



NOTE

Deviation from the Brownian motion expectation implies episodic adaptive divergences in traits of *Lithocarpus* species in Taiwan

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ABSTRACT: Adaptive trait divergence is usually episodic rather than universal in a phylogeny. To determine whether trait divergence reaches an extreme level (*i.e.* deviation from neutrality), the evolutionary rate of the species and intraspecific variation must be taken into account. Accordingly, we attempt to explore the trend of trait divergence over divergence times conditioned on the genetic distance. The Brownian motion (BM) model, a commonly used random-walk process for describing the neutral evolution of traits, is used to simulate the distribution of trait divergence under neutrality. The observed trait divergences are then compared to the BM expectations to detect outliers, which are considered to be selected. We assessed the ability of this method to characterize trait divergence under selection among 14 *Lithocarpus* species in Taiwan. The results are consistent with the previous inference of phylogenetic constraint based on tests of the phylogenetic signal, but further signals of adaptive and conserved trait divergences are detected between some lineages derived from particular nodes (common ancestors), indicating differential investments in growth rate and chemical defense among some endemic species in Taiwan. These results show that the adaptive divergence of environment-related traits is transient, episodic, and punctuated. Under strong selection pressure, the trait divergence after standard deviation correction will be more significant because the trait variance within the population decreases. However, it tends to be an overestimation of conservative evolution in older common ancestors due to the larger standard deviation produced from diverse descendants. Nevertheless, this study still provides a simple approach to detect adaptive divergence in a phylogenetic framework.

KEY WORDS: Adaptive divergence, Brownian motion model, Continuous trait, *Lithocarpus*, Phylogenetic constraint, Trait evolution.

INTRODUCTION

Trait divergence is usually environment-driven, whether adaptively or plastically (Ghalambor *et al.*, 2007, Turcotte and Levine, 2016, Funk *et al.*, 2017), but is also constrained phylogenetically (Lord *et al.*, 1995, Crisp and Cook, 2012). The divergence of these "response traits" (*i.e.* traits associated with the response of plants to environmental factors (Lavorel and Garnier, 2002)) is assumed to be linked to the variation of the resilience of species to environmental stresses (Funk *et al.*, 2017). The evolutionary resilience and phylogenetic constraints of heritable traits (especially "codependent traits") synergically determine the adaptability of species to the environment (Peiman and Robinson, 2017). There are several intrinsic causes of phylogenetic constraint, including physicochemical, developmental, and genetic constraints, that establish the pattern of phylogenetic niche conservatism (Crisp and Cook, 2012). If the pace of trait change is not synchronized with environmental change, species may become extinct. Consequently, to avoid moving toward a dead end in evolution, the pace of trait evolution is usually regulated, and shifts from neutral to excessive or inferior evolution do not occur

easily. Accordingly, we hypothesized that, like most selection hypotheses, deviations of trait evolution from neutrality are usually temporally transient in only certain taxa (*i.e.* punctuated evolution) instead of universal or phyletic gradualism (Gould and Eldredge, 1977, Beilharz *et al.*, 1993, Møller and Pomiankowski, 1993).

In this study, we focus on the adaptive divergence of continuous traits from a species-centered perspective. Since many ecophysiological traits are quantitative (*i.e.* function-valued traits (Gomulkiewicz *et al.*, 2018)), the evolution of these continuous characteristics is often predicted by the Brownian motion (BM) model (e.g. Pagel, 1999, Blomberg *et al.*, 2003, O'Meara *et al.*, 2006). BM is a continuous, directionless, stochastic diffusion process in which the expected value is constant through time. Therefore, BM is usually adopted as a null model to describe the neutral evolution of continuous traits and to test selection. Traits are characteristic of species, and thus the evolutionary rate of species, which is not always constant, may affect degrees of trait divergence. In addition, the degree of intraspecific variation of response traits may differ among species. Hence, it is necessary to consider both species evolution and intraspecific variation when investigating trait evolution. We use the BM model



as the null hypothesis to test the degree of trait divergence at different nodes (common ancestors) of a tree and attempt to determine the characteristics of adaptive divergence under the concept of evolutionary punctuation.

