

## Testing the Relationships between Diversification, Species Richness, and Trait Evolution

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**Abstract.**—Understanding which traits drive species diversification is essential for macroevolutionary studies and to understand patterns of species richness among clades. An important tool for testing if traits influence diversification is to estimate rates of net diversification for each clade, and then test for a relationship between traits and diversification rates among clades. However, this general approach has become very controversial. Numerous papers have now stated that it is inappropriate to analyze net diversification rates in groups in which clade richness is not positively correlated with clade age. Similarly, some have stated that variation in net diversification rates does not explain variation in species richness patterns among clades across the Tree of Life. Some authors have also suggested that strong correlations between richness and diversification rates are a statistical artifact and effectively inevitable. If this latter point is true, then correlations between richness and diversification rates would be uninformative (or even misleading) for identifying how much variation in species richness among clades is explained by variation in net diversification rates. Here, we use simulations (based on empirical data for plethodontid salamanders) to address three main questions. First, how is variation in net diversification rates among clades related to the relationship between clade age and species richness? Second, how accurate are these net diversification rate estimators, and does the age–richness relationship have any relevance to their accuracy? Third, is a relationship between species richness and diversification rates an inevitable, statistical artifact? Our simulations show that strong, positive age–richness relationships arise when diversification rates are invariant among clades, whereas realistic variation in diversification rates among clades frequently disrupts this relationship. Thus, a significant age–richness relationship should not be a requirement for utilizing net diversification rates in macroevolutionary studies. Moreover, we find no difference in the accuracy of net diversification rate estimators between conditions in which there are strong, positive relationships between clade age and richness and conditions in which these strong relationships are absent. We find that net diversification rate estimators are reasonably accurate under many conditions (true and estimated rates are strongly correlated, and typically differ by ~10–20%), but become more accurate when clades are older and less accurate when using incorrect assumptions about extinction. We also find that significant relationships between richness and diversification rates fail to arise under many conditions, especially when there are faster rates in younger clades. Therefore, a significant relationship between richness and diversification rates is not inevitable. Given this latter result, we suggest that relationships between richness and diversification should be tested for when attempting to explain the causes of richness patterns, to avoid potential misinterpretations (e.g., high diversification rates associated with low-richness clades). Similarly, our results also provide some support for previous studies suggesting that variation in diversification rates might explain much of the variation in species richness among major clades, based on strong relationships between clade richness and diversification rates. [Diversification; simulations; species richness.]

Understanding which traits drive species diversification (speciation minus extinction) is critically important for evolutionary biology and conceptually adjacent fields (e.g., biogeography, ecology). For example, testing for a relationship between diversification rates and species traits can help explain why some clades have more species than others. Similarly, analyses of diversification rates can address why clades in some regions or habitats have more species than others, and therefore why some regions or habitats may be particularly species rich (e.g., tropical vs. temperate regions; [Pyron and Wiens 2013](#); [Rolland et al. 2014](#); land vs. oceans; [Wiens 2015b](#)). This approach can also help test whether particular morphological, ecological, or behavioral traits promote diversification, possibly through their role in speciation (e.g., [Kozak and Wiens 2010](#); [Rabosky et al. 2013](#); [Rainford et al. 2014](#); [Weber and Agrawal 2014](#); [Gómez-Rodríguez et al. 2015](#)).

An important approach for revealing the relationships between species diversification and trait evolution is to estimate net diversification rates of clades and test

for relationships between these rates and particular phenotypic traits (or rates of phenotypic evolution). This approach is based on the idea that the age of a clade and its species richness can be used to estimate the clade's net rate of diversification (e.g., [Magallón and Sanderson 2001](#)). Using this approach, clades that are relatively old and have few extant species will have relatively low net rates, whereas those that are relatively young and have many living species will have higher net rates. Mathematically, this must be true regardless of changes in rates and patterns of speciation and extinction within each clade over time (therefore, we use the term “net diversification rate estimator” and not “constant rate estimator”: see also Discussion section). This approach is particularly useful because it does not require that all or even most species within a clade be included in a phylogeny. In fact, it only requires that one species per clade be sampled (at least for estimating rates based on stem-group ages). This approach is also useful in that it can help identify how much variation in diversification rates among clades a particular variable explains (e.g., using regression), and can allow testing

of multiple variables simultaneously to evaluate their relative impacts on diversification (e.g., with multiple regression; Wiens et al. 2015). Many studies have now utilized this approach, including studies focused on explaining patterns of species richness (e.g., Kozak and Wiens 2012; Hutter et al. 2013; Pyron and Wiens 2013; Wiens 2015a, 2015b) and on understanding the factors that drive speciation (e.g., Kozak and Wiens 2010; Rabosky and Matute 2013; Rabosky et al. 2013; Gómez-Rodríguez et al. 2015; Wiens et al. 2015).

Nevertheless, in recent years, this general approach of testing the correlates of net diversification rates has become very controversial. Rabosky (2009b) argued that it is inappropriate to analyze net diversification rates unless there is a strong, positive relationship between clade age and species richness among clades. This idea has been repeated in many subsequent papers (e.g., Rabosky and Adams 2012; Rabosky et al. 2012; Rabosky and Matute 2013) and followed by many authors (e.g., Title and Burns 2015). There are now numerous papers that have concluded that net diversification rates are uninformative or misleading about species richness patterns based on this idea, all utilizing a similar approach (e.g., Rabosky 2010; Rabosky and Adams 2012; Rabosky et al. 2012). We refer to this approach as the simulated richness approach (SR) for brevity. This SR approach involves simulating distributions of species richness among clades based on empirical values (derived from net diversification rate estimators) and then analyzing the relationship between clade age and species richness among clades in these simulated data sets. These simulations often show significant, positive relationships between clade age and species richness, whereas the empirical data sets often show weak or negative relationships. The inference is then made that there is something wrong with the estimates of net diversification rates (e.g., they assume “constant rates,” but see above). However, the specific errors or biases in these net diversification rate estimates are not addressed. In the most extensive SR analysis to date, Rabosky et al. (2012) repeated this SR approach across many eukaryote clades (including various plants, animals, and fungi), and concluded that net diversification rates were uninformative or even misleading about species richness patterns across the Tree of Life. But they did so without testing for a relationship between diversification rates and richness patterns among clades (leaving open the possibility that variation in diversification rates actually explained most of the variation in richness among clades across the eukaryotic Tree of Life).

There has been very little critical appraisal of this SR approach. Wiens (2011) argued that variation in diversification rates among clades should itself tend to erase the positive relationship between species richness and clade ages, making this a problematic test of the validity of estimated net diversification rates in a given study. Instead, Wiens (2011) proposed that variation in instantaneous diversification rates within clades over time could potentially uncouple patterns of species

richness and net diversification rates among clades (e.g., if only young, species-poor clades have high net diversification rates, and diversification slows in older clades). Further, he suggested that this particular problem could be addressed by testing for a relationship between species richness and diversification rates among clades. Specifically, if patterns of species richness are strongly and positively related to diversification rates among clades, then this uncoupling has presumably not occurred. However, it has not been explicitly tested whether this pattern of younger clades having higher rates of diversification (and older clades having lower rates) will actually decouple species richness and diversification rates.

Rabosky and Adams (2012) addressed the relationships between diversification rates, clade ages, and species richness in a study combining simulations and empirical analyses of plethodontid salamanders (the largest salamander family). First, they randomly generated species richness and clade age values among a set of clades (note here and throughout that the clades are distinct from each other, and not overlapping or nested inside each other). When they analyzed these simulated data sets, they found that diversification rates and species richness values were almost always significantly and positively correlated. Based on these results, they argued that the test proposed by Wiens (2011) was invalid. However, they did not actually simulate variation in rates within clades over time for this analysis, the specific problem that the test from Wiens (2011) was intended to address, and the focus of Rabosky (2009b). More generally, they did not simulate any non random patterns relating diversification rates and clade ages. They then repeated the SR approach of earlier papers. Again, their simulations of species richness patterns among plethodontid clades using estimated net diversification rates yielded a positive age–richness relationship, contrasting with the negative relationship observed in the empirical data. They concluded that this discrepancy between their simulations and empirical data sets was caused by the inaccuracy of the estimated net diversification rates, but without identifying the frequency, magnitude, or direction of those putative errors. In fact, to our knowledge, no studies have actually addressed the accuracy of these net diversification rate estimators, despite their widespread use (and widespread claims that they are inaccurate).

