EVOLUTIONARY ECOLOGY

The role of divergent ecological adaptation during allopatric speciation in vertebrates

Sean A. S. Anderson[1,2,3]* and Jason T. Weir[1,2,4]

After decades of debate, biologists today largely agree that most speciation events require an allopatric phase (that is, geographic separation), but the role of adaptive ecological divergence during this critical period is still unknown. Here, we show that relatively few allopatric pairs of birds, mammals, or amphibians exhibit trait differences consistent with models of divergent adaptation in each of many ecologically relevant traits. By fitting new evolutionary models to numerous sets of sister-pair trait differences, we find that speciating and recently speciated allopatric taxa seem to overwhelmingly evolve under similar rather than divergent macro–selective pressures. This contradicts the classical view of divergent adaptation as a prominent driver of the early stages of speciation and helps synthesize two historical controversies regarding the ecology and geography of species formation.

The geographic and ecological contexts in which new species arise are two subjects of historical controversy in evolution research. For much of the 20th century, biologists debated the geography of speciation and whether allopatry was required for the process to begin (*1*). By the turn of the 21st century, this debate shifted to whether speciation resulted from divergent adaptation to distinct ecological pressures (*2, 3*), as had been classically hypothesized by Darwin and Wallace (*4*). Today, biologists largely agree that most speciation events require an allopatric phase (*1, 5*), and it is clear that speciation can occur both in response to (*2, 6*), and in the absence of (*3, 7, 8*), adaptive ecological divergence. Yet the effects of geographic separation and ecological divergence have been largely studied in isolation, and it is unclear whether and how they combine to generate new species. Most classic model systems of speciation ecology are in fact co-occurring lineage pairs [e.g., lake stickleback, Galápagos finches, rift-lake cichlids, host-switching insects (*9–12*)], and although diversification is increasingly studied in non-model and primarily allopatric taxa (*8*), the ecological context of their speciation has not been systematically characterized. It is thus unknown whether allopatric speciation typically involves divergent adaptation to distinct ecological pressures or whether lineages tend to adapt to similar pressures in allopatry, with most ecological divergence occurring later (e.g., during range expansion and the establishment of sympatry) (*3*) (Fig. 1). This distinction is important, because the two scenarios

imply different limits to speciation rates (e.g., ecological opportunity versus time in allopatry) and thus different explanations for the buildup of biodiversity.

Addressing the general role of divergent adaptation in allopatry requires a broad-scale statistical approach. Here, we amass ecological trait data across a wide array of terrestrial vertebrates (birds, mammals, and amphibians) and create new statistical models to evaluate evidence for ecology-based divergent adaptation as a prominent evolutionary force during speciation's allopatric phase. Our approach is based on the prediction that if allopatric speciation is generally driven by divergent ecological adaptation, then speciating and recently speciated allopatric lineages (i.e., sister taxa) should tend to differ in ecologically relevant traits (e.g., body size, limb length, climate niche preferences) (Fig. 1A). However, some degree of trait differentiation between independently evolving lineages is inevitable over time and does not necessarily indicate adaptive divergence. We thus developed different evolutionary models of trait differentiation with and without divergent adaptation and compared the fit of these models to empirical distributions of trait differences in numerous sets of sister lineages.

We measured traits with well-established ecological importance in 129 allopatric sister pairs [i.e., speciating or recently speciated taxa, including taxonomic sister species and sister "phylogroups" (*13*) within species] of New World terrestrial birds from museum specimens and compiled an additional 14 published trait datasets for various groups of birds, mammals, and amphibians [see (*14*) for data criteria]. Each of the 15 datasets ultimately contained absolute trait differences and evolutionary age estimates for between 27 and 1001 allopatric sister pairs (median 87 allopatric pairs per dataset; Table 1). Most datasets contained measures of divergence for each sister pair in multiple ecologically re-

levant characters, including body size, feeding traits, appendage characteristics, and climate niche variables (Table 1 and table S1). We also included song data from birds, which is a social trait that is often strongly influenced by ecology (*15*). Some traits were not independent [e.g., limb length and principal component (PC) scores partly based on limb length], but they may yet have distinct functional relevance to the organisms (i.e., the interaction of several traits may have ecological relevance apart from that of any trait on its own). We therefore analyzed the statistical distributions of sister-pair divergence in each trait and composite trait from each of the 15 datasets (for a total of 130 separate analyses; Table 1 and table S1) to see how prevalent divergent adaptation appears to be among traits and across taxa [all data and code are deposited at (*16*)].