Species and traits tested in this study

Lithocarpus Bl. (Fagaceae) is widespread in the East and Southeast Asia ranging from the Northeast India, South China, South Japan, Taiwan, and the Greater and Lesser Sunda Islands (Nixon, 1989). Short genetic distances among species estimated by cpDNA and nrITS sequences implied the recent diversification of *Lithocarpus* in Southeast Asia (Cannon and Manos, 2003). A previous biogeographic inference indicated two major groups of these Asian stone oaks: the more ancestral Asian continental branch and the later southward-deriving Greater-Sunda-Islands branch (Yang *et al.*, 2018a). Comparing the phylogenetic position and the corresponding splitting times between Taiwan and Asian mainland, *Lithocarpus* species in Taiwan is suggested multiple-time and multiple-source origins (Yang *et al.*, 2018a). High endemism of *Lithocarpus* in Taiwan (~50%) also indicates the rapid evolutionary rate of these island species after colonization (Yang *et al.*, 2018a). The geographic distribution of these *Lithocarpus* species is diversified, with a latitudinal range of 21.95°N–25.20°N, longitudinal range of 120.15°E–121.90°E, and altitudinal range of 3–3016 m. In Taiwan, where the topography and local climate heterogeneity are sharp, adaptive physiological traits may be greatly different among species (Yang *et al.*, 2018b). The detailed measurements of these trait values of 14 *Lithocarpus* species are listed in Table S1.

Leaf phenolic acid content (PA) is considered an adaptive characteristic in pathogenic and phytophagous resistance (Lattanzio *et al.*, 2006); leaf carbon-nitrogen ratio (C/N) is a proxy of the potential growth rate because it reflects nutrient limitation (Wigley *et al.*, 2016); the phytochemical yield of photosystem II (YII) is an indicator of energy used in photochemistry by photosystem II under steady-state photosynthetic lighting conditions and is usually used as an index of photosynthetic efficiency. In our previous studies, we thought that the chemical defense characteristics of PA probably evolved in parallel with epicuticular wax crystals on the leaf surface, reflecting the phytophagous stress of the late-Miocene-to-Pliocene (Yang *et al.*, 2018b). Such synergistic evolution for these different traits may enhance the defense function against insect biting (Shepherd and Griffiths, 2006) or water repellency (Pierce *et al.*, 2001). In Yang *et al.* (2018b), the evolution of these three ecophysiological traits did not deviate from the BM stochastic process under a species tree (Pagel's $\lambda = 0.637$, 6.90×10^{-5} , and 6.90×10^{-5} , respectively, and Blomberg's $K = 0.326$, 0.184, and

0.267 for PA, C/N, and YII, respectively). These estimates indicated that although these trait values diverge, the overall trend of evolutionary rate of these traits does not deviate from the divergent rate of species.

However, the adaptive divergence of traits could not be tracked easily by phylogenetic-signal tests because the positive selection is usually local and transient instead of pervasive and permanent. To assess the performance of the concept and the method developed in this study, we collected three trait values of mature leaves from 14 *Lithocarpus* species in Taiwan from Yang *et al.* (2018b), including PA, C/N, and YII. In this case, we try to find out the episodic adaptive divergence of traits in the evolutionary trajectory behind the previous analysis of the phylogenetic signal.

METHODS

Conceptions

In the process of neutral evolution, the degree of trait divergence is expected to be proportional to the degree of species differentiation. Species divergence (D) is determined by the divergence time (T) and the evolutionary rate of species (R), which are usually denoted as $D = 2T \times R$. Species divergence is usually determined by genetic distance of neutrally evolving genes (GD). If a trait evolves neutrally, we assume the evolutionary rate of this trait will be proportion to R . However, the evolutionary rate of species (*i.e.* speciation rate) or trait may not be constant, so we divide the difference value of traits by GD between species to correct for the effect of an inconstant speciation rate. Thus, we can obtain the magnitude of trait change per unit of genetic distance, *i.e.* the ratio of the trait-change rate to the species evolutionary rate. Since the trait change is expected to be constant through time under a neutral random-walk process, we hypothesize a random distribution of the (corrected) trait change against the divergence time. Therefore, we can determine whether the trait change is prone to acceleration or deceleration by the deviation from the random distribution.

