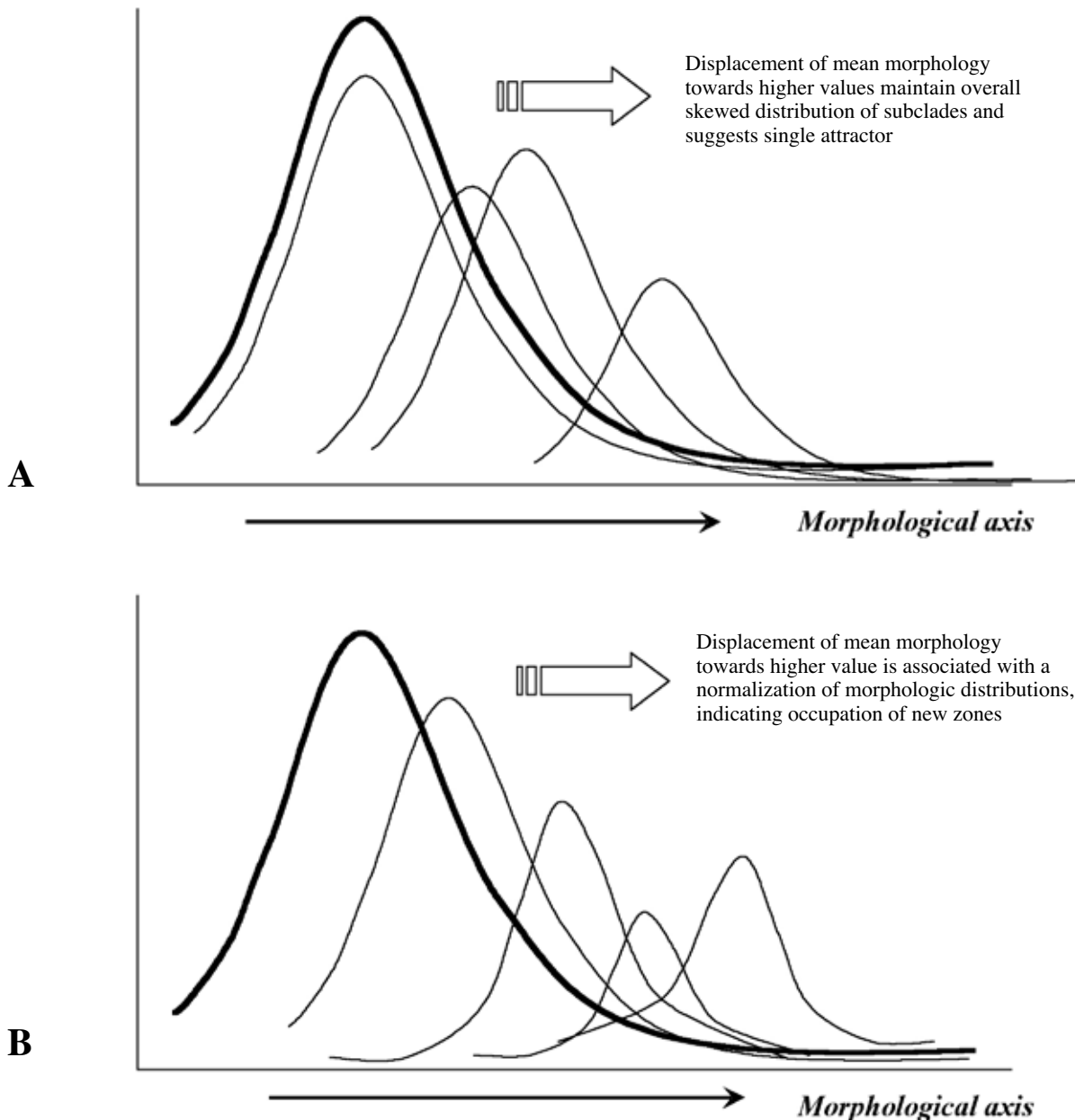
However, what if there is a right-skewed distribution of body sizes associated with more complex distributions in the occupation of morphological space by subclades within this group? Answering this question may facilitate our understanding of alternative models for morphological trends. McShea [16] proposed that such skewedness reflects a combination of the position of the ancestral species in morphological space and the existence of physical or physiological barriers close to this ancestral condition. For example, if a clade originates from a small species and there is a ‘minimum viable’ body size (a ‘barrier’, or bounded region in morphological space) [19], then the only possibility for evolving is towards a larger body size, even if this increase is not driven by selection (i.e., there is no ‘attractor’ in morphological space).

