

RH: Cretaceous-Palaeogene extinction does not affect mammalian disparity.

Cretaceous-Palaeogene extinction does not affect mammalian disparity.

THOMAS GUILLERME^{1,2*}, AND NATALIE COOPER^{1,2,3}

¹*School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland.*

²*Trinity Centre for Biodiversity Research, Trinity College Dublin, Dublin 2, Ireland.*

³*Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, UK.*

***Corresponding author.** *Zoology Building, Trinity College Dublin, Dublin 2, Ireland; E-mail: guillert@tcd.ie; Fax: +353 1 6778094; Tel: +353 1 896 2571.*

Abstract

1

2

3 (Keywords: disparity, diversity, punctuated equilibrium, gradualism, time slicing)

INTRODUCTION

Throughout history, life on Earth has suffered a series of mass extinction events resulting in drastic declines in global biodiversity (e.g. Raup, 1979; Benton and Twitchett, 2003; Renne et al., 2013; Brusatte et al., 2015). However, the long-term effects of mass extinctions are more varied (Erwin, 1998), and include increases in species richness in some clades (Friedman, 2010), species richness declines in others (Benton, 1985), changes in morphological diversity (Ciampaglio et al., 2001; Ciampaglio, 2004; Korn et al.) and shifts in ecological dominance (e.g. Brusatte et al., 2008b; Toljagic and Butler, 2013; Benson and Druckenmiller, 2014). These shifts are characterized by the decline of one clade that is replaced by a different unrelated clade with a similar ecological role (e.g. Brachiopoda and Bivalvia at the end Permian extinction Sepkoski 1981; Clapham et al. 2006 but see Payne et al. 2014). Shifts in ecological dominance are of particular interest because they are a fairly common pattern observed in the fossil record (e.g. Foraminifera; D'Hondt et al. 1996; Coxall et al. 2006; Ichtyosauria; Thorne et al. 2011; Plesiosauria; Benson and Druckenmiller 2014) and are often linked to major macroevolutionary processes such as adaptive (Losos, 2010) or competitive radiations (Brusatte et al., 2008b).

One classical example of a shift in ecological dominance is at the Cretaceous-Palaeogene (K-Pg) mass extinction 66 million years ago (Renne et al., 2013), where the non-avian dinosaurs went extinct, potentially leading to the “rise of the age of the mammals” (Archibald, 2011; Lovegrove et al., 2014). This is based on the idea

that placental mammals were able to diversify after the extinction of many terrestrial vertebrates at the K-Pg boundary (including the dominant non-avian dinosaur group; Luo 2007; Archibald 2011; O’Leary et al. 2013; Brusatte et al. 2015). Some authors suggest this reflects placental mammals filling the “empty” niches left after the K-Pg event (Archibald, 2011), others suggest it reflects a release from predation and/or competition (Lovegrove et al., 2014). However, evidence for the diversification of placental mammals after the K-Pg event is mixed. Thorough analysis of the fossil record (e.g. Goswami et al., 2011; O’Leary et al., 2013) supports the idea that placental mammals diversified after the K-Pg event as there are no undebated placental mammal fossils before the K-Pg event and many afterwards (Archibald, 2011; Goswami et al., 2011; Slater, 2013; O’Leary et al., 2013; Wilson, 2013; Brusatte et al., 2015). Conversely, evidence from molecular data suggests that the diversification of placental mammals started prior to the K-Pg extinction event without being drastically affected by it (e.g. Douady and Douzery 2003; Bininda-Emonds et al. 2007; Meredith et al. 2011; Stadler 2011 or Beck and Lee 2014 using morphological data as well). Therefore, whether the diversification of placental mammals began before the K-Pg event, or in response to the extinctions at the K-Pg event, is a matter of great debate (dos Reis et al., 2012; O’Leary et al., 2013; Springer et al., 2013; O’Leary et al., 2013; dos Reis et al., 2014).

There are two main reasons why there is still debate about the timing of the diversification of placental mammals. In this paper we focus on solving these issues as follows:

1. **Palaeontological and neontological data show different patterns.** As mentioned above, conclusions about when placental mammals diversified tend to be split depending on what kind of data are used: palaeontological data generally suggest that placental mammals diversified post K-Pg (e.g. O’Leary et al., 2013), whereas neontological data suggest that K-Pg event had little to no effect on mammalian diversification (Bininda-Emonds et al., 2007; Meredith et al., 2011; Stadler, 2011). Fortunately a recently successfully implemented method allows to use cladistic data for both living and fossil taxa along with molecular data for living taxa (the Total Evidence method; Eernisse and Kluge, 1993; Ronquist et al., 2012). This method can also be combined with the tip-dating method (Ronquist et al., 2012; Wood et al., 2013) to get more accurate estimates of diversification times for both fossil and living species (but see Arcila et al., 2015). Recently, two study have been published using the Total Evidence and tip-dating methods to study (1) variation in mammalian body mass (Slater, 2013) and (2) diversification rates (Beck and Lee, 2014) around the K-Pg boundary. Slater (2013) found good support for a shift in mammalian body mass evolution pattern before and after the K-Pg boundary suggesting a clear effect of the K-Pg boundary on mammalian body mass diversification. Whereas, Beck and Lee (2014) found mixed result on diversification rates supporting both a diversification of placental mammals before (“ancient dates” hypothesis) or after (“accelerated rates” hypothesis) the K-Pg boundary.

2. **Diversity can be defined in different ways.** Diversity is a difficult concept to define. In many studies it is measured as phylogenetic diversity or species richness (Stadler, 2011; Meredith et al., 2011; O’Leary et al., 2013), but often the more interesting aspect of diversity is related to the ecological niches the species occupy (Wesley-Hunt, 2005; Brusatte et al., 2008b; Toljagic and Butler, 2013), particularly if we want to make hypotheses about macroevolutionary processes (Pearman et al., 2008; Olson and Arroyo-Santos, 2009; Losos, 2010; Glor, 2010). Sometimes phylogenetic diversity is used as a proxy for other kinds of diversity, however, species richness can be decoupled from morphological diversity (Slater et al., 2010; Ruta et al., 2013; Hopkins, 2013), so phylogenetic diversity may not be the best proxy for ecological diversity. For example in Slater (2013), the diversity of mammalian body mass is studied instead as the sheer number of species. In this particular case, body mass diversity rather than species diversity can be a better proxy for describing mammalian diversity. One can also use morphological diversity, also known as disparity (e.g. Wills et al., 1994; Hughes et al., 2013), as a way to quantify changes in mammalian diversity that should relate to the ecology of the species. However some methods for measuring disparity are outdated and make inappropriate assumptions. Many studies quantifying changes in morphological diversity were proposed > 20 years ago (Foote, 1994; Wills et al., 1994) and are sometimes used without modifications (e.g., Brusatte et al., 2008a,b; Cisneros and Ruta, 2010; Thorne et al., 2011; Prentice et al., 2011; Brusatte et al.,

2012; Toljagic and Butler, 2013; Ruta et al., 2013; Benton et al., 2014; Benson and Druckenmiller, 2014), even when the statistical assumptions of the methods are violated (see Methods). Additionally, previous methods are based on an underlying assumption that changes in disparity occur by punctuated evolution (e.g. Wesley-Hunt, 2005) which is not always the case (Hunt et al., 2015). Finally, most studies of disparity through time use unequal time units based on biostratigraphy (Brusatte et al., 2008b, 2012; Toljagic and Butler, 2013). This can be tautological as biostratigraphy is already based on changes in fossil assemblages and morphology through time.