In the BM process, the changes of trait values over a time interval (*i.e.* the variance of a trait) are a function of the product of the evolutionary rate (*i.e.* σ^2 , the Brownian rate) and the time elapsed since the common ancestor (*i.e.* the length of time, τ) (cf. Pagel, 1999). Therefore, lineages with longer independent evolving times will have higher expected trait variance (e.g. larger variance of a trait in taxon C than in taxa A and B, Fig. 1B). Fig. 1A displays an evolutionary pattern of a trait with a simple phylogenetic relationship ((A, B), C). Under the BM model, the expected difference values of a trait can be obtained (*i.e.* $E(D_{AB})$ and $E(D_{(AB)C})$) and used as a null model to test whether the observed differences (*i.e.* $O(D_{AB})$ and $O(D_{(AB)C})$) deviate from expectations (Fig. 1B). If the



observed differences of trait values fall within the BM expectations, the trait evolution can be regarded as following species evolution, *i.e.* under phylogenetic constraint. By contrast, outliers are regarded as deviating from the neutral path of species evolution. The positive and negative outliers can be defined as adaptive and stabilized (or conserved) divergence of traits, respectively. For example, an observed trait difference between taxa A and B that is larger than the expectation (*i.e.* $O(D_{AB}) > E(D_{AB})$) suggests that the trait diverged adaptively in taxa A and B (Fig. 1B).

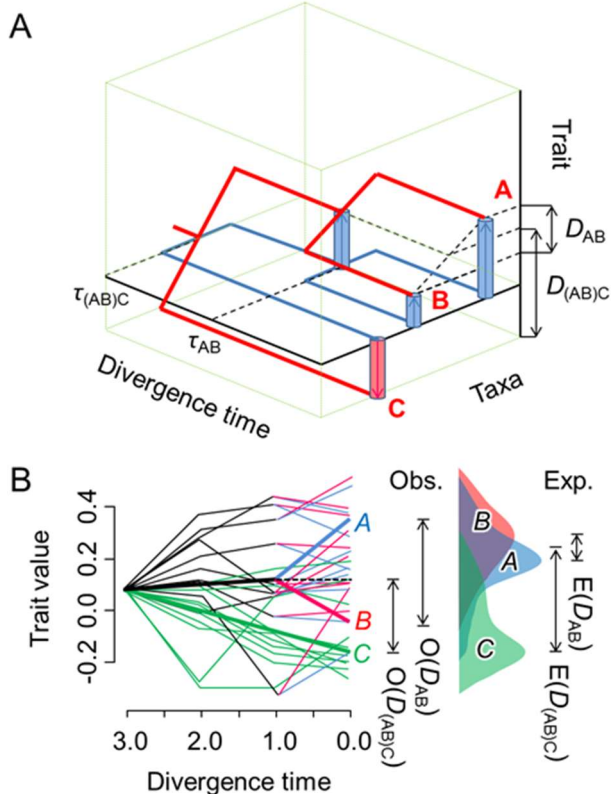


Fig. 1 The change patterns of continuous traits under the evolution of species. (A) Schematic diagram of the trait evolution model of three taxa. The blue bars are positive trait values compared to the common ancestor (trait value = 0), and the red bar denotes a negative trait value. D_{AB} and $D_{(AB)C}$ are the differences in trait values between taxa and nodes. τ_{AB} and $\tau_{(AB)C}$ are the divergence times among taxa. (B) Simulations (thin lines) of trait values among three taxa under the BM model. The thick lines in the left figure are the observed trait values. A density plot of the simulation is shown in the right panel. The observed and expected differences between taxa A and B are expressed as $O(D_{AB})$ and $E(D_{AB})$, respectively. In this schematic diagram, $O(D_{AB}) > E(D_{AB})$ but $O(D_{(AB)C}) \approx E(D_{(AB)C})$ indicates obvious trait differentiation caused by divergent selection between taxa A and B.

Performance evaluation using empirical data

The phylogenetic relationships of the 14 *Lithocarpus* species in Taiwan referred to Yang *et al.* (2018b), who used the Bayesian approach to reconstruct the species tree based on six neutral nuclear genes (*CAP*, *DGD*, *ESRK*, *FAD*, *SAHH*, and *SAM*, totaling 3090 bp). This

phylogenetic tree is rooted with *Quercus robur*, *Castanea mollissima*, and *Fagus sylvatica*, and dating using the calibration points of the origin time of Fagaceae (Manos and Stanford, 2001) and the divergence of *Fagus* and *Castanea* (Manos and Stanford, 2001) at 100 million years ago (Mya) and 60 Mya, respectively, under the lognormal relaxed molecular model (Yang *et al.*, 2018b).

Three ecophysiological measurements were obtained from Yang *et al.* (2018b), in which YII was measured using MINI-PAM-II Photosynthesis Yield Analyzer (Heinz Walz GmbH, Germany), PA was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi (1965), and C/N measurements was obtained from SGS Taiwan Ltd which used a protocol of Carter and Barwick (Carter *et al.*, 2011). The measurements were listed in Table S1. We calculated the pairwise differences of these trait values between species and their pairwise GD. To correct the trait divergence for the inconstant evolutionary rate of species, the trait difference was divided by GD (*i.e.* $\Delta(C/N)/GD$, $\Delta PA/GD$, and $\Delta YII/GD$), by which we can acquire the trait change values per genetic unit between lineages. Subsequently, these trait-change values were plotted against the divergence times to see if the trait evolves at different rates over a given time.

