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THE VARYING RATES OF PHENOTYPIC EVOLUTION AND HISTORICAL PROCESSES RECONSTRUCTION: CONSEQUENCES FOR THE METABOLIC THEORY OF ECOLOGY AND ADAPTIVE RADIATION

TASAS VARIABLES DE EVOLUCIÓN FENOTÍPICA Y LA RECONSTRUCCIÓN DE PROCESOS HISTÓRICOS: CONSECUENCIAS PARA LA TEORÍA METABÓLICA DE LA ECOLOGÍA Y LA RADIACIÓN ADAPTATIVA

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

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This thesis is dedicated to my family Llautureo Quinán, who are the pillars of all that I am and shall be.

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GENERAL ABSTRACT

The rate at which phenotypic evolution proceeds varies widely across the tree of life, and this variation is fundamental to make accurate inferences about the evolutionary mechanisms that explain the origin of present-day biodiversity. In this thesis I evaluated the evolutionary prediction of two universal theories that makes clear assumptions about the rate of phenotypic evolution in order to explain all diversity patterns and the striking number of species reached by some clades: The Metabolic Theory of Ecology (MTE; chapter one), and Adaptive Radiation (chapter two). The MTE attempts to predict biodiversity patterns by the Basal Metabolic Rate (*BMR*) of organisms. Assuming that *BMR* is a direct consequence of temperature, it also predicts that *BMR* and Body Temperature (T_b) should evolved at constant rates, and hence in a correlated fashion during mammalian radiation. On the other hand, Adaptive Radiation predicts that the impressive species richness reached by some clades is caused by frequent episodes of past disruptive selection and speciation.

I evaluated these predictions with new phylogenetic statistical methods that assumes evolution proceeds at variable rates. By accommodating shifts in the rate of phenotypic evolution across each branch of a time-calibrated phylogenetic tree, these methods have demonstrated that is possible to detect and reconstruct accurate historical evolutionary processes, even from data of extant species only.

Our results reveal that *BMR* and T_b were decoupled during the 160 million years of mammalian evolution because they evolved at contrasting rates across each branch of the phylogeny. The observed accelerated evolution of *BMR*, caused by the abrupt changes in the environmental temperature, was the pivotal process explaining this decoupled scenario. This demonstrates that neither the kinetic effect of T_b nor the functional mechanisms between both traits, constrained BMR to evolve as a direct response of T_b evolution. On the other hand, I find that the distinction between species originated by divergent and directional selection was fundamental to identify the proportion of species originated by an Adaptive Radiation in Sigmodontinae rodents, and these distinction was possible only by studying the rate of body size evolution, branch per branch in the phylogeny.

This thesis brings new empirical information that support the importance of variation in the rate of evolution to make appropriate evolutionary predictions expected under the MTE. Furthermore, this thesis provides a new theoretic-based approach that allows to detect the species in a clade originated by an adaptive radiation. These findings arise by the idiosyncrasy of rate variation within the lifespan of species. Consequently, any study that seeks to understand current biodiversity patterns by studying the historical process of phenotypic evolution, should evaluate to what extent the rate of phenotypic evolution varies in both ancestral and extant species, and therefore, in each branch of the phylogenetic relationships.

KeyWords: Phylogenetic Comparative Method, Macroevolution, Adaptive Evolution, Speciation Rates.

GENERAL INTRODUCTION

The premise of gradualism was pivotal to propose natural selection as the causal mechanism for the origin of both phenotypic and species diversities (Darwin, 1859; Gould, 2002). This idea states that evolutionary change or divergence should accumulates steadily and slowly as species succeeds the previous one in the struggle for life (Venditti & Pagel, 2008). From this follows that, since the origin of life, evolution have to occurs at both constant and low rates, making necessary just time for natural selection be able to accumulate both the phenotypic and species diversity we observe today.

Under gradual evolution, we expect to observe that the amount of phenotypic divergence that a species accumulates during its life, depends simply on the time elapsed since its origin. Consequently, sister lineages (phylogenetic lines originated from a common ancestor) should accumulate the same or equal amount of phenotypic divergence (Fig. 1A). However, since the latter part of the nineteenth century, the gradual view of evolution started to be incoherent to explain the phenotypic diversity that is observed in both living species and the fossil record (Fitch and Ayala, 1994). For example, how can gradual evolution explain the contrasting body sizes achieved by sister lineages in mammals, like Chiroptera (bats) and Cetartiodactyla (giraffes, whales), if both evolved during the same period of time from a common ancestor? (Fig. 1A; B). Why the naked mole rat (*Heterocephalus glaber*) has extremely low metabolic rates compared to its sister species (in such a way to be called the poikilothermic mammal; Lovegrove (2012a)) if they evolved during the same time from a common ancestor? How can gradual evolution explain the abrupt appearance of hypsodonty in the evolution of Equidae (Simpson, 1944)?

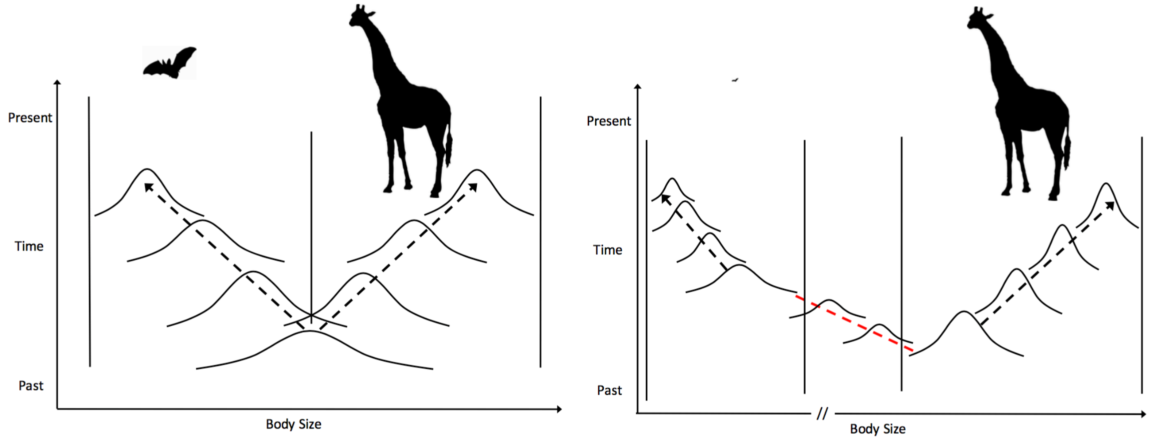


Figure 1. Expected phenotypic divergence achieved by sister lineages under a scenario of gradual evolution (A) and by a non-gradual one emerging by sudden shift in the rate of evolution (B). The common ancestry for Chiroptera and Cetartiodactyla is based on the Meredith et al. (2011) mammalian phylogeny.

The solution to this problem was not so simple because it was necessary to change the way many biologists thought about phenotypic evolution for decades, and more important, because the necessary consequence that evolution can occurs at sudden steps and at the expense of the gradual premise (Fig. 1B), could deny that natural selection plays the major role in the origin of biodiversity (Gould, 2002). However, George G. Simpson (1944), in his most important contribution to the evolutionary theory, resolved this problem by postulating new evolutionary principles in which natural selection can actually work at different intensities or variable rates – not limited to the steady and slowly changes preconceived by gradual evolution. For example, high rates of phenotypic evolution between generations are expected if environmental conditions to which organisms are adapted change suddenly, because great phenotypic changes shall be demand in order to allow organisms to survive in these new environments. On the other hand, the lowest rates are expected if the environmental conditions are stable because no phenotypic change is demanded for populations to survive and keep in these optimal conditions. Simpson also noticed that not just the rate of change gives valuable information about the evolutionary process, but also the direction. Natural selection can favour both extreme of the phenotypic variation by disruptive selection, which generate no net direction over time in a group of species, or can favour just one by directional selection, which generates evolutionary trends over time. Furthermore, just when the phenotypic change is disruptive, natural selection can be the dominant cause of speciation and its accumulation over time.

It is important to notice that this theoretical approach is not based exclusively in natural selection as the causal mechanism of evolution. Genetic drift can also play a major role in some cases (see below). However, this theoretical approach makes clear prediction of the particular scenarios in which natural selection can actually be the dominant cause of both phenotypic evolution and speciation. In this context, natural selection plays the major role in both phenotypic change and the origin of new species in a scenario in which selection is disruptive within a main zone or adaptive landscape (Fig. 2A). From this follows that phenotype evolves from low to medium rates and with a random net change, that is, without any particular direction. The justification for the expected low to medium rates of phenotypic evolution lies in that adaptation by disruptive selection does not demand great phenotypic changes to colonize the empty sub-zones within an adaptive zone (Fig. 2A). The expected random phenotypic change of the group under differentiation lies in the fact that the cumulative change by splitting determines both extreme of phenotypic variants in a population to have an equal probability to survive, which erases any trend over time (Fig. 2A). On the other hand, natural selection can play a minor role in speciation by splitting but a major one in phenotypic change when just one extreme of phenotypic variation is selected under pressures arising from a changing adaptive zone (Fig. 2B). Under this scenario, called Phyletic Evolution, evolution involves the sustained, directional shift of the mean phenotype from ancestral to derived species from slow to high rates. Selection is the dominant evolutionary force acting homogeneously over several species, and causing directional trends above the species level. Under Phyletic Evolution, speciation by population split is rare or “not typical”, because species accumulation occurs by replacement instead splitting over evolutionary time. This not means that those groups do not or rarely speciate by splitting, they do and frequently, but by other non-selective forces. The third mode, quantum evolution, identify the instances of phenotypic evolution between adaptive zones at highest rates (Fig. 2C), because great phenotypic changes are required to colonize a new adaptive zone. Quantum evolution is associated to the origin of taxa above species level (e.g. orders, phylum), where either natural selection or genetic drift can initiate and dominate the mode.

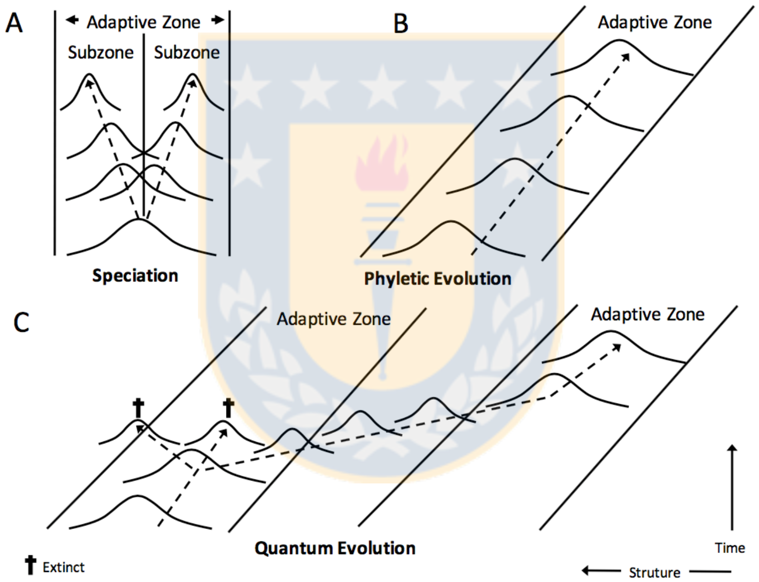


Figure 2. Three major modes of phenotypic evolution inferred by variable rates and direction of evolution. A: Speciation, B: Phyletic Evolution, C: Quantum Evolution. Figure extracted from *Tempo and Mode in Evolution* (Simpson, 1944).

Currently, abundant evidence demonstrate that the the rate of phenotypic evolution varies widely across the tree of life, even in single ancestral lineages (Eastman et al. 2011; Kratsch and McHardy, 2014; Landis et al. 2013; Rabosky 2014; Revell et al. 2012; Thomas and Freckleton 2012; Benson et al. 2014; Puttick et al. 2014; Venditti et al. 2011; Chira & Thomas

2016; Baker et al. 2016). Furthermore, it has also been demonstrated that speciation is not necessarily limited to natural selection but rather it is more likely the cause of multiple stochastic events in which each individually have the potential to cause a speciation event (Venditti et al. 2010). This cumulated facts and knowledge about rates of phenotypic evolution and causes of speciation invite us to reconsider or re evaluate long-standing problems that has been approached under both the assumptions of gradual evolution and selection-exclusive causes of speciation, for example, the evolution of basal metabolic rate during Mammalian radiation and the hypothesis of Adaptive Radiation (Simpson, 1953; Schluter, 2000).