Here, we propose an updated approach to test whether mammals diversified before or after K-Pg, using morphological disparity, measured as cladistic disparity (see Methods), as our proxy for diversity. We measured the disparity of living and fossil mammals taken from two previously published studies (Slater, 2013; Beck and Lee, 2014). Using a novel time-slicing approach we produce fine-grain estimates of disparity through time under two different models of morphological character evolution (either gradual or punctuated). Finally, to test whether mammals display significant changes in disparity after the K-Pg boundary, we compared the observed changes to two null models assuming purely stochastic or purely Brownian evolution. We found no significant increases in mammalian disparity after the K-Pg event; instead the disparity of placental mammals increased during the K-Pg event. These results suggest that the shift in dominant terrestrial vertebrate clades in the fossil record (from non-avian

dinosaurs to placental mammals) during the Tertiary was not a direct result of the K-Pg mass extinction.

METHODS

Cladistic data and phylogenies

We used the cladistic morphological matrices and the Total Evidence tip-dated trees (Ronquist et al., 2012) from Slater (2013, 103 taxa with 446 morphological characters) and Beck and Lee (2014, 102 taxa with 421 morphological characters). We chose these two data sets because they have a similar number of taxa and morphological characters. Slater (2013) ranges from 310 million years ago (Mya; Late Carboniferous) to the present and focuses on Mammaliamorpha at the family-level. Beck and Lee (2014) ranges from 170 Mya (Middle Jurassic) to the present and focuses on Eutheria at the genus-level. We used the first and last occurrences reported in Slater (2013) and Beck and Lee (2014) as the temporal range of each taxon in our analysis. Both phylogenies are illustrated in the supplementaries material (see Figure S1 and S2 @@@).

Estimating ancestral character states

For both datasets we used the re-rooting method (Yang et al., 1996; Garland and Ives, 2000) to get Maximum Likelihood estimates of the ancestral states for each character at every node in the tree, using the `rerootingMethod` function from the R package

phytools version 0.4-45 (Revell, 2012; R Core Team, 2015). Where there was missing character data for a taxon we followed the method of Lloyd (2015) and treated missing data as any possible observed state for each character. For example, if a character had two observed states (0 and 1) across all taxa, we attributed the multi-state “0&1” value to the taxon with missing data, representing an equal probability of being either 0 or 1. This allows the ancestral node of a taxon with missing data to be estimated with no assumptions other than that the taxon has one of the observed character states. To prevent poor ancestral state reconstructions from biasing our results, especially when a lot of error is associated with the reconstruction, we only included ancestral state reconstructions with a scaled Likelihood ≥ 0.95 . Ancestral state reconstructions with scaled Likelihoods below this threshold were replaced by missing data (“?”).

Building the cladisto-space

To explore variations in mammalian disparity through time (defined here as the variation in morphologies through time), we use a cladisto-space approach (e.g. Foote, 1994, 1996; Wesley-Hunt, 2005; Brusatte et al., 2008b; Friedman, 2010; Toljagic and Butler, 2013; Hughes et al., 2013). This approach is similar to constructing a morphospace based on continuous morphological data (e.g. Friedman, 2010), except a cladisto-space is an approximation of the morphospace based on cladistic data (i.e. the discrete morphological characters used to build a phylogenetic tree). Mathematically, a cladisto-space is an n dimensional object that summarizes the cladistic distances between the taxa present in a cladistic matrix (see details below). Note that because of

its inherent combinatorial properties, a cladisto-space is a finite theoretical object limited by the product of the number of character states. Thus a cladisto-space will be overloaded if the number of taxa is higher than the product of the number of character states, although this is not an issue in our study (our cladisto-spaces have maximal capacities of 1.9×10^{181} taxa; Slater, 2013, and 4.5×10^{159} taxa; Beck and Lee, 2014).

To estimate the cladisto-spaces for each of our datasets we first constructed pairwise distance matrices of length k , where k is the total number of taxa in the dataset. For each dataset separately, we calculated the $k \times k$ distances using the Gower distance (Gower, 1971), i.e. the Euclidean distance between two taxa divided by the number of shared characters. This allows us to correct for distances between two taxa that share many characters and could be closer to each other than to taxa with fewer characters in common (i.e. because some pairs of taxa share more characters in common than others, they are more likely to be similar). For cladistic matrices, using this corrected distance is preferable to the raw Euclidean distance because of its ability to deal with discrete or/and ordinated characters as well as with missing data (Anderson and Friedman, 2012). However, the Gower distance cannot calculate distances when taxa have no overlapping data. Therefore, we used the `TrimMorphDistMatrix` function from the `Claddis` R package (Lloyd, 2015) to remove pairs of taxa with no cladistic characters in common. This led us to remove 11 taxa from Slater (2013) and none from Beck and Lee (2014).

After calculating our distance matrices we transformed them using classical

multidimensional scaling (MDS; Torgerson, 1965; Gower, 1966; Cailliez, 1983). This method (referred to as MDS; e.g. Donohue et al. 2013; PCO; e.g. Brusatte et al. 2015; or PCoA; e.g. Paradis et al. 2004) is an eigen decomposition of the distance matrix. Because we used Gower distances instead of raw Euclidean distances, negative eigenvalues can be calculated. To avoid this, we first transformed the distance matrices by applying the Cailliez correction (Cailliez 1983; as used in Toljagic and Butler 2013) which adds a constant c^* to the values in a distance matrix (apart from the diagonal) so that all the Gower distances become Euclidean ($d_{Gower} + c^* = d_{Euclidean}$; Cailliez 1983). We were then able to extract n eigenvectors for each matrix (representing the n dimensions of the cladisto-space) where n is equal to $k - 2$, i.e. the number of taxa in the matrix (k) minus the two last eigenvector which are always null after applying the Cailliez correction. Contrary to previous studies (e.g Brusatte et al., 2008a; Cisneros and Ruta, 2010; Prentice et al., 2011; Anderson and Friedman, 2012; Hughes et al., 2013; Benton et al., 2014), we use all n dimensions of our cladisto-spaces and not a sub-sample representing the majority of the variance in the distance matrix (e.g. selecting only m dimensions that represent up to 90% of the variance in the distance matrix; Brusatte et al. 2008b; Toljagic and Butler 2013).

Note that our cladisto-spaces represent an ordination of all possible mammalian morphologies coded in each study through time. It is unlikely that all morphologies will co-occur at each time point, therefore, the disparity of the whole cladisto-space is expected to be \geq the disparity at any specific point in time.

Calculating disparity

Disparity can be estimated in many different ways (e.g. Wills et al., 1994; Ciampaglio, 2004; Thorne et al., 2011; Hopkins, 2013; Huang et al., 2015), however most studies estimate disparity using four metrics: the sum and products of ranges and variances, each of which gives a slightly different estimate of how the data fits within the cladisto-space (Foote, 1994; Wills et al., 1994; Brusatte et al., 2008a,b; Cisneros and Ruta, 2010; Thorne et al., 2011; Prentice et al., 2011; Brusatte et al., 2012; Toljagic and Butler, 2013; Ruta et al., 2013; Benton et al., 2014; Benson and Druckenmiller, 2014). The sum and products of ranges and variances are based on the ranges and variances of the eigenvectors calculated from a distance matrix. However, these metrics do not take into account the covariance among eigenvectors. This is only valid statistically if the eigenvectors are independent. In multidimensional scaling, all n eigenvectors are calculated from the same distance matrix and are therefore not independent, thus covariances among eigenvectors should be included when estimating disparity. In addition, because we include all n eigenvectors in the analysis (see above), the products of ranges and variances will tend towards zero since the scores of the last eigenvectors are usually really close to zero themselves. These features make using the sum and products of ranges and variances unfeasible in our study. Instead, we use a more intuitive metric for measuring the dispersion of the data in the cladisto-space: the distance from centroid (similar but not equivalent to Wills et al. 1994; Korn et al.;

210 Huang et al. 2015) calculated as:

$$Disparity = \frac{\sqrt{\sum_{i=1}^k (\mathbf{v}_{n_i} - Centroid_n)^2}}{k} \quad (1)$$

211 where:

$$Centroid_n = \frac{\sum_{i=1}^k (\mathbf{v}_{n_i})}{k} \quad (2)$$

212 k is the size of the distance matrix (i.e. the total number of taxa), \mathbf{v}_n is any of the n
213 eigenvectors (i.e. the n^{th} dimension of the cladisto-space), and $Centroid_n$ is the centroid
214 euclidean distance of the n^{th} eigenvector (equation 2). Note that we also calculated the
215 sum and products of ranges and variances to compare our results with previous studies
216 (see supplementary material @).

