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Investigating the timing of origin and evolutionary processes shaping regional species diversity: Insights from simulated data and neotropical butterfly diversification rates

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Different diversification scenarios have been proposed to explain the origin of extant biodiversity. However, most existing meta-analyses of time-calibrated phylogenies rely on approaches that do not quantitatively test alternative diversification processes. Here, I highlight the shortcomings of using species divergence ranks, which is a method widely used in meta-analyses. Divergence ranks consist of categorizing cladogenetic events to certain periods of time, typically to either Pleistocene or to pre-Pleistocene ages. This approach has been claimed to shed light on the origin of most extant species and the timing and dynamics of diversification in any biogeographical region. However, interpretations drawn from such method often confound two fundamental questions in macroevolutionary studies, tempo (timing of evolutionary rate shifts) and mode ("how" and "why" of speciation). By using simulated phylogenies under four diversification scenarios, constant-rate, diversity-dependence, high extinction, and high speciation rates in the Pleistocene, I showed that interpretations based on species divergence ranks might have been seriously misleading. Future meta-analyses of dated phylogenies need to be aware of the impacts of incomplete taxonomic sampling, tree topology, and divergence time uncertainties, as well as they might be benefited by including quantitative tests of alternative diversification models that acknowledge extinction and diversity dependence.

KEY WORDS: Amazonia, density-dependence, extinction, museum model, Neogene, Pleistocene refuges.

The Neotropics is one of the main reservoirs of terrestrial biodiversity on Earth due to its outstanding species richness and endemic diversity (Myers et al. 2000). Although the causes of this impressive radiation have been scrutinized since the times of Darwin, Humboldt, Bates, and Wallace, a lively debate continues today (e.g., Hoorn et al. 2011; Rull 2011b, 2015; Garzón-Orduña et al. 2015a). More recently, relaxed molecular clock models have advanced our understanding of the origin of extant Neotropical biodiversity. However, the macroevolutionary mechanisms driving lineage diversification are still subject to intensive research, and sometimes the topic is highly controversial. For instance, published meta-analyses of time-calibrated phylogenies have not offered a satisfactory synthesis for tropical America's biodiversity, but a number of contradictory interpretations of species divergences (e.g., Moritz et al. 2000; Weir 2006; Aleixo and Rossetti 2007; Rull 2008, 2011a; Antonelli et al. 2010; Hoorn et al. 2010; Barber and Jensen 2011; Turchetto-Zolet et al. 2013; Garzón-Orduña et al. 2014). Although such contrasting outcome might be expected owing to the intrinsic characteristics of each plant and animal lineage and their intricate evolutionary history, a critical issue is related to nonquantitative approaches that might have misled investigators' interpretation of species divergences.

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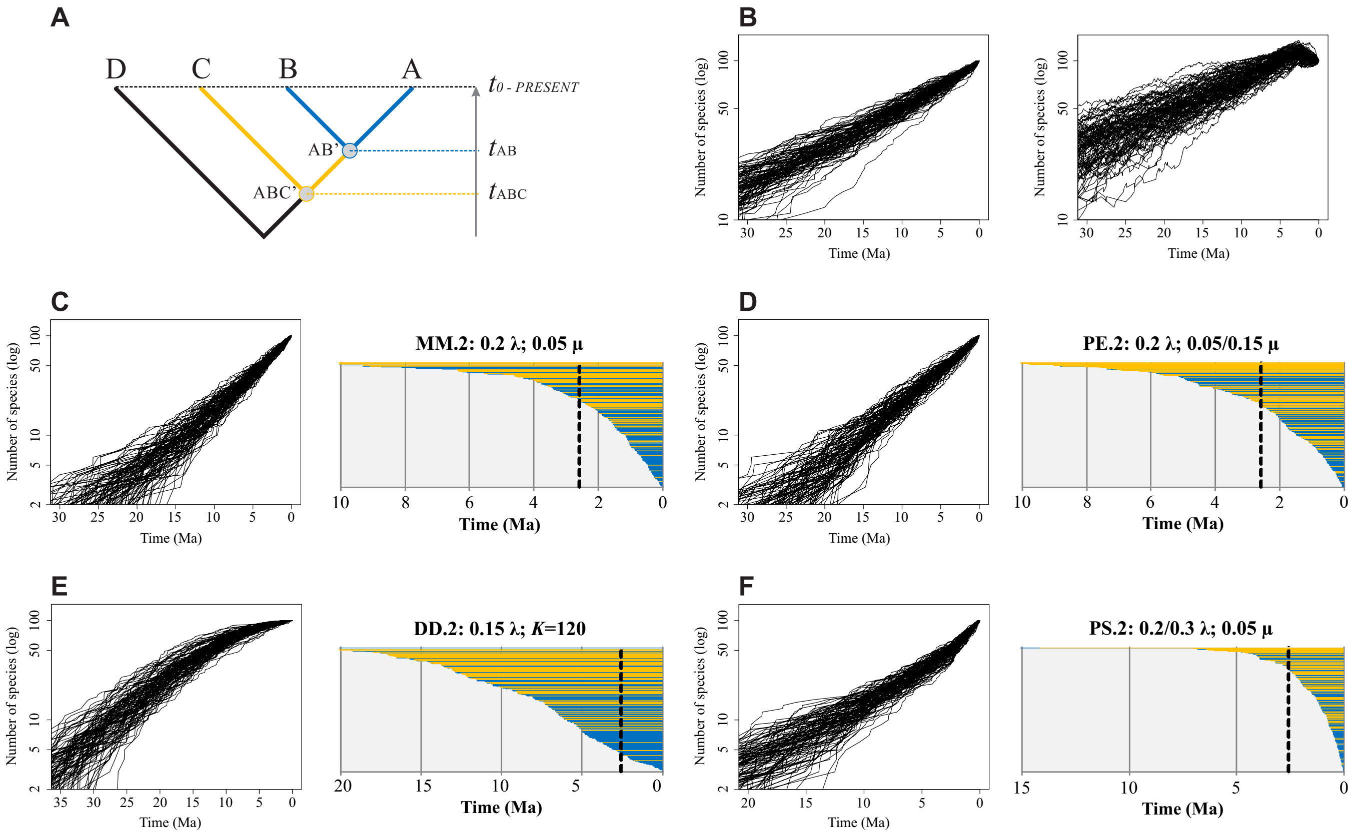

