

Antipredator defenses predict diversification rates

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The “escape-and-radiate” hypothesis predicts that antipredator defenses facilitate adaptive radiations by enabling escape from constraints of predation, diversified habitat use, and subsequently speciation. Animals have evolved diverse strategies to reduce the direct costs of predation, including cryptic coloration and behavior, chemical defenses, mimicry, and advertisement of unprofitability (conspicuous warning coloration). Whereas the survival consequences of these alternative defenses for individuals are well-studied, little attention has been given to the macroevolutionary consequences of alternative forms of defense. Here we show, using amphibians as the first, to our knowledge, large-scale empirical test in animals, that there are important macroevolutionary consequences of alternative defenses. However, the escape-and-radiate hypothesis does not adequately describe them, due to its exclusive focus on speciation. We examined how rates of speciation and extinction vary across defensive traits throughout amphibians. Lineages that use chemical defenses show higher rates of speciation as predicted by escape-and-radiate but also show higher rates of extinction compared with those without chemical defense. The effect of chemical defense is a net reduction in diversification compared with lineages without chemical defense. In contrast, acquisition of conspicuous coloration (often used as warning signals or in mimicry) is associated with heightened speciation rates but unchanged extinction rates. We conclude that predictions based on the escape-and-radiate hypothesis must incorporate the effect of traits on both speciation and extinction, which is rarely considered in such studies. Our results also suggest that knowledge of defensive traits could have a bearing on the predictability of extinction, perhaps especially important in globally threatened taxa such as amphibians.

escape-and-radiate | coevolution | speciation | extinction | amphibians

The idea that defensive traits determine macroevolutionary patterns was originally suggested in the plant literature (1) to explain heightened diversity. In this hypothesis, the presence of repellent chemical defenses was proposed to open up an “adaptive zone” of diverse ecological opportunities and hence promote speciation by adaptive radiation. This became known as the “escape-and-radiate” hypothesis (2). Similarly, it has been suggested that bright coloration can reduce the constraints of hiding, enabling niche expansion and promoting diversification in animal prey (3, 4). The escape-and-radiate hypothesis is influential in the adaptive radiation literature (2, 5, 6) but has been tested surprisingly rarely (6–8). The very few macroevolutionary studies on animal defenses focus only on coloration, are small-scale, and often only consider net diversification or comparisons of species richness in relation to the defensive trait of interest (5, 9, 10). For instance, one recent study on poison dart frogs included defense-based diversification analyses as part of an examination of acoustic divergence (10). However, that study was relatively small scale, as it focused on only one group of frogs with insufficient sample sizes for some aspects of those analyses (particularly extinction estimates). Diversification consists of two processes—speciation and extinction—and recent methodological developments (11, 12) enable us to investigate each of these in relation to the evolution of a phenotypic trait. By explicitly modeling the effects of chemical defense and color variation on speciation and extinction rates separately, we are able to test the escape-and-radiate prediction

that chemical defense leads to higher diversification rates and to examine whether escape-and-radiate is able to explain the macroevolutionary effects of defense variation.

Amphibians provide an exceptionally good case study with which to test the effects of defense variation on macroevolutionary trends. The phylogenetic history of this species-rich group is now relatively well resolved (13), they inhabit ecologically diverse habitats, and include species that use a wide range of antipredator strategies (14). These defenses include chemical defense, camouflage, and conspicuous (aposematic) coloration. Importantly, chemical defense and coloration strategies are not strongly dependent on each other. On the one hand, some conspicuous species may not be chemically defended but use mimicry or are conspicuous as a result of sexual selection. On the other hand, chemical defense may be effective without advertisement, as many such species are cryptic (e.g., many toads). This therefore allows us to tease apart the effect of these two types of defense on diversification. Furthermore, amphibians are currently facing severe threats from many different sources (15), and therefore information on predictors of susceptibility to extinction is timely.

Results and Discussion

To carry out to our knowledge the first large-scale, empirical test of the escape-and-radiate hypothesis in animals, we assembled a dataset of the presence/absence of chemical defense in amphibians from the literature. Of the 2,871 species investigated, 857 had available data on the presence/absence of chemical defense. We then fit a range of trait-dependent models of diversification and compared them using Akaike’s information criterion (AIC). Specifically, the set of models we fit were BiSSE (binary state

Significance

Prey use a variety of mechanisms to avoid the risk of predation, including chemical defense, camouflage, and conspicuous coloration. Here we show that variation in these forms of protection can have profound effects on macroevolutionary patterns in amphibians. Chemical defense and conspicuous coloration both increase speciation rates, in line with the “escape-and-radiate” hypothesis. However, chemical defense also increases extinction rates, lowering net diversification. We therefore show that alternative defensive strategies have important macroevolutionary consequences but that these are only partially consistent with standard escape-and-radiate interpretations. Hence, we suggest a conceptual expansion of this highly influential evolutionary hypothesis. In addition, our findings may have implications for endangered species conservation by allowing some predictability of extinction risk from knowledge of antipredator defenses.

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Table 1. Model selection for the influence of chemical defense on diversification

Model	K	AIC	logLik	Δ AIC	w	ER	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}
Full	6	8592.83	-4290.42	0	0.989	1	0.1412	0.2836	0.0846	0.2632	0.0227	0.0010
Null	3	8623.05	-4308.53	30.22	<0.001	3.66e+06	0.2229	—	0.1906	—	0.0061	—
Equal diversification	4	8602.95	-4297.48	10.12	0.006	157.65	0.2255	—	0.1934	—	0.0096	0.0037
Equal speciation	5	8604.93	-4297.46	12.10	0.002	423.64	0.2222	—	0.1900	0.1898	0.0095	0.0037
Equal extinction	5	8604.53	-4297.27	11.70	0.003	347.89	0.2181	0.2221	0.1876	—	0.0088	0.0038
Random trait (min; max)	6	8800.23; 8936.67	-4462.34; -4394.12	207.40; 343.84	<0.001; <0.001	1.09e+45; 4.62e+74						

ER, evidence ratios (evidence for the best model/evidence for each model); K, number of parameters; logLik, log likelihood; w, Akaike weights (model probabilities); λ , μ , and q , speciation, extinction, and transition rates, respectively, for species where chemical defense is absent (0) or present (1). Transition rates are denoted such that q_{ij} is the transition rate from state i to state j .

speciation and extinction) models that assumed different influences of the chemical defense on diversification.