In this study, we use simulations to address three main questions. First, how is variation in diversification rates among clades related to the relationship between clade age and species richness? For example, can we identify what actually causes the discrepancy between the age–richness relationship in empirical and simulated data sets that is observed using the SR approach? Second, how accurate are these net diversification rate estimators, and does the relationship between clade age and richness have any bearing on their accuracy? Third, is a strong, positive relationship between diversification rates and richness inevitable and therefore artifactual?

Or instead, can we use regression between species richness and diversification rates to quantify how much variation in species richness among clades is explained by diversification rates (and identify conditions where richness and diversification rate are uncoupled)? We base our simulations on empirical patterns of clade age, species richness, and diversification rates in the major clades of plethodontid salamanders (as in Rabosky and Adams 2012).

We find that there can be strong, positive relationships between clade age and species richness when there is no variation in diversification rates among clades. Thus, the SR approach suggests that it is only appropriate to analyze net diversification rates under conditions where (based on our simulations) diversification rates may actually be invariant and, therefore, uninformative. At the same time, we find that when variation in diversification rates is simulated among clades (but not within the clades), the significant, positive relationship between clade age and clade richness is frequently disrupted. These are the conditions under which (based on the SR approach) it is inappropriate to analyze net diversification rates. But these are actually the conditions where diversification rates will be the most variable, and therefore the most potentially informative. Perhaps most importantly, our simulations suggest that the age–richness relationship has no bearing on the accuracy of these net diversification estimators. These estimators appear to be reasonably accurate under the conditions simulated here (true and estimated rates typically differ by only ~10–20%, and are strongly correlated), and their accuracy increases with greater clade age and decreases when incorrect assumptions about extinction rates are made. Our results also reject the idea that a correlation between species richness and diversification rates is somehow inevitable or a mere statistical artifact. Instead, we find no consistent, positive relationship between species richness and diversification rates when simulated diversification rates are invariant, and when diversification rates are faster in younger clades. Therefore, testing for relationships between richness and diversification rates may be important for identifying conditions when richness and diversification rates are uncoupled (and therefore diversification rates may not explain the origins of richness patterns). Such relationships between richness and diversification rates may also be important for identifying how important diversification rates are for explaining patterns of species richness among clades across the Tree of Life.

#### METHODS

We used simulations to address five specific questions: (1) Will clade age and richness (and diversification rates and richness) be significantly and positively related when there is no variation in underlying diversification rates? (2) Will diversification rates and richness be related when species richness and clade ages are randomly shuffled and re-associated among clades (for both simulated and empirical data)? (3) Will clade age

and richness (and diversification rates and richness) be significantly and positively related when diversification rates vary randomly among clades? (4) Will clade age and richness (and diversification rates and richness) be significantly and positively related when diversification rates and clade ages vary non randomly among clades, such that younger clades have faster diversification rates? (5) How accurate are these net diversification rate estimators, and does the relationship between clade age and richness predict how accurate they will be?

We explored these questions using birth–death (speciation–extinction) simulations based on the estimated ages and diversification rates of the 16 plethodontid clades analyzed in Kozak and Wiens (2010; their Table 2), including all 15 clades analyzed by Rabosky and Adams (2012). For each clade, we simulated 1000 data sets using the `sim.bdtree` function in the R package GEIGER version 2.0.1 (Harmon et al. 2008). In these simulations, the clade's extant species richness was determined by its age and a given birth rate and given death rate. Clades were non nested and treated as evolving independently of each other.

For the simulations in which diversification rates were invariant among clades (Question 1 above), we initially used a birth (speciation) rate of 0.10 and a death (extinction) rate of 0. The resulting diversification rate (birth–death) roughly corresponds to the mean estimated net diversification rate among the 16 major plethodontid clades (0.094; Kozak and Wiens 2010). For each simulated data set, all 16 clades had the same birth rate, with only differences in clade age and stochastic variability in the birth–death process accounting for variability in species richness among clades. We then paired the SR values with their corresponding clade ages to estimate the net-diversification rate,  $r$ , using the crown-group method-of-moments estimator (Magallón and Sanderson 2001), following Kozak and Wiens (2010) and Rabosky and Adams (2012). Given that we simulated species richness under a pure birth model, we estimated the net diversification rate assuming a relative extinction fraction (epsilon, extinction rate/speciation rate) of 0.

We then estimated the relationships between species richness and clade age and between richness and diversification rate ( $r$ ) across each of the simulated data sets of 16 clades, using linear regression in R. We assessed how often clade age and richness are related to help address the idea that net diversification rate estimates should only be used when clade age and richness are significantly and positively correlated (e.g., Rabosky 2009b; Rabosky and Adams 2012; Rabosky and Matute 2013). We also assessed how often diversification rates and richness are related when no variation in diversification rates is present among clades (i.e., variation in species richness among clades is the result of stochasticity in the birth–death process alone), to address the idea that diversification rates and richness must be correlated as a statistical artifact (Rabosky and Adams 2012).

To test whether reshuffling the richness values among clades that have no variation in diversification rate

can generate significant relationships between rate and richness, we randomized the simulated richness values across the 16 focal clades and used the reshuffled species richness values and the original clade ages to compute the crown-group net diversification rate ( $r$ ) for each clade, and then estimated the relationship between richness and diversification rate for each data set. This latter approach assessed how often richness and diversification rate are significantly related when clade species richness values were originally generated using the same birth rate across clades, with the resulting richness values randomly redistributed among the focal clades.

Species richness and diversification rates are strongly and positively related among the clades of plethodontid salamanders ( $R^2 = 0.48\text{--}0.52$ ; see [Kozak and Wiens 2010](#)). We randomly redistributed the empirical richness values across clades (given that a strong relationship exists between these two variables) to see whether we could break up the strong relationship between rate and richness across the 16 clades. We tested this possibility by creating 1000 data sets in which we randomly reshuffled the empirical richness values among the 16 clades (while maintaining their original ages). We then calculated the diversification rate ( $r$ ) for each clade based on its new richness value (drawn from the empirical richness values) and the original clade age, and then estimated the relationship between these reshuffled richness values and newly estimated diversification rates across the 1000 randomized data sets. Again, we used a relative extinction fraction of 0 when estimating diversification rates. We also performed a limited set of randomizations based on simulations in which diversification rates varied among clades (see below further).

We also tested the relationships between richness and clade age and between richness and diversification rate when diversification rates varied randomly among clades. We used birth–death simulations combined with the empirical plethodontid clade ages to simulate variation in species richness among clades, with diversification rates that varied randomly among the 16 clades. We conducted three sets of simulations in which birth rates were randomly drawn from a uniform distribution and assigned to each clade. In each set of simulations, birth rates ranged from either 0.01 to 0.10, 0.01 to 0.20, or 0.01 to 0.30. These values are somewhat arbitrary, but were intended to give mean values of diversification rates that were broadly similar to the minimum, mean, and maximum values of diversification rates estimated among the 16 plethodontid clades ([Kozak and Wiens 2010](#)). Specifically, the range from 0.01 to 0.10 corresponds to a mean of  $\sim 0.05$ , and the observed minimum diversification rate of 0.03, the range from 0.01 to 0.20 corresponds to a mean of  $\sim 0.10$  and the observed estimated mean of 0.09, and the range from 0.01 to 0.30 corresponds to a mean of  $\sim 0.15$  and the observed estimated maximum value of 0.18. We note that using larger birth rates often generated extremely large clades that were very computationally challenging (especially when death rates were set to zero). For

each range of birth rates, the death rates were set at 0, 0.50 and 0.90 of the randomly chosen birth rate, corresponding to the standard, assumed epsilon values of 0, 0.5, and 0.9 used in many empirical studies. We then calculated the relationships between clade age and richness, and diversification rate ( $r$ ) and richness, for each of the simulated data sets of 16 clades. Diversification rates were estimated assuming relative extinction fractions of 0, 0.50, and 0.90. For simplicity, the relative extinction fractions used to estimate  $r$  generally matched the simulated values. However, we also explored the possible effects of using assumed extinction fractions that were very different from the simulated values. Specifically, we employed an epsilon of 0.9 when the actual epsilon was 0, and an epsilon of 0 when the actual epsilon was 0.9. We used the intermediate range of birth rates (0.01–0.20) for this latter set of simulations.