Figure 1. **Representation of branching events following distinct diversification scenarios. (A) Extant sister species divergence is represented by the age of the most recent common ancestor of species A and B (t_{AB}). Sister species and their closest extant relative shared a common ancestor whose divergence age is represented by t_{ABC}. (B) Lineage Through Time (LTT) plots reconstructed from 100 simulated trees following the Pleistocene extinction scenario PE.1. Diversity loss is only evidenced when extinct and extant species are included in the LTT curve (diagram on the right), whereas a quasi-constant diversification is recovered when the LTT plot accounts only for extant species (diagram on the left). (C–D) LTT shapes (left side) and species divergence ranks (right side) under four distinct diversification scenarios: (C) Museum model depicting constant-rate evolution; (D) increased Pleistocene extinction; (E) Neogene diversity-dependence model; and (F) increased quaternary speciation. In divergence ranks, colors follow panel A where sister species are depicted as blue bars, and sister species and their closest relative as yellow bars. Bars (divergence times) are sorted by age, and the beginning of the Pleistocene (2.6 Ma) is depicted with a vertical dotted line.**

Constructing time-calibrated species divergence ranks (for an example of these ranks, see Fig. 1) is a common exercise in meta-analyses (but see e.g., Zink et al. 2004; Weir 2006; Barber and Jensen 2011), but their conflicting interpretations might be related to shortcomings in this approach per se (Zink and Klicka 2006). The original use of such ranks (Klicka and Zink 1997, 1999) aimed to show that extant songbird divergences predated the Last Glacial Maximum, but it was not meant to, as explicitly stated, provide evidence in favor of any speciation model (e.g., ice ages driving allopatric speciation). Nonetheless, most published meta-analyses categorize cladogenetic events using divergence ranks to either pre-Pleistocene or Pleistocene ages, in an attempt to simultaneously shed light on the age of origin of extant diversity (tempo) and the potential mechanisms driving speciation (mode). Although tempo and mode must be clearly distinguished in macroevolutionary studies, meta-analyses have often ambiguously investigated "when" (tempo) and "how" (mode) the Neotropics became hyperdiverse. In general, studies based only on the visual inspection of divergence ranks (e.g., Johnson and Cicero 2004; Garzón-Orduña et al. 2014) might have been seriously biased by interpretations such as speciation driven by environmental and "microevolutionary processes" if most species divergences occurred in the Pleistocene, or driven by palaeogeographic reconfigurations if lineage divergence mostly occur within the Neogene Period.

The apparent dichotomy between "Pleistocene or Neogene events" shaping extant biodiversity is the main conflicting outcome of meta-analyses; and this dichotomy was recently discussed as the "paradigm shifting" handicap (Rull 2013, 2015). However, a major issue related to hypothesis testing across published meta-analyses of time-calibrated phylogenies remains to be addressed. Critically, the lack of quantitative approaches to understand diversification dynamics might have neglected important components of biodiversity, such as extinction and diversity-dependent diversification. Here, I aim to provide insights on the importance of including tests of alternative diversification models to study tempo and to rule out implausible modes of evolution in future meta-analyses, not only restricted to tropical America but also applicable to other biogeographical regions. I use simulations of chronograms under four different diversification processes (constant-rate, diversity-dependence, high extinction, and high speciation rates in the Pleistocene) to recreate the main hypotheses on the origin of Neotropical biodiversity (Box 1). Divergence ranks are also constructed with the aim of testing their efficiency to recover actual timing and diversification scenarios. Finally, I use an empirical dataset of published butterfly molecular chronograms (Garzón-Orduña et al. 2014) to provide insights on the advantages and limitations of utilizing diversification methods compared to divergence ranks.

BOX 1. Phylogenetic diversification models

The main hypotheses on the origin of Neotropical biodiversity recreated by the simulations of phylogenies in this study are briefly described. For extensive reviews of hypotheses, refer to, for example Haffer (2008); Antonelli and Sanmartín (2011).

A. *Museum model of diversification*

This hypothesis suggests that diversity is correlated with the time available for lineages to evolve, implying that speciation and extinction rates remain nearly constant over time (Wallace 1878; Fischer 1960; Stebbins 1974). Accordingly, groups with low diversification rates (speciation minus extinction) would require more time to accumulate species compared to lineages with higher diversification rates. The Museum hypothesis assumes a reduced probability of species extinction as a result of stable environmental conditions in the tropics; thus, older lineages in a clade may persist in time (Darwin 1859; Wallace 1878; Mittelbach et al. 2007). Palaeoecological reconstructions describing severe environmental changes over time undermine the stable environment hypothesis, but species may still have escaped extinction by dispersing to favorable habitats as suggested by the phylogenetic niche conservatism hypothesis (Wiens and Donoghue 2004).