We further simulated the trait evolution in a phylogenetic framework of the species tree under the BM model (Fig. 3A). We simulated 1000 steps from a uniform distribution in a limit of trait variance using the function fastBM of the package phytools (Revell, 2012) in R. One of the simulated trait values (YII) under the BM model is shown in Fig. 3B. We display five of 1000 simulated YII, PA, and C/N values in Fig. S1. The observed trait differences between the lineages derived from each node (*i.e.* $\Delta C/N$, ΔPA , and ΔYII) are compared to the null (neutral) distributions of 1000 simulated trait differences (Fig. 3F-H). To eliminate the effect of intraspecific variation, the observed difference values were divided by the standard deviation (SD) and plotted against the divergence times (Fig. 3C-E).

RESULTS AND DISCUSSION

Adaptive trait divergence in three species endemic to Taiwan

No significant deviation from a random distribution was detected in the corrected trait change against the divergence time ($P = 0.104, 0.683, 0.492$ in C/N, PA, YII, respectively, Fig. 2), suggesting a consistent evolutionary rate of the potential growth rate (C/N), chemical defense (PA), and photosynthetic efficiency (YII) with the divergence of *Lithocarpus* species in Taiwan, *i.e.* constant trait changes per unit time. This result also supports Yang *et al.*'s (2018b) inference of phylogenetic constraints in these traits by phylogenetic signal tests.