Because of the tendency to partly retain ancestral conditions, species will accumulate in the small body size classes, thereby generating the same pattern of skewness previously discussed for the overall group or clade. However, as long as new subclades arise and shift from this initial position so that the ancestral mean is greater than the overall clade mean and therefore distant from the ancestral barrier, their distribution in morphological space will become normal (Fig. 1B). In this case, new adaptive peaks may appear appear time after time, which suggests that there is more than one attractor in the morphological space (i.e., large-bodied species

with a greater selective advantage). This phenomenon is known as a passive trend because the variation is normally distributed in the absence of a barrier, and skewness in morphological space (and, consequently, the observed trend) results from a combination of the ancestral condition and the existence of a barrier to

morphological variation, rather than from specific processes that drive variation towards a particular body size class.

Based on this theoretical reasoning, McShea [12,15,16] proposed several approaches that can be used to assess whether a morphological trait evolved



**Figure 1.** A visual distinction between alternative models for macroevolutionary trends towards different (increasing) values of a morphological trait, e.g., body size. If continuous selection for larger body sizes generates a skewed distribution for an entire group of organisms along morphological space, then all subgroups (subclades) evolving within this large group will show a similarly skewed distribution. This is a driven process in which we can characterize an ‘attractor’ in morphological space (**A**). However, differential occupation of this morphological space could reflect adaptive radiation that may produce clades situated a given ‘distance’ from the ancestral peaks such that the adaptive process will position the species according to a Gaussian distribution ‘around’ the new adaptive peaks of morphological variation. In this case, the overall skew will reflect the position of the ancestral species close to the ancestral peak (**B**).

under a driven or passive macroevolutionary trend. The more intuitive of these (in terms of the foregoing discussion) is the so-called ‘subclade’ test, which is based on the relationship between means and skewness coefficients [17] measured in different subclades of a clade. In this case, the lack of a relationship between the means and skewness for multiple subclades indicates that groups far from the mean are not less skewed, and there is therefore no evidence of a barrier. An overall skewed distribution of the clade would probably then be determined by a morphological attractor (a driven trend throughout the entire clade). This test also has the advantage of not requiring an explicit phylogeny (but see Maurer [12]).

On the other hand, the presence of a monotonic or linear relationship between the mean and skewness indicates that subclade means become normal as long as they are greater than the overall (clade), which is further evidence of a barrier and that overall skewness in morphological space results from passive (random) diversification [12]. The term passive, in this case, does not indicate an absence of natural selection in general, but rather only the absence of a common selective agent throughout evolutionary time that generates a trend. Clearly, the appearance of new adaptive peaks involves a complex evolutionary process and usually includes key innovations that will eventually increase the absolute rates of diversification within a particular subclade [17].

This distinction allows us to partition the evolutionary trends of morphological diversification into passive, inter-subclade processes (adaptive radiation in a subset of clades outside of ancestral conditions) and driven, intra-subclade processes (an attractor in morphological space that drives all species, independently of the clade). However, these mechanisms are not mutually exclusive and both processes may be expected to be operate along an evolutionary hierarchy. Indeed, Wang [20] recently proposed a new approach that allows to this partitioning to be done statistically, using a procedure known as Analysis of Skewness (ANSKEW hereafter). This approach is analogous to the well-known analysis of variance (ANOVA) [18] and is discussed below.

### Analysis of Skewness

ANSKEW is analogous to ANOVA and was designed to allow the distribution of variation in skewness within and between subclades, together with a heteroscedasticity component, along a species’

position in a given (i.e., morphological) space [12]. These components quantify the proportion of variation in skewness within and between subclades that can distinguish between driven and passive processes of morphological diversification.