B. *Neogene diversification*

This hypothesis describes substantial lineage diversification in the Miocene followed by a deceleration of evolutionary rates in the Pleistocene, or in a broader sense the Quaternary (Hooghiemstra and van der Hammen 1998; van der Hammen and Hooghiemstra 2000; Wesselingh and Salo 2006; Hoorn and Wesselingh 2010; Hoorn et al. 2010). Two models might describe decreased Quaternary diversification: (1) speciation may have decreased in time due to diversity-dependence constraints (Rabosky 2009a,b), or (2) extinction may have increased during the Pleistocene causing net biodiversity loss after the Neogene (van der Hammen and Hooghiemstra 2000). Under the first diversity-dependence scenario, lineages may colonize new habitats and/or expand their ecological niches, and further diversify thanks to ecological opportunity. Constraints to clade size may eventually lead to slowdown in diversification rates once new niches are filled (e.g., Weir 2006; Barber and Jensen 2011; Fine et al. 2014). On the other hand, the second scenario of increased extinction rates in the Plio-Pleistocene has been proposed based only on fossil and palynological records (van der Hammen and Hooghiemstra 2000; Wesselingh 2008).

C. *Quaternary diversification*

This hypothesis has gained wide support in the past decades since the proposal of Pleistocene refuges as a mode of allopatric differentiation (Haffer 1969). However, other mechanisms driving speciation in the Pleistocene have been proposed for lowland taxa, such as the riverine barriers, disturbance-vicariance, and gradient hypotheses. Alternative speciation mechanisms were also proposed for highland species, such as elevational gradients and vertical speciation (Endler 1977; Hall 2005), montane range fragmentation (Weir 2006), and out-of-the highlands hypotheses (Fjeldså and Lovett 1997). Overall, the Quaternary model of diversification would suggest substantial increase of diversification rates within the past 2.6 Ma. This does not necessarily imply that most extant species originated in the Pleistocene (Haffer and Prance 2001; Haffer 2008), but that older lineages across clades accumulate at a slower rate than taxa of the Quaternary age.

Methods

SIMULATION OF PHYLOGENIES AND DIVERGENCE TIMES

I simulated chronograms under four diversification processes (Table 1). I used the R package TreeSim (Stadler 2011b, 2013; R Development Core Team 2015) to simulate trees under birth-death and diversity-dependence models using the functions *sim.bd.taxa* and *sim.rateshift.taxa*. Each simulation recovered a tree with 100 extant tips, and the root age was not fixed. One hundred topologies were simulated for each diversification scenario.

Table 1. **Simulated phylogenetic diversification models..**

	λ_0	μ_0	r_0 ($\lambda_0 - \mu_0$)	K	λ_1	μ_1	r_1 ($\lambda_1 - \mu_1$)	Pairs $\leq$ 2.6 Ma (%)	Triplets $\leq$ 2.6 Ma (%)
Museum model (MM)									
MM.1	0.1	0.05	0.05	–	–	–	–	51.8	16.5
MM.2	0.2	0.05	0.15	–	–	–	–	78.0	51.9
MM.3	0.4	0.1	0.3	–	–	–	–	93.8	81.2
Diversity-dependent (DD)									
DD.1	0.05	–	0.05	120	–	–	–	4.4	0.0
DD.2	0.15	–	0.15	120	–	–	–	21.6	2.4
DD.3	0.3	–	0.3	120	–	–	–	36.9	9.8
Pleistocene extinction (PE)									
PE.1	0.1	0.05	0.05	–	0.1	0.15	−0.05	41.1	14.0
PE.2	0.2	0.05	0.15	–	0.2	0.15	0.05	74.7	50.6
PE.3	0.4	0.1	0.3	–	0.4	0.2	0.2	93.3	80.0
Pleistocene speciation (PS)									
PS.1	0.1	0.05	0.05	–	0.2	0.05	0.15	76.6	43.2
PS.2	0.2	0.05	0.15	–	0.3	0.05	0.25	89.0	73.3
PS.3	0.4	0.1	0.3	–	0.5	0.1	0.4	94.4	88.5

Phylogenies were simulated under four types of diversification scenarios: (1) Museum model (scenarios MM); (2) Diversity-dependence diversification (scenarios DD); (3) High extinction rates in the Pleistocene (scenarios PE); and (4) High speciation rates in the Pleistocene (scenarios PS). λ_0, speciation rate before a rate shift at 2.6 Ma, and λ_1 after a rate shift at 2.6 Ma; μ_0, extinction rate before rate shift at 2.6 Ma, and μ_1 after rate shift at 2.6 Ma; *r*, diversification rate, speciation minus extinction; *K*, clade-level carrying capacity; Pairs $\leq$ 2.6, proportion of extant sister species of Quaternary age; Triplets $\leq$ 2.6 Ma, proportion of divergences among sister species and their closest extant relative of Quaternary age.

Museum model (scenarios MM)

Simulated trees were set to a constant diversification rate. Because the average diversification rate of megadiverse groups such as arthropods is estimated to be about 0.1–0.2 species/million years (Coyne and Orr 2004), slower, moderate, and faster rates were included in the models (Table 1).

Neogene diversification: Diversity-dependence (scenarios DD)

Speciation rate was set to decrease in a diversity-dependence fashion. The function *sim.rate.shift* in TreeSim assumes zero extinction under a model of diversity-dependence. The parameter K (clade-level carrying capacity) was set to 120 thus the number of extant tips in the simulated trees (100) would reach equilibrium due to constraints to clade size.

Neogene diversification: Higher Pleistocene extinction (scenarios PE)

Extinction rate was set to increase during the Pleistocene. A positive shift (increase) in extinction rate was set to 2.6 Ma to simulate decreasing diversification in the Pleistocene.

Quaternary diversification: Higher Pleistocene speciation (scenarios PS)

Speciation rate was set to increase during the Pleistocene. A positive shift in speciation rate was set to 2.6 Ma to simulate increasing diversification in the Pleistocene.