In our basic modeling design, differences in ecological traits begin to arise when a lineage splits into allopatric populations that start independently evolving. At this point, speciation has begun; if independent evolution continues long enough, reproductive isolation is inevitable. Trait differences develop over time as a stochastic process based on one of three underlying scenarios: (i) sister lineages adaptively diverge toward distinct trait optima [i.e., the divergent adaptation "DA" model (*17*), approximating the scenario shown in Fig. 1A]; (ii) lineages adapt about the same optimum as that of their allopatric sister (i.e., the shared optimum "SO" model, approximating the scenario shown in Fig. 1B); or (iii) both sisters in each pair evolve under phenotypic drift or fluctuating phenotypic selection (i.e., the Brownian motion "BM" model of trait differentiation). The three models differ in terms of the expected statistical distribution of trait differences through time (Fig. 2B); thus, in a given analysis, the models are each fit to an empirical distribution composed of the absolute differences in a particular trait for all of the sister pairs of a particular dataset. The relative fit of the different models to that empirical distribution is then judged using corrected Akaike information criterion (AICc).

In this basic framework, all sister pairs in a particular analysis are assumed to have diverged under the same evolutionary process, but our aim in this paper is to test alternative hypotheses for the relative contribution of divergent adaptation to allopatric divergence. We thus create two new mixture models that estimate the proportion of sister pairs in a dataset whose divergence in a given trait has occurred under alternative processes. These are the DA-SO mixture model, in which a proportion (P_{DA}) of sister pairs diverge under a DA process and the remaining proportion (P_{SO}) diverge under the SO process, and the SO-BM mixture model (Fig. 2, C to E). [A DA-BM model is also possible but was not used

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Table 1. Support for divergent adaptation in various traits from 15 datasets. Rows are in descending order of the number of allopatric pairs in a dataset. "Number of traits supporting DA-SO" is the number of traits in a dataset for which DA-SO was supported over SO-BM by a minimum of two AICc. "Maximum P_{DA}" is the highest P_{DA} estimate from all traits for which DA-SO was supported in a given dataset and is a key basis for interpreting the role of DA in allopatric divergence. "PCA 2D" refers to two-dimensional Euclidean distances in the PC1-PC2 plane (PCA, principal components analysis). Results for individual analyses are shown in table S1. Sources for trait datasets and trees that were used to calculate divergence times are listed in table S3. Full results with additional parameter estimates are in the supplementary results files (16). Ma, million years; NA, not applicable.

Taxa	Number of allopatric pairs	Median pair age (Ma)	Traits measured	Number of traits supporting DA-SO/number of traits	Maximum P_{DA} (trait)
Birds general	1001	2.8	Morphology PC1-PC3; bill-only PC1-PC3; bill PCA 2D; morphology PCA 2D	5/8	0.133 (bill-only PC1)
Mammals general	500	2.1	Body mass	0/1	NA
Birds general	381	4.4	Bill length, width, and depth; hand-wing index; body mass; bill PC1-PC2; bill PCA 2D	6/8	0.145 (bill length)
Rodents	242	2.6	Appendage lengths; midpoint latitude; six climate variables; morphology PC1-PC3; climate PC1-PC3; morphology PCA 2D; climate PCA 2D	10/25	0.129 (climate PC3)
Frogs general	146	4.5	Six climate variables; climate PC1 and PC2; climate PCA 2D	5/9	0.147 (annual precipitation)
New World land birds	129	2.6	Body PC1-PC3; bill PC1-PC3; bill length, width, and depth; wing, tail, and tarsus length	0/12	NA
New World land birds	111	2.7	Climate PC1-PC3; body mass	2/4	0.321 (climate PC3)
Emberizoid birds	87	1.8	Bill specialization; bill shape; range area and perimeter; PC1-PC3 of bill geometric morphometrics; bill PCA 2D	3/8	1.00 (PCA 2D)*
Amazonian birds	86	2.3	Morphology PC1-PC7; song PC1-PC2	1/9	0.041 (song PC1)
New World land birds	74	2.7	Song pitch	1/1	1.00 (song pitch)*
New World land birds	69	5.4	Song length and syllable diversity	1/2	0.358 (song length)
Neotropical ovenbirds (Furnariidae)	37	2.0	Song PC1-PC4; bill PC1; tarsus length; song PCA 2D	0/6	NA
Salamanders	30	6.4	Eight linear morphometrics; morphology PC1 and PC2; morphology PCA 2D	2/11	0.171 (snout-vent length)
Plethodontid salamanders	29	6.5	Surface area (SA); volume (V), and SA/V ratio; seven linear morphometrics; seven climate variables; morphology PC1-PC2; Climate PC1-PC3; morphology PCA 2D; climate PCA 2D	1/16	0.072 (mean annual precipitation)
Plethodontid salamanders	27	5.0	Seven linear morphometrics; morphology PC1 and PC2; morphology PCA 2D	1/10	0.808 (body width)
Total				**38/130**	