[Wiens \(2011\)](#) suggested that clade richness and rates of diversification might be uncoupled when diversification rates vary among clades such that younger clades have higher rates of diversification than older ones (as might be expected if rates are declining within clades over time due to “ecological limits,” or if younger clades have one or more key innovations or other traits that increase their diversification rates). To address this possibility, birth rates were drawn at random from a large uniform distribution (0.01–0.30) and assigned to clades so that diversification rates decreased with clade age (i.e., for each set of 16 clades, the lowest rate was assigned to the oldest clade, the next lowest rate was assigned to the next oldest clade, etc.). As described above, death rates were set to 0, 0.50, and 0.90 of the birth rate assigned to each clade. We then assessed the relationships between clade age and richness, and diversification rate ( $r$ ) and richness, for each of the simulated data sets of 16 clades.

Finally, we used these simulation to test the accuracy of the net diversification rate estimators. We specifically focused on evaluating whether the relationship between clade age and richness had any relevance for the accuracy of these estimators (i.e., the main idea behind the SR approach). We analyzed the accuracy of these estimators using the intermediate diversification rates (0.01–0.20), with 1000 replicates for each clade and each set of conditions. We analyzed conditions where diversification rates were unrelated to clade ages (i.e., rates randomly assigned to clades), and conditions where rates were inversely related to clade age (faster rates in younger clades; see above). These latter conditions are those where there is often a weak and/or negative relationship between clade age and richness (see Results section). We also examined the impact of using values of epsilon that matched the simulated values (low and high simulated and assumed epsilon values of 0 and 0.9), and values that were very different (i.e., assuming high extinction when there is no extinction, and no extinction when there is high extinction). For each set of conditions, we estimated the crown-group diversification rate for each clade. We then compared the estimated rate to the known, simulated



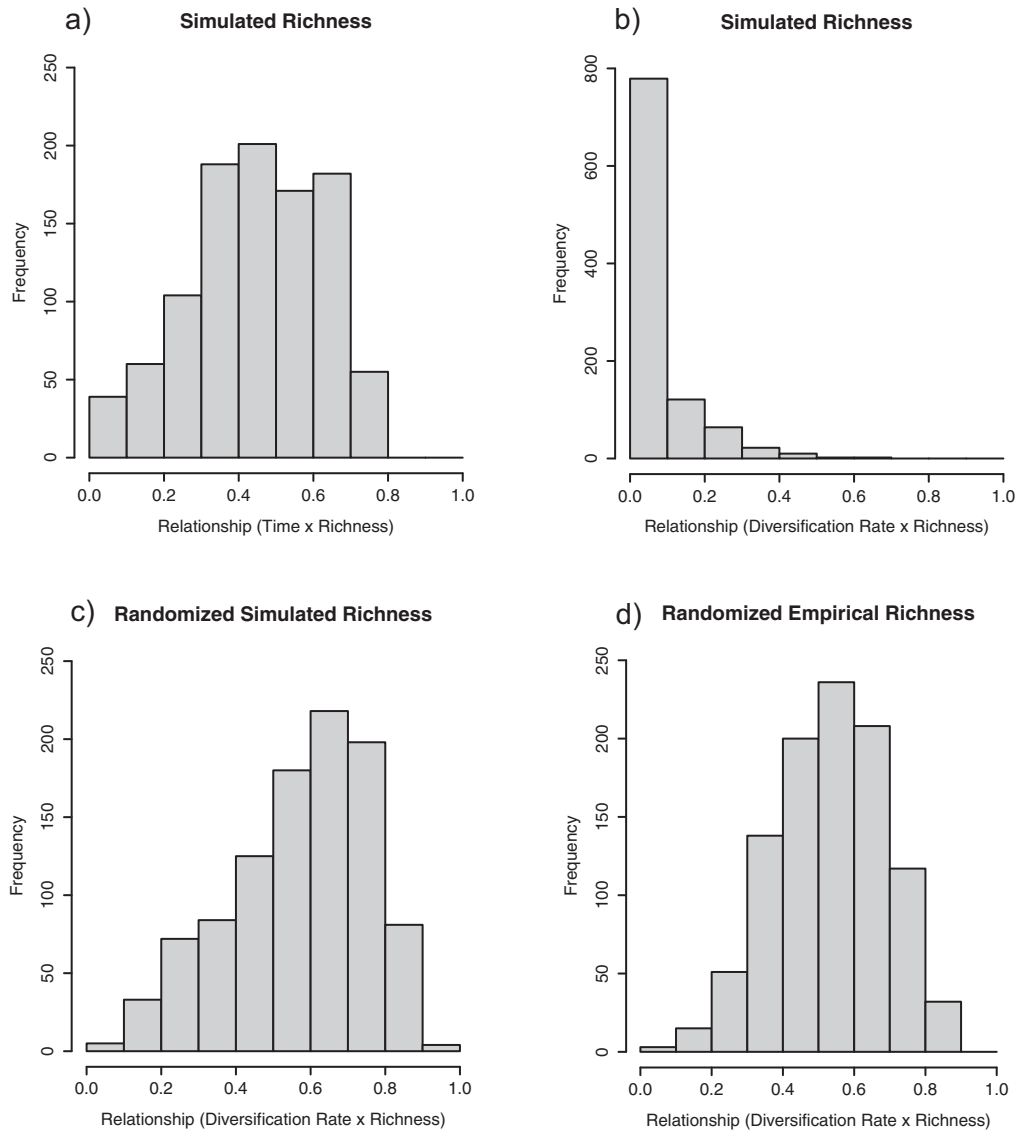


FIGURE 1. Relationships ( $R^2$ ) between clade age and richness (a) and diversification rates and richness (b–d), when diversification rates were simulated as invariant among clades (a, b), when richness values were simulated under invariant diversification rates and then randomized among clades with diversification rates then estimated from the randomized richness values (c), and when empirical richness values were randomized among clades (d).

rate and calculated the difference for each replicate for each clade. We then estimated the mean difference between the known and estimated rates for each clade for each set of conditions as the error. We used paired *t*-tests to evaluate whether the mean errors for each clade were significantly different for paired conditions (e.g., same extinction rates and epsilon values) when simulated diversification rates were random with respect to clade age (leading to a positive relationship between clade age and richness) and negatively sorted by clade age (leading to a weak and/or negative relationship between clade age and richness).

We also used regression to examine the relationships between the true (known, simulated) diversification rate and the estimated diversification rates across all 1000 replicates for each clade individually. These analyses

were performed for one representative set of conditions (birth rates vary randomly from 0.01 to 0.20 in each clade; epsilon values of 0 simulated and assumed, diversification rates random with respect to clade age).

## RESULTS

We found strong, positive relationships between clade age and richness for simulations in which clades varied in age, but had the same rate of diversification ( $P < 0.05$  in 850 of 1000 data sets; Fig. 1a). Thus, in our simulations, the only conditions in which estimates of net diversification rates would be considered consistently valid under the SR approach are conditions under which the underlying net diversification rates are actually invariant and therefore uninformative (Table 1).

In contrast, strong relationships between diversification rates and richness were not inevitable. Very few of these equal-rate simulations resulted in strong, positive relationships between diversification rates and species richness across clades ( $P \leq 0.05$  in only 31 of 1000 data sets; Fig. 1b), in contrast to the strong, positive relationship between diversification rates and species richness observed empirically among the 16 plethodontid clades.