ESTIMATION OF DIVERSIFICATION RATES

Simulated trees

I used the R package DDD (Etienne et al. 2012; Etienne and Haegeman 2013) to estimate speciation and extinction rates from the simulated trees by using the functions *bd_ML* (constant diversification), *dd_ML* (diversity-dependent diversification), and *dd_SR_ML* (shifts in speciation and extinction at a given time). Bias and precision were approximated by the 25th, 50th, and 75th percentiles of each estimated parameter over 100 simulated trees. Further analyses were conducted to test the accuracy in parameter estimation when the sampling of tips represented 75% and 50% of the total extant diversity. To recreate these undersampling scenarios, 25% and 50% of tips were randomly pruned from simulated phylogenies using the function *drop.random* of the R package Geiger (Harmon et al. 2008). New estimation of diversification rates using these pruned trees was carried out in DDD while acknowledging the number of missing tips in the phylogenies (Table S1).

Empirical butterfly chronograms

I tested the four diversification scenarios defined above on the time-calibrated butterfly phylogenies that were used in the latest meta-analysis of the group (Garzón-Orduña et al. 2014). Unsampled species diversity in such phylogenies was acknowledged based on the Neotropical butterfly checklist (Lamas 2004; Table 2). The relative probability of each diversification

Table 2. Diversification rate estimations by DDD of time-calibrated butterfly phylogenies.

Lymanopoda (66 described sp.; 40 in tree)	λ_0	μ_0	*K*	λ_1	μ_1	t_shift	loglik	AIC	nP	Akaike weights
a) Constant λ and μ (birth-death)	**0.207**	**0.000**					**−105.53**	**215.05**	**2**	**0.65**
b) Diversity-dependence	0.207	0.000	Inf.				−105.53	217.05	3	0.24
c) Birth-death with shift in λ	0.231	0.007	194	0.401	μ_0	0.9	−105.05	220.10	5	0.05
d) Birth-death with shift in μ	0.249	0.016	Inf.	λ_0	0.249	1.5	−105.04	220.08	5	0.05
Taygetis (73 described sp.; 52 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	0.202	0.000					−135.65	275.31	2	0.00
b) Diversity-dependence	0.403	0.000	90				−129.77	265.53	3	0.15
c) Birth-death with shift in λ	**0.295**	**0.000**	**248**	**0.000**	$\boldsymbol{\mu_0}$	**1.0**	**−126.08**	**262.16**	**5**	**0.81**
d) Birth-death with shift in μ	0.484	0.096	89	λ_0	0.000	1.2	−129.14	268.28	5	0.04
Morpho (29 described sp.; 21 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	0.135	0.000					−59.66	123.31	2	0.19
b) Diversity-dependence	**0.263**	**0.000**	**37**				**−57.78**	**121.57**	**3**	**0.45**
c) Birth-death with shift in λ	0.194	0.002	226	0.029	μ_0	2.6	−56.21	122.42	5	0.29
d) Birth-death with shift in μ	0.274	0.045	39	λ_0	0.000	4.7	−57.59	125.18	5	0.07
Phyciodina (107 described sp.; 65 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	0.095	0.000					−232.61	469.23	2	0.00
b) Diversity-dependence	0.189	0.000	134				−224.42	454.84	3	0.09
c) Birth-death with shift in λ	**0.159**	**0.003**	**239**	**0.011**	$\boldsymbol{\mu_0}$	**3.1**	**−220.14**	**450.28**	**5**	**0.89**
d) Birth-death with shift in μ	0.213	0.050	127	λ_0	0.000	11.9	−224.08	458.17	5	0.02

(Continued)

Table 2. Continued.

Troidini (47 described sp.; 30 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	**0.117**	**0.019**					**−96.72**	**197.44**	**2**	**0.67**
b) Diversity-dependence	0.117	0.019	Inf.				−96.72	199.44	3	0.25
c) Birth-death with shift in λ	0.100	0.000	723	0.146	μ_0	3.1	−96.38	202.77	5	0.05
d) Birth-death with shift in μ	0.123	0.031	1138	λ_0	0.000	2.6	−96.62	203.23	5	0.04
Napeogenes (25 described sp.; 24 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	0.158	0.000					−62.66	129.32	2	0.09
b) Diversity-dependence	**0.365**	**0.000**	**30**				**−59.57**	**125.13**	**3**	**0.69**
c) Birth-death with shift in λ	0.307	0.000	45	0.139	μ_0	2.8	−59.26	128.52	5	0.13
d) Birth-death with shift in μ	0.368	0.003	30	λ_0	0.000	3.3	−59.56	129.12	5	0.09
Ithomia (24 described sp.; 24 in tree)	λ_0	μ	*K*	λ_1	μ_1	t_shift	loglik	AIC		Akaike weights
a) Constant λ and μ (birth-death)	0.176	0.000					−59.79	123.58	2	0.16
b) Diversity-dependence	**0.360**	**0.000**	**31**				**−57.69**	**121.38**	**3**	**0.49**
c) Birth-death with shift in λ	0.259	0.001	871	0.070	μ_0	2.6	−56.24	122.49	5	0.28
d) Birth-death with shift in μ	0.365	0.000	31	λ_0	0.033	0.4	−57.67	125.34	5	0.07

The number of described species and the number of species included in the empirical trees are both referred next to each butterfly group's name. Four diversification models were compared: (a) Constant birth-death diversification; (b) Diversity-dependence constraints; (c) birth-death diversification with shift in speciation rate; and (d) birth-death diversification with a shift in extinction rate. When applicable: Speciation rate, λ0 before a rate shift, and λ1 after a rate shift; Extinction rate, μ0 before rate shift, and μ1 after rate shift; Clade-level carrying capacity, K; Time of rate shift in million years ago, t_shift; Log-likelihood, loglik; Akaike Information Criterion, AIC; number of parameters in the model of phylogenetic diversification, nP. Akaike weights is interpreted as the conditional probability of each model, and when log-likelihoods across models are about the same, the difference is likely driven by the number of additional free parameters that collectively do not improve data explanation. The most probable model is highlighted in bold.

scenario given the butterfly phylogenies was estimated using Akaike weights (Wagenmakers and Farrell 2004). Additionally, shifts in diversification rates were further evaluated using the function *bd.shifts.optim* in TreePar (Stadler 2011a), which allows the estimation of negative diversification rates, that is extinction higher than speciation (Table S2).