Due to not all phenotypic traits (specially those related to energetics) leave appropriate fossil record to study their evolution, it is necessary to make historical inferences using the abundant phenotypic data of extant species plus their well resolved phylogenetic relationship. New phylogenetic statistical methods, whose development was inspired on the basic principles of the evolutionary theory, for example, that rate of evolution can vary from clades to single lineages, now provide us with the opportunity to make inferences to the past from data of extant species. By accommodating shifts in the rate of phenotypic evolution across each branch of a time-calibrated phylogenetic tree (Fig. 3), these methods have demonstrated that is possible to detect and reconstruct accurate historical evolutionary processes, even from data of extant species only (Baker et al. 2015; 2016). Time-calibrated trees, that is, phylogenetic relationships of species in which branch length represent the amount of time between two events of speciation, can be used to evaluate if some phenotype has evolved in direct proportion to the branch length measured in time (i.e. the gradual view of evolution), or by variation in the rate of evolution across any branch of the phylogeny (Fig. 3). Furthermore, phylogenetic trees have information about past speciation events (i.e. nodes that determinate branch bifurcations; Fig. 3), which allow its integration with rates and direction of phenotypic evolution, to evaluate if they are linked as expected under the view of speciation by divergent natural selection (Fig 2A). This thesis was based on this theoretic and methodological framework to resolve two long-standing problems in macroevolution: the evolution of Basal Metabolic Rate (*BMR*) in mammals (chapter one); and the role of natural selection on the origin of Sigmodontinae species (chapter two).

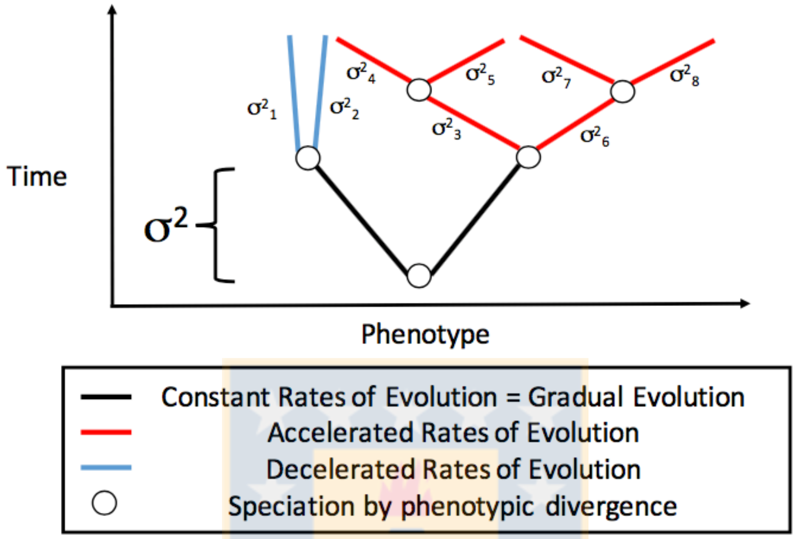


Figure 3. Conceptual diagram showing model parameterization of an evolutionary scenario of gradual evolution (Black lines; see Fig. 1A), and an evolutionary scenario that allows the rate of evolution to vary across every single branch of the phylogeny (red and blue lines; see Fig. 1C). Source: Own elaboration.

Thesis structure

Along the following chapters I evaluated the evolution of *BMR* during Mammalian radiation, and the link between body size size evolution and speciation in South American small mammals. Both chapters were based on Simpson's theoretic framework and using phylogenetic statistical method that allow the evaluation of different evolutionary scenarios. In the first chapter I studied the evolution of *BMR* under expectations from the first principle of the Metabolic Theory of Ecology (kinetic effects of temperature on metabolic rates; Brown et al. 2004), and from the Correlated Progression Model for the evolution of biological systems (Kemp, 2006; 2007). In this chapter I might expect to observe that *BMR* and body temperature (T_b) in mammals evolved gradually and tightly correlated (see Fig. 1 in Chapter 1), due to the kinetic effect of T_b on metabolism, and the functional integration between both physiological traits (metabolic rate is the source of heat). Translated into the statistical phylogenetic framework, it should be observed constant rates of *BMR* and T_b evolution, and a positive correlation between theirs rates of evolution across each branch of the mammalian phylogeny.

In the second chapter I evaluated the evolution of body size and its effect on speciation under the expectations of Adaptive Radiation (Simpson, 1953). This hypothesis has been essential to understand the particular scenario in which natural selection is the dominant force that cause the origin of striking species numbers in some clades. Adaptive radiation postulate that speciation is the consequence of frequent episodes of intensified divergent selection operating on a trait that allow organisms to survive and reproduce in new environments (Simpson, 1953; Skelton, 1993: Schluter, 2000). According to this hypothesis speciation is expected to be caused by natural selection only if phenotype evolves at accelerated rates and at random (without any directional trend) (Fig. 2A; Fig. 3 red lines).

Research hypotheses

The widely *BMR* and T_b diversity in mammals, and the Sigmodontinae species richness, was originated by the evolutionary process operating at variable intensities during the life span of ancestral and present-day species.

General objective

Estimate the rate of phenotypic evolution across every branch of the phylogenetic relationships, and use it to evaluate the theoretic expectations about correlated evolution and speciation by natural selection.

Specific objectives

a) Obtain a time-calibrated phylogeny for Mammals (Chapter 1) and Sigmodontinae rodents (Chapter 2).

b) Obtain phenotypic data for species. *BMR* and T_b for mammals (Chapter 1), and Body Size for Sigmodontinae rodents (Chapter 2).

c) Model the evolution of *BMR*, T_b, and Body Size, by inferring rates of evolution across every branch of the phylogeny, and evaluate potential trends across the phylogeny.

CHAPTER 1

Basal metabolic rate and body temperature are decoupled in mammalian evolution

(Article in preparation)

Abstract

The Metabolic Theory of Ecology (MTE) attempts to predict how *BMR* of organisms controls biodiversity by the kinetic effect of temperature on mass-corrected *BMR*. From this follows that the positive correlation between body temperature (T_b) and mass-corrected *BMR* in present-day endotherms is simply an extrapolation of this kinetic mechanism acting at the organismal level. Furthermore, *BMR* and T_b should also be linked owing to their functional integration, given that metabolic rate is the heat source to maintain a constant T_b in endotherms. Because of this, it is widely assumed that *BMR* and T_b were coupled and evolving in a correlated progression during mammalian evolution. Nevertheless, there are hints in the literature that suggest the story may not be so simple because *BMR* could be determined by the amount of energy needed to compensate the rate of heat lost to the environment, a response which is ultimately limited by ambient temperature (T_a) and resource availability.

Using a phylogenetic statistical method that allows the rates of evolution to vary across every branch of the Mammalian phylogeny, we find that *BMR* and T_b evolved independently in such a way to be decoupled during the 160 million years of the mammalian history. We find extremely more frequent rate variation in *BMR* than in T_b evolution. Specifically, 88.8% of branches across the mammalian phylogeny were detected with accelerated rates of evolution for *BMR,* versus 29.7% with accelerates rates for T_b. Furthermore, *BMR* evolutionary rates were correlated with rates of T_a evolution, demonstrating that *BMR* evolved in response to environmental conditions instead to the T_b of organisms.

These results bring new empirical information that support the importance of variations in the rate of evolution to make appropriate evolutionary predictions under the MTE. We support an

historical scenario in which the decoupled association between *BMR* and T_b provided mammals with the opportunities to adapt for diverse energetic modes of existence.

INTRODUCTION

The Metabolic Theory of Ecology (MTE; Brown et al. 2004) attempts to predict how metabolism of organisms controls biodiversity. This is because if we consider that metabolism is the flux and transformation of energy and material in organism, ecology is the interactions between organisms and their environments, and an interaction is an exchange of energy, material y/o information, then most ecological processes are fundamentally metabolic. Furthermore, given that metabolism sets powerful constraint on the processes of survival, growth, and reproduction, it is also postulated that metabolism is the fundamental biological rate that links the fitness of organism to the ecology of population, communities, ecosystems, and the evolution of biodiversity (Brown et al. 2004).

Based on first principles, the MTE master equation predicts a positive correlation between body temperature (T_b) and mass-corrected *BMR* in present-day endotherms, which is based on the fact that all biological rates scales positively with temperature (Brown et al. 2004). Furthermore, *BMR* and T_b should also be correlated owing to their functional integration, given that metabolic rate is the heat source to maintain a constant T_b in endotherms (Lovegrove, 2012a). Because of this, it is widely assumed that *BMR* and T_b were coupled and evolving in a correlated progression during mammalian evolution (Kemp, 2006; 2007; Lovegrove, 2012a). Nevertheless, there are hints in the literature that suggest the story may not be so simple because *BMR* could be determined by the amount of energy needed to compensate the rate of heat lost to the environment, a response which is ultimately constrained by ambient temperature (T_a) and resource availability (McNab, 2002; Clarke, 2004; Clarke & Fraser, 2004; O'Connor et al. 2007).

New phylogenetic statistical methods (e.g. Venditti et al. 2011, Revell et al. 2012; Rabosky, 2014) now provide us with the opportunity to formally test the idea that *BMR* and T_b have been linked throughout the evolutionary history of the Mammalia. By accommodating shifts in the rate of phenotypic evolution across each branch of a time-calibrated phylogenetic tree, these methods have demonstrated that is possible to detect and reconstruct accurate historical evolutionary processes, even from data of extant species only (Baker et al. 2015; 2016). Under this methodological approach, we expect to detect a positive correlation between rates of *BMR* and T_b evolution across every branch of the mammalian phylogeny (i.e. coupled evolution) in support to the MTE and the correlated progression model. In one scenario, *BMR* and T_b could be coupled by evolution at constant rates. If this prediction is valid, we would expect to find no significant rate variation relative to a background rate in any trait (Fig. 1A). In another scenario, if both traits were coupled, but evolutionary forces expanded their phenotypic variation by an intensification of evolutionary forces, we would expect to see a positive relationship arising by rates of evolution higher than the background rates across every branch (Fig. 1C). On the other hand, if both traits were coupled but evolutionary forces acted to reduce their phenotypic variation (e.g. some kind of stabilizing selection, or constraint, or optima as suggested by Nespolo et al. 2017), we would expect the inferred rates to be less than expected from the background rates (Fig. 1G). If both traits were coupled but evolutionary forces acted to reduce phenotypic variation in one trait (rate deceleration) and expand it in the other (rate acceleration), we would expect to see a negative relationship between both rates (Fig. 1I; E). Finally, if *BMR* and T_b evolved totally decoupled by evolutionary forces acting only in one trait, we expect to see constant rates in T_b and variable rates in *BMR* (Fig. 1H; D) or vice versa (Fig. 1B; F).

METHODS

We tested the expectation that *BMR* and T_b have been coupled throughout evolution using the *variable-rates regression model* in a Bayesian framework (Baker et al. 2016). This model is designed to automatically detect shifts in the rate of evolution in a continuous trait after accounting for a relationship with another trait or traits (e.g. *BMR* with body mass M or T_b with *BMR*). The method allows for simultaneous estimation of both an overall relationship between the trait of interest and other characters but also any shifts in rate that apply to the phylogenetically structural residuals errors. The rate of phenotypic change that occurs in a branch is divided in two components: a rate parameter which assume changes are draw from an underlying Brownian process (σ^2_b), and a second set of parameters that identify branch-specific rate shifts by adjusting the lengths of a branch in a time-calibrated tree (stretch or compress a branch is similar to increase or decrease the phenotypic rate of change relative to the underlying Brownian rates). Rate shifts are detected by a set of branch-specific scalars r ($0 < r < \infty$) which transform each branch in order to optimize the phenotypic rate of change to a Brownian process ($\sigma^2_v = \sigma^2_b\, r$). If phenotypic change occurred at accelerated rates in a specific branch of the tree, then $r > 1$ and the branch is stretched. Decelerated rates of evolution are detected by $r < 1$ and the branch is compressed. Constant rates of evolution ($r = 1$) therefore does not modify the branch lengths of the time-calibrated tree.

We used BayesTraits v3.0 (Pagel et al. 2004) to detect the magnitude and location of rate scalars (r) for *BMR* and T_b in a Bayesian Markov chain Monte Carlo (MCMC) reversible-jump, which generates a posterior distribution of trees with scaled branches lengths. Our dataset comprises 502 mammal species for which data was available (Clarke et al. 2010), and it include representatives from 16 orders (Afrosoricida, Artiodactyla, Carnivora, Chiroptera, Didelphimorphia, Diprodontia, Eulipotyphla, Lagomorpha, Macroscelidea, Notoryctemorphia, Peramelemorphia, Pholidota, Primates, Rodentia, Scandentia, Tubulidentata and Xenarthra). Rates were estimated in two independent analyses while accounting for the effect of main covariates (M and T_b for *BMR; M* and *BMR* for T_b).