More formally, ANSKEW is an improved subclade test [6,12,14-16] that allows a quantitative evaluation of the driven and passive trends involved in macroevolutionary patterns. The total clade skewness sum of cubes (SCT) in a group is given by

$$SCT = \sum_i \sum_j (Y_{ij} - Y_G)^3$$

where  $Y_{ij}$  is the quantitative variable of interest (i.e., the species’ value along the morphological axis) for the  $j$ -th species in the  $i$ -th subclade, and  $Y_G$  is the mean among all species in all subclades (the grand mean). However, it is also possible to partition this SCT into between-subclade (SCB) and within-subclade (SCW) skewness, by defining

$$SCB = \sum_i \sum_j (Y_{Ci} - Y_G)^3$$

and

$$SCW = \sum_i \sum_j (Y_{ij} - Y_{Ci})^3$$

where  $Y_{Ci}$  is the mean of the  $i$ -th subclade. There is also a subclade heteroscedasticity component (SCH), given by

$$SCH = 3 \sum_i \sum_j (Y_{Ci} - Y_G) (Y_{ij} - Y_{Ci})^2$$

that combines the variability of the  $i$ -th subclade about the mean (the second term of SCH) weighted by the ‘distance’ to the overall mean (the first term of SCH). Hence, SCH will be large when subclades in the tail of the overall distribution are more variable than subclades close to the grand mean.

In general, SCB, SCW and SCH can be expressed as proportions of SCT. A purely driven trend in the group under study would be characterized by a high relative value for SCW (close to 1.0) since the overall skewness is largely determined by within-subclade processes. Alternatively, under a purely passive process, most of the overall skewness will be attributable to shifts in the subclade means such that most of the variation will be in the SCB component, which will tend to unity.

Wang [20] argued that passive drives should be viewed as a null model against which driven trends can be tested, in which case it would be necessary to devise an evolutionary scenario to generate null expectations and to define how new adaptive peaks (i.e., clade means) move in morphological space in evolutionary time. However, in a purely statistical

framework, if the positions of species in morphological space are randomized with respect to subclades, then the relative values for SCW will approach 1.0 since the overall skewness will be entirely attributable to within-subclade variation or, more precisely, to the random allocation of all species in the morphological space, independently of the subclade, thereby resulting in similar patterns of skewness. We have used bootstrap samples [11] to obtain a confidence interval for SCB components by assuming that all evolution results from a given species' responses which are governed by a morphological attractor. For this, the overall vector corresponding to the body sizes of the species was re-sampled (with replacement) and randomly assigned to one of the groups, with the ANSKEW components then being calculated from this dataset. This procedure was repeated 5,000 times and provided a critical value for SCB under a purely driven trend. The null statistical hypothesis being tested was the absence of adaptive radiation outside the ancestral morphological conditions, and was established by determining the critical magnitude of the passive component (SCB/SCT) expected by chance alone under a purely driven trend.

### An Application of ANSKEW

To illustrate the application of ANSKEW to the study of evolutionary patterns in body size, we used a small dataset from Vanin [19] for variation in a morphological character (body size, expressed as maximum body length, in mm) in 75 species of Erodiscini (Coleoptera: Curculionidae: Otidoccephalinae) beetles. Although this sample group was small and uniform (because of the relatively low hierarchical level chosen), it was used here to illustrate how ANSKEW functions with a commonly encountered dataset. ANSKEW analyses and bootstraps were done in a software written in QBASIC, a copy of which is available from the main author upon request.

The body size of the 75 species varied from 2.9 to 11.6 (coefficient of variation, 26%), with a significant right skew equal to 0.842 ( $\pm 0.277$ ;  $t = 3.039$ ;  $P = 0.003$ ) (Fig. 2A). This asymmetrical distribution was analyzed with ANSKEW by examining the distribution of body sizes in different subclades of Erodiscini. For this analysis, we assumed that the four genera (*Erodiscus*, *Pimelerodius*, *Prosicoderus* and *Sicoderus*) within the tribe were monophyletic or, according to Wang [21], that they represented

coherent evolutionary units. Consequently, the data were partitioned into four subclades. A few species of these genera were not considered because no body size data were available for them.