Remarkably, randomizing the simulated species richness values across clades and then re-estimating their diversification rates based on these new richness values and their original ages yielded many data sets in which diversification rates and species richness were strongly related ( $P \leq 0.05$  in 943 of 1000 data sets; Fig. 1c). This is particularly striking because there is no underlying variation in diversification rates in this case. Randomizing the empirical species richness values across plethodontid clades also produced many data sets in which diversification rate and richness remain strongly related ( $P \leq 0.05$  in 956 of 1000 data sets; Fig. 1c). These results suggest that randomizing richness values among clades (and recalculating the clade diversification rates from the randomized values) can lead to spurious relationships between rates and richness.

In simulations where rates of diversification varied among clades, these two overall patterns were generally reversed (Table 1). First, with variable, random diversification rates among clades, clade age and richness were significantly related in only about half of the simulation replicates, with exact values depending on the specific birth and death rates employed (Fig. 2a, c, e; Table 1). These significant relationships were positive when present. Across these simulations, the  $R^2$  for the relationship between time and richness was highly variable but was most frequently very low (0–0.10; Fig. 2). Thus, our simulations show that the requirement that clade age and richness must be significantly and positively related to use estimates of net diversification rates (i.e., the SR approach) is unnecessary and potentially misleading: we find that clade age and richness are strongly related when diversification rates are invariant, and are often not significantly related when diversification rates vary among clades (but without any variation in diversification rates *within* clades).

Second, when diversification rates varied randomly among clades, we found that diversification rates and clade richness were almost always significantly and positively related, regardless of the combination of birth and death rates that were assigned to clades (Fig. 2b, d, f; Table 1). This remained true even when diversification rates were estimated with relative extinction fractions (epsilon values) that were very different from the simulated values. However, the relationship was weakened when high extinction rates were simulated but were then assumed to be low when estimating diversification rates (Table 1).

When simulated rates were varied non-randomly among clades such that younger clades had faster rates than older clades, we found that clade age and

TABLE 1. Results of simulations in which diversification rates were varied among the 16 focal clades.

Birth rate	Death rate	Age × richness	Rate × richness
0.01–0.10	0.0	480 (0.25 ± 0.19)	987 (0.52 ± 0.16)
0.01–0.10	0.5	351 (0.20 ± 0.17)	998 (0.58 ± 0.15)
0.01–0.10	0.9	349 (0.21 ± 0.18)	1000 (0.71 ± 0.10)
0.01–0.20	0.0	593 (0.26 ± 0.18)	954 (0.34 ± 0.17)
<b>0.01–0.20</b>	<b>0.0 (0.9)</b>	<b>593 (0.26 ± 0.18)</b>	<b>997 (0.54 ± 0.15)</b>
0.01–0.20	0.5	453 (0.23 ± 0.18)	1000 (0.44 ± 0.18)
0.01–0.20	0.9	389 (0.21 ± 0.19)	1000 (0.71 ± 0.11)
<b>0.01–0.20</b>	<b>0.9 (0.0)</b>	<b>389 (0.21 ± 0.19)</b>	<b>865 (0.44 ± 0.17)</b>
0.01–0.30	0.0	495 (0.18 ± 0.18)	996 (0.29 ± 0.12)
0.01–0.30	0.5	464 (0.24 ± 0.17)	990 (0.36 ± 0.17)
0.01–0.30	0.9	512 (0.22 ± 0.18)	1000 (0.62 ± 0.15)
0.01–0.30	0	42 (0.05 ± 0.08)	221 (0.11 ± 0.17)
(sorted by clade age)			
0.01–0.30	0.5	44 (0.06 ± 0.09)	236 (0.15 ± 0.18)
(sorted by clade age)			
0.01–0.30	0.9	56 (0.07 ± 0.10)	307 (0.23 ± 0.21)
(sorted by clade age)			

Notes: Shown are the simulated birth and death rates and the number of simulated data sets (out of 1000) in which clade age and richness, and diversification rate and richness, were significantly related at  $P < 0.05$ . The mean and standard deviation of the  $R^2$  values for these relationships across the simulation replicates are shown in parentheses. For age and richness, the relationship is consistently positive, unless diversification rates are sorted by clade age, with faster rates in younger clades (in which case the relationship is consistently negative). The rate–richness relationship is always positive. Note that diversification rates were estimated assuming a value of epsilon that matched the simulated value, except for two cases (boldfaced) in which the assumed epsilon (in parentheses) was very different from the simulated value.

richness were not significantly related in >94% of the simulated data sets (Table 1; Fig. 2g). In those cases in which the relationship between clade age and richness was significant, the relationship was negative rather than positive. Furthermore, as predicted by Wiens (2011), diversification rates and richness were not significantly related in the majority of these replicates (Table 1; Fig. 2h). Thus, we show that certain patterns of variation in diversification rates among clades can potentially uncouple diversification rates and richness patterns. Importantly, the weak relationships between age and richness and diversification rates and richness in these simulations did not actually arise because there were “ecological limits” on richness within these clades. Instead they arose because different clades were diversifying at different (constant) rates in a non-random pattern with respect to clade age.

We found that the age–richness relationship had little relevance to the mean accuracy of net diversification rate estimates (Tables 2 and 3). Instead, accuracy was determined more by the ages of clades, and whether the assumed epsilon values (relative extinction fraction) matched those that were simulated (Tables 2 and 3). First, across all paired conditions, we found no significant difference in the accuracy of the estimated net diversification rates between conditions in which the simulated diversification rates were random with respect to clade age (leading to a significant, positive age–richness relationship) and those in which