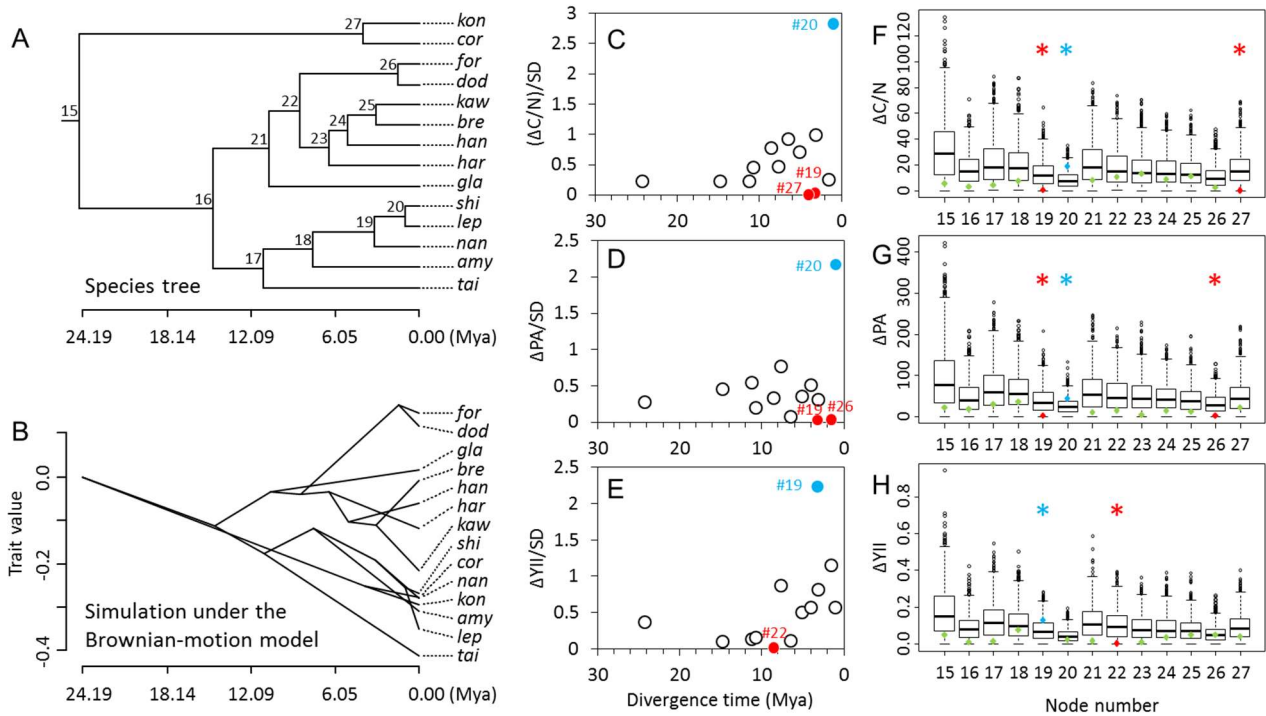


Fig. 3 Summary results of the divergence of ecophysiological traits between derived lineages of nodes. (A) Species tree of *Lithocarpus* species in Taiwan. The number near the node is the node number corresponding to the x-labels of (G)~(H). The divergence time of each node is listed in Table S2. (B) One of the 1000 simulation trees under the Brownian motion (BM) model. The trees were simulated with the mean and SD of the YII values of 14 *Lithocarpus* species. This simulation graph shows how a trait evolves stochastically under the phylogenetic constraint under BM model. The simulated trees of C/N and PA values are shown in Fig. S1. (C)~(D) Differences in trait values between the derived lineages of nodes with their corresponding divergence times. Red dots are negative outliers (<2.5% quantiles) deviating from BM expectations, and blue dots are nodes with higher difference values than the 75% quantile of the BM simulations. (G)~(H) Box plots of the differences in trait values under the BM simulations with the corresponding observed differences (green dots). Red and blue dots indicate the nodes with observed values <2.5% quantile and >75% quantile of the BM simulations, respectively, which are also denoted by asterisks.

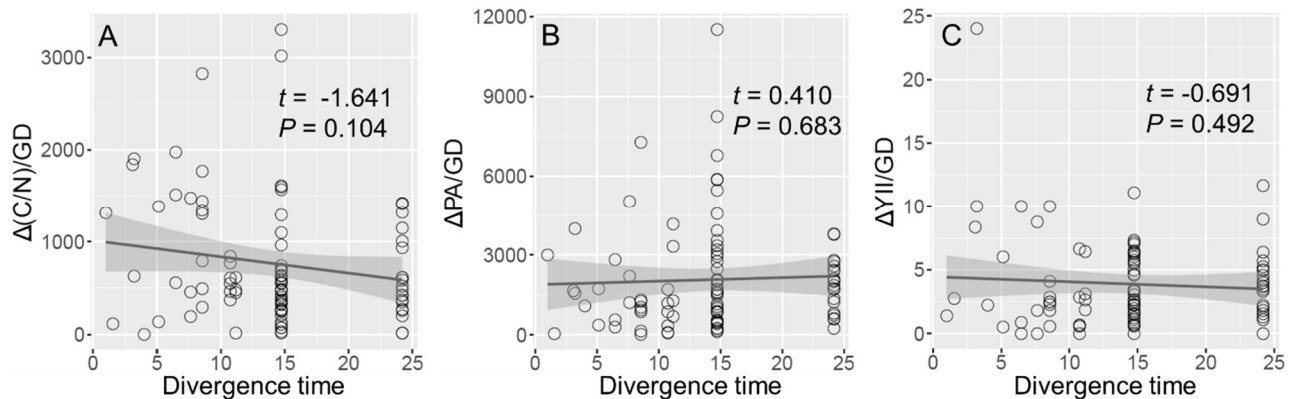


Fig. 2 Plots of the trait differences against divergence times. (A)~(C) display the trait differences in leaf C/N ratio ($\Delta C/N$), phenolic acid content (ΔPA), and photosynthetic efficiency (ΔYII), respectively. All trait differences were divided by the pairwise genetic distance (GD) to correct for degrees of species differentiation.

However, although no positive outliers were observed, high observed values of $\Delta C/N$, ΔPA , and ΔYII that were greater than the 75% quantiles of the BM simulations were shown between lineages derived from node 20, node 20, and node 19, respectively (Fig. 3F-H).

The species divergence time at nodes 19 and 20 are 3.21 Mya (95% highest posterior density (HPD): 0.21 – 6.98 Mya) and 1.00 Mya (95% HPD: 0 – 3.65 Mya), respectively (Table S2). This high differentiation became more obvious when the effect of intraspecific



variation was eliminated by conditioning on the SD (Fig. 3C-E). This result suggests an underlying adaptive divergence of these traits between *L. shinsuiensis* and *L. lepidocarpus* and between *L. nantoensis* and the common ancestor of *L. shinsuiensis* and *L. lepidocarpus*.