217 *Estimating disparity through time*

218 Changes in disparity through time are generally investigated by calculating the
219 disparity of taxa that occupy the cladisto-space during specific time intervals (e.g
220 Cisneros and Ruta, 2010; Prentice et al., 2011; Hughes et al., 2013; Hopkins, 2013;
221 Benton et al., 2014; Benson and Druckenmiller, 2014). These time intervals are usually
222 defined based on biostratigraphy (e.g. Cisneros and Ruta, 2010; Prentice et al., 2011;
223 Hughes et al., 2013; Benton et al., 2014) but can also be arbitrarily chosen time periods
224 of equal duration (Hopkins, 2013; Benson and Druckenmiller, 2014). However, this
225 approach suffers from two main biases. First, if biostratigraphy is used to determine

the time intervals, disparity may be distorted towards higher differences between time intervals because biostratigraphical periods are geologically defined based on differences in the morphology of fossils found in the different strata. Second, this approach assumes that all characters evolve following a punctuated equilibrium model, because disparity is only estimated once for each interval resulting in all changes in disparity occurring between intervals, rather than also allowing for gradual changes within intervals (Hunt et al., 2015).

To address these issues, we use a “time-slicing” approach that considers subsets of taxa in the cladisto-space at specific equidistant points in time, as opposed to considering subsets of taxa between two time points. This results in even-sampling of the cladisto-space across time and at a finer grain than using time intervals, and permits us to define the underlying model of character evolution (as punctuated or gradual). In practice, time-slicing considers the disparity of any element present in the phylogeny (branches, nodes and tips) at any point in time. When the phylogenetic elements are nodes or tips, the eigenvector scores for the nodes (estimated using ancestral state reconstruction as described above) or tips are directly used for estimating disparity. When the phylogenetic elements are branches we inferred the eigenvector score for the branch using one of two evolutionary models:

1. **Punctuated evolution.** This model selects the eigenvector score from either the ancestral node or the descendant node/tip of the branch regardless of the position of the slice along the branch. Similarly to the time interval approach, this reflects

a model of punctuated evolution where changes in disparity occur either at the start or at the end of a branch over a relatively short time period (Gould and Eldredge, 1977). We applied this model in three ways:

- (i) selecting the eigenvector score of the ancestral node of the branch
- (ii) selecting the eigenvector score of the descendant node/tip of the branch
- (iii) randomly selecting either the eigenvector score of the ancestral node or the descendant node/tip of the branch

Method (i) assumes that changes always occurs early on the branch (accelerated transition, ACCTRAN) and (ii) assumes that changes always occur later (delayed transition, DELTRAN). We prefer not to make either assumption so we report the results from (iii), although the ACCTRAN and DELTRAN results are available in the Supplementary Information @.

2. **Gradual evolution.** This model also selects the eigenvector score from either the ancestral node or the descendant node/tip of the branch, but the choice depends on the distance between the sampling time point and the end of the branch. If the sampling time point falls in the first half of the branch length the eigenvector score is taken from the ancestral node, conversely, if the sampling time point falls in the second half of the branch length the eigenvector score is taken from the descendant node/tip. This reflects a model of gradual evolution where changes in disparity are gradual and cumulative along the branch.

We applied our time-slicing approach to the two cladisto-spaces calculated from Slater (2013) and Beck and Lee (2014), time-slicing the phylogeny every five million years from 170 Mya to the present resulting in 35 sub-samples of the cladisto-space. For each sub-sample, we estimated its disparity assuming punctuated (ACCTRAN, DELTRAN and random) and gradual evolution as described above. To reduce the influence of outliers on our disparity estimates, we bootstrapped each disparity measurement by randomly re-sampling with replacement a new sub-sample of taxa from the observed taxa in the sub-sample 1000 times. We then calculated the median disparity value for each sub-sample along with the 50% and the 95% confidence intervals.

We also reported the number of phylogenetic elements (branches, nodes and tips) in each sub-sample. Disparity may be higher in sub-samples with more phylogenetic elements simply because there are more taxa represented. To test whether our analyses were biased in this way, we rarefied our sub-samples during the bootstrap procedure by randomly re-sampling a fix number of taxa across each sub-sample. In Slater (2013), the minimum number of taxa in each sub-sample from 170 to present was 8. In Beck and Lee (2014), the minimum number of taxa however was 3, however, from 150 Mya until the present, the minimum number of taxa is 8. To make both data sets comparable, we used 8 as a minimum number of taxa for the rarefied bootstrap measurements in Beck and Lee (2014) ignoring therefore the sub-sample between 170 and 150 Mya. We report both results of the bootstrapped measurements and the rarefied bootstrap measurements.

To compare our results to previous studies we also repeated our analyses using two time interval approaches; one based on biostratigraphy (e.g. Cisneros and Ruta, 2010; Prentice et al., 2011; Hughes et al., 2013; Benton et al., 2014) using each geological stage from the Middle Jurassic to the present. We report the results of these analyses in the Supplementary Materials.

Finally, to assess if the K-Pg boundary had a significant effect on mammals disparity, we performed Anderson (2001)'s Permutational Analysis of Variance (also referred to as PERMANOVA or NPANOVA; e.g. Brusatte et al., 2008a; Ruta et al., 2013) to test whether there was a significant effect of time on our calculated disparity. We calculated the euclidean distance of the ordinated data with 1000 permutations on both data sets and on both evolutionary scenario using the `adonis` function from the R package `vegan` (Oksanen et al., 2015). When a significant effect of time on disparity was measured, we ran a series of *post-hoc* t-test between the time sub-samples (Anderson and Friedman, 2012; Zelditch et al., 2012; Smith et al., 2014) to test whether there was a significant effect between of the K-Pg boundary. We measured the difference between the last sub-sample of the Cretaceous (65 Mya) to all the slices of the Cenozoic to test whether there was either:

1. no effect of the K-Pg event: no significant difference between the last Cretaceous sub-sample and any of the Cenozoic sub-samples.
2. a direct effect of the K-Pg event: significant difference between the last Cretaceous sub-sample and the first Cenozoic sub-sample.

3. a lag effect of the K-Pg event: a significant difference between the last Cretaceous sub-sample and some sub-samples during the Cenozoic.

Because these *post-hoc* t-tests involve multiple p-values (13 comparisons), we corrected each p-value by multiplying them by the number of comparisons (Holm-Bonferonni correction; Holm, 1979).

RESULTS

Both data sets do not display a change in species richness after and before the K-Pg boundary (Figure 1): respectively a decrease in Eutherians (data from Beck and Lee, 2014) and an increase in Mammaliaformes (data from Slater, 2013). However, this variations in species richness are not linked with changes in disparity (Figure 2). We measured a significant effect of time on disparity in Eutherians under both gradual and punctuated evolution model and in Mammaliaformes only under the gradual evolution model (table 1). Regarding the effect of the K-Pg boundary, we detected a lag effect on disparity in Eutherians under both gradual and punctuated evolution model (table 2) but not any more after taking species richness into account (i.e. rarefaction; table 3). For the Mammaliaformes under gradual evolution model, there was no effect of the K-Pg boundary in both the raw and the rarefied data (table 4).