RESULTS AND DISCUSSION

The *variable-rates regression model* significantly improved the fit to the data over an equal rates model in both cases (BF=106 and BF=94, respectively). Unexpectedly, we find extremely more frequent rate variation in *BMR* than in T_b evolution (Fig. 2A; B). Specifically, 88.8% of branches (773 of 870) across the mammalian phylogeny were detected with rate acceleration ($1 <$ median $r \leq 130.15$) for *BMR,* versus 29.7% with rate acceleration for T_b ($1 <$ median $r \leq 62.7$), both in more than 50% of the posterior sample of trees with scaled branch lengths. The relationship between *BMR* and T_b rates across every branch shows constant rates for both traits ($r = 1$) in only 10.6% of branches (92 of 870; Fig. 2C, white circle). In 60.2 % of branches (524 of 870) the rates were totally decoupled by acceleration just in *BMR* (59.7%; Fig. 2, horizontal black circles) or T_b (0.5%; Fig. 2C, vertical black circles). Despite we found accelerated rates of evolution ($r > 1$) in both traits in 29.2% of branches (254 of 870; Fig. 3, grey circles), they are not statistically associated (mean slope = 0.005, 95% HPD = -0.017, 0.13; P = 7.5).

Looking at the accelerates rates of *BMR* evolution into mammalian orders, all branches within the orders Artiodactyla, Carnivora, Chiroptera, Eulipotyphla, Lagomorpha, Pholidota, Primates, Rodentia, Scandentia and Xenarthra were identified with accelerates rates (Fig. 2A). Within Afrotheria, rate acceleration was found just in the order Afrosoricida, and within Marsupialia, less than half of their branches showed rate acceleration. These results suggest that, during the radiation of most mammalian orders, the accelerated divergence of *BMR* was a frequent response of ancestral lineages to some selective pressure, which is line with the idea that metabolic diversity opens the opportunity to new modes of existence by adjusting energy expenditure under different environmental conditions (McNab, 2002). We propose that the main selective pressure acting on *BMR* could be the ambient temperature (T_a) that mammals experience through their evolution. This idea is based in at least three facts: (1) *BMR* correlates negatively with T_a in present-day mammals (McNab, 2002; Lovegrove, 2003; Naya et al. 2013; White & Kearny, 2013; Fristoe et al. 2015), given that to maintain the T_b constant, endotherms balance the rate of heat production (*BMR*) according to the rate of heat loss to the environment, which increase at lower T_a (Scholander et al. 1950). (2) Since the Mesozoic, ancestral mammals have dispersed over different thermal environments to the extent to colonize almost all environments in the earth (Kemp, 2005; Springer et al, 2011; Goin et al. 2016). (3) During most the evolutionary history of mammals, they were exposed to a high T_a variability because the global temperature has undergone a complex change with gradual trends of warming and cooling, periodic cycles, rare rapid shifts and extreme climate transients (Zachos et al. 2001).

Taking these facts together, we propose that the accelerated expansion of *BMR* diversity was a response to the challenges imposed by ancestral thermal environments that mammals experience during their evolution. Because T_a could be the selective factor promoting *BMR* divergence at accelerated rates, we might expect to find the inferred accelerated rates of *BMR* evolution to be linked with rates of thermal niche evolution across each branch of mammalian phylogeny. We evaluate this idea inferring the rates of T_a evolution from data of extant thermal niches of mammals (Cooper et al. 2011) to correlate it with the already inferred *BMR* rates. Given the latitudinal gradient of temperature, we used the *variable-rates regression model* to estimate rates of T_a evolution accounting simultaneously for the effect of latitude (centroid of range of distribution). *The variable-rates regression model* significantly improved the fit to the data over the equal rates model (BF = 564). T_a rates of evolution were accelerated ($r > 1$) in 74.6% of branches (Fig. 3B), and in more than 50% of the posterior sample of trees with scaled branch lengths. The relationship between *BMR* and T_a rates shows that in 70% of branches they were positively correlated (mean slope = 0.35; 95% HPD = 0.29, 0.4; P = 0; Fig 3C; red circles), supporting that most of the metabolic diversity expansion at accelerated rates was a response to the changing thermal condition that mammals faced through the evolution.

Because the faster rates of T_a were inferred for those lineages that lives in coldest environments today, we expect to find a negative trend in the evolution of thermal niches in Mammalian phylogeny (i.e. we expect to find higher rate of evolution linked to lower values of T_a across the mammalian phylogeny), and consequently, a positive trend in the evolution of mammalian *BMR* in order to compensate heat lost in these colder environments. To evaluate this expected negative trend, we study the relationship between T_a of present-day species and path-wise rates (*PWR*) of T_a (i.e. the sum of all the rate-scaled branches along the evolutionary path of a species, leading from the root to the tip). We included *PWR* as an additional covariate in our phylogenetic linear model for T_a, using the phylogeny with scaled branch lengths to account for shared history. The same analysis was done to study the expected positive trend in *BMR* evolution*.* As expected, we found a negative trend in evolution of T_a (Fig. 4), given the negative effect *PWR* across all mammals (mean slope = -0.001; 95% HPD = -0.0012, -0.0008; P = 0). However, we did not find evidence for any trend in *BMR* evolution. This means that, while mammals experienced colder environments during their radiation (Fig. 4), both high a lower *BMR* were favoured. This results can be explained by the energetic cost associated to *BMR* elevation in response to colder environments. Higher metabolic rates can be attained only when adequate amount of resources are continuously available in the environment, so an increase in *BMR* could be possible just when this was energetically viable (Grigg et al. 2004). Consequently, the best alternative to survive in colder environments without adequate amount of resources, is decrease the energetic cost or *BMR*, as several mammals' species do in the present (McNab, 2002).

The alternative scenario of stabilizing selection acting on *BMR* (i.e. a resistance to change) after an optimum value was attained in early mammals about 160 million years ago (Nespolo et al. (2017), is less likely to explain our results and incoherent with the fact about the widespread variation in metabolic diversity that is widely observed in present-day mammals (McNab, 2002).

If a particular optimal-*BMR* level was favoured by natural selection in ancestral lineages, then we should expect lineages with *BMR*s away this optimum to be eliminated by natural selection and, therefore, to infer decelerated rates of evolution (i.e. the residual variance in *BMR* to be reduced after accounting for the effect of *M* and T_b). However, there was no evidence for rate deceleration (i.e. $0 < r < 1$) in any branch - the residual variance was not reduced neither across all mammals and in any particular group. This means there is no evidence of a pull toward the 'optimum' *BMR* in any group.

Regarding to the decoupled evolution of T_b related to *BMR* (Fig. 2, 3), we find constant rates in most Mammalian T_b evolution (Fig. 2B). However, some accelerations were found in branches leading to groups within Chiroptera, Rodentia, and Marsupialia (Fig. 2B), which are species that in average have T_b closer to the lower category of mammalian T_b (i.e. basoendotherms; *sensu* Lovegrove, 2012a; b). If these particular shifts in the rate of T_b were the consequence of intensified evolutionary forces favouring lower T_b during Mammalian evolution, we expect to find that longer scaled branches are linked to lower T_b across the phylogeny. To evaluate this expectation, we study the relationship between T_b of present-day species and *PWR* of T_b. We included *PWR* as an additional covariate in our phylogenetic linear model for T_b. As expected, we find a significant and negative effect of *PWR* s across all mammals (mean slope = -0.0042; 95% HPD = -0.0087, 5.44E-4; P = < 5%; Fig 5), supporting the idea that lineages that diverge at faster rates led to colder bodies. This directional model predicts a mean T_b of 39.3 °C (95% HPD = [37.9 – 40.7]) for the most recent common ancestor of mammals, suggesting that early mammals could attain T_b similar to the warmer present-day species.

This result is not agreement with the idea that ancestral mammals had T_b lower than 35 ºC (Crompton et al. 1978; Grigg et al. 2004; Lovegrove, 2012b; 2017). The argumentation for this idea lies in the fact that T_b maintenance becomes more expensive energetically when the rate of heat lost to the environment increase at colder T_a, and this energetic demand could not be compensated when resources in the environment are scarce (McNab, 1982; 1992b). The problem becomes more acute at smaller sizes, because smaller organisms (like early mammals: Ji Q et al, 2002; Luo, 2007; Baker et al. 2015) have a relative largest surface to volume ratio and less fur, which also accelerates rates of heat lost to the environment (Riek & Geiser, 2013). However, the mechanistic basis of this idea is based on the Scholander-Irving model (Scholander et al. 1950) for endotherms thermoregulation ($BMR = C\ (T_b - T_a)$), and in the same line of the model, another way for an ancestral mammal to maintain a high and constant T_b could also be possible if T_a is high enough to reduce the temperature differential with T_b and hence the rate of heat lost to the environment. In this context, we expect an ancestral mammal with about 39.3 °C of T_b to live in a warm climate in order to reduce temperature differential, and consequently, energetic expenditure (*BMR*). According to our directional model describing the thermal niche evolution in mammals (Fig. 4), we predicted an ancestral environment with 28.74 °C in average (95% HPD = 27.1, 30.48), which determines a differential of 10.56 °C respect to the inferred T_b of the ancestral mammal. Current evidence demonstrate that this ancestral scenario is very conservative compared with current T_b-T_a differences observed, for example there are small mammals with contrasting ecologies and T_b higher than 39 °C (e.g. *Microdipodops pallidus*, *Erophylla sezekorni*) that can survive in colder environments (6 °C in average) today (T_b-T_a = 33 °C).

Our results reveal that *BMR* and T_b evolved independently in such a way to be decoupled during the 160 million years of the mammalian history. Natural selection exploited effectively the diversity of *BMR* promoting their divergence away from any constrain in order to solve the problem of reproduction and survival in diverse, often extreme thermal environments. The story for T_b was a different one, it divergence was a relative less frequent response to survive in ancestral environments and, just in some groups and near to the present, the accelerated evolution towards colder bodies was advantageous. These results bring new empirical information that encourages the inclusion of variations in the rate of evolution to make appropriate evolutionary predictions under the MTE, because *BMR* and T_b are far more free to vary over time than expected from the kinetic mechanism of temperature on metabolic rates. From this follows that the evasion of functional constraint was the most effective response of organisms compare to the alternative evolutionary scenario of correlated progression. We support an historical scenario in which the decoupled evolution between *BMR* and T_b provided mammals with the opportunities to adapt for diverse energetic modes of existence.

FIGURE LEGENDS

Figure 1. Conceptual diagram outlining the predicted relationships between rates of *BMR* and T_b evolution across each branch of the mammalian phylogeny, and indicating coupled to uncoupled scenarios of evolution. **A** indicates no significant rate variation in any trait relative to the background rates across every branch of mammalian phylogeny, which means *BMR* and T_b were coupled and diverging at random. **B**, **D**, **F** and **H** are decoupled scenarios of evolution by variable intensities of evolutionary forces operating in one trait only. **C**, **E**, **G**, and **I** are coupled scenarios arising by variable intensities of forces operating in both traits. Source: Own elaboration.

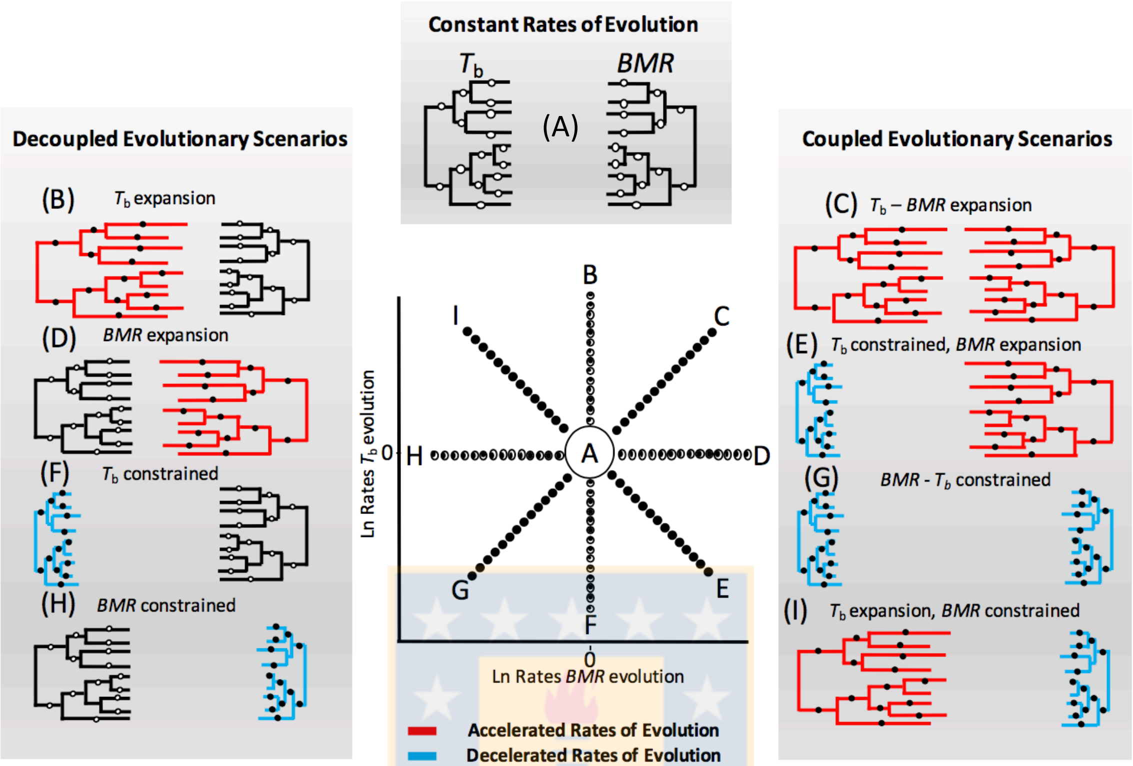
Figure 2. Accelerated rates of *BMR* (**A**, 89.5% of branches) and T_b (**B**, 29.8% of branches) evolution represented by red branches across the mammalian phylogeny. Black branches indicate the background constant rates of evolution according to a Brownian motion model. **C.** Rates of *BMR* and T_b evolution were totally decoupled in 60.2% of branches in mammalian phylogeny (black circles). Despite accelerated rates of evolution ($r > 1$) were inferred for both traits in the remainder 29.2% of branches (red circles), they were not statistically correlated. We find no significant rate variation ($r = 1$) in 10.6% of branches (white circle). Source: Own elaboration.