Results and Discussion

PRECISION AND BIAS OF DIVERSIFICATION ESTIMATIONS ON SIMULATED CHRONOGRAMS

Overall, the estimation of speciation rates, clade-level carrying capacity, and the timing of rate shifts were well-recovered by the program DDD (Table S1). Nonetheless, the estimation of extinction rates in the diversification scenarios with high extinction in the Pleistocene (PE scenarios) showed significant biases, particularly when extinction surpasses speciation (i.e., negative diversification as in PE.1) or counterbalances speciation as in PE.2. In such scenarios, DDD estimated quasi-constant diversification rates over time; a similar pattern was also recovered in LTT plots (Fig. 1 and Fig. S1). In the scenario PE.3, the program recovered an increase in extinction after 2.6 Ma, but extinction rate was somewhat underestimated. On the other hand, DDD accurately estimated shifts in speciation rates in the scenarios describing high speciation in the Pleistocene (scenarios PS), although extinction was underestimated in the scenario PS.3.

Incomplete taxonomic sampling may represent another potential limitation for phylogenetic diversification methods. Sampling the entire species diversity in empirical phylogenies can be logistically challenging, and even more problematic when dealing with cryptic and undescribed diversity. While biases in macroevolutionary inferences based on incomplete phylogenetic trees have long been recognized (e.g., Pybus and Harvey 2000; Heath et al. 2008; Cusimano and Renner 2010; Davis et al. 2013), some approaches account for incomplete sampling as long as it is randomly distributed across the tree (e.g., Stadler 2009, 2011a; Etienne et al. 2012). In this study, the estimation of speciation rates and the timing of rate shifts using incomplete phylogenies were well-recovered using the method of Etienne et al. (2012). However, when incomplete sampling was substantial (50%), extinction rates were underestimated in most diversification scenarios. The strongest biases in extinction rate estimations were found in the scenarios of net diversity loss in the Quaternary (scenarios PE). A better sampling of extant tips in the phylogeny (75%), nonetheless, recovered increased extinction only in PE.3, suggesting that a better taxonomic sampling may at least improve the detection of extinction shifts. Nonrandom sampling is another problematic issue often encountered in empirical phylogenies. It has been shown that nonrandom sampling increases the type-I error of diversification tests (e.g., Pybus and Harvey's (2000) Monte Carlo constant rates test) (Cusimano and Renner 2010; Brock et al. 2011). However, given the limited space of this brief communication, evaluating the impact of nonrandom taxonomic sampling in meta-analyses' conclusions is an area of future work.

Extinction might also hinder the actual diversification process in a clade, making it difficult to infer the mechanisms that are responsible for slowdowns in diversification rates (Cusimano and Renner 2010). Fossil and palynological records of Neotropical taxa have been discovered in the past decades, and our understanding of palaeoecological processes in the Cenozoic has notably improved (e.g., van der Hammen and Hooghiemstra 2000; Jaramillo et al. 2006; Hoorn and Wesselingh 2010). Unfortunately, fossils of ecologically important and species-rich groups such as insects are not yet sufficient to draw well-supported palaeoecological inferences nor to estimate confident extinction rates (Mendes and Pinto 2001; Antoine et al. 2006; Petrulevičius et al. 2011). Given that the estimation of extinction is critical to understand the origin of extant biodiversity (Ricklefs 2007; Morlon 2014), future meta-analyses of empirical time-calibrated phylogenies might benefit from the inclusion of quantitative tests of alternative diversification models. For instance, increased regional extinction during the Plio-Pleistocene has been suggested based on a number of Neotropical fossil and palynological records (van der Hammen and Hooghiemstra 2000; Behling et al. 2010; Wesselingh et al. 2010), but this hypothesis has not been favored, or even explicitly tested, using time-calibrated molecular phylogenies.

SPECIES DIVERGENCE RANKS VERSUS DIVERSIFICATION RATE ESTIMATIONS

Tempo and mode of evolution are two primary subjects defined in the modern synthesis of evolutionary theory (Simpson 1944). In the study of macroevolution, tempo encompasses the estimation of evolutionary rates and its dynamics over time, whereas mode determines the mechanisms of speciation (the "how" and "why"), which can only be inferred in the light of tempo (Gould and Eldredge 1977). In a phylogenetic context, time-calibrated molecular distances among extant lineages might address the estimation of speciation and extinction rates, providing a valuable tool to infer biodiversity dynamics over time even for groups lacking a comprehensive fossil record (Nee et al. 1992; Ricklefs 2007; Morlon 2014). It is under this phylogenetic framework that constructing species divergence ranks may only partially address tempo, as diversification rates are never estimated, while any inferred mode of species evolution might therefore be unwarranted. I further discuss the implications of using divergence ranks to unravel tempo and mode of evolution by exploring two main methodological limitations: (1) arbitrarily chosen cladogenetic events, which might have favored distinct "timing of lineage evolution", and (2) neglected evolutionary processes, which might have hindered tempo and mode patterns.