In the best-supported model from our analyses, both speciation and extinction rates differ between chemically defended and nonchemically defended amphibians (Table 1). The speciation rate for chemically defended lineages is approximately twice as high as that for nonchemically defended lineages, supporting a major prediction of the escape-and-radiate hypothesis. Unexpectedly, however, extinction rates are three times higher in lineages with chemical defense than without it (Fig. 1). As a consequence of this, we found that chemically defended lineages had a reduction in net diversification rate compared with nonchemically defended lineages. This result is not anticipated by the escape-and-radiate hypothesis, suggesting that the hypothesis may be adequate to predict effects on speciation but inadequate to predict effects on diversification as a whole. We further note that chemical defense was gained at a rate an order of magnitude (\sim 10- to 20-fold) higher than it was lost (Fig. 1 and Table 1). Our results are consistent with the observation that many species-rich amphibian families with a large proportion of threatened species also have a large number of chemically defended species (e.g., Bufonidae and Ambystomatidae) (15).

To enable an investigation of protective coloration strategies in addition to chemical defenses, we assembled a dataset of cryptic versus conspicuous coloration in amphibians from the

literature. We also included polymorphism in our dataset for species that had both cryptic and conspicuous color patterns within or between populations. Of the 2,871 species investigated, 2,683 had available data on coloration. We fit a similar range of trait-dependent models of diversification as with chemical defense, and again compared them using AIC. In this case the models we fit were MuSSE (multistate speciation and extinction) models, because our dataset had more than the two categories allowed for BiSSE.

The best-supported model is one in which coloration influenced only speciation rate but not extinction rate (Table 2). The speciation rate for conspicuous species is two to three times higher than that for species with cryptic coloration (Fig. 2). Because extinction rates were equal between coloration strategies, we found that net diversification rate was also higher in conspicuous than cryptic species (Fig. 2). Polymorphic species had speciation rates equivalent to that of cryptic species (Fig. 2). Hence, the macroevolutionary consequences of cryptic coloration and polymorphism are approximately equal. These results strongly support the hypothesis that conspicuous coloration increases speciation and net diversification rates compared with other forms of protective coloration. Our results for our coloration dataset are therefore in line with predictions from the escape-and-radiate hypothesis (3–5, 9).

It is perhaps counterintuitive to find higher rates of speciation and diversification in conspicuous compared with cryptic lineages, considering that a relatively small proportion of amphibians are unambiguously conspicuous (*Materials and Methods*). However, this is explained by the transient nature of conspicuousness. While it is present it increases diversification, but it is usually short-lived and reverts to cryptic frequently. In support of this interpretation, we found that transition rates away from conspicuous coloration were higher than transition rates to conspicuous coloration by a factor of 2 when shifts are to or from polymorphism and a factor of 50 when shifts are to or from cryptic coloration (*SI Appendix, Fig. S1 and Table S2*). Furthermore, transition rates from polymorphic to cryptic coloration are 3.5-fold higher than from polymorphic to conspicuous coloration, indicating that polymorphic lineages are 3.5 times more likely to lose the conspicuous morph than the cryptic ones. Consequently, the rarity of conspicuous species is a result of frequent shifts back to cryptic coloration. However, while the lineage is conspicuous it is expected to experience radiations, although these are usually relatively small scale due to limited time in this state. This is likely to have been the case in groups that include many of the conspicuous species in our dataset, such as Dendrobatidae and *Mantella* (48 and 10% of unambiguously conspicuous species, respectively). Nevertheless, we stress that despite a predominance in certain groups, conspicuousness has arisen many times across amphibian phylogeny including in caecilians, salamanders, and frogs.

Dendrobatidae are therefore unusual among conspicuous species only in the extent of their radiation. This in itself could be

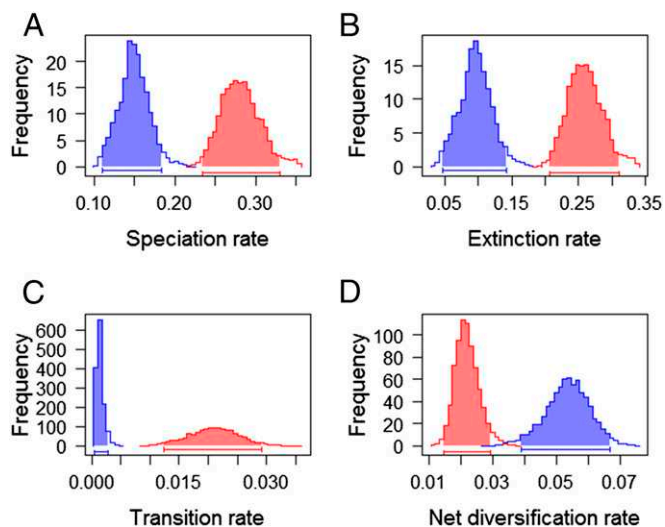


Fig. 1. Posterior distributions of parameter estimates for speciation (A) and extinction (B) rates of chemically defended (red) and nonchemically defended (blue) amphibians and for transition rates for gain (red) and loss (blue) of chemical defense in amphibians (C). Net diversification rate (colors as in A and B) is shown (D). Lines immediately beneath each distribution are 95% confidence intervals.

Table 2. Model selection for the influence of coloration on diversification

Model	K	AIC	logLik	Δ AIC	w	ER	λ_0	λ_1	λ_2	μ_0	μ_1	μ_2
Full	20	24969.67	-12464.8	3.62	0.141	6.12	0.0614	0.0535	0.1472	0.0000	0.0027	0.0658
Null	3	25364.48	-12679.2	398.42	<0.001	3.29e+86	0.0675	—	—	0.0143	—	—
Equal diversification	14	25138.05	-12555.0	172.00	<0.001	2.23e+37	0.0676	—	—	0.0145	—	—
Equal speciation	17	25065.12	-12515.6	99.07	<0.001	3.26e+21	0.0636	—	—	0.0010	0.0515	0.0000
Equal extinction	17	24966.05	-12466.0	0	0.859	1	0.0614	0.0493	0.1093	0.0000	—	—
Random trait (min; max)	17	25476.80; 25774.77	-12870.4; -12721.4	510.75; 808.72	<0.001; <0.001	1.32e+110; 6.68e+174						

λ and μ , speciation and extinction rates for cryptic (0), polymorphic (1), and conspicuous (2) species. Transition rates are not shown here for clarity; see *SI Appendix, Table S2* for further details. Diversification parameters for uncertain states are not shown but were estimated in the models.

explained by mere stochasticity, or alternatively by certain characteristics of this lineage that may have acted to enhance diversification further in dendrobatids. For instance, the interaction of natural and sexual selection with a dynamic demographic history including expansion and population isolation has been suggested to influence diversification patterns in these frogs (16, 17). In addition, many of these species have specialist diets that have been considered to have a diversifying effect on the group (18, 19). Nevertheless, we used sister clade analyses to rule out the possibility that our coloration results are driven largely by particular attributes in clades such as Dendrobatidae that dominate the conspicuous category. These tests are not susceptible to false positives resulting from single large clades, and are discussed in the following paragraph.