*Support for DA-SO is contingent on an obvious outlier being removed (14).

because of performance testing issues; fig. S1 (14).] Our main analysis then consisted of fitting the two mixture models to each of the 130 empirical distributions of sister-pair trait differences, which allowed us to generally char-acterize model support and generate a distribution of P_{DA} estimates. With mixture models, we can address the central question of this paper: If divergent adaptation is generally the predominant process driving allopatric diver-gence, then (i) the DA-containing model (DA-SO) should be generally well supported over the SO-BM model and (ii) DA-like patterns of trait differences should predominate (i.e., $P_{DA} > 50\%$) in at least some eco-logically

relevant traits in each of the 15 sets of sister pairs.

We find instead that only 38 of the 130 analyses strongly support the DA-SO model, and only a minor proportion of sister pairs from each dataset tend to show patterns of divergence consistent with DA in each trait (Fig. 3, Table 1, and table S1). The prevalence of

DA was low and varied little across traits and datasets (Fig. 3A); the median P_{DA} from the 38 analyses in which DA-SO was well supported was 9.9% (bolded rows in table S1 show the trait-dataset combinations that support DA-SO). Critically, DA was well supported as the predominant driver of trait divergence (i.e., P_{DA} exceeded 50%) for just one trait each in just 3 of the 15 datasets in our study: a sample of Plethodontid salamanders (for the trait body weight), a sample of New World passerines (for the trait song pitch), and a sample of Emberizoid birds (for the trait bill shape) (Table 1). In other words, in 12 of the 15 groups of sister pairs that we analyzed, including global datasets of birds and mammals, DA was not supported as the prevailing driver of divergence in any of several ecologically relevant traits measured for those taxa (Fig. 