Estimating the deviation of trait differences from the BM expectation in each node provided sharper perspectives on trait evolution (Fig. 3). For instance, for the species derived from node 20, *L. shinsuiensis* has a higher leaf C/N (38.70) and PA content (103.10 mg/mL) than *L. lepidocarpus* (C/N = 20.297 and PA = 61.06 mg/mL) (Yang *et al.*, 2018b). The high C/N and PA content reflect the adaptation of *L. shinsuiensis* to the warm and humid climate and pest-prone areas in low-altitude southern Taiwan; by contrast, *L. lepidocarpus* grows relatively slowly in cooler middle altitudinal areas, thereby permitting reduced investment in phytophagous resistance. The divergence between *L. shinsuiensis* and *L. lepidocarpus* may therefore reflect differential investments in growth rate and chemical defense. YII is lower in *L. nantoensis* (0.629 ± 0.025) than in *L. shinsuiensis* (0.745 ± 0.032) and *L. lepidocarpus* (0.770 ± 0.025) (Yang *et al.*, 2018b). Although the adaptive factors underlying the lower photosynthetic efficiency of *L. nantoensis* are unknown, the differences in YII reflect the rapid adaptive divergence in the utilization of light energy among these endemic species in Taiwan, even between the morphologically similar *L. nantoensis* and *L. shinsuiensis*. These examples illustrate that adaptive divergence that may not be evident from the overall trend of trait evolution but can be detected by testing the deviation from BM in individual nodes.

Conserved evolution in co-adapted traits

In addition to those underlying adaptive divergences, two negative outliers were detected in Δ C/N (nodes 19 and 27), two in Δ PA (nodes 19 and 26), and one in Δ YII (node 22) (Fig. 3F-H), indicating highly conserved trait evolution derived from these common ancestors. Such conservative trait evolution is probably due to a strong selective constraint under the same or similar environmental pressures (Losos, 2011). In fact, the regrowth of tissue damage or the herbivory defense could reduce the resource allocation on photosynthetic rate (Rosenthal and Kotanen, 1994) due to the limitation of intracellular CO₂ concentration after leaflet injury (Haile *et al.*, 1998). Similarly, herbivory is also a selective pressure to increase growth rate due to an adaptive response to the removal of apical dominance (Hjalten *et al.*, 1993). Such co-adaptive characteristic of photosynthesis efficiency, growth potential, and herbivory tolerance/resistance lead these ecophysiological traits relatively conserved (Rosenthal and Kotanen, 1994, Strauss and Agrawal, 1999).

Interestingly, some relatively conservative characters were detected between species with very

limited distribution. For example, *L. dodonaeifolius* and *L. formosanus* (derived from node 26) share close common ancestral polymorphisms and have entered southern Taiwan after the glacial period (Chiang *et al.*, 2004), which may lead to their very similar chemical defense characteristics (PA = 44.53 and 45.33, respectively, Table S1) to adapt to warm and humid environment. However, the two negative outliers, Δ PA at node 26 and Δ YII at node 22, represent the conservative evolution of long-ago traits (4.01 Mya and 8.55 Mya, respectively). Since the older lineage has longer coalescent interval, it may lead to relatively stable SD of trait divergence (Fig. S2). That is, the variation of trait divergence is relatively small per unit time in old lineages. Reducing missing taxa may increase unnecessary false negative. In fact, most nodes have large SDs (Fig. 3F-H). Nevertheless, these high SDs may reflect the fact that the extant species are distributed widely or diversely. Although the high variance of traits may be interpreted in turn to be more conducive to the widespread distribution of descendant lineages, the conservative evolution of traits should be interpreted more cautiously.

More sensitive to detecting episodic positive selections

Yang *et al.*'s (2018b) used two phylogenetic indices, Pagel's λ and Blomberg's K , to test the relationships between the evolution of these ecophysiological traits and the species tree and the gene trees associated with the leaf epicuticular wax crystals. Yang *et al.*'s (2018b) result showed that although some estimates are small ($\lambda = 0.637$, 6.90×10^{-5} , and 6.90×10^{-5} and $K = 0.326$, 0.184 , and 0.267 for PA, C/N, and YII, respectively), there is no deviation between the observed data of these traits and the BM stochastic process under a species tree inferred by Pagel's λ and Blomberg's K (*i.e.* $P > 0.05$). Since the phylogenetic signal only tests the overall trend of trait evolution, it is difficult to detect the difference value within the range of variance within the ingroup. However, the adaptive selection is usually local or instantaneous rather than pervasive, so this approach we developed could be more sensitive to the local selection at each node. The case study of *Lithocarpus* shows higher differences of ecophysiological trait values among three endemic species: *L. shinsuiensis*, *L. lepidocarpus*, and *L. nantoensis*. No such adaptive divergence was detected by the phylogenetic signal tests in Yang *et al.* (2018b), which shows that our method is more sensitive to episodic adaptive divergence. Such adaptive change could drive the species to a new phenotypic optimum via initial adaptive plasticity under the non-adaptive variation in the new environment (Ghalambor *et al.*, 2007). This process is usually environment-induced, initially plastic, and later genetically assimilated, and is always transient and rapid in the evolutionary trajectory (Via *et al.*, 1995, Levis and