Figure 3. Accelerated rates of *BMR* (**A**, 89.5% of branches) and thermal niche evolution (**B**, 74.6% of branches), represented by red branches across the mammalian phylogeny. Black branches indicate the background constant rates of evolution according to a Brownian motion model. **C.** Rates of *BMR* and T_a evolution were coupled in 70% of branches in mammalian phylogeny (red circles). In 23.5% of braches rates of evolution were decoupled (black circles). We find no significant rate variation ($r = 1$) in 6.5% of branches (white circle). Source: Own elaboration.

Figure 4. Faster path-wise rates (cumulative change) have led to lower T_as across all mammals. The dark blue-line indicates the posterior predicted mean slope. Clear lines represent the posterior distribution of slopes. Source: Own elaboration.

Figure 5. Faster path-wise rates (cumulative change) have led to lower T_bs across all mammals. The dark-blue line indicates the posterior predicted mean slope. Clear lines represent the posterior distribution of slopes. Source: Own elaboration.

Figure 1


Constant Rates of Evolution
T_b
BMR
(A)
Decoupled Evolutionary Scenarios
(B) T_b expansion
(D) BMR expansion
(F) T_b constrained
(H) BMR constrained
Coupled Evolutionary Scenarios
(C) T_b – BMR expansion
(E) T_b constrained, BMR expansion
(G) BMR - T_b constrained
(I) T_b expansion, BMR constrained
Ln Rates T_b evolution
Ln Rates BMR evolution
0
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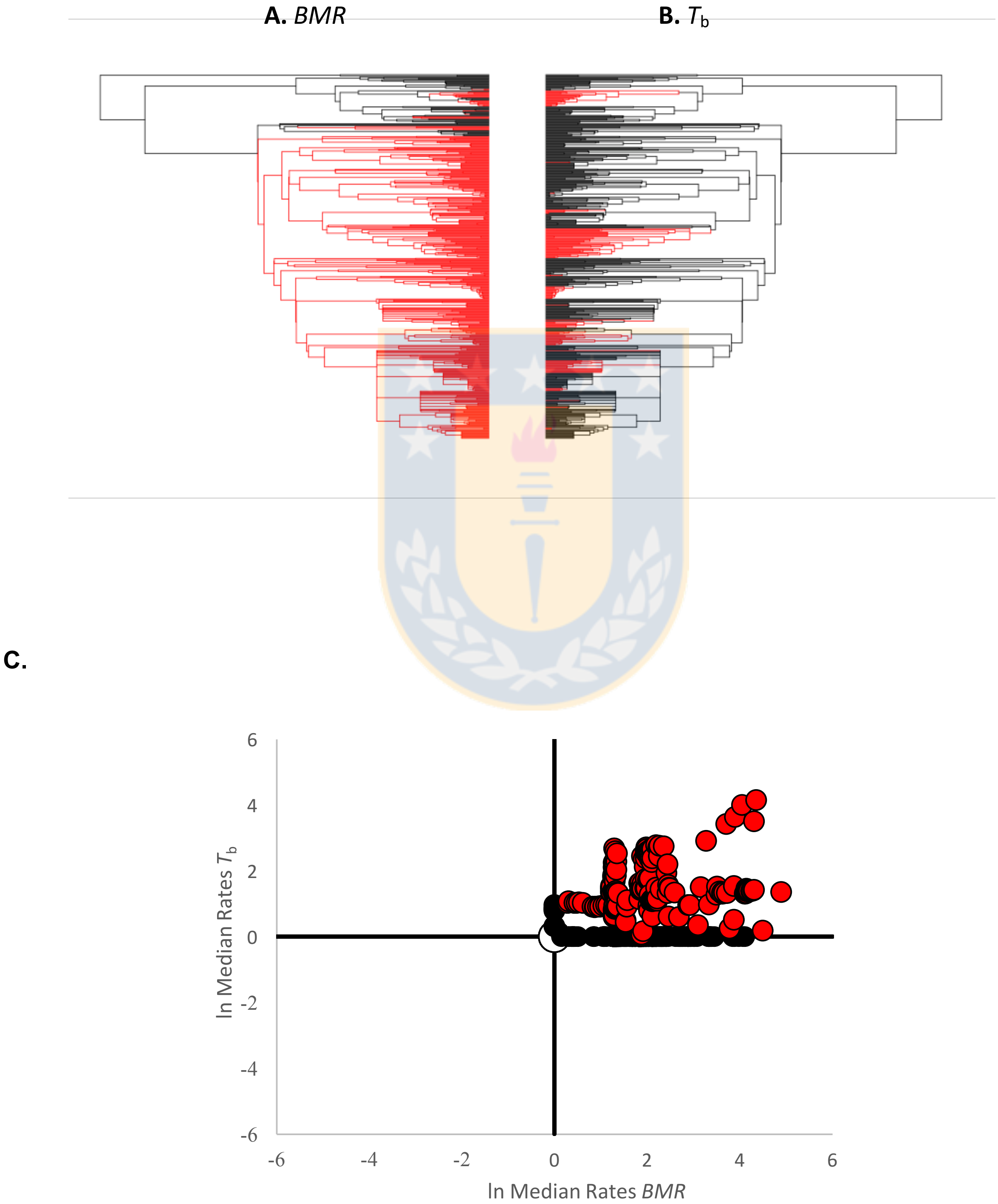
🟥 Accelerated Rates of Evolution
🟦 Decelerated Rates of Evolution

Figure 2


A. BMR
B. T_b
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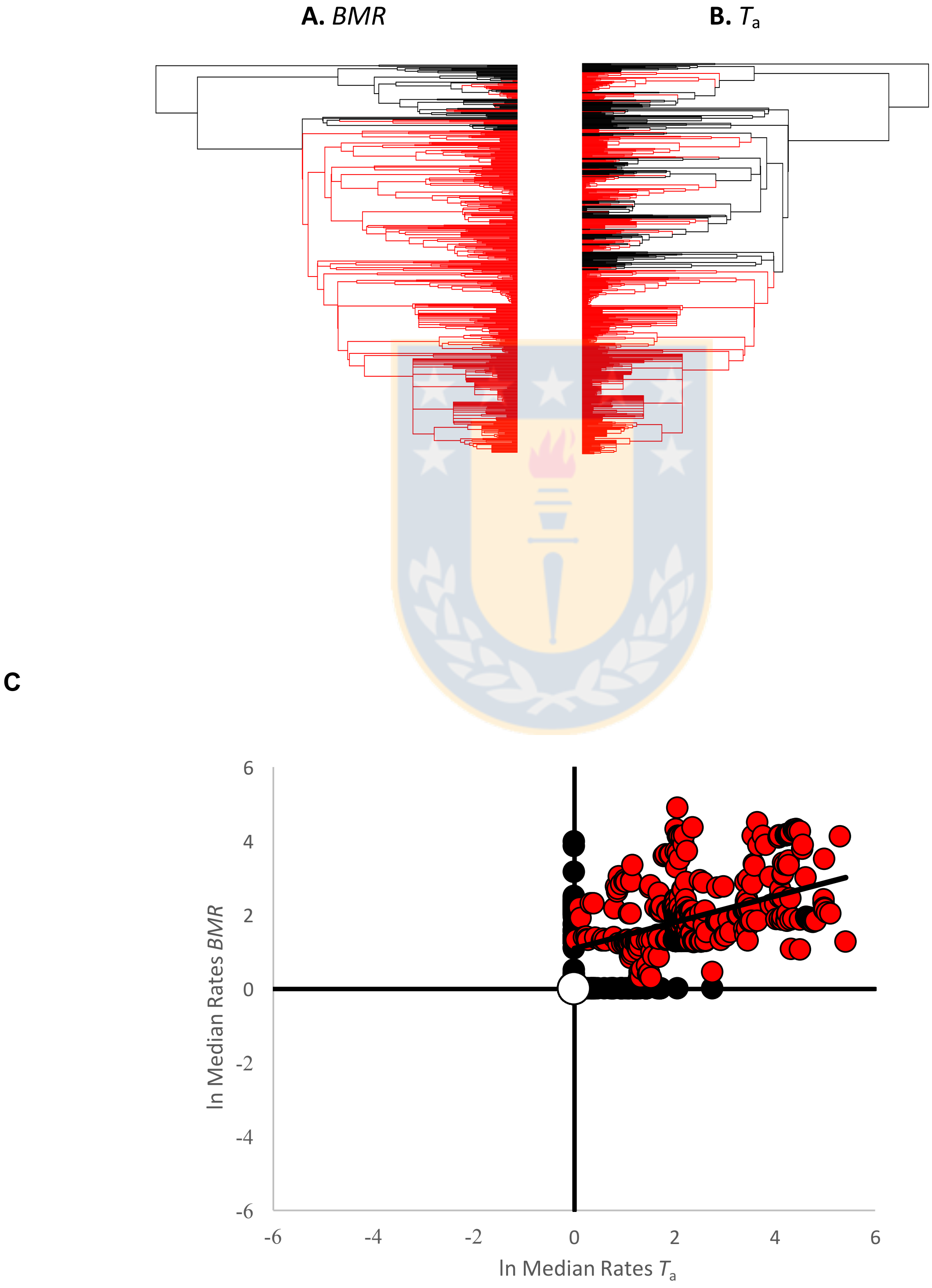
ln Median Rates BMR

Figure 3


A. BMR
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ln Median Rates T_a

Figure 4

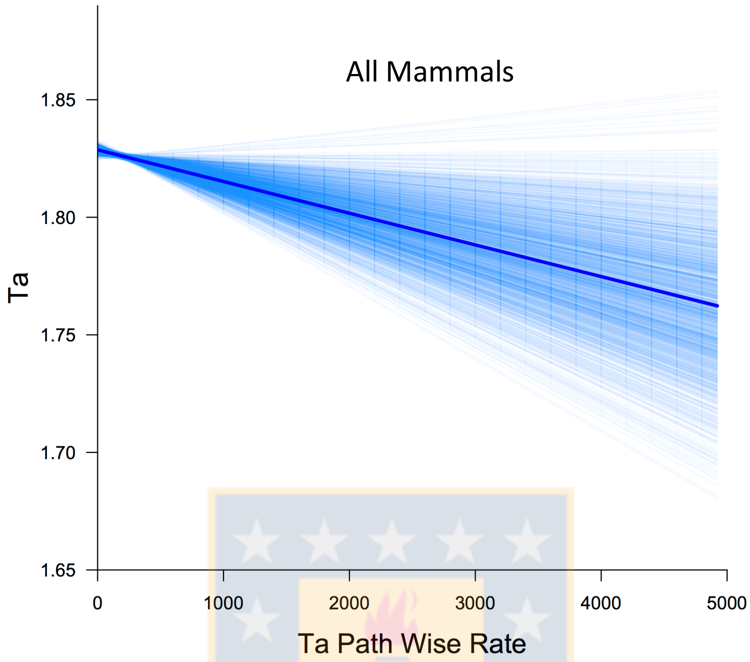
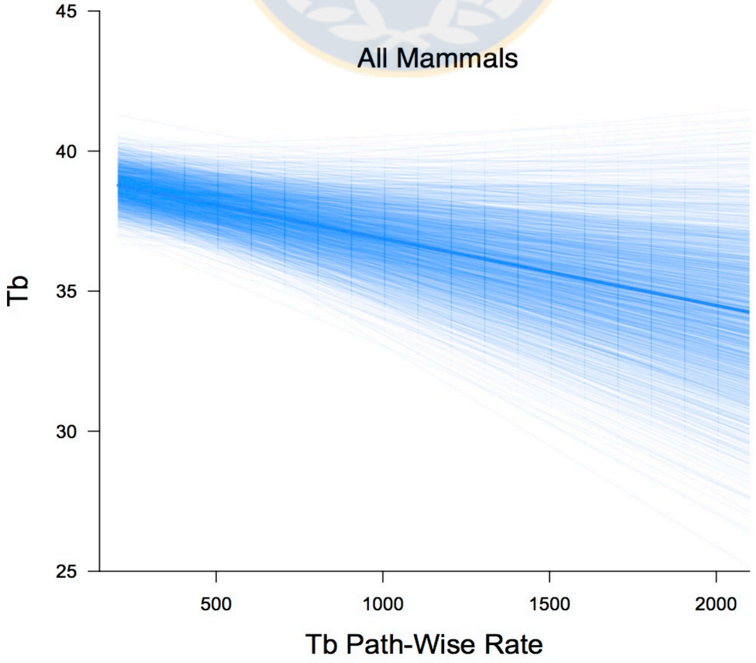


Figure 5



CHAPTER 2

Rates and trends of body size evolution reveals the link between natural selection and speciation in Sigmodontinae radiation

(Article in preparation)

Abstract

Adaptive radiation postulates that the high species numbers of some clades is the consequence of frequent episodes of speciation associate to intensified divergent selection. Recent studies, based on molecular phylogenies of extant species, seeks evidence for adaptive radiation by revealing slow-down on speciation rates and its positive association with the rate of phenotypic evolution inferred by clades. However, the speciation process by divergent selection lies within the lifespan of a species. Consequently, to get a precise picture of adaptive radiation, the rate and direction of phenotypic evolution arising from each branch of the phylogenetic relationships should be evaluated.