We also note that BiSSE and MuSSE analyses may falsely find an effect of a trait if the pattern is driven by only one or a few large clades and can be susceptible to different diversification patterns across the tree (20). To ensure our results are robust to these potential issues, we corroborated our results with a relatively powerful sister group analysis: the richness Yule test (see *SI Appendix* for further details). This method compares sister clades in which one clade contains only species with the trait of interest and its sister contains only species that lack the trait. Sister group analyses consider each clade as a single data point and so are not vulnerable to results dominated by a few large clades, as they require a consistent finding across multiple clades to yield a significant result. In all cases, we find that our results are supported ($P < 0.05$) by these analyses, which strengthens our conclusions derived from BiSSE and MuSSE modeling. Specifically, sister group analyses found that chemically defended clades had lower diversification rates than nonchemically defended clades, and conspicuous clades had higher diversification rates than cryptic clades.

Analyzing defensive traits separately as we have done leaves open the possibility that chemical defense and coloration interact to affect diversification rates. This could occur due to

synergetic benefits of chemical defense combined with conspicuous “warning” coloration in an aposematic display. However, when we explicitly model an interaction between chemical defense and coloration, we find that the best models support limited or no interactions on diversification (*SI Appendix, Table S1*). Specifically, there are two best models. Neither of these has an interactive effect on extinction rates, and the difference between them is that one also lacks an interactive effect on speciation rates (*SI Appendix, Table S1*). In addition, although we found a positive correlation between the presence of chemical defense and conspicuous coloration (*SI Appendix*), these traits influence diversification in different ways (cf. Figs. 1 and 2). Combined, this evidence strongly indicates that our results for chemical defense are not being driven by conspicuous coloration and vice versa. Our results are consequently the first, to our knowledge, to reveal that different antipredator strategies can have independent effects on speciation and extinction.

The escape-and-radiate hypothesis predicts that chemical defense should lead to higher diversification rates. Our results show that the hypothesis, which is widely cited and used, requires revision because of its failure to account for effects on extinction rates. We therefore propose that escape-and-radiate should be seen as one component of a more general hypothesis for the macroevolutionary effects of antipredator defense that include both speciation and extinction. In this framework, the escape-and-radiate hypothesis is a special case when an increased extinction rate conferred by the trait is of smaller magnitude than the increase in speciation rates, or when there is either no effect on extinction rate or it is decreased. However, when a trait leads to a greater increase in extinction than speciation rates, as we find for amphibian chemical defense, net diversification rate is decreased in contrast to predictions from the escape-and-radiate hypothesis. Therefore, our observations in the present study suggest that the escape-and-radiate hypothesis can be misleading if the extinction component of diversification is ignored.

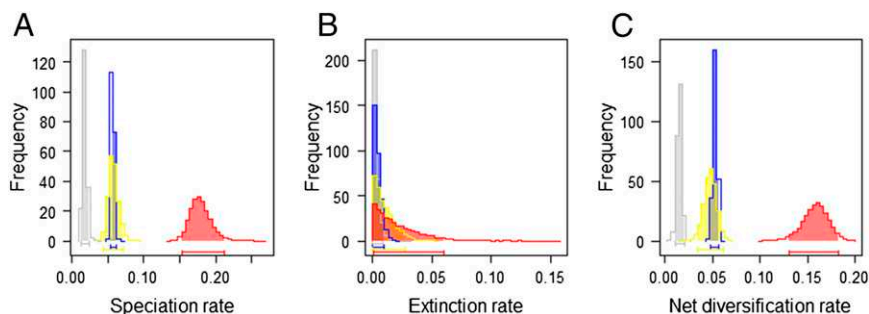


Fig. 2. Posterior distributions of parameter estimates for diversification of amphibians using different protective coloration strategies: Conspicuous species are in red, cryptic species are in blue, polymorphic species (those with both conspicuous and cryptic forms within or between populations) are in yellow, and species with uncertain strategies are in gray (these were not used for inference). (A) Speciation rates. (B) Extinction rates. (C) Net diversification rates. Lines immediately beneath each distribution are 95% confidence intervals.

The escape-and-radiate hypothesis predicts that effective chemical defense opens up adaptive zones in terms of more niches available for colonization, which ultimately leads to raised speciation rates. The addition of bright coloration implies that prey show a reduction in predator-induced “hiding” behavior and hence greater use of opportunities in a habitat. This in turn may explain the rise in speciation rates, because ecological opportunity can promote speciation (8, 21). In effect, conspicuousness could be an indicator of this mechanism rather than directly influencing diversification. Alternatively, bright coloration is often associated with mate choice such that sexual selection can act on variation in particular color patterns to drive heightened speciation via conspicuous sexual signals (22). For example, in the polymorphic dendrobatid frog *Dendrobates pumilio*, males in conspicuously colored populations are more aggressive in male–male contests and more explorative than those in cryptically colored populations (23). This could readily facilitate divergence via intrasexual competition and intersexual mate preferences. A recent study on birds has also shown that color polymorphic lineages have higher speciation rates (24), further highlighting the role that sexually selected color patterns can play in raising speciation rates.

How can defenses lead to heightened extinction rates? We suggest four mechanisms that could account for this result. First, chemical defense may impose particular kinds of costs that render prey populations vulnerable to other kinds of enemies such as infectious diseases. This is illustrated in the case of a nymphalid caterpillar in which investment into chemical antipredator defense decreases immune function (25). Second, chemically defended species may radiate by moving into habitats with low carrying capacities. Such habitats may be resource-poor, and effective defenses could help animals to better exploit such environments by reducing the need to avoid predators. Although hypothetical, if this is the case, then moving into areas with low carrying capacities may make species intrinsically vulnerable to extinction. Third, recent comparative work shows that chemical defense may cause a shift toward slower life histories. This could in turn weaken the resilience of the population to detrimental environmental change (26, 27) and raise extinction rates. Fourth, ecological correlates of chemical defense such as diet specialization in Dendrobatidae (28) may be linked to a greater propensity for extinction. This would lead to raised extinction rates in chemically defended species as a corollary, as suggested for specialization in other taxa (29). At present, the data required to tease these potential explanations apart are not available in a compiled format that would allow this to be done. However, the alternatives above provide a plausible set of hypotheses to explain the strongly supported patterns we find here.