Species divergence ranks based on arbitrarily chosen cladogenetic events

It has been argued that the ideal datasets to investigate the origin of extant biodiversity must consist only of pairs of sister species because crown group ages may overestimate pre-Quaternary events (Garzón-Orduña et al. 2014, 2015a). Here, I retrieved the ages of sister species pairs and their closest extant relative from 5 random trees simulated under each diversification scenario. Species divergence ranks were constructed using these branching events to highlight the shortages of this approach to infer "timing of biodiversity origin" (Fig. 1 and Fig. S1). Without any distinction of tempo and mode, it is likely that this exercise may be acknowledged as gathering only rough evidence to help uncover the period of time when most extant diversity originated. However, these datasets represented about 50–60% of all extant tips in the simulated trees, and the average age of sister species pairs was about 1–5 Ma. The remaining extant tips may be expected to be older surviving lineages that have further diversified given the background speciation and extinction rates.

Datasets consisting of arbitrarily chosen cladogenetic events, for example sister species divergences or crown group ages, seem to be the main reason for the large discrepancies found across meta-analyses. The crown group approach might indeed favor a pre-Pleistocene origin of Neotropical hyperdiversity because recent speciation events are ignored (Rull 2011a), but the sister-species approach may equally mislead data interpretation toward an apparent recent origin of biodiversity, because older diversification events are neglected. In the simulations conducted here, sister taxa were in most cases younger than 2.6 Ma regardless of diversification scenario and the timing of the evolution of lineages (Table 1, Fig. 1, Fig. S1). Even under models of high extinction during the Pleistocene (models PE), about 75–93% of all extant sister species are of Quaternary age, and when extinction is higher than speciation (negative diversification as in model PE.1), about 41% of all extant sister species were of Pleistocene ages (Table 1).

In this brief communication, I do not attempt to refute nor contradict empirical data from meta-analyses, but to address a severe limitation of the divergence rank approach and the usage of arbitrarily selected phylogenetic branching events to infer tempo and to evaluate modes of diversification. As reported to date, crown ages may mostly be of Neogene times while sister species divergences may have predominantly occurred within the Quaternary. But this empirical evidence needs to be studied in a phylogenetic framework to avoid relative measurements of the apparent origin of lineages which, in turn, would shed more light on the mechanisms shaping extant biodiversity. Meta-analyses commonly do not only report the timing of cladogenetic events across groups in the form of species divergence ranks, but also attempt to generalize patterns and to provide evidence on the tempo (e.g., increased speciation at certain periods of time) and, indirectly, on modes of speciation (e.g., Pleistocene refuges).

Evolutionary processes may obscure tempo and mode patterns

Species divergence ranks that fully account for all branching events in an extant species phylogeny might also suffer from biases due to macroevolutionary processes, such as high extinction and diversity-dependent diversification. Figure 2 depicts one randomly picked simulated phylogeny under the PE.1 scenario displaying either only extant tips (Fig. 2A) or the complete tree including extinct lineages (Fig. 2B). In this example, the number of cladogenetic events resulting in pairs of extant sister species is 33, with 15 being younger than 2.6 Ma (i.e., about 45% of all such events). Neither this significant proportion of sister species divergences of Quaternary age nor crown groups of mostly Neogene/Paleogene ages approximates well the simulated tempo of evolution; that is high extinction in the past 2.6 Ma.

The effectiveness of graphical methods, such as divergence ranks or Lineage Through Time (LTT) plots, to approximate tempo using extant species divergences might be severely diminished by increased extinction, reduced speciation, or simply by net rate of diversification close to zero as in diversity-dependence diversification (Rabosky and Lovette 2008). Continuing with the example discussed above, the log-linear LTT plot shape recovered with 100 simulated trees under the PE.1 scenario appeared to follow constant diversification over time, which was also the false-positive scenario recovered by DDD. It is only when extinct lineages are added to the plot that increased extinction at 2.6 Ma is evident (Fig. 1B). Etienne et al. (2012) showed the importance of observing LTT plot shapes as a preliminary step before estimating diversification rates. Log-linear shapes might be caused by nonidentifiable scenarios, whereas S-shapes might usually indicate that phylogenies contain adequate information to carry out diversification rate analyses. In the present study, the most precise rate estimates occur for LTT plots displaying S-shape curves (scenarios DD and PS), reinforcing the evaluation of LTT plots as a crucial exercise to identify datasets with sufficient signal for slowdown or acceleration of rates (Matzke, in prep.).

Because incorporating the complete set of extinct lineages in phylogenetic reconstructions is virtually impossible, well-sampled, accurately dated phylogenies, along with methods that account for extinction and diversity-dependence processes are essential in future meta-analyses dealing with the origin and dynamics of biodiversity in any biogeographical region (Cusimano and Renner 2010; Rull 2011a; Moen and Morlon 2014). The models described here are only a subset of the proposed diversification scenarios for the Neotropics, but these might be further modified to account for other events and also for other regions