Pfennig, 2019). Therefore, it is better to detect the trait divergence at each node than to track the overall trend of trait evolution to reflect the adaptability of trait innovation.

CONCLUSIONS

Many methods have been designed to test the deviation from a random-walk or conditional random-walk process to determine the adaptive divergence of a trait, e.g. Hernández *et al.* (2013), Ingram *et al.* (2013), Nourmohammad *et al.* (2017), Molina-Venegas *et al.* (2018), and Revell *et al.* (2018). However, most of these methods merely explore the overall trend of trait change. The method we used here under the BM process is similar to these previously reported methods but facilitates the detection of adaptive trait divergence between branches of particular nodes (common ancestors) with specific growth environments or at specific divergence times. A *post-hoc* correlation between the trait divergence with the geographic or environmental distances among taxa (or populations) could help in determining the sources of selective pressure in the follow-up research.

For the example of *Lithocarpus* in Taiwan, the evolutionary trends of the examined traits were not skewed from a random-walk process (Fig. 2 and Yang *et al.* (2018b)). However, we detected strong selective constraints on C/N and PA in lineages deriving from node 19, with subsequent extensive divergence between lineages deriving from node 20 as well as adaptive divergence of YII between lineages deriving from node 19 (Fig. 3). The derived lineages of nodes 19 and 20 diverged approximately since Pliocene or Pleistocene (Table S2), when the climate changed dramatically. Such trait divergence probably reflects the urgent niche partitioning of incipient species, especially those with only limited space in an island. Divergent functional traits are beneficial to the rapid adaptation of incipient species. However, the conservatism of co-adaptive traits, similar evolutionary trajectory, and the type-II error caused by large standard deviation could lead to overestimation of the conservative evolution of traits in our method. Therefore, it should be more cautious in explaining conservative traits. Our results not only illustrate the particularity of the ecophysiological adaptation of the three endemic species (*L. nantoensis*, *L. shinsuiensis*, and *L. lepidocarpus*) in the example but also show that the adaptive divergence of traits is usually episodic during the evolutionary process and cannot be revealed merely by examining the overall evolutionary trend.

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SUPPLEMENTARY DATA

Table S1 List of 14 *Lithocarpus* species and three ecophysiological measurements and the altitudinal distribution. This table is excerpted from Yang *et al.* (2018a).

Species	YII±STD	PA	C/N	Altitude
<i>L. amygdalifolius</i>	0.7709±0.0267	47.70	36.552	250m~2000m
<i>L. brevicaudatus</i>	0.6814±0.0181	44.11	29.961	200m~2350m
<i>L. cornea</i>	0.6995±0.0382	72.68	24.170	100m~1400m
<i>L. dodonaeifolius</i>	0.7457±0.0326	44.53	38.397	350m~1600m
<i>L. formosanus</i>	0.7006±0.0206	45.33	36.320	100m~550m
<i>L. glaber</i>	0.7376±0.0273	28.65	24.504	450m~1050m
<i>L. hanceii</i>	0.7438±0.0245	26.72	16.110	100m~2700m
<i>L. harlandii</i>	0.7266±0.0255	30.00	32.745	350m~600m
<i>L. kawakamii</i>	0.7345±0.0403	34.03	18.893	350m~2350m
<i>L. konishii</i>	0.6646±0.0541	52.87	24.220	100m~1150m
<i>L. lepidocarpus</i>	0.7702±0.0245	61.07	20.297	600m~2230m
<i>L. nantoensis</i>	0.6290±0.0250	83.09	29.146	550m~1300m
<i>L. shinsuiensis</i>	0.7451±0.0315	103.1	38.697	300m~1200m
<i>L. taiтоensis</i>	0.7236±0.0468	36.46	28.848	700m~1300m

Table S2 Species divergence time inferred by six nuclear genes (Yang *et al.* 2018b).