We also suggest that conspicuousness confers a benefit to chemically defended lineages on an evolutionary timescale by offsetting the increased extinction risk. Because effects of chemical defense and conspicuous coloration on diversification are independent, when a chemically defended species becomes conspicuous the increased diversification rate conferred by the coloration may compensate for the reduced diversification rate conferred by the chemical defense. This could partly explain the positive correlation between the presence of the two traits (*SI Appendix, Fig. S2 and Table S4*), because chemically defended lineages without such “diversification compensation” are more likely to die out than those that also possess conspicuousness. Therefore, a higher proportion of extant chemically defended species would be conspicuous than we would expect based on independent evolution of the traits without this diversification compensation effect, as such species are less likely to have gone extinct. Under this scenario, the disproportionate distribution of conspicuousness among chemically defended species should manifest itself in the positive correlation that we observe.

Our results also suggest that chemically defended species, all else being equal, may justifiably be targeted by conservation programs due to their higher extinction rates. This is perhaps especially the case for those species lacking conspicuous coloration. However, practical application of such a strategy relies on the background extinction rates estimated herein being representative of contemporary extinction risks. This may or may not be the case, and likely depends on the underlying explanations for the patterns observed in macroevolutionary extinction rates. For instance, if the cause is a slowdown in life history in chemically defended species, then when those species are faced with a threat such as habitat loss or disease they may particularly struggle to recover their populations in the aftermath. Consequently, in that situation, they may require more conservation effort than an equivalent nonchemically defended species. Nevertheless, there is considerable further research needed to establish whether our results could be used to inform conservation plans.

Many traits and processes are likely to influence diversification in any animal group. For instance, previous studies have found that factors such as latitude (13, 30) and the rise of angiosperm forests (31) (and consequent diversification of prey) are among those that have influenced the diversification of amphibians. We acknowledge that if these traits substantially covary with defensive traits, then the contributions of each trait to diversification would be difficult to disentangle, a caveat of all studies of this kind. However, we note that our aim here was not to investigate the relative importance of antipredator defense as an influence on amphibian diversification compared with other traits. Rather, we set out specifically to examine predictions of a highly influential macroevolutionary hypothesis for such defenses. We therefore do not wish to claim that antipredator strategies are the only, or most important, factor influencing diversification, but rather that it is a contributing factor that sheds light on our understanding of the evolutionary consequences of natural enemy interactions.

In summary, we report to our knowledge the first large-scale test of the diversification predictions of the escape-and-radiate hypothesis in animals. Although there is strong support for its central prediction of heightened speciation rates, we find that it is a special case within a more general framework of defense-driven diversification that incorporates both speciation and extinction. Specifically, predictions of the escape-and-radiate hypothesis hold for antipredator defenses, providing that the defensive trait does not raise extinction rates or that it raises extinction rates by a smaller margin than it raises speciation rates. Finally, we stress that, overall, antipredator defense often consists of multiple components, which can have independent and contrasting effects on diversification. Only large-scale investigations such as this study will be able to elucidate the overall impact on evolutionary diversification.

Materials and Methods

We used a recent, relatively comprehensive, and well-resolved phylogeny of amphibians (13) for this study that includes over 40% of currently known amphibian diversity spanning all major clades. We then assembled a large dataset by searching published literature for information on the presence or absence of chemical antipredator defenses and for images from which to assess coloration for each of the 2,871 species contained in the phylogeny. Chemical defense data were available for 857 species and coloration data were available for 2,683, providing a large and well-sampled dataset for all analyses contained herein.

Data Collection. We used both searchable literature and hard-copy books to obtain data on chemical antipredator defense for all amphibian species for which we had phylogenetic data (i.e., that were included in the tree). For searchable literature, we used the following search terms on a range of online literature databases and search engines such as Web of Science and Google Scholar (where “species” was substituted for the name of each species in turn): (“species” OR “synonyms”) AND (“chemical defense” OR toxin OR venom OR poison OR “skin secretion”).

Because amphibian taxonomy has been revised a great deal, we included all nomenclatural synonyms in the first part of the search term, replacing “synonyms” in the search term above. Synonymy lists were obtained from the Amphibian Species of the World database. Our search term was designed to generate a broad search so as not to exclude any literature. Every hit was then inspected manually to extract any information on the presence or absence of chemical defenses contained in each publication. In other words, we recorded data from literature where species were found to possess a chemical defense or where no such defenses were found after investigation by a set of authors. Of the 857 species for which we found data on chemical defense, 35% were found not to possess chemical antipredator defense whereas 65% did. The defenses themselves can be either biosynthesized by the amphibians, sequestered from the diet, or both. A full list of all publications from which we obtained data is available in *SI Appendix*.

Judgments of coloration can be dependent on the observer’s visual system and other biological and situational factors, making them unavoidably subjective to some degree. However, visual classification by human observers is a commonly used method to study animal color patterns in the literature (10, 19, 28). Perhaps most notably, coding of coloration based on the human visual system cannot account for UV colors (32), although we acknowledge that UV could also contribute to diversification dynamics. The detailed spectrographic data required to investigate this fully were neither available nor feasible to collect on this scale, but it is likely that useful information can be obtained from visible (to the human eye) colors. Indeed, the fact that we found associations between our color categories and diversification in this study strongly suggests that our human-biased scorings are meaningful. Nevertheless, we took steps to ensure our coloration scores were as robust as possible. First, we began with an extensive a priori definition of our basic coloration categories (cryptic or conspicuous), which was as follows.

A species is deemed to have conspicuous coloration if it possesses bright or contrasting patterns that create a distinctive appearance that draws the attention of the observer, at least at close range. This often involves a combination of black with bright yellow, red, green, blue, or white, although single bright colors may also be judged conspicuous if they appear to make the animal stand out against its typical environment. In contrast, species were rated as cryptic when their color pattern renders them subjectively camouflaged, often consisting of brown, green (in arboreal species), or mottled patterns. Because some bright patterns may function as disruptive coloration and thus provide camouflage, where this is suspected, coloration is recorded as “uncertain” to remain conservative. Similarly, polymorphic species with both cryptic and conspicuous morphs were recorded as such.