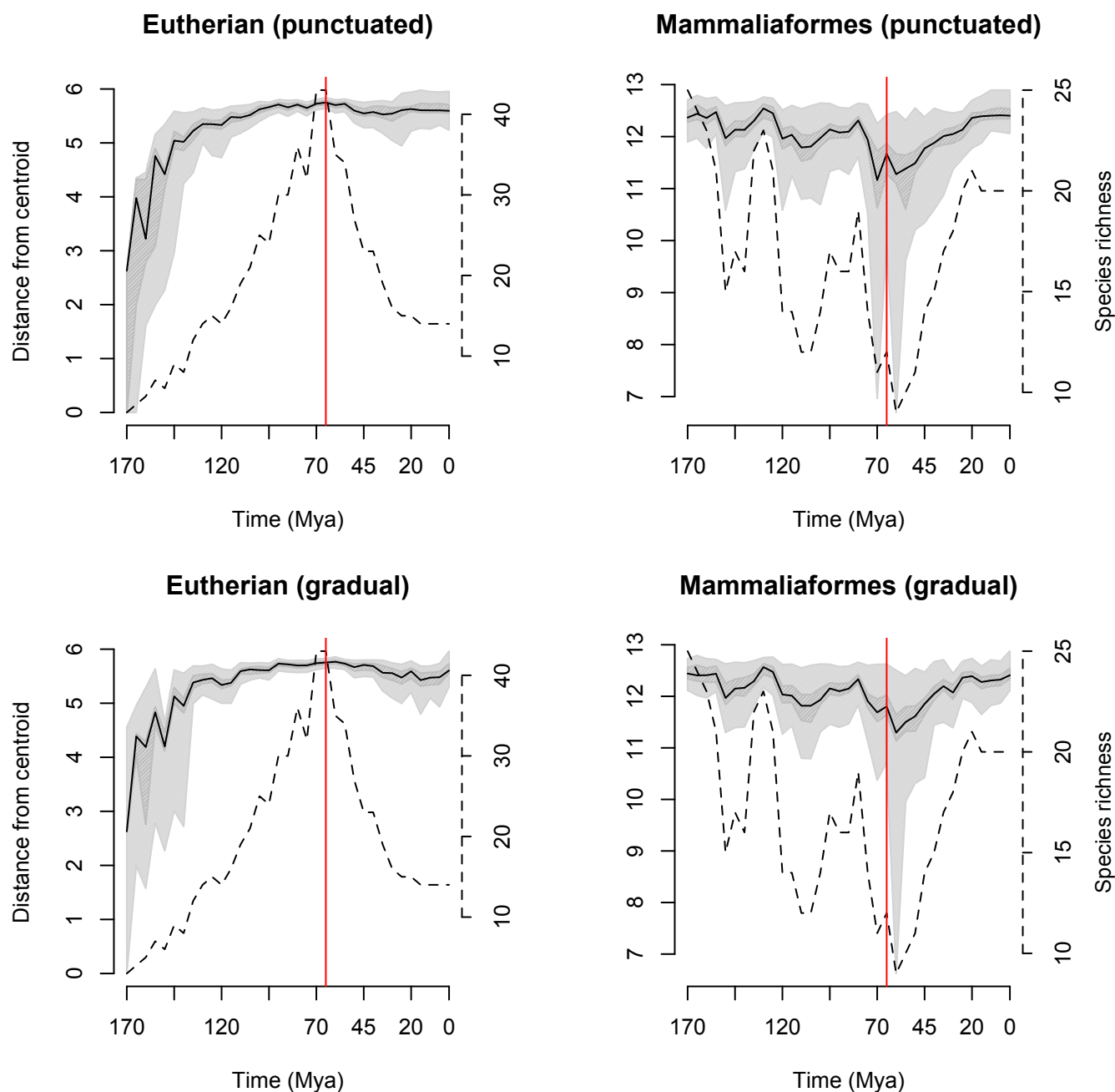


Figure 1: Observed variations of disparity through time among Eutherian and Mammaliaformes with a punctuated or gradual evolution model. The x axis represents the time in Million of years ago (Mya). The y axis represents the disparity measured as the median distance from centroid per sub-sample. The solid black lines is the mean disparity; the confidence intervals (CI) are represented by the grey polygons (50% CI in dark grey and 95% CI in light grey). The dashed line represent the species richness in each sub-sample (values are reported on the right hand side of each graphs). The red vertical

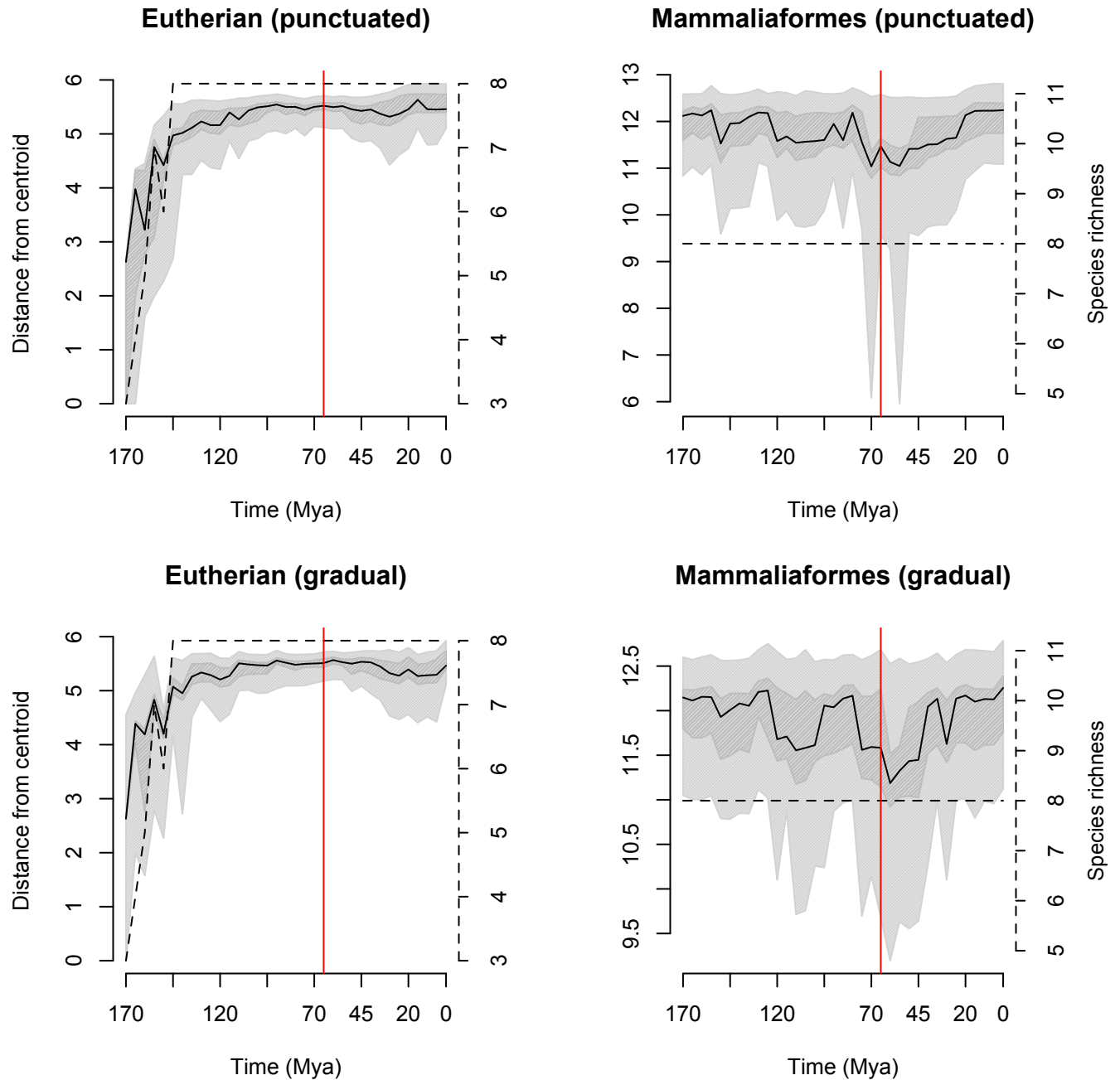


Figure 2: Rarefied variations of disparity through time among Eutherian and Mammaliaformes with a punctuated or gradual evolution model. The x axis represents the time in Million of years ago (Mya). The y axis represents the disparity measured as the median distance from centroid per sub-sample. The solid black lines is the mean disparity; the confidence intervals (CI) are represented by the grey polygons (50% CI in dark grey and 95% CI in light grey). The dashed line represent the species richness in each sub-sample (values are reported on the right hand side of each graphs). The red vertical line