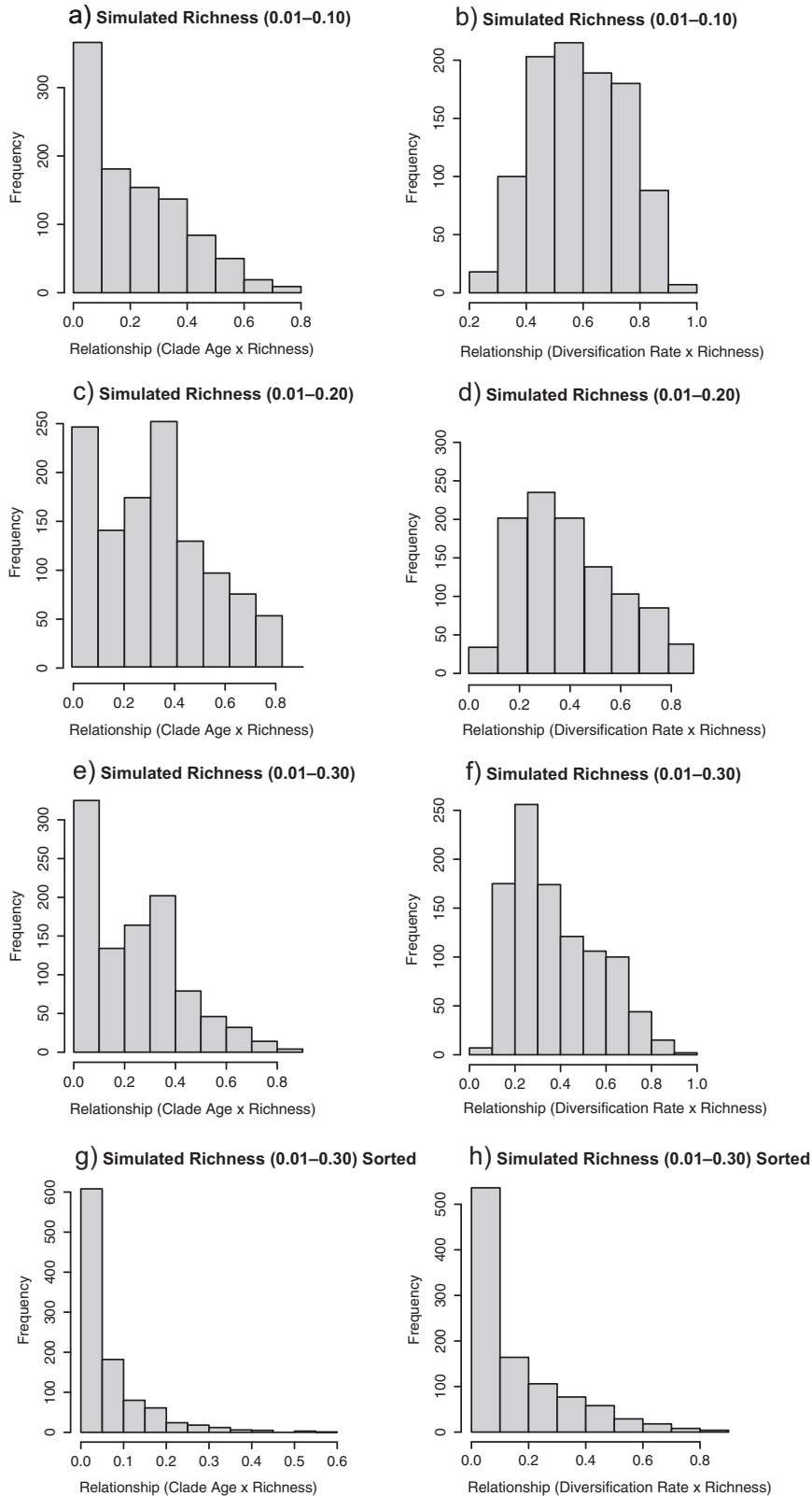


FIGURE 2. Relationships ( $R^2$ ) between clade age and richness (a, c, e, g) and diversification rate and richness (b, d, f, h) from simulations in which diversification rates were varied among clades. Rates for each clade were chosen from uniform distributions of birth rates: (a, b) 0.01–0.10, (c, d) 0.01–0.20, and (e–h) 0.01–0.30. In (g) and (h) rates and clade ages were sorted so that diversification rate was inversely proportional to clade age. For the results shown, net diversification rates were simulated (and estimated) using an epsilon of 0.5. Results based on other values for epsilon are summarized in Table 1.

TABLE 2. The mean difference between the known diversification rate and the estimated net rate, when true diversification rates vary randomly with respect to clade age.

Clade	Age (Ma)	Mean rate difference (0, 0)	Mean rate difference (0, 9)	Mean rate difference (9, 0)	Mean rate difference (9, 9)
<i>Desmognathus</i>	36.9	0.0065 ± 0.0014	0.0416 ± 0.0007	-0.0473 ± 0.0007	-0.0165 ± 0.0004
<i>Batrachoseps</i>	36.5	0.0071 ± 0.0014	0.0435 ± 0.0007	-0.0481 ± 0.0007	-0.0164 ± 0.0004
<i>Aneides</i>	30.4	0.0073 ± 0.0014	0.0470 ± 0.0008	-0.0519 ± 0.0008	-0.0170 ± 0.0004
<i>Pseudoeurycea</i>	27.6	0.0106 ± 0.0016	0.0528 ± 0.0009	-0.0549 ± 0.0008	-0.0172 ± 0.0004
<i>Gyrinophilus, Pseudotriton, Stereochilus</i>	23.4	0.0087 ± 0.0018	0.0551 ± 0.0009	-0.0588 ± 0.0009	-0.0178 ± 0.0005
<i>Eurycea</i>	22.7	0.0093 ± 0.0018	0.0574 ± 0.0010	-0.0597 ± 0.0010	-0.0180 ± 0.0005
Western <i>Plethodon</i>	20.5	0.0011 ± 0.0021	0.0601 ± 0.0010	-0.0604 ± 0.0011	-0.0181 ± 0.0005
<i>Plethodon wehrlei-welleri</i> group	19.9	0.0103 ± 0.0021	0.0606 ± 0.0010	-0.0598 ± 0.0011	-0.0182 ± 0.0005
<i>Magnidigitata, Oaxakia, Pachymandra</i>	19.4	0.0111 ± 0.0021	0.0606 ± 0.0011	-0.0649 ± 0.0011	-0.0183 ± 0.0005
<i>Bolitoglossa, Mayamandra, Nanotriton</i>	18.8	0.0099 ± 0.0022	0.0601 ± 0.0010	-0.0621 ± 0.0011	-0.0191 ± 0.0005
<i>Plethodon cinereus</i> group	18.1	0.0012 ± 0.0023	0.0632 ± 0.0011	-0.0624 ± 0.0012	-0.0181 ± 0.0005
<i>Oedipina</i>	18.0	0.0080 ± 0.0022	0.0595 ± 0.0010	-0.0646 ± 0.0012	-0.0177 ± 0.0005
<i>Chiropterotriton</i>	16.6	0.0136 ± 0.0025	0.0655 ± 0.0011	-0.0683 ± 0.0012	-0.0175 ± 0.0005
<i>Eladinea</i>	16.3	0.0108 ± 0.0023	0.0647 ± 0.0011	-0.0654 ± 0.0012	-0.0182 ± 0.0005
<i>Plethodon glutinosus</i> group	15.7	0.0122 ± 0.0024	0.0647 ± 0.0012	-0.0696 ± 0.0013	-0.0188 ± 0.0006
<i>Nototriton</i>	13.5	0.0126 ± 0.0027	0.0693 ± 0.0012	-0.0704 ± 0.0014	-0.0190 ± 0.0006
Mean error		0.0088	0.0579	-0.0605	-0.0174
Age-error ( $R^2$ )		0.098	0.973	0.944	0.737
Age-error ( $P$ )		0.2375	<0.0001	<0.0001	<0.0001

Notes: For each of 1000 simulations a birth rate was randomly chosen from a uniform distribution with values ranging from 0.01 to 0.20. Each column shows the mean difference between the two rates (calculated by subtracting the estimated rate from the true rate) across the 1000 simulations ± two standard errors. The values shown in parentheses show the actual value of epsilon used for the simulations, followed by the value of epsilon that was used to calculate the net rate from the simulated richness values. Clades are ordered from oldest to youngest. At the bottom of the table, mean errors across clades are shown, along with  $R^2$  and  $P$ -values from regressions of clade age against error among clades.

TABLE 3. The mean difference between the known diversification rate and the estimated net rate, when true diversification rates vary inversely with respect to clade age.

Clade	Age (Ma)	Mean rate difference (0, 0)	Mean rate difference (0, 9)	Mean rate difference (9, 0)	Mean rate difference (9, 9)
<i>Desmognathus</i>	36.9	0.0032 ± 0.0004	0.0152 ± 0.0003	-0.0167 ± 0.0005	-0.0042 ± 0.0002
<i>Batrachoseps</i>	36.5	0.0041 ± 0.0005	0.0222 ± 0.0003	-0.0252 ± 0.0005	-0.0064 ± 0.0002
<i>Aneides</i>	30.4	0.0054 ± 0.0006	0.0294 ± 0.0004	-0.0312 ± 0.0006	-0.0080 ± 0.0002
<i>Pseudoeurycea</i>	27.6	0.0071 ± 0.0007	0.0362 ± 0.0005	-0.0327 ± 0.0006	-0.0099 ± 0.0003
<i>Gyrinophilus, Pseudotriton, Stereochilus</i>	23.4	0.0082 ± 0.0008	0.0436 ± 0.0005	-0.0455 ± 0.0007	-0.0118 ± 0.0003
<i>Eurycea</i>	22.7	0.0091 ± 0.0009	0.0496 ± 0.0006	-0.0522 ± 0.0007	-0.0141 ± 0.0003
Western <i>Plethodon</i>	20.5	0.0104 ± 0.0010	0.0564 ± 0.0006	-0.0588 ± 0.0008	-0.0156 ± 0.0004
<i>Plethodon wehrlei-welleri</i> group	19.9	0.0108 ± 0.0011	0.0615 ± 0.0007	-0.0639 ± 0.0008	-0.0175 ± 0.0004
<i>Magnidigitata, Oaxakia, Pachymandra</i>	19.4	0.0115 ± 0.0011	0.0669 ± 0.0007	-0.0686 ± 0.0009	-0.0191 ± 0.0004
<i>Bolitoglossa, Mayamandra, Nanotriton</i>	18.8	0.0133 ± 0.0012	0.0730 ± 0.0008	-0.0745 ± 0.0009	-0.0207 ± 0.0005
<i>Plethodon cinereus</i> group	18.1	0.0142 ± 0.0012	0.0784 ± 0.0008	-0.0794 ± 0.0009	-0.0223 ± 0.0005
<i>Oedipina</i>	18.0	0.0134 ± 0.0013	0.0817 ± 0.0009	-0.0841 ± 0.0009	-0.0242 ± 0.0005
<i>Chiropterotriton</i>	16.6	0.0149 ± 0.0014	0.0890 ± 0.0009	-0.0911 ± 0.0010	-0.0264 ± 0.0005
<i>Eladinea</i>	16.3	0.0143 ± 0.0014	0.0817 ± 0.0009	-0.0947 ± 0.0010	-0.0284 ± 0.0006
<i>Plethodon glutinosus</i> group	15.7	0.0122 ± 0.0012	0.0926 ± 0.0010	-0.0696 ± 0.0012	-0.0304 ± 0.0006
<i>Nototriton</i>	13.5	0.0188 ± 0.0017	0.1099 ± 0.0011	-0.1136 ± 0.0012	-0.0326 ± 0.0007
Mean error		0.0107	0.0617	-0.0626	-0.0178
Age-error ( $R^2$ )		0.890	0.895	0.853	0.854
Age-error ( $P$ )		<0.0001	<0.0001	<0.0001	<0.0001