ANOVA showed that the mean body size differed among these four subclades ( $F = 9.181$ ;  $P < 0.01$ ). However, to evaluate the mechanisms underlying these differences, the entire shape of the distributions within and among subclades must be evaluated. Because of the small number of subclades, it was difficult to directly evaluate the relationship between the coefficients of skewness and mean body size. Nevertheless, ANSKEW provided a satisfactory means of defining driven and passive trends in the evolution of body size. The average within-genus skew was  $0.328 \pm 0.320$ , and ranged from  $-0.066$  (in *Erodiscus*) to  $0.714$  (*Pimelerodius*); only *Erodiscus* has a non-skewed distribution.

ANSKEW showed that 19.9% of the body size variation in Erodiscini was attributable to driven trends (i.e., a morphological attractor), whereas 80.1% of the variation was attributable to the occupation of different new regions of morphological space by distinct subclades, under a passive process. The latter variation consisted of components from the normalization of distributions outside the minimum body size (50.3%) and heterogeneity in the variances of groups departing from this minimum (29.8%). These values agreed with the skew variation among genera compared with the total skew, as defined above. Also, the magnitude of the passive component was much higher than expected by the randomization (bootstrap) approach under the null hypothesis of a purely driven trend in body size evolution. In only 0.4% of the 5,000 simulations were the simulated driven components lower than the 20% observed under a purely driven process. The departure from a driven process was therefore highly significant ( $P < 0.01$ ). The heteroscedasticity component was not particularly low, indicating that there was some important heterogeneity in the variability among subclades, although this may be difficult to interpret because of the relatively low and variable sample sizes in the different subclades.

Because this interpretation of heteroscedasticity could reflect the heterogeneous sample sizes (richness) of the four different genera, we repeated the foregoing analyses for the genus *Sicoderus*, which contains 46 species, using 12 'groups of species' as subclades. The results were qualitatively similar,

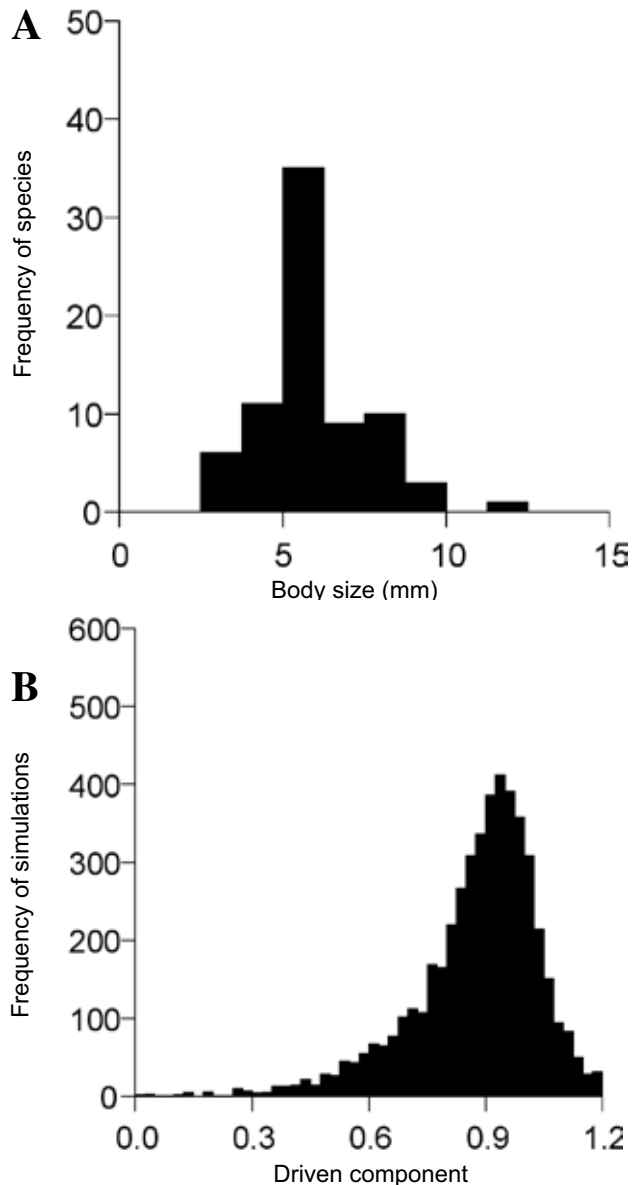
but the relative value of the driven components was much closer to 1.0 (0.986). This finding indicated that even at a lower hierarchical level there was a smaller component of driven trend and that body size in each species or group of species tended to be adapted to its own conditions.