We evaluated these theoretical expectations using a phylogenetic method that accommodate shifts in the rate of phenotypic evolution across each branch of a time-calibrated phylogenetic tree. Then we used the estimated rates of phenotypic evolution to obtain the direction of the cumulative change from the root of tree for each species. We used this information to detect those groups evolving at random (Speciation mode by divergent selection) and with directional trends (Phyletic modes by directional selection), in order to evaluate the expected link between phenotypic evolution and speciation rates just in a random evolutionary scenario.

Our results reveal that natural selection on body size was the cause of speciation just when it favoured both extremes of size diversity (Speciation by divergent selection). In the scenario of Phyletic evolution by directional selection, its effect on speciation by splitting was rare or not typical. These results are consistent with the Adaptive Radiation predictions, indicating that to understand to what extent natural selection could be associated to the origin of species it is necessary to study the multiple scenarios in which natural selection provide to organisms with

the opportunity to adapt, rather than assuming the evolutionary process to work homogeneously across the entire group under study or sub clades.

INTRODUCTION

The hypothesis of adaptive radiation is essential to understand how some clades have reached a large number of species. Under the premise that divergent natural selection leads to speciation, adaptive radiation postulates that the striking species numbers is the consequence of frequent episodes of intensified selection operating on a trait that allow organisms to survive and reproduce in new environments (Simpson, 1953; Skelton, 1993; Schluter, 2000). Recent studies, based on molecular phylogenies of extant species, seeks positive evidence for adaptive radiation by revealing slow-down on speciation rates and the positive correlation between speciation and the rate of phenotypic evolution by clade-averaged rates (e.g. Glor, 2010; Weir & Mursleen, 2012; Piincheira-Donoso et al. 2015; Maestri et al. 2016b; Seeholzer et al. 2017). However, the speciation process by divergent selection lies within the life span of a species, from its birth until a new speciation event. Consequently, to get a precise inference about the role of adaptive radiation in the origin of rich clades, the rate and direction of phenotypic evolution arising from each branch of the phylogenetic relationships might be evaluated.

In order to study the extent of divergent natural selection as the dominant force causing phenotypic divergence and speciation, we follow the Simpson's (1944; 1953) theoretical framework. This allows the distinction between Speciation by divergent selection and Phyletic Evolution by directional selection (modes of evolution). These modes are inferred on the basis of particular rates (tempo) and evolutionary trends for a given trait. Accordingly, Speciation by natural selection arises by local split of two or more populations within a widespread ancestral species. Under this scenario, the rate of phenotypic evolution is slow to medium and the expected evolutionary trend is typically random given the adaptation of populations to divergent local conditions leads to the evolution of phenotypes that erases any trend. On the other hand, Phyletic Evolution involves the sustained, directional shift of the mean phenotype from ancestral to derived species from slow to high rates. Under this mode, selection is the dominant evolutionary force acting homogeneously over several species, and causing directional trends above the species level. Under Phyletic Evolution, speciation by population split is rare or “not typical”, because species accumulation occurs by replacement instead splitting over evolutionary time. The third mode, quantum evolution, is relevant for the rapid origin of higher taxa (e.g. families, orders) where either natural selection or genetic drift can initiate and dominate the mode.

According to Simpson’s framework, if natural selection has dominated the phenotypic historical change in a clade adaptively radiated, then we might observe accelerated rates of evolution. This can be evidenced by departures from a background constant evolutionary rates, in which phenotype evolves by random walks and there is no dominant evolutionary force (Fig. 1A). If natural selection disproportionally favoured any extreme of phenotypic variation of a clade in such a way that generates a historical trend, and from low to high rates (Fig. 1B; 1C; red and green cases), then species accumulation caused by natural selection should be rare or non significant (Fig. 1D; same cases). On the other hand, if the phenotype evolves from low to medium rates, and at random, that is without any particular trend (Fig. 1B; 1C; blue case), then natural selection on phenotype might be associated to high speciation rate by splitting as Adaptive Radiation predicts (Fig. 1D; same case).

Study model

Cricetid rodents of the subfamily Sigmodontinae provides an interesting species-rich test case for Simpson's theoretical predictions. First, they experience an explosive radiation after a single lineage invasion into South America from North America (Leite et al. 2014), to the extent to reach a taxonomic diversity comprised by ~ 400 species included in 84 genera and 10 tribes (Patton et al. 2015; Salazar-Bravo et al., 2016). This numbers are impressive when compared to the 10 species belonging to their sister Central American subfamily Tylomyinae. Second, the South American landscape went through a rapid new environments differentiation after ancestral lineages reached a widespread distribution in South America (Leite et al. 2014), therefore is likely that frequent episodes of intensified divergent selection caused most of the speciation events within this continental landscape as adaptive radiation predicts (Engel et al. 1998; Steppan et al, 2004). Third, there is no study that evaluate the adaptive radiation hypothesis taking into account the two components of the selective process: rate and direction of phenotypic evolution during the life of ancestral and present-day species. Currently, the long standing proposals of Sigmodontinae adaptive radiation (Hershkovitz, 1962; Reig, 1986) has been replaced by models where range fragmentation by physical barriers could be the main trigger of Sigmodontinae speciation events (Parada et al., 2015; Maestri et al. 2016c). However, these recent proposals are based on absence of correlation between rates of phenotypic evolution and speciation rates. Also, these studies do not determine the potential signatures of lineages evolving according to Phyletic mode because the rate of phenotypic evolution was evaluated using phylogenetic models which assumes a homogeneous process within subgroups (tribes and dietary categories; Maestri et al. 2016c). In fact, the Phyletic mode should be part of Sigmodontinae radiation, because some lineages of Sigmodontinae has evolved towards extreme body sizes and consequently away from the average size of their clade. For example, *Microakodontomys transitorious*, is such small rodent (12 g) that has been called an aberrant form that belong to Oryzomyini, the tribe with biggest species in average (143.4 g; Maestri et al. 2016a). The same if for *Kunsia tomentosus*, the rare biggest species (maximum mass 654 g) that belongs to Akodontini, a tribe with average mass of 40 g. (Patton et al. 2015; Maestri et al. 2016a). Finally, the alternative geographical model has not been properly evaluated. According to Pigot et al. (2010), there should be a negative correlation between range size of present-day species and speciation rates, given that lineages who accumulated more species by frequent range fragmentation should reach the smaller range size in the present.

In this study we evaluated these theoretical expectations using a phylogenetic method that accommodate shifts in the rate of phenotypic evolution across each branch of a time-calibrated phylogenetic tree. We used the estimated rates of phenotypic evolution to obtain the direction of the cumulative change from the root of tree for each species, and this information was used to detect those groups evolving according to the Speciation (Fig. 1; blue cases) and Phyletic (Fig. 1; red and green cases) modes.

METHODS

Sigmodontinae phylogeny, phenotypic and range size data.

We inferred the phylogenetic relationships for sigmodontines using the last concatenated sequences available in Parada et al. (2015) and replicating their analyses to get a time-calibrated tree (Supp. Information). To obtain a proxy of Sigmodontinae interaction between phenotype and environment, we compiled a database containing the maximum body sizes (head-body length) for species with molecular data (Supplementary Table S1; 235 species among 259 used for phylogeny). We selected maximum body size because the following four reasons: (1) it is less temporally variable than other metrics such as body mass; (2) it is robustly related to overall body size distribution, and hence to mean and median body size (Trites and Pauly 1998); (3) because there are many morphological and life history traits that allometrically scale with body size, especially those traits related with energy use which are extremely important for small endotherms as Muroidea rodents (Stanley, 1973; Schmidt-Nielsen, 1984; McNab, 2002); and, most importantly (4) because body size has the highest variability among other traits available (e.g. skull morphology) which is the fundamental premise for natural selection be able to create phenotypic change.

To obtain a proxy of range size of sigmodontines, we compiled a database containing the distribution area for species with available molecular data (Supplementary Table S1; 235 species among 259 used for phylogeny). Geographic area was estimated using ESRI shapefiles polygons available from the IUCN (2015). To obtain a surface metric in Km^2, we projected the data to Behrmann spatial reference system using ArcGis 10.2 (ESRI, 2014). Finally, body and range size were log-transformed for subsequent analyses.

Rates of phenotypic evolution, the intensity of natural selection, and evolutionary trends.

In order to obtain rates of body size evolution considering their potential heterogeneity caused by natural selection across individual branches or monophyletic sub clades, we used the *Variable Rates* model (Venditti et al. 2011). This model is designed to automatically detects instances of phenotypic rates shifts in different regions of a time-calibrated phylogenetic tree. The rate of phenotypic change that occurs in a branch is divided in two components: a rate parameter which assume changes are draw from an underlying Brownian process (σ^2_b; which is the background rate) – and a second set of parameters that identify branch-specific rate shifts by adjusting the lengths of a branch in the time-calibrated tree (stretch or compress a branch is similar to increase or decrease the phenotypic rate of change relative to the underlying Brownian rates). Rate shifts are detected by a set of branch-specific scalars r ($0 < r < \infty$) which transform each branch in order to optimize the phenotypic rate of change to a Brownian process ($\sigma^2_v = \sigma^2_b\ r$). If phenotypic change occurred at accelerated rates in a specific branch of the tree, then $r > 1$ and the branch is stretched. Decelerated rates of evolution are detected by $0 < r < 1$ and the branch is compressed. Constant rates of evolution ($r = 1$), therefore, does not modify branch lengths and the phenotypic variation is predicted by the time-calibrated tree.

To detect the intensities of natural selection acting on body size evolution, we quantified how much rate variation in each single branch of the tree can be attributed to exceptional instances of positive selection (Baker et al. 2016). We detected those instances according to the magnitude in which rate variation departs from the background rate, specifically, when the optimized rate is more than two fold the expected background rate (i.e. $r > 2$). We also based our inferences according to certainty about the estimated instances of positive selection in a particular branch, *i.e.* when those instances were present in more that 95% of the posterior sample of trees with scaled branch lengths (Baker et al. 2016). We inferred the magnitude and location of rate scalar (*r*) in a Bayesian Markov chain Monte Carlo (MCMC) reversible-jump framework, generating posterior distribution of trees with scaled branches lengths. MCMC ran for 25 million iterations, sampling every 12500 iterations to ensure independence in model parameters. Analysis was conducted with BayesTraits v3.0 (Pagel et al. 2004), and model's parameter inspection was done online via http://www.evolution.reading.ac.uk/VarRatesWebPP/.