Notes: For each of 1000 simulations a birth rate was randomly chosen from a uniform distribution with values ranging from 0.01 to 0.20. These birth rates were then assigned to clades such that clade age was inversely proportional to birth rate (i.e., younger clades have higher rates than older clades). Each column shows the mean difference between the two rates (calculated by subtracting the estimated rate from the true rate) across the 1000 simulations ± two standard errors. The values shown in parentheses show the actual value of epsilon used for the simulations, followed by the value of epsilon that was used to calculate the net rate from the simulated richness values. Clades are ordered from oldest to youngest. At the bottom of the table, mean errors across clades are shown, along with  $R^2$  and  $P$ -values from regressions of clade age against error among clades.

diversification rates were sorted to be inversely related to clade age (leading to a weak and/or negative age-richness relationship). Specifically, for these latter conditions (rates = 0.01–0.20, rates negatively related to

clade ages), there is typically no significant relationship between age and richness (epsilon = 0: mean  $P$  = 0.10; mean  $R^2$  = 0.25; epsilon = 0.9: mean  $P$  = 0.14; mean  $R^2$  = 0.22), and observed age–richness relationships



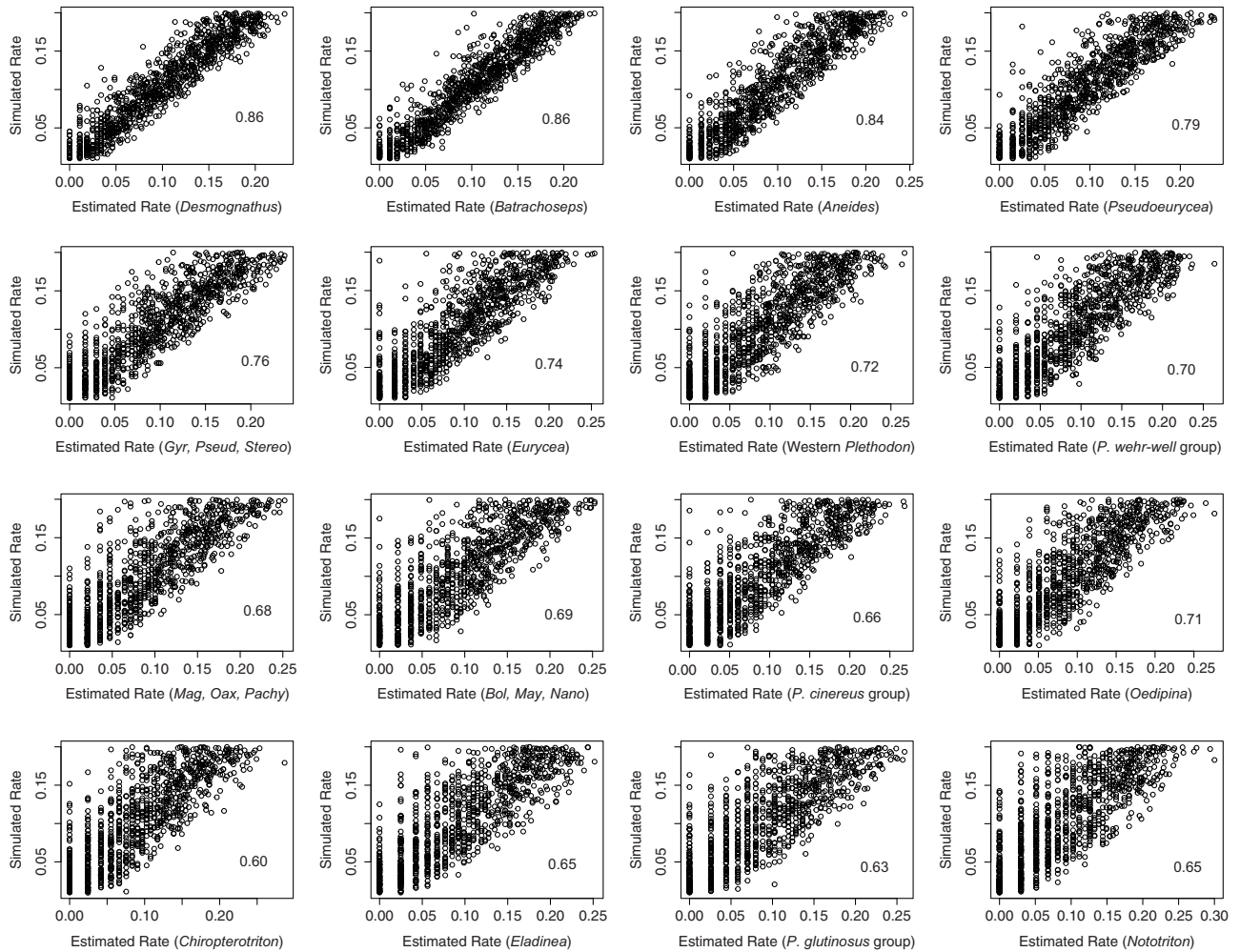


FIGURE 3. Relationships ( $R^2$ ) between the true diversification rates (used for simulating species richness) and the diversification rate estimated using the method-of-moments estimator for each of 16 clades. The simulations depicted used a birth rate ranging (uniformly) from 0.01 to 0.20 and a constant death rate of 0. When estimating the rates from simulated richness, an epsilon of 0 was used.  $R^2$  values for the relationship are shown inside each plot. Clade age decreases from the top left, to the bottom right. The full name and age of each clade is given in Table 2.

are predominantly negative (e.g., for epsilon = 0, negative coefficients in 978 of 1000 replicates). For all four comparisons of accuracy for paired clades for matched conditions (simulated/assumed epsilon values of 0/0, 0/0.9, 0.9/0, and 0.9/0.9) with positive versus negative age–richness relationships (Tables 2 vs. 3), all  $P$ -values from  $t$ -tests were  $>0.12$ . This result strongly reinforces our conclusion that a significant, positive age–richness relationship is irrelevant to the accuracy of these estimators, and should not be required for their use. Second, we found that across almost all conditions the mean accuracy of these estimators was strongly and positively related to the ages of clades (Tables 2 and 3), such that estimates for older clades were more accurate. Third, we found that accuracy also depended on whether the assumed epsilon value matched the simulated epsilon value. Specifically, when a low epsilon was simulated and assumed (epsilon = 0 and 0), then rates tended to be underestimated relative to the true values, by only ~10% on average (i.e., given a mean diversification rate

of ~0.10, the difference between the true and estimated values was 0.009 to 0.011). When the simulated extinction rate was low but a high rate was assumed, net rates were more strongly underestimated (~60%). When the simulated extinction rate was high and a high rate was assumed, then diversification rates were overestimated, by ~18%. When the simulated extinction rate was high and a low extinction rate was assumed, then diversification rates were overestimated more strongly (~60%). Our analyses across all replicates for each clade confirmed that there was a general relationship between true and estimated rates across all rates and clades, but that accuracy was much higher for older clades (Fig. 3), with much stronger relationships between true and estimated diversification rates. The only notable difference in the accuracy of the estimated diversification rates between the random diversification rates (Table 2) and the age-sorted rates (Table 3) is that for the age-sorted rates, the relationship between clade age and accuracy is more dramatic, even though the mean accuracies are similar.