3B and table S1). Instead, the SO process was consistently prominent; the median P_{SO} from the best-supported model in each of the 130 analyses was 93.2% (Fig. 3B). Thus, in each dataset, the vast majority of pairs have diverged under similar rather than divergent macro–selective pressures or drift-like processes in most or all ecological traits analyzed. These results are robust to alternative range
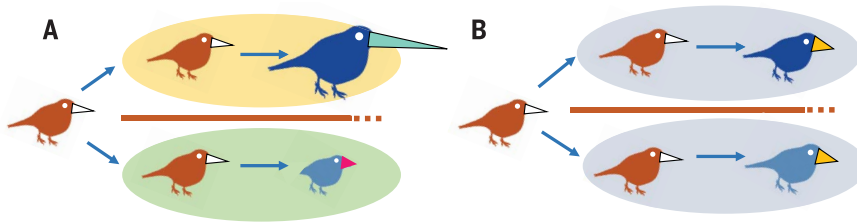
Fig. 1. Two modes of adaptation in allopatry. (**A** and **B**) A lineage splits into two allopatric populations separated by a geographic barrier. Over time, the populations evolve into new species. Body color differences symbolize that species are distinct. In (A), differently colored ovals represent allopatric habitats with distinct ecological characteristics. Speciation results as lineages adapt to these disparate environments, and divergent adaptation generates substantial differences in ecologically important traits (body size, beak size and shape, and tail length). In (B), same-colored ovals represent allopatric habitats that are ecologically alike. Lineages adapt in parallel to these like environments, and speciation results from factors unrelated to ecological divergence. Ecological traits may or may not evolve during this time, but if evolution occurs, it occurs in similar directions in the two lineages such that trait differences remain slight (beak shape). Taxa that speciate under the scenario shown in (A) may be better able to co-occur in sympatry once lineages expand their ranges and come into contact. Taxa from the scenario shown in (B) might competitively exclude one another or be forced to undergo character displacement. The two scenarios suggest different limiting factors in the build-up of biodiversity. Whether the scenario shown in (A) or (B) predominates in nature is unknown.
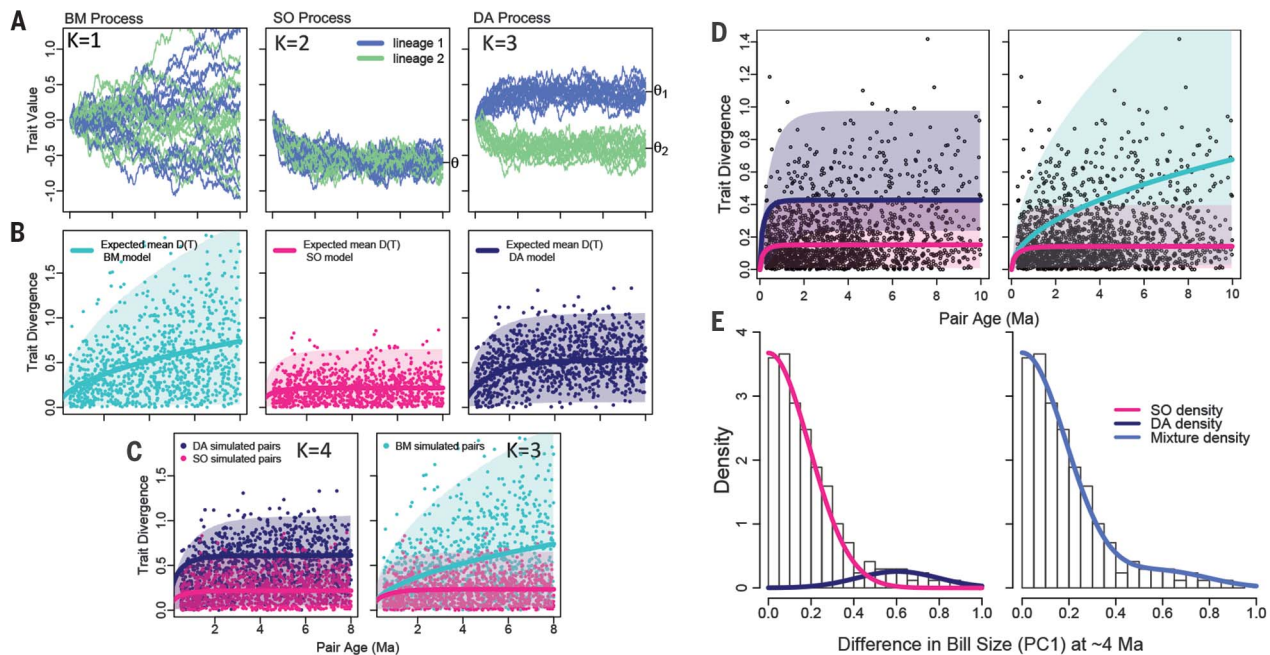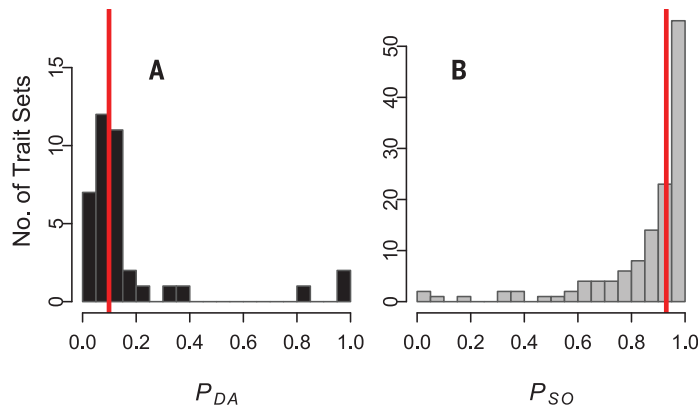