To get the cumulated change in body size evolution for each Sigmodontinae species, we sum all of the rate-scaled branches along the evolutionary path of each species (leading from the root to the tips) in the Bayesian consensus tree with the mean scaled branch lengths. These summed branches equate to *path-wise rates*, a measure of the total changes in rate a species has experienced during the course of its evolution (Baker et al. 2015). Therefore, if natural selection disproportionally favoured any extreme of phenotypic variation of various species in such a way to generate a historical trend, and from low to high rates (i.e. phyletic evolution), we expect to find that longer *path-wise rates* are linked to higher or lower sizes across several species (Baker et al. 2016). We evaluated this expectation regressing body size as a response of *path-wise rates* using the MCMC Generalized Linear Mixed Model approach (GLMMs), which is implemented in the MCMCglmm R package (Hadfield, 2010). We assume a Gaussian distribution for the response variable (i.e. body size) and phylogenetic relationships were included as the random effect, in the form of an inverse phylogenetic variance-covariance matrix to account for shared ancestry (Hadfield, 2010). This was our *All Species Model*, which evaluates the directional trend of body size evolution for all Sigmodontinae. Then, we allowed for different intercepts and slopes given tribes to evaluate the potential evolutionary trends in those groups (Avaria-Llautureo et al. 2012). This was our *Tribe Model*. We considered different slopes for the *Tribe Model* when sample size was higher than ten - remaining tribes with less than ten data points were considered together as reference group in the model. Finally, we also allowed for different intercepts and slopes given the two types of molar morphology (the number of buco-lingual enamel folds), that is, Tetralophodont (generally hypsodont) and Pentalophondont (generally brachydont). This was our *Molar Model*. This is because those species, specially Tetralophodont, should follow specific evolutionary trends given that evidence indicates that hypsodonty might assist the survival of large mammals over time (Simpson, 1944; 1953). We also evaluated the interaction between the *Tribe and Molar Models*, and this was our *Tribe-Molar Model.*

Model selection was based on deviance information criterion (DIC) and inspection of model parameters significance. We selected the best fit model given the lowest DIC score and a difference compared with a base model that is greater than 4 (Sakamoto et al. 2016). When DIC difference was less than four, we selected the best fitted model given the significant parameters (non-significant parameters of the regression were removed). We considered statistical significance when the estimated value of the parameter crossed zero less than 5%, or when "pMCMC" values calculated in MCMCglmm were less than 0.05.

The effect of phenotypic evolution and geographic range size on net speciation rates.

To get an estimation of speciation rates for each Sigmodontinae lineage and include it as the response variable in our speciation model, we used the number of nodes (node count) for each species leading from the root to the tip (excluding the root) in the time-calibrated tree. This represent the mean net-speciation rate for each species (speciation minus extinction) when total evolutionary time (path length) for each species is the same (Freckleton et al. 2008).

We used *path-wise rates* of body size evolution and range size as the main covariates to evaluate the effect of phenotypic evolution and range dynamics as triggers of speciation in Sigmodontinae as a whole. We modelled node count with MCMCglmm approach (Hadfield, 2010). The node count for each species was treated as a variable with Poisson distribution and phylogenetic relationships were included as the random effect, in the form of an inverse phylogenetic variance-covariance matrix to account for shared ancestry (Hadfield, 2010). Following the early proposals of Schenk et al. (2013) and the previous studies for the group (Parada et al. 2015) we also evaluated the slowdown in speciation rates by adding a quadratic term for *path wise rates* of body size evolution and range size. MCMC ran for 25 million iterations, sampling every 12500 iterations to ensure independence in model parameters.

Finally, to evaluate the speciation mode according to Simpson's theoretic framework and, therefore, the adaptive radiation hypothesis, we included in our speciation model those groups who evolved at random and with directional trends (i.e. tribes and/or molar morphology groups). If just lineages who evolved at random from low to medium rates have a significant effect on speciation rates, then we can identify this as the group of species originated by an adaptive radiation.

RESULTS

Rates of body size evolution, the intensity of natural selection, and evolutionary trends.

The model that allowed the rate of body size evolution to vary across each branch of the phylogeny, improved the marginal likelihood in 67.9 log-units when compared to the constant single-rate model, and got strong support by a Bayes Factor > 10. The estimated *r* values in the posterior distribution were greater than one for all individual branches in the phylogeny (*r* > 1), demonstrating that the rate of body size evolution was accelerated during the ~9 million years of Sigmodontinae history. Furthermore, six branches were identified with *r* > 2 in more than 95% of the posterior distribution or phylogenetic trees with scaled branch lengths, which indicates those accelerated rates of evolution were caused by exceptional instances of positive selection (Baker et al. 2016). Those branches correspond to (1) the single branch leading to the common ancestor of *Akodon mollis*, *A. orophilus* and *A. torques*, (2) the single branch leading to the common ancestor of *Calomys callidus and C. callosus*; and the terminal branches corresponding to (3) *Akodon kofordi*, (4) *Calomys callidus*, (5) *Calomys callosus,* and (6) *Mickoakodontomys transitorious*, all species who have evolved towards extreme body sizes within their clades.

On the other hand, we do not find evidence for Phyletic evolution across all species, because the relationship between body size and *path-wise rates* in the *All Species Model* was not significant. However, the *Tribe Model* showed that body size and *path-wise rates* were positively associated in Akodontini and negatively in Oryzomyini tribe, which support Phyletic evolution toward larger and smaller size in these two tribes (Table 1). The *Molar Model*, which categorize all species according to tetralophodont and pentalophodont molar morphology, also show evidence for Phyletic evolution toward larger and smaller size (Table 1).

Finally, the *Tribe-Molar Model*, the model allowing for the interaction between tribe and molar morphology, showed that only akodontines-tetralophodont, and oryzomyines-pentalphodont species evolved toward toward larger and smaller size (Fig. 3B; Table 1). The remaining species - who evolved from low to medium rates, belong to more than one tribe, and have both types of molar morphology - did not show any particular trend, implying random body size evolution in those species (Fig. 3B blue colour).

The effect of body size evolution and geographic range size on net speciation rates.

Our speciation model which includes body size *path-wise rates* and range size as main covariates, indicates that just body size *path-wise rates* had a significant effect (Table 2). This result suggests that the origin of new species during Sigmodontinae radiation was associated to the accelerated evolution of body size, and that range dynamic did not have a significant effect on speciation. However, the best fitted model included a negative quadratic term that predicts a curvilinear relationship between speciation rates and body size *path-wise rates* (Table 2, Fig. 3A). According to this quadratic model, lineages that evolves from low to medium rates had a positive effect on speciation rates but lineages that evolves at highest rates had a negative effect. This is also consistent with the common slowdown diversification pattern.

When we include the *Tribe-Molar Model* covariates into our speciation model, we find a positive association between node count and *path-wise rates* in those 44% of species in which body size evolved at random from low to medium rates (Fig. 3C blue colour, Table 3). The remain 56% of species who showed Phyletic size increase and decrease did not have a significant effect on node count (Fig. 3C, red and green colour; Table 3). Furthermore, the intercepts of the Phyletic groups were significant and higher than the group containing the remaining species (Table 3), suggesting that, despite the speciation events of the Phyletic-evolving groups were not caused by natural selection on body size, other evolutionary forces caused their high and constant rates of speciation.

DISCUSSION

Recent studies hint that adaptive radiation play a minor role in the origin of rich clades with widespread Neotropical distributions like Sigmodontinae rodents (Maestri et al. 2016c). This conclusion is based on the lack of evidence for the correlation between speciation rates and rates of phenotypic evolution evaluated by clade-averaged rates. However, accelerated speciation by divergent selection, the mechanistic basis of Adaptive Radiation (Simpson, 1953; Schluter, 2000), lies within the life span of species. In a phylogeny, ancestral and present-day species are represented by internal and terminal branches, and we demonstrated that the study of evolutionary rates at this scale revealed the portion of Sigmodontinae species originated by an Adaptive Radiation.

The evaluation of body size evolutionary rates, across each branch of the Sigmodontinae phylogeny, allowed the identification of three species groups according to their particular evolutionary histories. Two of them are species whose body size showed Phyletic size increase and decrease, and the third one is conformed by species whose body size evolved at random (Fig. 2; 3B). This distinction has important implication for the study of adaptive radiation, because it predicts species accumulation by natural selection just when the process is accelerated and disruptive (Simpson, 1944, Schluter, 2000). In this line, we captured the proportion of species originated by an adaptive radiation, corresponding to the 44% of species whose body size evolved at both accelerated rates and random, representing members of the tribe Abrotrichini, Phyllotini, Sigmodontini, Thomasomyini, and Oryzomyini (with tetralophodont molars only) (Fig. 2; ·3B). These results also imply that the subsidiary role attributed to natural selection as the cause of Sigmodontinae speciation events, could be due to not consider the valuable information about rates and direction of the evolutionary process present in each branch of the phylogeny. This is because, the rate of phenotypic evolution was averaged by groups given clades, dietary categories, or other discrete traits relevant for Sigmodontinae evolution in previous studies (Maestri et al. 2016c). Consequently, our results support the long-standing assumed idea about the Sigmodontinae Adaptive Radiation (Hershkovitz, 1962; Reig, 1986; Engel et al. 1998; Steppan et al, 2004), by giving the first empirical evidence that evaluated the evolutionary causal mechanisms.

The remaining 56% of species, defined as the non-adaptive Sigmodontinae radiation, are composed by two Phyletic size increase and decrease groups, represented by akodontines species with tetralophodont molar morphology, and oryzomines species with pentalophodnt molars. This means that the 56% of species included in those two groups evolved by directional selection on body size from their common ancestors, and during the ~ 6 million years of their evolution in South America (Leite et al. 2014; Parada et al. 2015). The selective processes underlying these two contrasting trends at accelerated rates are not straightforward to infer but some insights can be inferred from the integrations of three facts: (1) both tribes inhabit contrasting environments (Patton et al. 2015; Maestri et al. 2016b), (2) they had different centres of origin and routes of historical dispersal (Patton et al. 2015; Maestri et al. 2016b), and (3) their molar morphology have markedly different functions (Simpson, 1944; Hershkovitz, 1962; Patton et al. 2015). First, it is likely that akodontines, the Phyletic size increase group, have had originated in south-eastern South America, because the oldest fossil record is found there (Patton et al. 2015). From this follows that most ancestral species disperse towards the north and west in South America in order to determine its current spatial distribution (Maestri et al. 2016b). The akodontine historical dispersal could be associated to the emergence and expansion of open grass-dominated habitats (Werneck, 2011; Patton et al. 2015; Maestri et al. 2016b), because akodontines has tetralophodont molars, which is an adaptation to generally feed on the abundant abrasive vegetation in grassland ecosystems (Simpson, 1944; Patton et al. 2015). We propose that the historical dispersal and the higher energetic impute associated to tetralophodont molars in grass ecosystem, favoured disproportionally bigger sizes throughout the time and, therefore, can explain the Phyletic size increase supported by the interaction between Akodontine tribe and tetralophodont molars in our *Tribe-Molar Model*.

On the other hand, oryomyines, the Phyletic size decrease group, originated in the northern South America (Valencia-Pacheco et al. 2011; Patton et al. 2015). This imply that the ancestral lineages were well adapted to those high-energy environments in Amazonia, where the biggest species of the tribe lives today. From this follows that most historical dispersal events were towards low-energy environments from the Amazonia, principally to both high altitudes in Andes mountains and high latitudes in South America (Valencia-Pacheco et al. 2011), where the smaller species lives today (Maestri et al. 2016a). These facts make likely the idea that the Phyletic size decrease in oryzomines was caused by the historical dispersal towards low-energy environments, in which organisms with the ability to survive with less energy could have been disproportionally favoured. Considering that body size correlates positively with energetic expenditure (McNab, 2002), it is conclusive that smaller organism were actually selected in those poor energy environments. Under this scenario, pentalophodont molars could increase the survival probabilities of smaller species when some type of food was scarce. This is because pentalophodont molars allows sigmodontines to obtain energy from multiple types of food e.g. animals, seeds, fruits, or fungus (Hershkovitz, 1962; Patton et al. 2015). Therefore, the historical dispersal of Oryzomyini and the benefits associated to pentalophodont molars in low-energy environments, can explain the Phyletic size decrease supported by the interaction between Oryzomyini tribe and pentalophodont molars in our *Tribe-Molar Model*.

In these two Phyletic-evolving groups, rates of body size evolution were not associated to net-speciation rates (Fig. 3C), implying that their species were not originated by an Adaptive Radiation. However, those groups had the highest rates of speciation because our speciation model estimated the highest and significant intercepts for them (2.23 and 2.35 respectively, Table 3). This suggest that, despite their speciation events were independent of body size evolution by natural selection, they generate new species at constant rates and by other non-selective processes. A plausible hypothesis for this phenomena can be deduced by the fact that constant rates of speciation emerge in groups that generates new species by frequent stochastic events (Venditti et al. 2010). These - also called accidental events - can be polyploidy, hybridization, chromosome rearrangement and physical factors associated to range fragmentation by vicariance. In the case of the two Phyletic-evolving groups, we did no find evidence for the expected association between range size and speciation as predicted under a scenario of range fragmentation and speciation (Pigot et al. 2010). However, several genera of these groups have species limits defined by diploid number, whereas other genera have species with variable chromosome complement (*e.g.* Agrellos et al., 2012; Fagundes et al. 2000; Patton et al. 2015). Therefore, chromosomal evolution might bring valuable information about the causes of past speciation events in those two Phyletic-evolving groups.

We acknowledge that our findings are limited to the radiation of Sigmodontinae. However, we think these findings might help to find the role of accelerated divergent selection in other radiations. This is because we detected the portion of species originated by an adaptive radiation just by considering the lineages who evolved with different directions at variable rates, and evolutionary trends and variation in the rate of phenotypic evolution are common and widespread in the tree of life.