Finally, we conducted randomizations of the relationship between diversification rates and richness under two conditions in which rates varied among clades (i.e., randomizing richness among clades, recalculating diversification rates with the new richness values, and then re-testing the relationship between rates and richness using the new values). First, we examined the case in which diversification rates varied randomly among clades (0.01–0.20), with an intermediate epsilon (0.5; both simulated and assumed). This yields a consistently significant and positive relationship between rates and richness (1000 out of 1000 replicates: Table 1). Second, we examined the case in which diversification rates varied more strongly among clades (0.01–0.30) and are sorted negatively with respect to clade age (again with an intermediate epsilon simulated and assumed). This only yielded a significant, positive relationship between diversification rates and richness in 24% of the replicates (Table 1). In the first case (random rates from 0.01–0.20), randomizations yielded a significant positive relationship between diversification rates and richness in 963 of 1000 replicates, with a mean  $R^2$  higher than in the original, non randomized data sets (0.59 vs. 0.44). Similarly, in the second case (rates from 0.01–0.30, sorted by clade age), relationships between rates and richness were stronger and more often significant in the randomized data sets than in the original data sets (mean  $R^2 = 0.15$  [original] vs. 0.59 [randomized];  $P$ -values significant in 236 [original] vs. 967 [randomized] data sets). These results further reinforce the idea that randomizing richness values among clades to test the validity of the rate–richness relationship can lead to spurious conclusions. In the first simulation, diversification rates clearly underlie richness, but the randomizations suggest that they do so no more than at random. In the second simulation, randomization yields a strong rate–richness relationship when such a relationship is typically absent in the original data.

## DISCUSSION

Estimates of net diversification rates are a potentially invaluable tool for testing the evolutionary and ecological drivers of speciation and species richness patterns, especially when species sampling within clades is incomplete. However, some authors have suggested that these estimates should only be used when there is a strong, positive, relationship between species richness of clades and their ages (e.g., Rabosky 2009b; Rabosky and Adams 2012; Rabosky et al. 2012). Further, some authors have suggested that it is not useful to test whether diversification rates are related to richness patterns, since a significant, positive relationship between richness and diversification rates is effectively inevitable (Rabosky and Adams 2012).

Here, we show that both of these conclusions are highly problematic. First, we show that significant and positive relationships between species richness and

clade age arise in the absence of any variation in diversification rates (i.e., constant rates among clades; Fig. 1a), whereas realistic variation in diversification rates among clades can cause this relationship to frequently disappear (Table 1; Figs. 1, 2). Thus, requiring such a positive age–richness relationship to analyze variation in net diversification rates is unnecessary and potentially misleading.

More importantly, we show that the mean accuracy of the net diversification rate estimators is similar regardless of whether the simulated conditions show a strong, positive age–richness relationship or a weak or negative one (Tables 2, 3). Again, none of the SR studies addressed how often net diversification rate estimates were incorrect (i.e., were rate estimates incorrect for every clade in a given data set, or only some?), by what magnitude (were rate estimates incorrect by 200%, 50%, or only 1%?), or in what direction (were they overestimated or underestimated relative to the true values?). Our results here may be the first exploration of the accuracy of these estimators. We discuss the details of their accuracy below.

Second, we also show that a significant relationship between diversification rates and species richness is not an inevitable, statistical artifact. We show that a significant relationship between diversification rates and richness is typically absent when we simulate no variation in diversification rates among clades (Fig. 1b) and when we vary rates among clades such that the young clades have higher rates than older ones (Fig. 2h).

### *Testing the Relationship Between Diversification Rates and Richness and the Perils of Randomization*

Given our results, it may seem surprising that Rabosky and Adams (2012) found a strong relationship between richness and diversification rates so consistently in their results. We speculate that their procedure of randomly assigning species richness and clade age values to clades seemingly generated significant variation in diversification rates, which were then (non artifactually) related to richness patterns. More generally, given the design of their simulations, we think that there was no clear reason to expect diversification rates and richness to be uncorrelated in their results. In their simulations, those authors drew clade species richness values from a uniform distribution (with no specific values given) and drew clade ages from a geometric distribution (again with no specific values given). We note that assigning different clades large differences in species richness values and small differences in clade ages should lead to data sets where differences in richness will be strongly correlated with variation in diversification rate (i.e., clades of very similar age should only differ in richness due to differences in diversification rates). In other words, given that estimated diversification rates are effectively  $r = \ln(\text{species richness})/\text{clade age}$ , and if variation in clade ages is small relative to variation in  $\ln(\text{species richness})$ , then  $r$  and  $\ln(\text{species richness})$  should be strongly correlated in every data set, but not

through any kind of error or statistical artifact. We cannot tell if this is true in the study of Rabosky and Adams (2012), since no values for the distributions of these variables were given. Nevertheless, we reiterate that it is unclear why diversification rates and richness would be expected to be uncorrelated in their results, given their design.

Our results also suggest that randomized associations between richness and clade ages should not be used to dismiss observed relationships between diversification rates and richness. If there is little or no variation in clade ages among taxa, then randomizing richness values (or clade ages) among clades and then recalculating diversification rates (as we did here) can lead to strong relationships between diversification rates and richness in the randomized data sets. Remarkably, we found that randomizing richness values among clades can lead to strong relationships between diversification rates and richness even in the absence of any underlying variation in diversification rates (Fig. 1), even though testing for a relationship between the original (non randomized) rates and richness correctly inferred that diversification rates were unrelated to richness in this case (Fig. 1b). Similarly, we found that randomizing richness values among clades that have significant (simulated) rate variation can lead to randomized data sets that also have a strong rate–richness relationship among clades. However, this does not mean that diversification rates are meaningless for interpreting richness patterns. Instead, it indicates that the randomization was problematic, because we know that diversification rates do actually explain richness patterns under these simulated conditions. We hypothesize that randomizing richness values among clades often generates associations between clade ages and species richness that could not be observed unless there actually was variation in diversification rates among the clades. Again, we strongly caution against using randomizations to dismiss observed relationships between diversification rates and richness.

On the other hand, our results do not show that strong relationships between richness and net diversification rates indicate that these net diversification rate estimators are necessarily “valid.” This claim was ascribed to Wiens (2011) by Rabosky and Adams (2012). However, Wiens (2011) merely proposed this test to deal with the potential uncoupling of diversification rates and richness due to variation in diversification rates within clades over time. In other words, despite the claims made by Rabosky and Adams (2012), Wiens (2011) merely proposed testing the richness–diversification relationship to address the richness–diversification relationship.

#### *Rate Variation in Clades Over Time*

In our simulations, all variation in diversification rates was among clades, and diversification rates are actually constant within these clades. Thus, the lack

of a significant relationship between clade age and richness in many of our simulations (Table 1) clearly did not indicate slowdowns in diversification rates within clades over time (in contrast to the conclusions of Rabosky (2009a) and subsequent papers). As an aside, we note that some authors refer to estimators of net diversification rates as “constant rate” estimators, and they might do so because of a conflation of the idea of rate constancy among clades with the idea of rate constancy within clades. We recommend that this “constant rates” terminology be abandoned to avoid further confusion. As noted in the introduction, net diversification rate estimators are actually agnostic about variation in diversification rates within clades over time (i.e., given that the net rate depends only on the clade’s age and extant richness, the net rate will be the same regardless of whether rates within the clade are constant, rapidly increasing, slowly decreasing, or some other pattern).