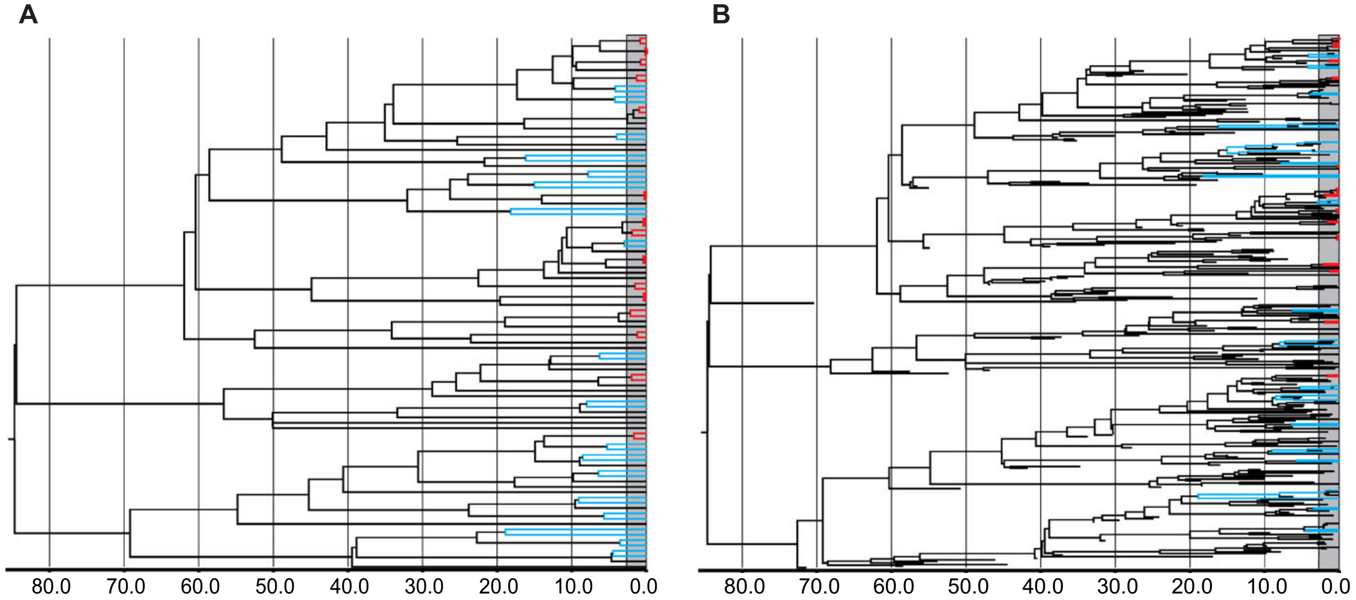

Figure 2. Randomly picked simulated chronogram from the scenario PE.1 (increased extinction at 2.6 Ma). Phylogeny displaying only extant tips is depicted in panel A, whereas the same phylogeny including both extant and extinct is shown in panel B. Extant sister species are colored either in red if their divergence is younger than 2.6 Ma or in blue if divergences are older than 2.6 Ma. The Quaternary Period is displayed as gray shadows in both phylogenies. Time scale in the *x*-axis represents million of years ago (Ma).

with distinct geological, palaeoenvironmental, or hydrological histories.

CASE STUDY: BUTTERFLY DIVERSIFICATION IN THE NEOTROPICS

Butterflies may exemplify the "paradigm shifting" viewpoint of Rull (2013, 2015) that distinct authors favor either Quaternary or Neogene origin of Neotropical biodiversity. In the past decades, a range of butterfly speciation mechanisms (modes) taking place mostly during the Pleistocene were proposed for lowland (Mallet 1993; Turner and Mallet 1996), including forest refuges (Brown et al. 1974), and for montane faunas (Shapiro 1992; Hall 2005). With the advent of molecular clock approaches, the significance of Neogene over Pleistocene events in the origin and diversification of butterflies was proposed based on time-calibrated phylogenies (e.g., Elias et al. 2009; Espeland et al. 2015). However, a recent meta-analysis suggested that extant Neotropical butterflies are mostly of Pleistocene origin, but the interpretation that the authors of the study gave to this finding was a congruence between timing of butterfly divergence and the refuge hypothesis predictions (i.e., mode of evolution) (Garzón-Orduña et al. 2014, 2015a).

Garzón-Orduña et al. (2014) showed that 72% of sister species pairs and 64% of sister species and their closest relative are of Quaternary age. The simulations conducted here are also in agreement with such empirical information, that is that extant sister species divergences are mostly younger than 2.6 Ma regardless of diversification scenario. However, these results can hardly be interpreted as evidence in favor of high speciation during the Quaternary (tempo) or that the timing of extant butterfly diversity is consistent with the predictions of the Pleistocene forest refuge hypothesis (mode). Such interpretations have been clearly biased by the overestimation of species divergence ranks. High proportion of sister species divergences younger than 2.6 Ma may also be expected even in the opposite scenario of increased extinction during the Pleistocene (e.g., in scenario PE.2, sister species pairs and their closest extant relative were of Quaternary age in 75% and 51% of the cases, respectively).

In the diversification rate estimations conducted here using eight empirical butterfly phylogenies (taxon sampling encompasses 60–100% of described diversity), most groups showed either constant speciation rates over time or decelerated diversification during the Quaternary. The hypothesis of increased diversification in the Pleistocene (tempo) was thus not supported by any butterfly clade. As noted by Haffer (2008), the "refuge hypothesis refers to a particular mode of allopatric speciation, but not to a particular time of differentiation" (pp. 930). Haffer (2008) also believed that most extant diversity in Amazonia probably originated during the entire Cenozoic (Tertiary and Quaternary, i.e., the last 60 Ma). Thus, claiming that the timing of sister species divergences is consistent with the temporal (and mode) predictions of the refuge hypothesis is another over interpretation of species divergence ranks.

Origin of extant Neotropical butterfly diversity

Montane butterfly diversity was likely driven by the major orogeny of the Andes and the availability of new habitat and niche spaces during the Miocene and Pliocene. Fragmentation of butterfly vertical and horizontal geographic ranges might have promoted the diversification of *Lymanopoda* (Satyrinae: Pronophilina) during the Andean uplift in the late Miocene (Casner and Pyrcz 2010). Its diversification may have continued in the Pleistocene as well, possibly due to vicariance along altitudinal ranges triggered by glacial/interglacial cycles. In contrast, a diversity-dependence model may better explain the diversity of *Ithomia* and *Napeogenes* (Danainae: Ithomiini). Slowdown in their diversification rates can potentially be linked to niche saturation of their larval host plants in the family Solanaceae (Mallarino et al. 2005; Elias et al. 2009).