Table 1: Permanova results of testing the effect of time on the ordinated distance matrix with 1000 permutations based on euclidean distance. Data: Eutherian (data from Beck and Lee, 2014); Mammaliaformes, (data from Slater, 2013). Model: evolutionary model. Significant effects are highlighted in bold: one star (*) signifies a p-value between 0.05 and 0.005; two stars between 0.005 and 0.0005 and three stars < 0.0005 .

Data	model	terms	Df	Sum of squares	Mean sum of squares	F Model	R^2	p-value	
Eutherian	gradual	time	34	1825.92	53.703	1.5784	0.0769	0.0009	***
		residuals	644	21911.65	34.024		0.9231		
	punctuated	time	34	1597.07	46.973	1.3693	0.0674	0.0009	***
		residuals	644	22092.28	34.305		0.9326		
Mammaliaformes	gradual	time	34	6525.61	191.930	1.1660	0.0663	0.0009	***
		residuals	558	91852.55	164.610		0.9337		
	punctuated	time	34	5741.25	168.860	1.0167	0.0583	0.2248	
		residuals	558	92672.75	166.080		0.9417		

DISCUSSION

Our results show that there is a significant effect of time on changes in disparity under the assumption of gradual evolution in Mammaliaformes and Eutherians as well as under the assumption of punctuated evolution for Eutherians (table 1). However, regardless the taxonomic level (i.e. family *vs.* genus) and regardless the evolutionary model (i.e. gradual or punctuated evolution), there is no effect of the K-Pg event on mammalian disparity (Figure 2). In fact, after correcting for species richness, we did not detect any significant difference between the last sub-sample of the Cretaceous and any of the sub-samples of the Cenozoic. This shows that, within the frame of our data set, there is no significant changes in mammalian disparity across the K-Pg boundary. In fact the disparity seems to reach a plateau at the end of the Jurassic (150 Mya) for Eutherians and during the late Triassic (Norian; 220 Mya) for the Mammaliaformes (see figure S4 @@@).

Effect of the K-Pg boundary on mammalian disparity

These results are consistent with previous studies on mammalian disparity and on disparity in metazoans in general. In fact, as previously shown, both focal taxonomic levels (i.e. family *vs.* genus) reaches maximal disparity early in their history (Hughes et al., 2013). The patterns of disparity seems to plateau at their maximum disparity early in their history (approximatively after 25% and 12% of their history in respectively Mammaliaformes and Eutherians). Additionally, disparity display a

pattern clearly decoupled from taxonomic diversity (Slater et al., 2010; Ruta et al., 2013; Hopkins, 2013). The results for the Mammaliaformes data-set disparity variation are in accordance with a recent study from Close et al. (2015) showing a peak of Mammalian disparity during the Early to Middle Jurassic. However, our results differ from Grossnickle and Polly (2013) that showed a significant decrease in disparity during the late Cretaceous (but see Wilson et al., 2012). These differences can be due to the different input data to calculate disparity (morphometric data in Grossnickle and Polly 2013; and cladistic data in the present study; but see Hetherington et al. 2015), the different method to calculate disparity through time (time bin in Grossnickle and Polly 2013; and time slicing in the present study) or the different focal morphological aspect (dental morphology in Grossnickle and Polly 2013; and gopal - including dental - morphology in the present study). Finally, we found a fundamental difference with Slater (2013) which shows solid evidences for a change in mode of body mass evolution at the K-Pg boundary. This difference can be due to the number of traits used in Slater (2013) and the present study. In this study we look at an aggregate of discrete traits (the 446 morphological characters) in opposition of one continuous trait (body mass in Slater, 2013). However, when using the two independent data sets with two focal taxonomic level, we still find no evidences for a significant effect of the K-Pg boundary on changes in mammalian disparity through time.

Few caveats can be underlined however. Firstly, both our data sets are limited and do not represent the full known mammalian taxonomic diversity, especially in the

Neogene (23 Mya) where no fossils were represented in our data set. However, this might not cause a serious under-sampling problem since Mammaliaformes diversity has been shown to peak at the latest Cretaceous (Newham et al., 2014). Additionally Raia et al. (2012) have shown that mammalian diversification rates declined throughout the whole Cenozoic. In our study, these findings could suggest that an effect of the K-Pg boundary would be more likely detected during the Palaeogene when mammalian diversification rates were still high.

Secondly, the core of the debate in mammalian evolution is whether placental mammals originated before or after the K-Pg boundary (dos Reis et al., 2012; O’Leary et al., 2013; Springer et al., 2013; O’Leary et al., 2013; dos Reis et al., 2014). The traditional definition of the infraclass Placentalia is “the least inclusive clade that includes all extant placentals” (Beck and Lee, 2014). However, part of the dating debate might be due to the lack of clear characters that can be used to define early placental mammals (Bininda-Emonds et al., 2012; Beck and Lee, 2014). Cartmill (2012) also argues that the use of higher taxa definition in general might be obsolete since “there is only a long, geologically slow cascade of accumulating small apomorphies”. Therefore, in this study, we made the deliberate choice to focus on the taxonomic levels (genus *vs.* family) rather than on the higher clades definitions. We argue that if a significant change in disparity occurred at the K-Pg boundary in any of such infraclass (Placentalia, Marsupialia, etc...) it would be detectable even at a higher level (i.e. changes in Placentalia correspond by definition to changes in Eutheria and Mammaliaformes).

Also, using Total Evidence tip-dated trees provides more accurate estimates of diversification times (Ronquist et al. 2012; Wood et al. 2013; Beck and Lee 2014; but see Arcila et al. 2015) and allows the opportunity to look at changes in disparity for both living and fossil species. eментарies.

Methodology improvements for measuring disparity

Additionally, throughout this paper, we propose several incremental changes to the classic ways to measure disparity. This is how we think they improve the whole yoke.

- Using all axis
- Does not create a subjective cut-off
- Centroid distance
- Does not violate co-variance thingy
- Is more decoupled from diversity
- Time slicing:
- Allows model choice (gradual vs. constant)
- Allows continuous and independent sample, not based on geological ages (and therefore not based on fossil data)
- Evolutionary model

Differences between Gradual and Punctuated are probably due because the truth is a mix of both Hunt et al. (2015). See differences with Grossnickle

Howevers, biases:

- Ancestral states reconstruction can be tricky

But the use of fossils helps a lot (David Polly, 2001; Finarelli and Flynn, 2006; Albert et al., 2009; Slater, 2013)

- Might be sensitive to sample size (but does not seem to much)
- Link between phylogeny and cladistic disparity

CONCLUSION

When looking at pattens of diversification, it is important to look at both phylogenetic diversification (i.e. species richness) and phenotypic diversification (i.e. disparity).