Neither we nor Rabosky and Adams (2012) explicitly addressed the problem of variation in diversification rates within these separate clades over time. However, we think that the most serious consequence of such temporal variation would be to uncouple richness patterns and diversification patterns (e.g., if fast rates occur only in young clades, such that high initial rates of diversification fail to yield high species richness over time). We performed a set of analyses in which we associated the youngest clades with the fastest diversification rates in each replicate (and the slowest rates with the oldest clades), before simulating richness patterns within each clade. As predicted by Wiens (2011), this pattern of variation in diversification rates and clade ages frequently uncoupled diversification rates and clade richness (Table 1). Therefore, we argue that this test should be useful for evaluating whether diversification rates are indeed related to richness patterns, since there are simulated conditions that can potentially uncouple these variables, as well as empirical ones (e.g., fast diversification rates but low richness in the high Andes, see Hutter et al. 2013).

#### *Beyond Plethodontids*

We acknowledge that our simulation results were based on the plethodontid example in terms of clade ages and diversification rates. However, we see no reason why these results should not apply more broadly, especially to older clades (note: plethodontid clades range from 14 to 37 myr old). For example, recent studies among animal phyla, major vertebrate clades, and insect orders suggest that variation in net diversification rates explains most of the variation in species richness among these clades ( $r^2 = 0.85–0.89$ ,  $0.85–0.88$ ,  $0.62–0.89$ , respectively; Wiens 2015a, 2015b; Wiens et al. 2015), with clade ages in these studies ranging from >200 myr old to >800 myr old. Thus, even if estimates of net diversification rate estimates are not perfect, they still can explain most of the variation in clade richness among some



of the most species-rich clades across the Tree of Life. This is important, because the supposed inability of net diversification rates to predict empirical richness patterns among clades was another major criterion used by Rabosky (2009a, 2010) and Rabosky et al. (2012) to reject these estimators. Our simulations here also show that such strong relationships between rates and richness are by no means inevitable.

#### *Implications for Other Studies Utilizing the SR Approach*

Our results have other important implications for studies using the SR approach. For example, the main conclusion of Rabosky (2009b) is that there are weak relationships between clade richness and clade age that are observed among many clades that cannot be explained by variable net diversification rates among clades. Similar conclusions were drawn by Rabosky (2010) and Rabosky et al. (2012) for various groups of organisms, based on simulations of net diversification rates that generated strong, positive relationships between age and richness among clades. Here, we commonly found weak relationships between age and richness of clades simply by simulating variable net diversification rates among clades (Fig. 2; Table 1). What explains the difference in these results? We most frequently found weak or negative relationships between clade age and species richness when we varied diversification rates non-randomly among clades, such that younger clades had higher rates (Table 1). In other words, we created a negative relationship between diversification rates and clade ages. Importantly, such negative relationships appear to be common in empirical data sets. For example, among the 16 plethodontid clades analyzed here, there is a strong, negative relationship between clade ages and diversification rates ( $r^2=0.337$ ;  $P=0.0185$ ; using linear regression of data from Kozak and Wiens 2010). Similarly strong, negative relationships are present across animal phyla ( $r^2=0.233$ ;  $P=0.0092$ ; using Tree1 and  $\epsilon=0.5$ ; Wiens 2015b) and insect orders ( $r^2=0.293$ ;  $P=0.0017$ ; using the Misof tree and  $\epsilon=0.5$ ; Wiens et al. 2015), but are weaker across major vertebrate clades ( $r^2=0.079$ ;  $P=0.3756$ ; Tree1,  $\epsilon=0.5$ ; Wiens 2015a). These four groups also show no significant relationships between clade age and richness (plethodontids:  $r^2=0.020$ ;  $P=0.6023$ ; animal phyla:  $r^2=0.031$ ;  $P=0.3698$ ; insect orders:  $r^2=0.042$ ,  $P=0.2700$ ; vertebrate clades:  $r^2=0.010$ ;  $P=0.7531$ ). As far as we can tell, all papers using the SR approach have simply distributed net diversification rates randomly among clades with respect to their clade ages. Had they incorporated a non-random distribution of rates among clades, they might have found a much greater match between their simulations and the empirical data sets by their criterion (i.e., no strong, positive relationships between clade age and richness). Instead, they concluded that there were errors in the net diversification rate estimators (but without actually specifying or quantifying those errors).

We note that some might conclude that the negative relationships observed between diversification rates and clade ages indicate that there are widespread “ecological limits” on richness within these groups that causes slowing diversification over time, and that this conclusion is supported by our simulations. Indeed, our simulations were intended to mimic slowing diversification over time among clades (i.e., older clades with slower rates). However, we reiterate that, in our simulations, rates within these clades are actually constant over time, not slowing. Thus, it may be problematic to infer patterns of slowing *within* clades over time based only on patterns of variation in diversification rates *among* clades. Furthermore, in the four groups mentioned above (plethodontids, metazoans, insects, and vertebrates) and the associated studies, ecological factors were identified that explained significant variation in diversification rates among their clades, including colonization of species-poor tropical regions (plethodontids), land (metazoans and vertebrates), and plants (insects). Thus, there may simply be accelerated diversification in certain younger clades that have colonized these regions, habitats, and diets (rather than slowing in older clades). Further, we recognize that limited resources (and competition for them) can have important consequences for the diversification and richness of clades (e.g., sympatry between clades slows diversification; Kozak and Wiens 2010). We merely maintain that “ecological limits” do not necessarily make net diversification rate estimators incorrect or invalid (Wiens 2011).

#### *Accuracy of Net Diversification Rate Estimators*

Our results may offer the first direct exploration of the accuracy of these net diversification rate estimators. We find several interesting results. First, these estimators appear to be more accurate when applied to older clades (Fig. 3). This pattern may occur because in younger clades the species richness expected from a particular diversification rate may not be achieved because of the stochasticity of the simulated diversification process. This stochasticity may then be ameliorated over longer time scales in older clades. Of course, “old” is relative, and these 16 plethodontid clades may be quite young relative to many other higher taxa. We also found that these estimators were more accurate when the assumed  $\epsilon$  value matched the simulated value. Unfortunately, the actual  $\epsilon$  value is rarely (if ever) known. However, it is standard practice to conduct analyses across a broad range of assumed  $\epsilon$  values (e.g., 0, 0.5, and 0.9). Our results also show some biases in these estimators, such that rates are underestimated when extinction rates are low and overestimated when extinction rates are high. Using the correct  $\epsilon$  value reduces these biases, but does not eliminate them completely. An important but unresolved question from our results is whether the apparent accuracy of these estimates is “good enough.” Our results suggest that



estimated rates are broadly correlated with the true rates (Fig. 3), such that low true rates are estimated to be low and high rates are estimated to be high, especially for older clades. This may be adequate for testing whether particular phenotypic traits are correlated with variation in diversification rates, a common application of these estimates. However, we caution against overinterpreting small differences in rates among clades, especially given the levels of error observed here (~10–20% with a correct epsilon, and ~60% without).

We note several important topics that should be addressed in future studies on the accuracy of these estimators. First, the accuracy of the stem-group estimator should be addressed, although we expect it to be broadly similar to that of the crown-group estimator analyzed here. Second, the robustness of these estimators to a broader range of conditions should be addressed (including more extreme ages and rates), and variability in diversification rates within clades (among subclades and over time). Third, the accuracy of these estimators should be compared to other relevant methods.

Finally, despite the potential shortcomings of these estimators, we note that it is unclear that a strong alternative approach is presently available that can be used instead to test the relationship between traits and diversification rates among clades, particularly when there is limited phylogenetic information within one or more clades. In support of this idea, Rabosky and Matute (2013) and Rabosky et al. (2013) used the same net diversification rate estimators (from Magallón and Sanderson 2001) that were strongly stated as being problematic by Rabosky and Adams (2012) and Rabosky et al. (2012).

#### CONCLUSIONS

In this study, we used simulations to show that strong, positive relationships between species richness and clade age should not be a requirement for using estimates of net diversification rates in evolutionary studies. Indeed, we found that the accuracy of these net diversification rate estimators was similar regardless of the age–richness relationship among clades. Further, we showed that strong, positive relationships between species richness and diversification rates are not universal or inevitable. Therefore, it is important to test whether diversification rates and richness patterns are aligned when making inferences about the causes of richness patterns. The strong relationships observed between richness and net diversification rates among clades in several recent empirical studies suggest that variation in diversification rates may be very important for explaining species richness patterns across the Tree of Life.

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