Speciation rate in the *Taygetis* clade (Satyrinae: Euptychiina) appears to have decreased during the Pleistocene, whereas the early radiation of *Taygetis* may be linked to shifts in larval host plant (Matos-Maraví et al. 2013). The genus *Forsterinaria* might have colonized and diversified in the Andes thanks to its adaptation to feed on *Chusquea*, which is an abundant and widespread bamboo genus occurring mostly in montane habitats (Peña and Lamas 2005). Contrary to *Lymanopoda* butterflies, whose larval host plants are also montane woody bamboos, the diversification slowdown recovered for *Forsterinaria* might be related to physiological constraints. Whereas *Lymanopoda* is a representative of the subtribe Pronophilina, which typically occur from 1000 to 4000 MASL, the genus *Forsterinaria* belongs to the subtribe Euptychiina, which are commonly known in the lowlands and up to ~2000 MASL.

The charismatic *Morpho* butterflies (Satyrinae: Morphini) began their radiation in the middle Miocene, and their success has also been linked to their adaptation to feed as larvae on eudicots (Penz et al. 2012). However, its phylogenetic diversification appears to have declined in the Pleistocene, possibly caused by diversity-dependence (Table 2). The out-of-the-Andes hypothesis might partially explain the evolution of *Morpho*. Its ancestral range was likely to have been the eastern slope of the Andes but lineages may have been "pushed" to the lowlands due to the uplift of mountains in the Miocene, and further diversified in Amazonia, Central America, and the Southern Brazilian Atlantic forest (Penz et al. 2012).

Slowdown in speciation during the Pliocene and Quaternary was also inferred for the subtribe Phyciodina (Nymphalidae: Nymphalinae). These butterflies arrived in South America from the Nearctic region during the early Miocene, and palaeogeographical rearrangements might have triggered their radiation (Wahlberg and Freitas 2007). Examples of such rearrangements are the termination of the long-lived lake Pebas (Wesselingh et al. 2002), the uplift of the Andes (Gregory-Wodzicki 2000; Garzione et al. 2008), and the establishment of the Amazon river (Lundberg et al. 1998). Finally, the Museum Model of diversification in the lowlands was supported by three New World genera within the tribe Troidini (Papilionidae). Landscape reconfigurations in Amazonia that happened during both the Neogene and the Pleistocene may have been important in its diversification, acting together as a museum and a cradle for Troidini diversity (Condamine et al. 2012).

Overall, butterfly diversification in the Neotropics cannot be explained by a single mechanism or historical event. Instead, their evolution is the result of intricate processes, including geographical changes, climatic fluctuation, and ecological innovations such as shifts in larval host plants (Kozak et al. 2014). In fact, future studies of biodiversity may start from this general point rather than with a preconceived idea that a particular period of time or a single evolutionary process have predominantly shaped the extant biodiversity. Most butterfly groups have moderate to high diversification rates during the Miocene (*ca.* 15–20 Ma) followed by diversity-dependence and/or constant-rate evolution during the Pliocene and the Pleistocene. Nonetheless, further studies are needed to disentangle the causes for slowdown in diversification rates recovered for some butterfly groups. Both protracted speciation and unrecognized cryptic diversity may add artefacts to diversification rate estimations, resulting in a tendency to decelerated rates toward the present (Cusimano and Renner 2010; Moen and Morlon 2014).

Furthermore, an important note of caution has been made on the accuracy of butterfly divergence time estimations based on secondary calibrations (Garzón-Orduña et al. 2015b). Most time-calibrated phylogenies reported here were taken from studies that used secondary calibrations from the Nymphalidae chronogram of Wahlberg et al. (2009). Although butterfly divergences may be substantially younger than previously inferred, increased speciation in the Pleistocene as postulated by Garzón-Orduña et al. (2014) may still not be recovered. In the recalibration of the Clearwing butterfly phylogeny (Ithomiini), the genera *Ithomia*, and *Napeogenes* seem to be about half the age of previous estimates (Garzón-Orduña et al. 2015b). However, given that the chronogram used in the present study includes all described species in both genera, the inferred diversification process may remain essentially unaltered, that is origin of the clades in the Miocene followed by diversity-dependence or decreased diversification rates toward the present. Phylogenetic (topology) and time estimates (branch lengths) uncertainties may also further decrease confidence in diversification rate estimations. Although software that perform macroevolutionary analyses do not directly account for uncertainty associated with dated phylogenies, authors may find it useful to conduct analyses on a set of sampled trees resulting from Bayesian inference. For instance, Peña and Espeland (2015) found dramatic differences in diversification rate estimation and rate shifts across the phylogeny of nymphalid butterflies, depending on the tree of choice, either maximum clade credibility or a random sample of posterior trees.

The timing of butterfly sister-species divergences should reinforce the Quaternary as a period of continuing diversification that began long ago, but this does not necessarily mean that the palaeoenvironmental changes in the Quaternary were the main contributors of tropical America's hyperdiversity. Recent and rapid diversification has for instance been reported for *Adelpha* (Limenitidinae: Limenitidini), but its radiation was mostly driven by larval host plant shifts to the toxic Rubiaceae rather than related to the glacial/interglacial cycles of the Pleistocene (Ebel et al. 2015). On the other hand, environmental fluctuations during the Pleistocene might have structured populations by modifying species geographical ranges, which in turn may have promoted population differentiation (Whinnett et al. 2005; Turchetto-Zolet et al. 2013). Although forest expansions/contractions are commonly invoked as a mechanism driving diversification in the Pleistocene, other speciation mechanisms have been proposed; for instance, riverine barriers (Ribas et al. 2012), dispersal corridors (Wüster et al. 2005), elevational gradient ecozones (Willmott et al. 2001), or parapatric speciation in mountain systems (Hall 2005).

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DATA ARCHIVING

Data is archived in Dryad doi: 10.5061/dryad.d0m4r.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Rate estimations for each diversification model by the program DDD.
Table S2. Shifts in diversification rates as estimated by TreePar for each butterfly group.
Figure S1. Composite figure of all phylogenetic diversification simulations.