DATA AVAILABILITY AND REPRODUCIBILITY

ACKNOWLEDGMENTS

Thanks to Graeme Lloyd, Andrew Jackson, Gavin Thomas and Sive Finlay for their useful comments on measuring disparity.

FUNDING

This work was funded by a European Commission CORDIS Seventh Framework Programme (FP7) Marie Curie CIG grant (proposal number: 321696).

*

References

- Albert, J. S., D. M. Johnson, and J. H. Knouft. 2009. Fossils provide better estimates of ancestral body size than do extant taxa in fishes. *Acta Zoologica* 90:357–384.
- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32–46.
- Anderson, P. S. and M. Friedman. 2012. Using cladistic characters to predict functional variety: experiments using early gnathostomes. *Journal of Vertebrate Paleontology* 32:1254–1270.

- 424 Archibald, J. D. 2011. Extinction and radiation: how the fall of dinosaurs led to the rise
425 of mammals. JHU Press.
- 426 Arcila, D., R. A. Pyron, J. C. Tyler, G. Ort, and R. Betancur-R. 2015. An evaluation of
427 fossil tip-dating versus node-age calibrations in tetraodontiform fishes (teleostei:
428 Percomorphaceae). *Molecular Phylogenetics and Evolution* 82, Part A:131 – 145.
- 429 Beck, R. M. and M. S. Lee. 2014. Ancient dates or accelerated rates? Morphological
430 clocks and the antiquity of placental mammals. *Proceedings of the Royal Society B:
431 Biological Sciences* 281:1–10.
- 432 Benson, R. B. J. and P. S. Druckenmiller. 2014. Faunal turnover of marine tetrapods
433 during the JurassicCretaceous transition. *Biological Reviews* 89:1–23.
- 434 Benton, M. J. 1985. Mass extinction among non-marine tetrapods. *Nature* 316:811–814.
- 435 Benton, M. J., J. Forth, and M. C. Langer. 2014. Models for the rise of the dinosaurs.
436 *Current biology* : CB 24:R87–R95.
- 437 Benton, M. J. and R. J. Twitchett. 2003. How to kill (almost) all life: the end-permian
438 extinction event. *Trends in Ecology & Evolution* 18:358 – 365.
- 439 Bininda-Emonds, O., R. Beck, and R. D. MacPhee. 2012. Rocking clocks and clocking
440 rocks: a critical look at divergence time estimation in mammals. *From bone to clone:
441 the synergy of morphological and molecular tools in paleobiology* Pages 38–82.
- 442 Bininda-Emonds, O. R., M. Cardillo, K. E. Jones, R. D. MacPhee, R. M. Beck, R. Grenyer,

- 443 S. A. Price, R. A. Vos, J. L. Gittleman, and A. Purvis. 2007. The delayed rise of
444 present-day mammals. *Nature* 446:507–512.
- 445 Brusatte, S., R. J. Butler, A. Prieto-Márquez, and M. A. Norell. 2012. Dinosaur
446 morphological diversity and the end-Cretaceous extinction. *Nature Communications*
447 3:804–804.
- 448 Brusatte, S. L., M. J. Benton, M. Ruta, and G. T. Lloyd. 2008a. The first 50 Myr of
449 dinosaur evolution: macroevolutionary pattern and morphological disparity. *Biology*
450 *Letters* 4:733–736.
- 451 Brusatte, S. L., M. J. Benton, M. Ruta, and G. T. Lloyd. 2008b. Superiority, competition,
452 and opportunism in the evolutionary radiation of dinosaurs. *Science* 321:1485–1488.
- 453 Brusatte, S. L., R. J. Butler, P. M. Barrett, M. T. Carrano, D. C. Evans, G. T. Lloyd, P. D.
454 Mannion, M. A. Norell, D. J. Pepe, P. Upchurch, and T. E. Williamson. 2015. The
455 extinction of the dinosaurs. *Biological Reviews* 90:628–642.
- 456 Cailliez, F. 1983. The analytical solution of the additive constant problem.
457 *Psychometrika* 48:305–308.
- 458 Cartmill, M. 2012. Primate origins, human origins, and the end of higher taxa.
459 *Evolutionary Anthropology: Issues, News, and Reviews* 21:208–220.
- 460 Ciampaglio, C. N. 2004. Measuring changes in articulate brachiopod morphology
461 before and after the permian mass extinction event: do developmental constraints
462 limit morphological innovation? *Evolution & Development* 6:260–274.

- Ciampaglio, C. N., M. Kemp, and D. W. McShea. 2001. Detecting changes in morphospace occupation patterns in the fossil record: characterization and analysis of measures of disparity. *Paleobiology* 27:695–715.
- Cisneros, J. C. and M. Ruta. 2010. Morphological diversity and biogeography of procolophonids (amniota: Parareptilia). *Journal of Systematic Palaeontology* 8:607–625.
- Clapham, M. E., D. J. Bottjer, C. M. Powers, N. Bonuso, M. L. Fraiser, P. J. Marenco, S. Q. Dornbos, and S. B. Pruss. 2006. Assessing the ecological dominance of phanerozoic marine invertebrates. *PALAIOS* 21:431–441.
- Close, R., M. Friedman, G. Lloyd, and R. Benson. 2015. Evidence for a mid-jurassic adaptive radiation in mammals. *Current Biology* Pages –.
- Coxall, H. K., S. D'Hondt, and J. C. Zachos. 2006. Pelagic evolution and environmental recovery after the cretaceous-paleogene mass extinction. *Geology* 34:297–300.
- David Polly, P. 2001. Paleontology and the comparative method: Ancestral node reconstructions versus observed node values. *The American Naturalist* 157:pp. 596–609.
- D'Hondt, S., T. D. Herbert, J. King, and C. Gibson. 1996. Planktic foraminifera, asteroid, and marine production: Death and recovery at the cretaceous-tertiary boundary. *Geological Society of America Special Papers* 307:303–317.

482 Donohue, I., O. L. Petchey, J. M. Montoya, A. L. Jackson, L. McNally, M. Viana,
 483 K. Healy, M. Lurgi, N. E. O'Connor, and M. C. Emmerson. 2013. On the
 484 dimensionality of ecological stability. *Ecology Letters* 16:421–429.

485 dos Reis, M., P. C. J. Donoghue, and Z. Yang. 2014. Neither phylogenomic nor
 486 palaeontological data support a palaeogene origin of placental mammals. *Biology*
 487 *Letters* 10.

488 dos Reis, M., J. Inoue, M. Hasegawa, R. J. Asher, P. C. J. Donoghue, and Z. Yang. 2012.
 489 Phylogenomic datasets provide both precision and accuracy in estimating the
 490 timescale of placental mammal phylogeny. *Proceedings of the Royal Society of*
 491 *London B: Biological Sciences* .

492 Douady, C. J. and E. J. Douzery. 2003. Molecular estimation of eulipotyphlan divergence
 493 times and the evolution of insectivora. *Molecular Phylogenetics and Evolution* 28:285
 494 – 296.

495 Eernisse, D. and A. Kluge. 1993. Taxonomic congruence versus total evidence, and
 496 amniote phylogeny inferred from fossils, molecules, and morphology. *Molecular*
 497 *Biology and Evolution* 10:1170–1195.

498 Erwin, D. H. 1998. The end and the beginning: recoveries from mass extinctions. *Trends*
 499 *in Ecology and Evolution* 13:344 – 349.

500 Finarelli, J. A. and J. J. Flynn. 2006. Ancestral state reconstruction of body size in the

caniformia (carnivora, mammalia): The effects of incorporating data from the fossil
record. *Systematic Biology* 55:301–313.

Foote, M. 1994. Morphological disparity in ordovician-devonian crinoids and the early
saturation of morphological space. *Paleobiology* 20:320–344.