We then randomly selected 50 amphibian species using a random number generator and obtained photos of each of these. This set of 50 photos was given to three observers to score independently as either cryptic or conspicuous, along with the definition above. Note that only these two codings were allowed, not uncertain, and so this initial assessment of interobserver variability is less conservative than the actual procedure used during data collection. Nevertheless, full agreement was found for ~95% of species, and so coding of coloration was at least consistent among human observers and therefore not overly subjective. Consequently, data collection for both chemical defense and coloration was undertaken by one observer. Photos were obtained from various literature and online sources as well as directly from live animals as available for each species. As many photos as possible were obtained for each species to assess the variability within a species. This was to ensure that we were able to detect polymorphism, which we recorded as such if some color forms were conspicuous and others were cryptic. Although we used a diverse search strategy, major online sources of images included AmphibiaWeb, Arkive, www.iucnredlist.org, and Google Images. Of the 2,683 species for which we found data on coloration, 88% were judged cryptic, 4% were conspicuous, 6% were polymorphic, and 2% were considered uncertain. For some supplementary analyses to ensure results were robust to uncertainty in evaluation of color categories, we recoded ambiguous species first as cryptic and then as conspicuous (see below for details). Coding ambiguous species as cryptic gave 96% cryptic and 4% conspicuous species in the dataset, whereas coding ambiguous species as conspicuous gave 88% cryptic and 12% conspicuous species. Results from these alternative codings, which represent both extremes of bias, are consistent with our main results (see below and *SI Appendix, Table S3*). This suggests that our findings are robust to such uncertainty in color pattern coding. Data used in the analyses in this paper are available as a csv file from [dx.doi.org/10.6084/m9.figshare.1248938](https://doi.org/10.6084/m9.figshare.1248938).

Diversification Models. We used a model-based inference approach (*sensu* ref. 33) based on extensions and modifications of binary state speciation and extinction models (11). The extensions of the BiSSE model used in this study

allow analyses of characters with more than two states (multistate speciation and extinction), testing for interactions of different traits on diversification and accounting for incomplete sampling (12). All diversification models were fit in R package *diversitree* version 0.9-7 (12).

BiSSE models fit speciation (λ) and extinction (μ) rates for each state of a binary character, giving four diversification parameters in total: λ_0 , λ_1 , μ_0 , and μ_1 . Transition rate parameters are also estimated for the rate of shift from state 0 to state 1 (q_{01}) and from state 1 to state 0 (q_{10}). Therefore, the full BiSSE model contains six parameters, which can then be constrained to test particular hypotheses. MuSSE models are simple extensions to BiSSE whereby speciation and extinction rates are estimated for more than two states and transition rates are estimated for shifts between all states.

MuSSE multitrait models, which we use here to test for interactive effects of chemical defense and coloration on diversification, use a different parameterization that is more akin to a general linear model framework. A “background” rate for speciation and extinction equivalent to the intercept in a linear model is estimated. Coefficients representing changes attributable to trait 1 (i.e., chemical defense) and trait 2 (i.e., coloration) are estimated, as are coefficients representing an interaction between the two traits. Finally, as with BiSSE and MuSSE models, transition rates between each combination of states are also estimated.

There has been a call for caution when estimating extinction rates from molecular phylogenies (34). However, all of the models we implemented have been shown to give accurate estimates in simulations, provided that sample sizes are sufficiently large (over ca. 400 species for extinction rates, which are the most difficult parameters to estimate) (11, 35). Sample sizes for all analyses in this paper were far in excess of those required to derive accurate estimates, as we had 2,871 species in the phylogeny in total, 2,683 with data for coloration and 857 with data on chemical defense.

For both chemical defense (BiSSE) and coloration (MuSSE) data, we fit a set of five models designed to test whether and how these traits influence diversification of amphibian lineages: (i) a “full” model including all parameters with no constraints; (ii) a “null” model assuming no influence of the trait and equal transition rates with all speciation, extinction, and transition rates constrained to be equal; (iii) an “equal diversification” model assuming no influence on speciation or extinction but allowing unequal transition rates with all speciation and extinction rates constrained to be equal; (iv) an “equal speciation” model assuming an influence on extinction but not speciation with all speciation rates constrained to be equal; and (v) an “equal extinction” model assuming an influence on speciation but not extinction with all extinction rates constrained to be equal. In all cases, we accounted for missing species by including information on the proportion of amphibian species sampled and for missing data by assuming that the proportion of species in each state for a given trait was equal to the observed frequencies. This approach is commonly used in such analyses, but assumes random sampling of species. To assess whether this assumption is reasonable, we tested for a correlation between total species richness and sampled species richness across the 74 currently recognized families of amphibians. In line with this assumption, we found strong correlations of these measures in both our chemical defense ($r = 0.76$, $t = 9.78$, $P = 7.6e-15$) and coloration ($r = 0.94$, $t = 23.19$, $P < 2.2e-16$) datasets.

Each of the above models was fit with maximum likelihood (ML), and comparisons were made within each model set using an information theory approach. The evidence for each model was quantified using Δ AIC scores (difference in Akaike information criteria between each model and the “best” one as defined as that with the lowest AIC score), model probabilities (or “Akaike weights”), and evidence ratios (the ratio of model probabilities for the best model compared with each other model in turn). Although there is limited consensus on exact values, a Δ AIC of 3–5 is often considered reasonable support for one model over another, 5–10 is often considered strong support, and >10 is often considered very strong support. This approach provides an explicit and ready means of comparing the strength of support for the models within our model sets.

Because a large phylogeny will almost always have high heterogeneity in diversification rates (36), it is possible that any “multirate” model (such as BiSSE or MuSSE) will be favored over a single-rate model such as the null models described above, even for an arbitrary trait. To rule out this possibility and provide additional evidence that our results are indeed a consequence of the traits in question, we used a randomization approach. Specifically, we randomly distributed a trait with the same properties (i.e., frequency distribution and number of states) as our observed data over the tips of the phylogeny 100 times. For each of these 100 randomly distributed traits, we fit the best model and list the minimum and maximum values obtained for our model comparison statistics. Such an approach makes use of an identical trait in all senses other than its association with diversification rates (as a result of decoupling

the trait from the tree structure) to separate arbitrary preference of multirate models from the preferred multirate model for our observed data.

To ensure our coding of coloration was robust given the subjective element of judging conspicuousness, we recoded all ambiguous species first as though they were cryptic and then as though they were conspicuous. We then followed the same analytical procedure as above using BiSSE models. This enabled us to investigate both extremes of bias: toward either cryptic or conspicuous judgments. The results from these analyses were qualitatively identical to the MuSSE models using our original coding scheme, and are presented in *SI Appendix, Table S3*.

To incorporate uncertainty in parameter estimates and therefore allow a more robust inference from our models, we also fit the full models using Markov chain Monte Carlo (MCMC). Our MCMC analyses essentially followed the guidelines in the diversitree manual and help files (cran.r-project.org/web/packages/diversitree) and used an unbounded prior but with informative starting parameters (the ML estimates). We initially ran a Markov chain for 1,000 steps to optimize the step size and subsequently used this optimized value in the final MCMC run of 15,000 steps. We conservatively discarded the first 5,000 posterior samples for further analysis and so used the posterior distributions of the last 10,000 MCMC samples for inference. However, we note that qualitatively identical results were achieved using the entire posterior sample in both the BiSSE and MuSSE models. The posterior distributions were then visualized along with their 95% confidence intervals to allow intuitive and robust interpretation of the results.