Fig. 2. Understanding trait differentiation models. Simulations are shown in (A) to (C), and real data are shown in (D) and (E); "K" denotes parameter count. (**A**) Replicate evolutionary walks (*n* = 15) of a continuous trait in sister lineages under BM, SO, and DA processes; θ_x is the trait optimum for lineage *x*. The SO model is agnostic as to whether the optimum shared between sisters differs from that of their common ancestor (as illustrated) or is the same. (**B**) Points are simulated trait differences for pairs of various ages. Models were fit to these simulated data, and their expectations were plotted. Model parameters determine the expected distribution of trait differences. In (B) to (D), colored lines show the mean and shaded areas show the 95% confidence intervals of this distribution. D(T) is the trait difference at time T. (**C**) DA-SO and SO-BM mixture models fit to simulated mixed datasets. (**D**) DA-SO and SO-BM mixture models fit to empirical bill-size differences from (*31*); DA-SO is the better-fit model from AICc (table S1). Figure S16 shows this style of graph for every dataset. In (B) to (D), colored lines show the mean, and shaded areas show the 95% confidence intervals of this distribution. (**E**) Model fits to data at a slice in time. Curves are proportion-weighted component densities (left) and the corresponding DA-SO mixture model density (right) expected at 4 million years. Histograms are bill size differences for pairs 3 million to 4.5 million years diverged (*n* = 339 pairs). Ma, million years.

Fig. 3. Proportion of pairs diverging under alternative evolutionary processes. A "trait set" is a set of differences in a given trait for all of the sister pairs of a particular dataset (each row in table S1 is a trait set). Trait sets thus differed based on the trait and/or the set of sisters being analyzed. P_{DA} is the estimated proportion of sister pairs in a trait set whose divergence conforms to the DA component of a DA-SO mixture model; P_{SO} is the proportion that conforms to the SO component of either the DA-SO or SO-BM model. (**A**) P_{DA} estimates from the 38 trait sets for which DA-SO was well supported. (**B**) Frequency of P_{SO} estimates from the best-fit model for all 130 analyses. Red lines indicate median estimates of the proportion parameter [9.9 and 93.2% in (A) and (B), respectively]. The y axes have different scales. The histograms illustrate little variation across traits and datasets: DA was consistently a minor process and SO the major process driving trait divergence between speciating and recently speciated allopatric lineages.

overlap thresholds used for classifying lineages as allopatric (*14*) (fig. S2).

The consistently low prevalence of the DA process and high prevalence of the SO process are unlikely to be artifacts of model inadequacy or of assumption violations. Simulation-based model performance tests show that estimates of P_{DA} and P_{SO} are accurate over a wide and relevant range of parameter space and dataset sizes (*14*) (figs. S3 to S5). Moreover, when we intentionally violated model assumptions in our simulations, we were unable to generate errors that falsely recreated the empirical results (*14*) (figs. S6 to S10). We can also exclude "species sorting" [i.e., a bias in which sister pairs with more pronounced trait differences become sympatric at a higher rate (*18*)] as a key driver of our results, because simulations show that this process consistently generates a statistical artifact (a negative correlation between trait divergence and sister-pair age) that is largely absent from empirical datasets (*14*) (figs. S11 to S14). Also, although it is possible that a few of the sister pairs in our datasets were previously sympatric, the common pattern of exaggerated trait differences observed between sympatric close relatives (*18, 19*) would suggest that unseen sympatry-to-allopatry state transitions would, if anything, tend to inflate rather than suppress support for the DA process. Similarly, although the assumption that trait differences accurately reflect ecological differences is violated when different traits have similar functions [i.e., "many-to-one mapping" (*20*)], such a violation causes functional divergence to be overestimated rather than underestimated and thus cannot account for the low P_{DA} values that we observed.