Foote, M. 1996. Ecological controls on the evolutionary recovery of post-paleozoic
crinoids. *Science* 274:1492–1495.

Friedman, M. 2010. Explosive morphological diversification of spiny-finned teleost
fishes in the aftermath of the end-Cretaceous extinction. *Proceedings of the Royal
Society B: Biological Sciences* 277:1675–1683.

Garland, J., Theodore and A. R. Ives. 2000. Using the past to predict the present:
Confidence intervals for regression equations in phylogenetic comparative methods.
The American Naturalist 155:346–364.

Glor, R. E. 2010. Phylogenetic insights on adaptive radiation. *Annual Review of
Ecology, Evolution, and Systematics* 41:251–270.

Goswami, A., G. V. Prasad, P. Upchurch, D. M. Boyer, E. R. Seiffert, O. Verma,
E. Gheerbrant, and J. J. Flynn. 2011. A radiation of arboreal basal eutherian mammals
beginning in the late cretaceous of india. *Proceedings of the National Academy of
Sciences of the United States of America* 108:16333–16338.

Gould, S. J. and N. Eldredge. 1977. Punctuated equilibria: The tempo and mode of
evolution reconsidered. *Paleobiology* 3:pp. 115–151.

521 Gower, J. C. 1966. Some distance properties of latent root and vector methods used in
522 multivariate analysis. *Biometrika* 53:325–338.

523 Gower, J. C. 1971. A general coefficient of similarity and some of its properties.
524 *Biometrics* 27:pp. 857–871.

525 Grossnickle, D. M. and P. D. Polly. 2013. Mammal disparity decreases during the
526 cretaceous angiosperm radiation. *Proceedings of the Royal Society of London B:*
527 *Biological Sciences* 280.

528 Hetherington, A. J., E. Sherratt, M. Ruta, M. Wilkinson, B. Deline, and P. C. Donoghue.
529 2015. Do cladistic and morphometric data capture common patterns of
530 morphological disparity? *Palaeontology* 58:393–399.

531 Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian*
532 *journal of statistics* Pages 65–70.

533 Hopkins, M. 2013. Decoupling of taxonomic diversity and morphological disparity
534 during decline of the cambrian trilobite family pterocephaliidae. *Journal of*
535 *Evolutionary Biology* 26:1665–1676.

536 Huang, S., K. Roy, and D. Jablonski. 2015. Origins, bottlenecks, and present-day
537 diversity: Patterns of morphospace occupation in marine bivalves. *Evolution* .

538 Hughes, M., S. Gerber, and M. A. Wills. 2013. Clades reach highest morphological
539 disparity early in their evolution. *Proceedings of the National Academy of Sciences*
540 110:13875–13879.

Hunt, G., M. J. Hopkins, and S. Lidgard. 2015. Simple versus complex models of trait evolution and stasis as a response to environmental change. *Proceedings of the National Academy of Sciences* 112:4885–4890.

Korn, D., M. J. Hopkins, and S. A. Walton. ????. Extinction spacea method for the quantification and classification of changes in morphospace across extinction boundaries .

Lloyd, G. T. 2015. Claddis: Measuring Morphological Diversity and Evolutionary Tempo. R package version 0.1.

Losos, J. B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *The American Naturalist* 175:pp. 623–639.

Lovegrove, B. G., K. D. Lobban, and D. L. Levesque. 2014. Mammal survival at the cretaceous–palaeogene boundary: metabolic homeostasis in prolonged tropical hibernation in tenrecs. *Proceedings of the Royal Society of London B: Biological Sciences* 281.

Luo, Z.-X. 2007. Transformation and diversification in early mammal evolution. *Nature* 450:1011–1019.

KEY: lu02007

ANNOTATION: 10.1038/nature06277

Meredith, R., J. Janečka, J. Gatesy, O. Ryder, C. Fisher, E. Teeling, A. Goodbla, E. Eizirik, T. L. Simão, T. Stadler, D. Rabosky, R. Honeycutt, J. Flynn, C. Ingram, C. Steiner,

561 T. Williams, T. Robinson, B. Angela, M. Westerman, N. Ayoub, M. Springer, and
 562 W. Murphy. 2011. Impacts of the Cretaceous terrestrial revolution and KPg extinction
 563 on mammal diversification. *Science* 334:521–524.

564 Newham, E., R. Benson, P. Upchurch, and A. Goswami. 2014. Mesozoic mammaliaform
 565 diversity: The effect of sampling corrections on reconstructions of evolutionary
 566 dynamics. *Palaeogeography, Palaeoclimatology, Palaeoecology* 412:32 – 44.

567 Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L.
 568 Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2015. *vegan: Community
 569 Ecology Package*. R package version 2.3-0.

570 O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L.
 571 Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S.
 572 Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P. M.
 573 Velazco, M. Weksler, J. R. Wible, and A. L. Cirranello. 2013. The placental mammal
 574 ancestor and the postK-Pg radiation of placentals. *Science* 339:662–667.

575 Olson, M. E. and A. Arroyo-Santos. 2009. Thinking in continua: beyond the adaptive
 576 radiation metaphor. *BioEssays* 31:1337–1346.

577 OLeary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L.
 578 Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini,
 579 Z. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding,
 580 P. M. Velazco, M. Weksler, J. R. Wible, and A. L. Cirranello. 2013. Response to

comment on the placental mammal ancestor and the postk-pg radiation of placentals.

Science 341:613.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.

Payne, J. L., N. A. Heim, M. L. Knope, and C. R. McClain. 2014. Metabolic dominance of bivalves predates brachiopod diversity decline by more than 150 million years. *Proceedings of the Royal Society B: Biological Sciences* 281.

Pearman, P. B., A. Guisan, O. Broennimann, and C. F. Randin. 2008. Niche dynamics in space and time. *Trends in Ecology and Evolution* 23:149 – 158.

Prentice, K. C., P. Ruta, and M. J. Benton. 2011. Evolution of morphological disparity in pterosaurs. *Journal of Systematic Palaeontology* 9:337–353.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

Raia, P., F. Carotenuto, F. Passaro, P. Piras, D. Fulgione, L. Werdelin, J. Saarinen, and M. Fortelius. 2012. Rapid action in the palaeogene, the relationship between phenotypic and taxonomic diversification in coenozoic mammals. *Proceedings of the Royal Society of London B: Biological Sciences* 280.

Raup, D. M. 1979. Size of the permo-triassic bottleneck and its evolutionary implications. *Science* 206:217–218.

Renne, P. R., A. L. Deino, F. J. Hilgen, K. F. Kuiper, D. F. Mark, W. S. Mitchell, L. E.
 Morgan, R. Mundil, and J. Smit. 2013. Time scales of critical events around the
 Cretaceous-Paleogene boundary. *Science* 339:684–687.

Revell, L. J. 2012. phytools: An r package for phylogenetic comparative biology (and
 other things). *Methods in Ecology and Evolution* 3:217–223.

Ronquist, F., S. Klopstein, L. Vilhelmsen, S. Schulmeister, D. Murray, and A. Rasnitsyn.
 2012. A total-evidence approach to dating with fossils, applied to the early radiation
 of the Hymenoptera. *Systematic Biology* 61:973–999.

Ruta, M., K. D. Angielczyk, J. Fröbisch, and M. J. Benton. 2013. Decoupling of
 morphological disparity and taxic diversity during the adaptive radiation of
 anomodont therapsids. *Proceedings of the Royal Society of London B: Biological
 Sciences* 280.

Sepkoski, J., J. John. 1981. A factor analytic description of the phanerozoic marine fossil
 record. *Paleobiology* 7:pp. 36–53.

Slater, G. J. 2013. Phylogenetic evidence for a shift in the mode of mammalian body size
 evolution at the cretaceous-palaeogene boundary. *Methods in Ecology and Evolution*
 4:734–744.