For our MuSSE multitrait models, we adopted a different model set to reflect our different aim, that is, to assess evidence for an interactive effect between chemical defense and coloration on diversification rather than to infer each trait's influence. We fit a set of four models using ML wherein constraints were only imposed on the interaction coefficients: (i) a model

including all interactions with no constraints; (ii) a model assuming no interactive effect on diversification with the interaction coefficients for speciation and extinction rates constrained to equal zero; (iii) a model assuming no interactive effect on speciation but allowing for one on extinction with the interaction coefficient for speciation constrained to equal zero; and (iv) a model assuming no interactive effect on extinction but allowing for one on speciation with the interaction coefficient for extinction constrained to equal zero. Models were compared using the same information theoretic approach as for our BiSSE and MuSSE models described earlier.

We note that a recent paper has highlighted that significant results can be obtained with the BiSSE class of models when the trait has only one or a few origins in the phylogeny, even when no significant effect of the trait on diversification exists (20). However, chemical defense has originated many times independently across the phylogeny (*SI Appendix, Fig. S3*), and coloration similarly has many origins. Such a distribution, combined with our sister group analyses, which are not susceptible to this issue, suggests that our analyses are robust to the problems highlighted here. We also note that we used information theory rather than *P* values for inference, and so our results are less likely to be misinterpreted based on highly significant but not very informative model outputs, a problem in any large dataset.

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Supplementary Material for "Antipredator Defences Predict Diversification Rates"

Table S1 - Model selection table for MuSSE multitrait models looking for an interactive effect of chemical defence and colouration on diversification.

Table S2 - Transition rate parameters from the full MuSSE model investigating the influence of different colouration strategies on diversification.

Table S3 - Model selection table for BiSSE models on colouration when ambiguous species were coded as either cryptic or conspicuous in order to examine the robustness of our results to our coding scheme.

Figure S1 - Posterior distributions of the transition rate parameters from MCMC analysis of the full MuSSE model investigating the influence of different colouration strategies on diversification.

Correlated evolution between chemical defence and colouration - additional analyses demonstrating the correlated evolution of these traits, despite their independent effects on diversification.

Sister group comparisons - results from richness Yule tests comparing the diversity of sister clades in which one sister has a trait and the other lineage does not.

Figure S3 - Ancestral state reconstruction of chemical defence.

Data references - References from which the data were obtained.

Table S1 – Model selection table for the interactions between chemical defence and colouration (cryptic versus conspicuous) on diversification. K = number of parameters; AIC = Akaike information criteria; Δ AIC = difference in AIC from the best model; w = Akaike weights (model probabilities); ER = evidence ratios (evidence for the best model/evidence for each model).

Model	K	AIC	logLik	ΔAIC	w	ER
All interactions	15	8807.67	-4388.84	52.36	<0.001	2.35E+11
No diversification interaction	13	8757.31	-4365.65	2	0.269	2.71
No speciation interaction	14	8824.80	-4398.40	69.49	<0.001	1.23E+15
No extinction interaction	14	8755.31	-4363.66	0	0.731	1

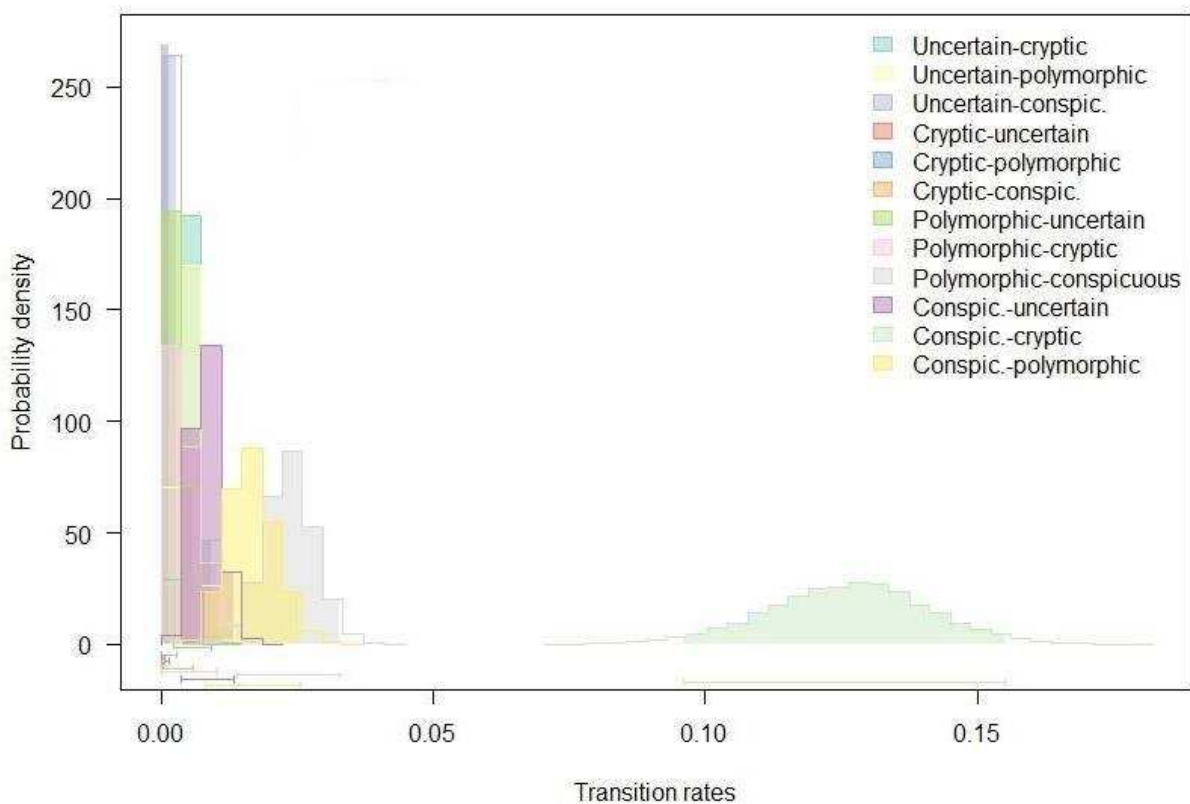
Table S2 – Maximum likelihood estimates of the transition rate parameters estimated in the full MuSSE model for colouration. See Figure S1 for posterior distributions of these same parameters as estimated by MCMC.