Our results reveal that natural selection has been the creative force of body size evolution in Sigmodontinae, being also the cause of speciation when it works favouring both extreme of size diversity (i.e. divergent selection). In the cases that natural selection favours disproportionally one extreme of the size variation among several species (i.e. Phyletic evolution) its effect on speciation by splitting was rare or not typical. These results are consistent with the evolutionary theory predictions, indicating that to understand to what extent natural selection could be associated to the origin of species it is necessary to study the multiple scenarios in which natural selection provide to organisms with the opportunity to adapt, rather than assuming the evolutionary process to work homogeneously across the entire group under study or sub clades.

Table 1. Body size predicted by *path wise rates* for all Sigmodontinae species, and allowing for different intercept and slopes given tribes, molar morphology and the interaction between them. Source: Own elaboration.

MCMCglmm Regression Model	Posterior mean	Lower 95% CI	Upper 95% CI	Effective sample	% Crossed Zero (x 2)	DIC
All Species Model						-845.4
Intercept	4.95	4.951	4.96	1999	0*	
Slope	0.0002	7.80E-05	0.0004	1999	93.6	
Tribe Model						-838.1
Intercept reference group	5.47	5.455	5.49	1724	0*	
Intercept Akodontini	4.59	4.586	4.6	1999	4.5*	
Intercept Oryzomyini	4.59	4.59	4.6	1660	68	
Intercept Phyllotini	4.95	4.937	4.98	1652	5.2	
Intercept Thomasomyini	5.27	5.267	5.28	1999	42	
Slope reference group	-0.03	-0.039	-0.036	1844	21.2	
Slope Akodontini	0.013	0.013	0.014	1851	1.2*	
Slope Oryzomyini	-0.016	-0.017	-0.016	1846	1*	
Slope Phyllotini	0.009	0.008	0.009	1699	26.7	
Slope Thomasomyini	-0.0081	-0.009	-0.006	1999	79.3	
Molar Model						-840.08
Intercept Tetralophodont (ref. Group)	4.83	4.82	4.837	1874	0*	
Intercept Pentalophodon	5.188	5.18	5.19	1999	1*	
Slope Tetralophodont	0.0099	0.0097	0.01	1999	4.3*	
Slope Pentalophodont	-0.0161	-0.0164	-0.015	1999	0.8*	
*Tribe*Molar Model*						-840.4
Intercept Remainder (ref. Group)	4.92	4.91	4.93	1999	0*	
Intercept Akodontini*Tetralophodont	4.658	4.65	4.66	1999	16.2	
Intercept Oryzomyini*Pentalophodont	5.22	5.21	5.23	1999	10	
Slope Remainder	0.0032	0.0029	0.0036	1999	67.6	
Slope Akodontini*Tetralophodont	0.013	0.013	0.014	1999	1.4*	
Slope Oryzomyini*Pentalophodont	-0.0167	-0.017	-0.0165	1999	0.4*	

Table 2. Net-speciation rates predicted by body size path-wise rates and range size for all Sigmodontinae species. Source: Own elaboration.

	Posterior mean	Lowe 95% CI	Upper 95% CI	Effective sample	pMCMC
Intercept	1.79	0.78	2.58	676	<0.001**
Range Size	0.00092	-0.14	0.16	660.1	0.978
(Range Size)^2	0.00018	-0.0068	0.0068	545.8	0.927
Body Size Path Wise rate	0.033	0.016	0.051	900	<0.001**
(Body Size Path Wise Rate)^2	-0.00038	-0.00063	-0.00017	900	<0.001**

Table 3. Net-speciation rates predicted by body size *path wise rates* and their interaction with akodontines-tetralophodont and orymines-pentalophodont. Source: Own elaboration.

MCMCglmm Regression Model	Posterior mean	Lower 95% CI	Upper 95% CI	Effective sample	% Crossed Zero (x 2)	DIC
Speciation model including the Tribe Molar Model covariates*						1098.8
Intercept Remainder (ref. Group)	1.79	1.79	1.803	1872	0*	
Intercept Akodontini*Tetralophodont	2.23	2.23	2.244	1999	2.6*	
Intercept Oryzomyini*Pentalophodont	2.35	2.34	2.36	1999	0*	
Slope Remainder	0.01773	0.01774	0.018	1850	0.4*	
Slope Akodontini*Tetralophodont	0.000075	0.00054	0.00097	1999	87.6	
Slope Oryzomyini*Pentalophodont	-0.0037	-0.0039	-0.0035	1999	42.6	

FIGURE LEGENDS

Figure 1. Conceptual figure outlining the expected results of the Adaptive Radiation hypothesis. **(A)** Phylogeny of all species in the group under study in which branch lengths are in unit of absolute time and therefore representing the expected phenotypic change given a Brownian Motion process. **(B)** Phylogeny with scaled branch lengths by branch-specific rates ($\sigma^2_{\mathrm{v}i}$), as expected under a scenario in which present-day phenotype of species rises by accelerated rates of phenotypic evolution relative to a Brownian motion process (σ^2_{b}). **(C)** Relationship between present-day phenotype and their cumulative change from the root. A positive (red line) or negative (green line) relationship indicates phyletic evolution. Consequently, random evolution is indicated by no relationship between present-day phenotype and their cumulative change from the root (blue line). **(D)** Relationship between speciation rates (node count for each species from the root) and the cumulative change of phenotype. Species in which phenotype evolved at random and accelerated rates (blue dotted line) have a positive effect on speciation (i.e. species origin by an adaptive radiation process), while species evolving phyletic trends (green and red dotted lines) does not have any effect on speciation. Source: Own elaboration.

Figure 2. Sigmodontinae phylogeny with scaled branch lengths according to the rates of body size evolution. All branches were stretched ($r > 1$) and hence all instances of rates variation in body size evolution were associated to accelerations. Red branches represent the Akodontine tribe with tetralophodont molars who evolved towards big sizes. Green branches represent the Oryzomyini tribe with pentalophodont molars who evolved towards small sizes. Blue branches are remaining species with both molars whose body size evolved at random, and represent members of the tribe Abrotrichini, Phyllotini, Sigmodontini, Thomasomyini, and Oryzomyini (with tetralophodont molars only). Source: Own elaboration.

Figure 3. (**A**) Net-speciation rates predicted by a quadratic function of body size evolutionary rates without considering the potential Phyletic-evolving species. **(B)** Faster rates of body size evolution lead to larger sizes in akodontines-tetralophodont (red lines; Phyletic size increase) and smaller sizes in oryzomines-pentalophodont species (green lines; Phyletic size decrease). Remain species evolved at random (blue lines). **(C)** Just species who evolved at random had a positive effect on net-speciation rates. Dark and light lines represent the mean and posterior distribution of the regression slopes obtained by Bayesian inference. Source: Own elaboration.

Figure 1

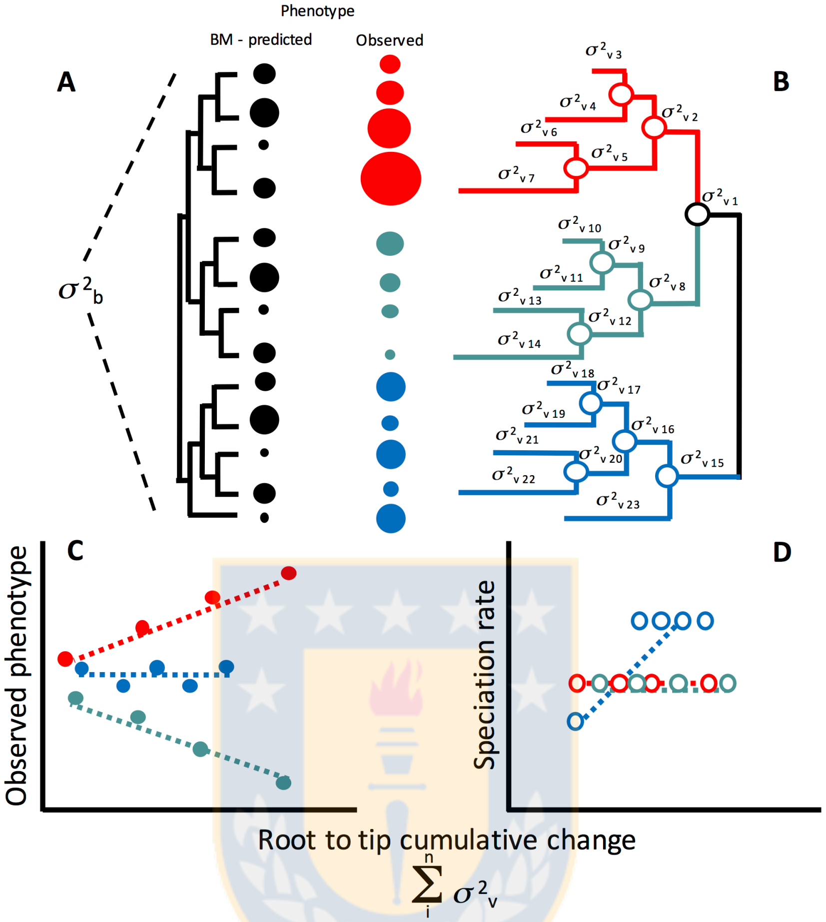
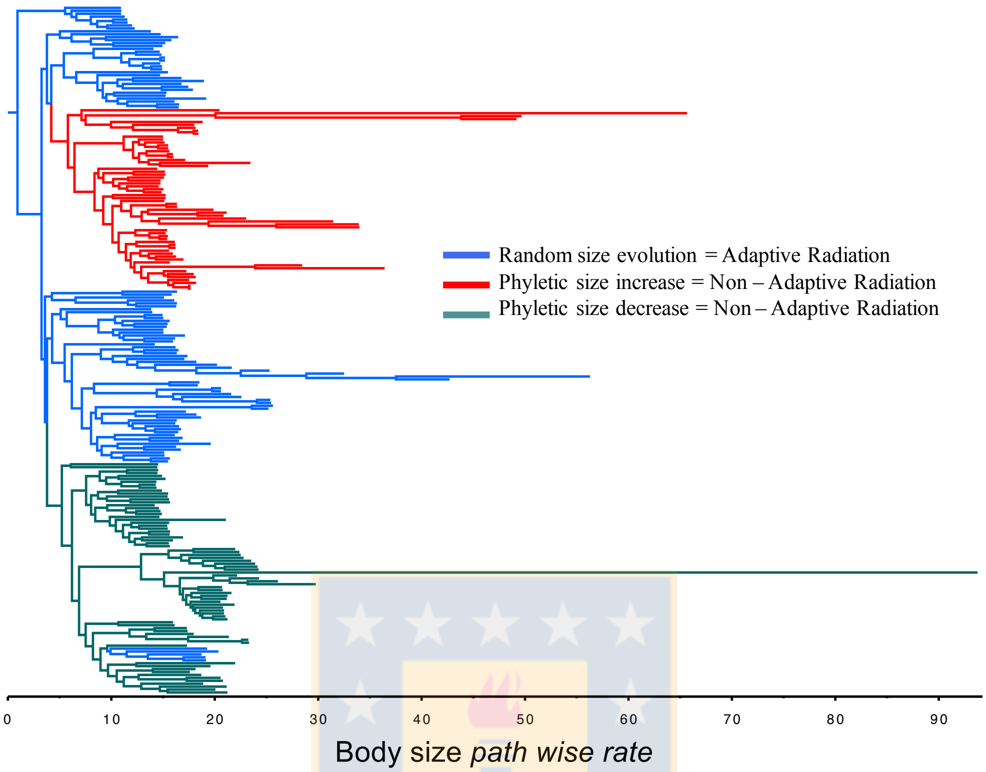
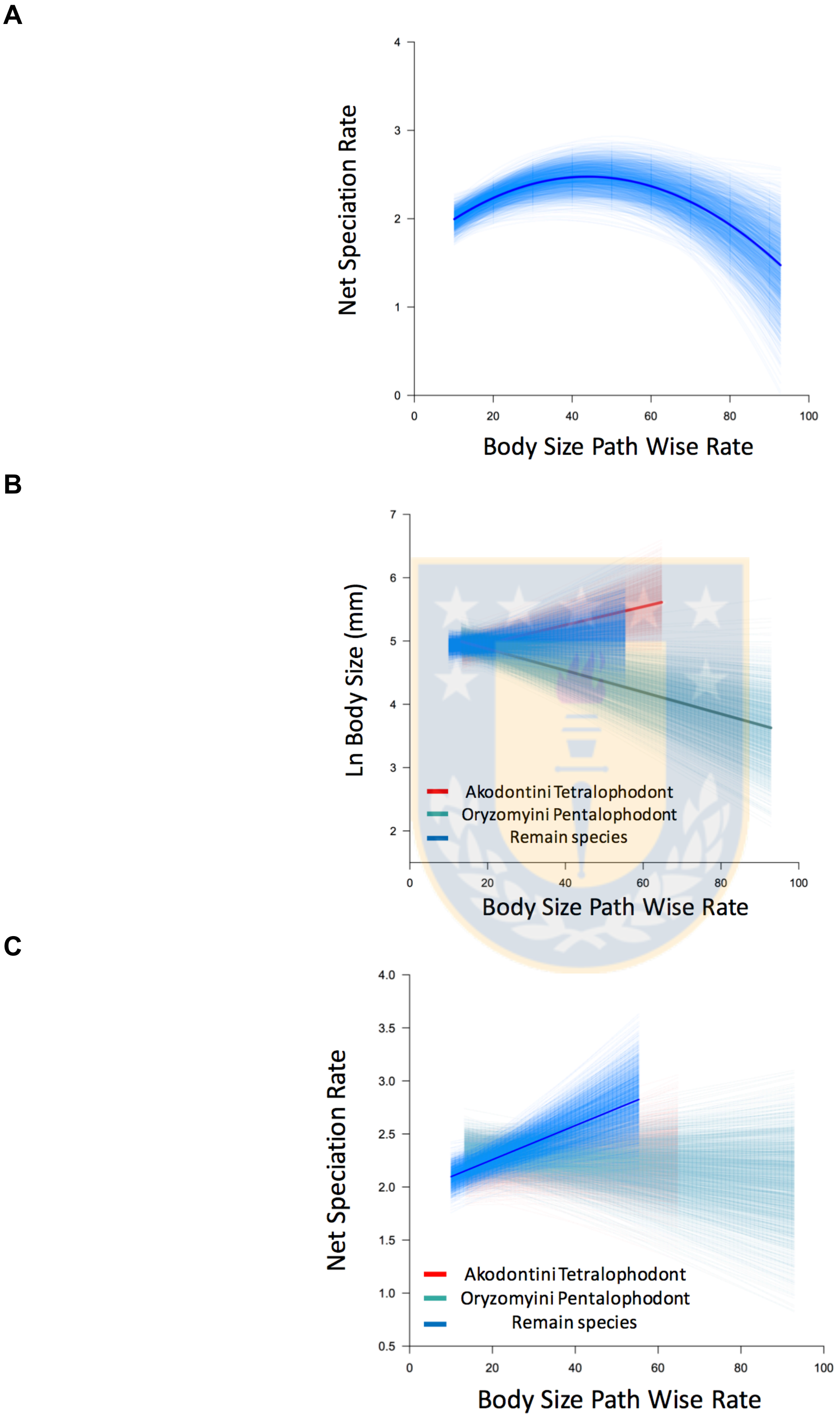