Slater, G. J., S. A. Price, F. Santini, and M. E. Alfaro. 2010. Diversity versus disparity and
 the radiation of modern cetaceans. *Proceedings of the Royal Society of London B:
 Biological Sciences* .

620 Smith, A. J., M. V. Rosario, T. P. Eiting, and E. R. Dumont. 2014. Joined at the hip:
 621 Linked characters and the problem of missing data in studies of disparity. *Evolution*
 622 68:2386–2400.

623 Springer, M. S., R. W. Meredith, E. C. Teeling, and W. J. Murphy. 2013. Technical
 624 comment on the placental mammal ancestor and the postk-pg radiation of placentals.
 625 *Science* 341:613.

626 Stadler, T. 2011. Mammalian phylogeny reveals recent diversification rate shifts.
 627 *Proceedings of the National Academy of Sciences* 108:6187–6192.

628 Thorne, P. M., M. Ruta, and M. J. Benton. 2011. Resetting the evolution of marine
 629 reptiles at the Triassic-Jurassic boundary. *Proceedings of the National Academy of*
 630 *Sciences* 108:8339–8344.

631 Toljagic, O. and R. J. Butler. 2013. Triassic-Jurassic mass extinction as trigger for the
 632 mesozoic radiation of crocodylomorphs. *Biology Letters* 9.

633 Torgerson, W. S. 1965. Multidimensional scaling of similarity. *Psychometrika*
 634 30:379–393.

635 Wesley-Hunt, G. D. 2005. The morphological diversification of carnivores in north
 636 america. *Paleobiology* 31:35–55.

637 Wills, M. A., D. E. G. Briggs, and R. A. Fortey. 1994. Disparity as an evolutionary index:
 638 A comparison of cambrian and recent arthropods. *Paleobiology* 20:93–130.

- 639 Wilson, G. P. 2013. Mammals across the k/pg boundary in northeastern montana, u.s.a.:
640 dental morphology and body-size patterns reveal extinction selectivity and
641 immigrant-fueled ecospace filling. *Paleobiology* 39:429–469.
- 642 Wilson, G. P., A. R. Evans, I. J. Corfe, P. D. Smits, M. Fortelius, and J. Jernvall. 2012.
643 Adaptive radiation of multituberculate mammals before the extinction of dinosaurs.
644 *Nature* 483:457–460.
- 645 Wood, H. M., N. J. Matzke, R. G. Gillespie, and C. E. Griswold. 2013. Treating fossils as
646 terminal taxa in divergence time estimation reveals ancient vicariance patterns in the
647 palpimanoid spiders. *Systematic Biology* 62:264–284.
- 648 Yang, Z., S. Kumar, and M. Nei. 1996. A new method of inference of ancestral
649 nucleotide and amino acid sequences. *Genetics* 141:1641–50.
- 650 Zelditch, M. L., D. L. Swiderski, and H. D. Sheets. 2012. Geometric morphometrics for
651 biologists: a primer. Academic Press.

Table 2: Results of the *post-hoc* t-tests for comparing the disparity at the last sub-sample of the Cretaceous (65 Mya) to all the sub-samples of the Cenozoic for the Eutherians (data from Beck and Lee, 2014). Sub-samples: reference sample (65 Million years ago; Mya) to Cenozoic sample (from 60 Mya to present). Gradual: gradual evolution; punctuated: punctuated evolution. Difference: mean sub-sample difference; Df: degrees of freedom; T: T statistic; p-value: adjusted p-value using Holm-Bonferroni correction. Significant differences are highlighted in bold: one star (*) signifies a p-value between 0.05 and 0.005; two stars between 0.005 and 0.0005 and three stars < 0.0005.

Sub-samples (Mya)	Gradual				Punctuated			
	Difference	Df	T	p.value	Difference	Df	T	p.value
65:60	0.06	76	1.055	1	0.04	76	0.760	1
65:55	0.05	75	0.999	1	0.16	75	3.145	0.0310 *
65:50	0.15	68	2.412	0.2413	0.08	68	1.403	1
65:45	0.21	64	3.016	0.0478 *	0.18	64	2.685	0.1200
65:40	0.18	64	2.579	0.1590	0.13	64	2.173	0.4354
65:35	0.23	60	2.840	0.0800	0.21	60	2.962	0.0568
65:30	0.27	57	2.927	0.0639	0.29	57	3.810	0.0044 **
65:25	0.22	56	2.500	0.1999	0.28	56	3.544	0.0104 *
65:20	0.16	56	1.922	0.7762	0.25	56	3.117	0.0374 *
65:15	0.14	55	1.819	0.9670	0.30	55	3.567	0.0098 **
65:10	0.14	55	1.843	0.9203	0.42	55	4.540	0.0004 ***
65:5	0.14	55	1.790	1 39	0.30	55	3.377	0.0176 *
65:0	0.14	55	1.818	0.9692	0.17	55	2.250	0.3705

Table 3: Results of the *post-hoc* t-tests for comparing the disparity at the last sub-sample of the Cretaceous (65 Mya) to all the sub-samples of the Cenozoic for the rarefied Eutherians (data from Beck and Lee, 2014). Column heads explained same as given in table 2.

Sub-samples (Mya)	Gradual				Punctuated			
	Difference	Df	T	p.value	Difference	Df	T	p.value
65:60	0.04	76	0.218	1	0.01	76	0.064	1
65:55	0.04	75	0.213	1	0.14	75	0.797	1
65:50	0.11	68	0.553	1	0.04	68	0.224	1
65:45	0.15	64	0.716	1	0.13	64	0.600	1
65:40	0.11	64	0.544	1	0.07	64	0.358	1
65:35	0.15	60	0.627	1	0.12	60	0.572	1
65:30	0.15	57	0.636	1	0.17	57	0.772	1
65:25	0.10	56	0.423	1	0.16	56	0.697	1
65:20	0.03	56	0.131	1	0.13	56	0.555	1
65:15	0	55	0.005	1	0.16	55	0.674	1
65:10	-0.01	55	-0.034	1	0.27	55	1.129	1
65:5	0.01	55	0.029	1	0.15	55	0.640	1
65:0	0	55	0.005	1	0.02	55	0.071	1

Table 4: Results of the *post-hoc* t-tests for comparing the disparity at the last sub-sample of the Cretaceous (65 Mya) to all the sub-samples of the Cenozoic for the Mammaliaformes (data from Slater, 2013) under gradual evolution model. Raw data: bootstrapped data without rarefaction; Rarefied data: rarefied bootstrapped data. Other column heads explained same as given in table 2.

Sub-samples (Mya)	Raw data				Rarefied data			
	Difference	Df	T	p.value	Difference	Df	T	p.value
65:60	0.49	19	0.826	1	0.26	19	0.365	1
65:55	0.45	20	0.734	1	0.31	20	0.428	1
65:50	0.13	21	0.267	1	0.03	21	0.042	1
65:45	-0.05	24	-0.109	1	0.03	24	0.051	1
65:40	-0.22	25	-0.543	1	-0.08	25	-0.118	1
65:35	-0.33	27	-0.858	1	-0.19	27	-0.321	1
65:30	-0.37	28	-0.973	1	-0.21	28	-0.335	1
65:25	-0.48	30	-1.358	1	-0.25	30	-0.394	1
65:20	-0.69	31	-2.030	0.6625	-0.44	31	-0.711	1
65:15	-0.76	30	-2.201	0.4620	-0.53	30	-0.906	1
65:10	-0.86	30	-2.666	0.1593	-0.66	30	-1.241	1
65:5	-0.85	30	-2.668	0.1585	-0.63	30	-1.197	1
65:0	-0.86	30	-2.678	0.1548	-0.62	30	-1.133	1