The patterns observed here were expected in the sense that most evolution in body size occurs under strong directional selection because of its adaptive nature and intrinsic correlation with many other components (ecological, physiological and behavioural) of an organism's life-history (despite the usually strong phylogenetic component in body size). Hence, shifts from a minimum body size will allow the occupation of new adaptive zones. In these new zones, the adaptation of new species follows an adaptive radiation beyond ancestral conditions, with a relatively small amount of inertia.

Analyses of evolutionary trends are usually done using log-transformed data, on the assumption that the evolution of body size and the origin of trends result from a multiplicative process. In this case, a change is considered to be 'proportional' to the ancestral state such that evolution towards a larger body size will, for example, occur by doubling the ancestral size. On a log-scale, this shift will not cause asymmetry and will not necessarily characterize a driving process in which the relative magnitude of the effect increases throughout evolution.

The log-transformation of the data used here qualitatively affected our conclusions because the distribution of body size was normal on this scale (skew =  $0.048 \pm 0.277$ ;  $t = 0.17$ ;  $P \gg 0.05$ ) and the ANSKEW components became meaningless because the SCB component was strongly negative. This finding suggested that partitioning of the components of skewness and the presence of evolutionary trends are valid only under an additive model of body size evolution. Indeed, it is difficult to evaluate the importance of these processes in generating trends on small time scales, as in our example (i.e., at a generic level), and most studies of trends have been done on much broader evolutionary scales. On smaller scales, body size may indeed evolve under additive processes such that on a log scale the distribution of body size becomes normal. This situation also tends to occur on different spatial scales, with the distribution of body sizes becoming normal in local assemblages and shifting to strongly skewed distributions on continental scales [1,13]. Further studies dealing with a broader evolutionary

scale, e.g., an entire family, could help to clarify this issue, with additional analyses of the applicability of ANSKEW to log-transformed data.



**Figure 2.** (A) The distribution of body length (in mm) of 75 species of Erodiscini studied using ANSKEW. Note the significant right skewness ( $g_1 = -0.842$ ) and the aggregation of species at low body lengths. (B) The distribution of the driven component obtained after 5,000 bootstraps of ANSKEW showing that under the null hypothesis total skewness was attributable to variation within subclades. In a purely 'driven trend' situation, ANSKEW generated an aggregation of passive components of ~10% such that the observed value of 20% was significantly lower than the 99.6% expected for these simulated values. This discrepancy indicated a significant passive (adaptive radiation) component in the evolution of body size in Erodiscini beetles.

The method applied here to Erodiscini beetles was relatively simple and can be used even in the absence of more detailed phylogenetic hypotheses. However, if a more explicit phylogenetic hypothesis is available, further analyses can be done to map the ancestral states, thereby allowing the direction, magnitude and idiosyncratic nature of evolutionary trends in multiple subclades to be determined [12,13].

The partitioning of the components of macroevolutionary trends in Erodiscini described here is still only preliminary, for two reasons. First, because of the relatively small evolutionary scale used, it is necessary to assume that the processes driving body size evolution act additively rather than multiplicatively, as is usually the case. Second, there is no information about the ancestral conditions of the trait studied, although such information is crucial for understanding the processes that drive body size evolution. For example, although ANSKEW does not explicitly assume knowledge about the phylogenetic patterns in body size variation, the overall reasoning of evolutionary trends makes sense only if the group's ancestor is a small-bodied species, with the driven and passive components producing a trend towards larger body sizes.

Despite these two shortcomings, we have shown here that there is a balance in the within- and among-subclade components that affect the evolution of body size in Erodiscini. We have also described a method that can be used in more detailed studies of body size evolution on broader evolutionary scales. This approach can provide a starting point for improving our understanding of adaptive variation at multiple hierarchical levels and of the relationship between variation at these levels and other ecological, physiological and behavioural aspects.

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