Does the ecology of allopatric speciation change with the ecological theater? Estimates of higher speciation rates at higher absolute latitudes (*21, 22*) have inspired the hypothesis that temperate-zone speciation may be driven to a greater extent by adaptive ecological divergence than is speciation in the tropics (*23*) because of the greater availability of underexploited resources (i.e., "ecological opportunity") at temperate latitudes (*24*). To test this hypothesis, we compiled latitude data from digital range maps for each sister pair in each dataset and created new mixture models that permit the proportion of pairs evolving under a given process (i.e., P_{DA}, P_{SO}, or P_{BM} in DA-SO or SO-BM models) to vary with latitude (*14*) (fig. S15). Reanalyzing all sets of trait divergence from 14 of the 15 datasets [122 analyses total; we excluded one dataset because all pairs were Amazonian birds that together covered only a small latitudinal range (*14*)], we find that the estimated proportion of allopatric sister pairs evolving under a particular evolutionary process is, with few exceptions, latitudinally constant (table S2). Thus, although rates of trait evolution may change across latitudes, the underlying evolutionary process driving allopatric divergence appears to vary little. We speculate that reported estimates of faster evolutionary rates for high-latitude taxa are driven in some cases by "character displacement" [in the sense of (*25*)] in sympatry (i.e., geographic overlap), and it is the establishment of secondary sympatry rather than allopatric trait divergence that is generally faster at high latitudes (*26*). This dynamic was implicated for patterns of divergence in avian plumage coloration (*27*).

We note that ecological adaptation can drive evolution in a number of traits that were not included in our analysis. Adaptive differences in phenotypes such as behavior, cellular attributes, sensory complexes, and life history characteristics are generally not captured in trait datasets. It is therefore possible that adaptive ecological divergence has occurred on undetected trait axes in the sister-pair groups of our study. We also note, however, that most textbook examples of "ecological speciation" in vertebrates exhibit unambiguous adaptive differences in common aspects of adult external morphology (e.g., body size in most taxa, bill shape or size divergence in birds) (*2, 28*), whereas we find only minor support for divergent adaptation as a prominent driver of divergence in these very same phenotypes and each of many additional traits. If adaptive ecological divergence is a major process during allopatric speciation in these pairs, then it is either much subtler than that observed in vertebrate model systems, there is greater variability in the trait axes on which different related pairs adaptively diverge, or it generally occurs on different and as-yet-unidentified trait axes for reasons that are unclear. The current most parsimonious interpretation is that our results reflect a genuine biological phenomenon: Allopatric divergence is most generally characterized by adaptive evolution to similar selective pressures.

Our study unites two historical controversies in evolution research—the roles of geographic and of ecological divergence in the evolution of new species. Using new models to analyze sister-pair trait differences, we find that adaptive ecological divergence in allopatry appears to be the exception rather than the rule in vertebrates. This result contradicts the classical idea that divergent adaptation initiates the earliest stages of speciation, and it supports an emerging picture in which new species commonly arise despite minimal ecological divergence (*8*). We suggest that it is notable that many textbook model systems of speciation ecology are sympatric taxa, because their pronounced ecological differences may have primarily evolved during or after the establishment of sympatry. Such differences may then be required more for ecological coexistence than for speciation per se. It is likewise possible that a previously reported correlation between ecological divergence and reproductive isolation in a diverse group of sister taxa (*29*) was mainly driven by sympatric pairs (sympatric and allopatric pairs were pooled in that analysis). This notion seems plausible because only premating (not postzygotic) isolation was correlated with ecological divergence in the study, and both ecological disparity and the strength of premating barriers are hypothesized to accelerate in sympatry [through character displacement and "reinforcement" (*30*), respectively]. A key implication of our result is that speciation in allopatry does not generally require lineages to exploit new resources or otherwise adapt to distinct

ecological pressures but relies instead on their prolonged geographic separation. Our findings leave open the possibility that pronounced ecological divergence is generally important after allopatric speciation as lineages expand their ranges and begin to overlap (*3*, *18*).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S16
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Science

The role of divergent ecological adaptation during allopatric speciation in vertebrates

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Similar but separate species

Speciation often requires a period of allopatry, when populations are separated long enough to diverge into distinct species. Sister species may occupy different niches, but whether ecological divergence occurs during or after allopatric speciation is poorly understood. Anderson and Weir used trait data on more than 1000 pairs of sister taxa, including birds, mammals, and amphibians, to model trait divergence over time. They found few examples of clear divergent adaptation, with greater support for a model of sister taxa evolving under similar selective pressures toward similar trait optima. —BEL

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