Transition from	Transition to	ML estimate
Uncertain	Cryptic	0.0055
	Polymorphic	0.0048
	Conspicuous	0.0001
Polymorphic	Uncertain	0.0036
	Cryptic	0.0209
	Conspicuous	0.0059
Cryptic	Uncertain	0.0006
	Polymorphic	0.0015
	Conspicuous	0.0003
Conspicuous	Uncertain	0.0079
	Polymorphic	0.0113
	Cryptic	0.0150

Table S3 – Model selection table for the influence of colouration on diversification when ambiguous species are coded as cryptic (con0 model set) or conspicuous (con1 model set). K = number of parameters; AIC = Akaike information criteria; Δ AIC = difference in AIC from the best model; w = Akaike weights (model probabilities); ER = evidence ratios (evidence for the best model/evidence for each model); λ , μ , q = speciation, extinction, and transition rates for species where colouration is cryptic (0) or conspicuous (1). Transition rates are denoted such that q_{ij} is the transition rate from state i to state j.

Model (con0)	K	AIC	logLik	ΔAIC	w	ER	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}
Full	6	23670.98	-11829.49	112.07	<0.001	2.17E+24	0.0594	0.1714	0.0000	0.1648	0.0013	0.0023
Null	3	23799.82	-11896.91	240.92	<0.001	2.07E+52	0.0656	-	0.0126	-	0.0007	-
Equal diversification	4	23719.27	-11855.63	160.36	<0.001	6.64E+34	0.0653	-	0.0121	-	0.0007	0.0242
Equal speciation	5	23695.63	-11842.82	136.73	<0.001	4.89E+29	0.0606	-	0.0010	0.0470	0.0010	0.0058
Equal extinction	5	23558.90	-11774.45	0	1	1	0.0592	0.1818	0.0239	-	0.0015	0.1473
Model (con1)	K	AIC	logLik	ΔAIC	w	ER	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}
Full	6	24547.92	-12267.96	0	0.998	1	0.0612	0.0985	0.0000	0.0812	0.0040	0.0044
Null	3	24705.56	-12349.78	157.64	<0.001	1.70E+34	0.0658	-	0.0128	-	0.0027	-
Equal diversification	4	24602.86	-12297.43	54.94	<0.001	8.51E+11	0.0658	-	0.0128	-	0.0021	0.0169
Equal speciation	5	24560.83	-12275.41	12.90	0.002	633.80	0.0616	-	0.0000	0.0384	0.0034	0.0067
Equal extinction	5	24610.30	-12300.15	62.38	<0.001	3.51E+13	0.0518	0.1419	0.0185	-	0.0051	0.1102

Figure S1 – Posterior distributions of transition rates from MCMC analysis of the full MuSSE model for colouration. Lines immediately beneath each distribution are 95% confidence intervals and, as well as being colour matched, are in the same vertical order as the legend. Note in particular the relatively high rate of loss of conspicuousness by reverting straight to a cryptic state. Furthermore, the 95% confidence intervals of some transitions overlap zero (including cryptic-conspicuous and polymorphic-cryptic, although the latter has a wider range). Taken together, these transition rates imply that cryptic lineages first shift to a polymorphic state, from which they commonly change to conspicuous only. Once conspicuous, reversions to polymorphism can occur but a direct reversion to cryptic colouration is far more common.



Correlated Evolution Between Chemical Defence and Colouration

We tested for correlated evolution between our two traits of interest (chemical defence and colouration) since they are often reported to coevolve (including in poison dart frogs; Summers and Clough, 2001) and understanding the relationship between the traits may inform discussion and interpretation of analyses which use both traits. We used two alternative methods and find evidence for correlated evolution with both. Firstly, we fit Pagel's (1994) models of correlated and independent evolution in the corHMM package in R (Beaulieu et al., 2014), and assessed the fit of these models using AICc scores (the best model considered to be that with the lowest AICc). Since these models require binary traits, we first converted our colouration data to binary format. Because 'ambiguous' (e.g. polymorphic) lineages tend to show evolutionary patterns indistinguishable from cryptic lineages, we considered those as cryptic, and only those which we coded as such to be conspicuous in the following analyses. However, we also ran models with the alternative extreme coding ('ambiguous' colouration considered to be conspicuous) and obtained qualitatively identical results. The best model represented correlated evolution (AICc = 1057.694, cf. AICc = 1088.119 for an independent evolution model) and is visually displayed in Figure S2 using flow diagrams - a standard way to present such models (Pagel and Meade, 2006).