Phenotype
BM - predicted
Observed
A
B
σ^2_b
$\sigma^2_{v\,3}$
$\sigma^2_{v\,4}$
$\sigma^2_{v\,2}$
$\sigma^2_{v\,6}$
$\sigma^2_{v\,5}$
$\sigma^2_{v\,7}$
$\sigma^2_{v\,1}$
$\sigma^2_{v\,10}$
$\sigma^2_{v\,9}$
$\sigma^2_{v\,11}$
$\sigma^2_{v\,8}$
$\sigma^2_{v\,13}$
$\sigma^2_{v\,12}$
$\sigma^2_{v\,14}$
$\sigma^2_{v\,18}$
$\sigma^2_{v\,17}$
$\sigma^2_{v\,19}$
$\sigma^2_{v\,16}$
$\sigma^2_{v\,21}$
$\sigma^2_{v\,20}$
$\sigma^2_{v\,15}$
$\sigma^2_{v\,22}$
$\sigma^2_{v\,23}$
C
D
Observed phenotype
Speciation rate
Root to tip cumulative change
$\sum_{i}^{n} \sigma^2_v$

Figure 2



Random size evolution = Adaptive Radiation
Phyletic size increase = Non – Adaptive Radiation
Phyletic size decrease = Non – Adaptive Radiation
0
10
20
30
40
50
60
70
80
90
Body size *path wise rate*

Figure 3


A
Net Speciation Rate
0
1
2
3
4
0
20
40
60
80
100
Body Size Path Wise Rate
B
Ln Body Size (mm)
2
3
4
5
6
7
0
20
40
60
80
100
Body Size Path Wise Rate
Akodontini Tetralophodont
Oryzomyini Pentalophodont
Remain species
C
Net Speciation Rate
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
20
40
60
80
100
Body Size Path Wise Rate
Akodontini Tetralophodont
Oryzomyini Pentalophodont
Remain species

GENERAL DISCUSSION AND CONCLUSION

The history of life has been shaped by frequent instances of phenotypic evolutionary burst, which demonstrates that nature has demanded organisms for rapid evolution in order to survive and diversify across different environments (Eastman et al. 2011; Kratsch and McHardy, 2014; Landis et al. 2013; Rabosky 2014; Revell et al. 2012; Thomas and Freckleton 2012; Benson et al. 2014; Puttick et al. 2014; Venditti et al. 2011; Chira & Thomas 2016; Baker et al. 2016). The idiosyncrasy of this phenomenon lies within the life span of a species, as one species succeeds the previous one in the struggle for the existence, from its birth to the death or a new speciation event. Consequently, any study that seeks to understand current biodiversity patterns by studying the historical process of phenotypic evolution, should evaluate to what extent the rate of phenotypic evolution varies in both ancestral and extant species, and therefore, in each branch of the phylogenetic relationships. Based on this premise, this thesis brings new empirical information that support the importance of variations in the rate of evolution to make appropriate evolutionary predictions expected under the MTE (Brown et al. 2004) (Chapter 1). Furthermore, this thesis provides a new theoretic-based phylogenetic approach to detect the portion of species originated by accelerated disruptive selection (Chapter 2).

As an efficient universal theory (Marquet et al. 2014), the MTE propose that *BMR* of organisms is the key to explain all biodiversity patterns. The building block of this theory is that mass-corrected *BMR* of organisms depend directly on the temperature of the system (e.g. T_b in endotherms) given the universal kinetic effect of temperature on biological rates (Brown et al. 2004). Furthermore, their dependence should also be caused by functional mechanisms in endotherms, because *BMR* is the heat source to maintain the T_b constant (Lovegrove, 2016). Given their association by kinetic mechanisms, and their constraint to keep the functional organism's integration, is is expected that their evolution occurs only by small steps (low rates), and that the magnitude of changes (rate) should be the same in both traits. However, in this thesis I do not find evidence for neither low rates of evolution for *BMR* and T_b, nor a positive correlation between rates during mammalian radiation. Rather than, the analyses support that *BMR* and T_b were decoupled, showing contrasting rates of evolution across each branch of the phylogeny during the 160 million years of mammalian evolution. The observed accelerated evolution of *BMR* caused by the environmental temperature of organisms, was the pivotal process that explain this decoupled scenario. This demonstrates that neither the kinetic effect of temperature, nor the functional mechanisms, constrained the evolution of the *BMR*. Therefore, if the first principle of the MTE is a positive correlation between *BMR* and T_b, can we understand macroevolution under the light of this theory? Yes, but only if the evolution of both traits is gradual and coupled throughout the time, but it is not the case. Under the alternative decoupled scenario, as the case of Mammalian radiation, the current formulation of the MTE does not have the predictive power to explain the phenomena observed at this Macroevolutionary scale, and should consider the variation in the rate of *BMR* evolution as the pivotal evolutionary principle to explain the evolutionary phenomena in endotherms.

On the other hand, the second Chapter, based on the Simpson's theoretic framework, predicted that natural selection can initiate speciation in a population if selection on a particular phenotype (affecting survival and reproduction) is intense and disruptive. Therefore, in order to evaluate to what extent natural selection caused the origin of species-rich taxa, it is necessary to consider both the rate and direction of the phenotypic change that promote new diversity. When these expectations were evaluated in order to explain the explosive radiation of Sigmodontinae, a long-standing problem in the macroevolution of South American mammals, I find that the only 44% of species whose body size evolved at accelerated rates and at random (i.e. without a particular trend), were originated by divergent selection operating on body size. Furthermore, and in coherence with the view of speciation as a multiple-causal process, the remainder 56%

of species, whose body size evolved at accelerated rates with a general tendency (i.e. phyletic evolution *sensu* Simpson, 1944), were likely originated by the operation of alternative causal mechanisms to natural selection. Therefore, I propose that to understand the scenario in which natural selection could be associated to the origin of species, it is necessary to study the multiple scenarios (e.g. directional, disruptive) and intensities (rate) in which natural selection operates, rather than assuming that a particular evolutionary process works homogeneously across a clade.

The results of both Chapter are consistent with the theoretic generalities inferred from Simpson's (1944) ideas based on the fossil record. This also support the long standing proposal that the rate of evolution gives clues about the intensity of evolutionary forces, and furthermore, which one can dominate the process (Darwin, 1859; Simpson, 1944; 1953; Eldredge and Gould, 1972). Therefore, besides to encourages a more rigorous abstraction of theoretic predictions, this thesis suggests to pay attention in evolutionary model assumptions. This is because model assumption made clear statement about how evolution proceeds and, unfortunately, most assumption in the abundant methods available has not been inspired on basic principles (Cooper et al. 2016). Evolution is a complex process, so if we deal with it under robust theories and complemented with appropriate phylogenetic statistical methods – that can get the fundamental emergent characteristic of the evolutionary processes - then it will be more likely the achievement of precise or unbiased answers about the origin of biodiversity.

SUPPLEMENTARY INFORMATION

Analytic details for rates of *BMR*, body and ambient temperature (Chapter 1).

We used the mammalian time-calibrated tree (Fritz et al. 2009) and data for *BMR*, T_b, Body mass, from Clarke et al. (2010). Data for Ambient Temperature (T_a) was obtained from Cooper et al. (2011). This data was analysed with BayesTraits v3.0 software (http://www.evolution.rdg.ac.uk/SoftwareMain.html), with the *Variable rates regression model*. We used this model because *BMR* is predicted principally by body mass and T_b of species, while T_b is predicted principally by body mass and *BMR*. Therefore, under this situation it is desirable to infer rates of *BMR* and T_b evolution after accounting for main covariates. 25.000.0000 million iterations were ran using Bayesian inference, sampling every 25000 to ensure independence in model parameters.

We estimated the consensus tree for *BMR* and T_b from the posterior sample of trees with scaled branch length. Then we got the the length of each branch (rate of evolution) from the consensus tree in the software BayesTrees v 1.3. (http://www.evolution.rdg.ac.uk/SoftwareMain.html). These branch lengths were divided by the branch lengths in time units (in Microsoft Excel), in order to get the rate of evolution per unit time. Rates of evolution were transformed to natural logarithm. Then we evaluated the association between *BMR* and T_b rates using a phylogenetic regression model in BayesTraits v3.0. (http://www.evolution.rdg.ac.uk/SoftwareMain.html), with a simulated tree and setting the Lambda parameter equal to zero (no phylogenetic signal). The regression was evaluated for those branches that showed variation in the red of evolution in both *BMR* and T_b (see red dots, Figure 2C, Chapter 1). The simulated tree was obtained with the R package phytools (https://cran.r-project.org/web/packages/phytools/index.html), with tips numbers equal to the number of branches in the Mammalian phylogeny for which rates of *BMR* and T_b were variable.

The same analyses were conducted to evaluated the relationship between *BMR* and T_a. Rates of T_a were inferred using the *Variable rates regression model* in BayesTraits v3.0, using latitude as main covariate. We also included a quadratic term for latitude given that T_a decrease towards the poles.

Analytic details for rates of body size evolution, directional trends, and its effect on speciation rates (Chapter 2).

We used the consensus time-calibrated tree and the body size in the natural logarithm of millimetres, in order to estimate its rates of evolution using the *Variable rates model* in BayesTraits v3.0 software. 25.000.0000 million iterations were ran using Bayesian inference, sampling every 25000 to ensure independence in model parameters. Then we used the sample of tress with scaled branch length (which represent the posterior distribution of evolutionary rates) to get the consensus trees in the software BayesTrees v1.3. From this consensus tress we got the cumulative change for each species by summing all branches from the root to the species, and using the patristic option of the function distRoot in the R package adephylo (https://cran.r-project.org/web/packages/adephylo/index.html).

The cumulative change was used as fixed effect to predict the body size of species using the R package MCMCglmm (https://cran.r-project.org/web/packages/MCMCglmm/index.html). Then, we included additional dummy variables that represented the tribes and molar morphology, in order to evaluated the association between body size and the cumulative change by tribes and/or molar morphology. Our *Tribe-Molar model* (see Chapter 2) correspond to the phylogenetic regression for body size and the interaction between (1) rates of body size evolution, (2) Tribes and (3) Molar Morphology as main covariates. The final model (Table 3), is the *Tribe-Molar model* but without considering the interaction between tribes and molar morphology that were not statistically significant.

We then used the same interaction between (1) rates of body size evolution, (2) Tribes and (3) Molar Morphology, but to predict the speciation rates for each species in the tree using the MCMCglmm package (see methods Chapter 2). Speciation rates for each species were estimated as the number of nodes from the root to the species (excluding the root). The number of nodes were calculated in the R package adephylo, and using the nNode option of the distRoot function.

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