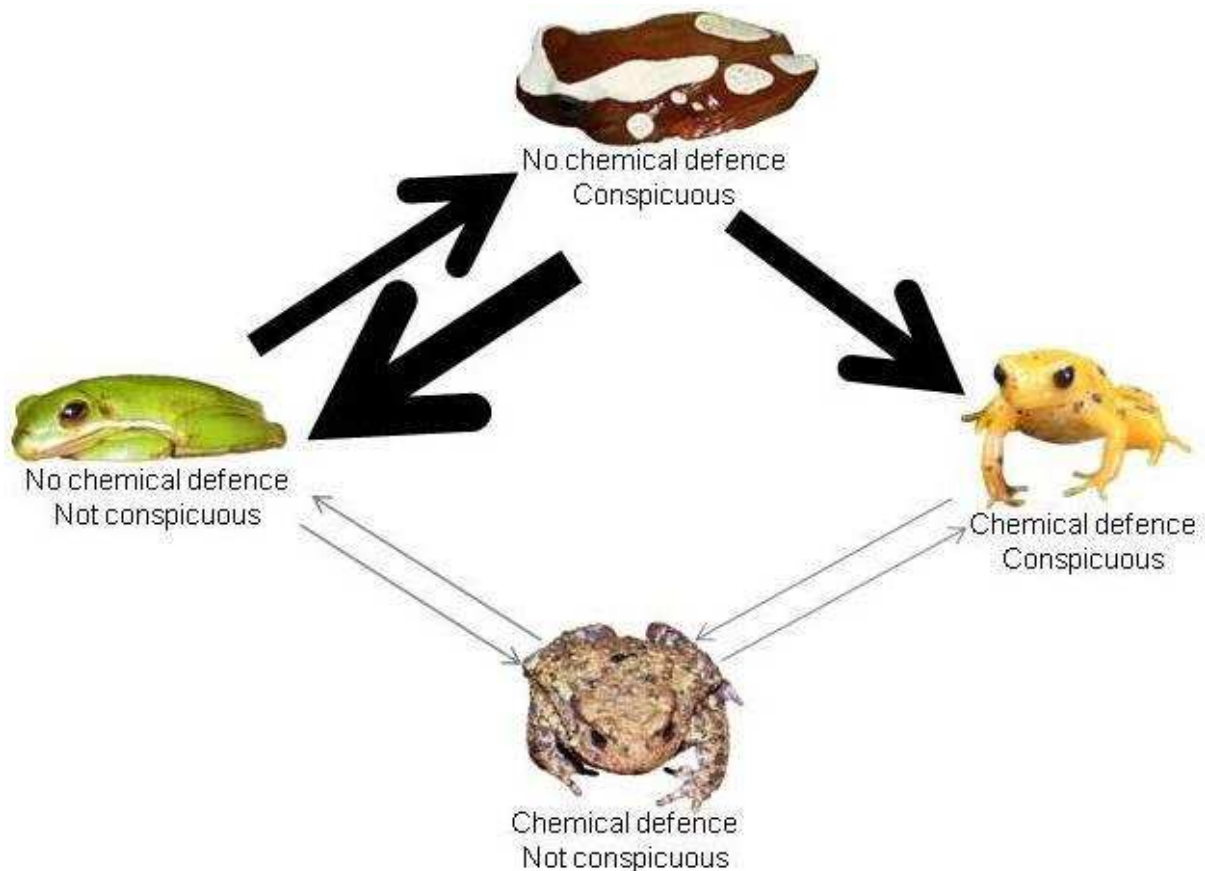


Figure S2 - Flow diagram showing the correlated evolution of chemical defence and conspicuousness in amphibians. Arrow thickness is related to transition rate (thicker arrows, higher transition rate) and grey arrows represent very small (but non-zero) transition rates.

Secondly, we fit generalised estimating equation (GEE) models to account for phylogeny, with colouration as the response variable and chemical defence as the explanatory variable. A null model (intercept only) was also fit to assess whether the first model explained the data well. The models were compared using QIC - an equivalent criteria to AIC for GEE models which similarly gives a lower score for better models). GEEs were fit in the *ape* package in R (Paradis et al., 2004). Consistent with the Pagel's models, we found that chemical defence was a significant predictor of conspicuous colouration (Table S4), and that this model was far better than the null model (QIC = 440.00, cf. QIC = 492.47 for the null model).

Table S4 – Results from a phylogenetic GEE model with conspicuousness as the response variable and chemical defence as the explanatory variable. Model was run on all species for which we had data on both colouration and chemical defence (N = 857).

Model term	Coefficient (Std Err)	t	P
Intercept	-6.381 (1.003)	-6.363	4.57e-9
Chemical defence	4.234 (1.011)	4.189	5.65e-5

Both of these analyses strongly support a model of correlated evolution between colouration and chemical defence wherein chemically defended species are more likely to be conspicuous and vice versa. However, there are also many exceptions and hence we were still able to recover independent effects of these two traits on diversification in amphibians (see main text).

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Sister Group Comparisons

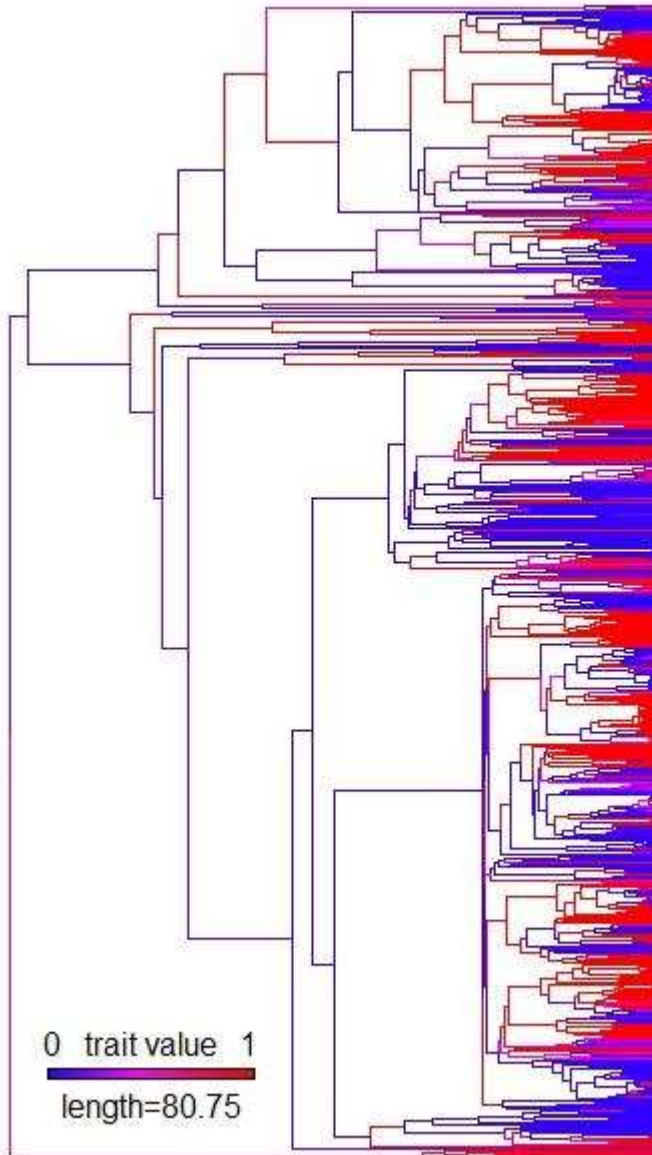
In order to check whether our results obtained from BiSSE and MuSSE models could be corroborated by other methods, we used sister groups analysis to compare the diversity in sister lineages which differ by trait. Paradis (2011) developed a new sister group method (the 'richness Yule test') and compared it to existing alternatives. He found the richness Yule test to be more powerful than other approaches and so we used this method to ask whether we find the same effects of chemical defence and colouration on net diversification as we did using BiSSE and MuSSE models.

Lineages with chemical defence had fewer species than sister groups that did not ($\chi^2 = 7.7269$, $df = 1$, $P = 0.0054$). The method requires binary traits, so colouration was coded as for the correlation analyses in the previous section. Nevertheless, conspicuous lineages had more species than cryptic lineages whether ambiguous species were treated as cryptic ($\chi^2 = 4.0311$, $df = 1$, $P = 0.0447$) or conspicuous ($\chi^2 = 11.9799$, $df = 1$, $P = 0.0005$). Therefore, in all cases, results were the same between the richness Yule tests and the BiSSE/MuSSE models.

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Figure S3 – Ancestral state reconstruction for chemical defence based on the parameters from the best BiSSE model. Colours refer to the probability that chemical defence was present in the branch. Note that chemical defence has evolved many times across the phylogeny.



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