Node	Time (Mya)		
	Median	95% HPD lower	95% HPD upper
15	24.19	18.62	28.95
16	14.73	10.78	19.42
17	11.16	6.04	16.58
18	7.64	2.44	12.49
19	3.21	0.21	6.98
20	1.00	0	3.65
21	10.74	6.79	15.30
22	8.55	4.73	12.64
23	6.47	2.89	10.49
24	5.13	1.66	9.09
25	3.09	0	6.75
26	1.55	0	4.97
27	4.01	0	13.69

95% HPD: 95% highest posterior density

YII±STD: phytochemical yield of photosystem II and the standard deviation, denotes the photosynthesis efficiency
 PA: contents of phenolic acids, denotes the intensity of chemical defense
 C/N: leaf C/N ratio, denotes the potential growth rate

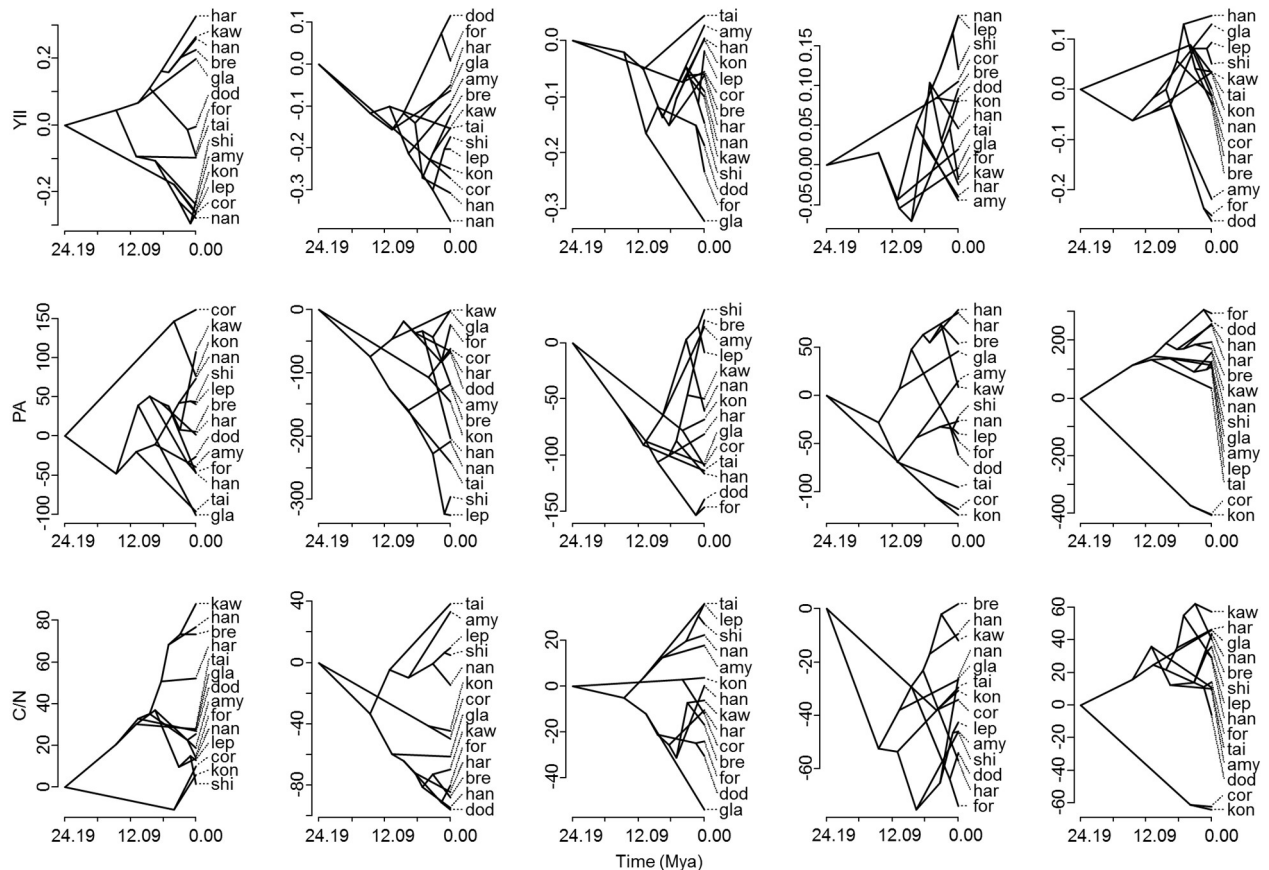


Fig. S1 Simulation trees of YII (the upper five plots), PA (the middle five plots), and C/N (the bottom five plots) under the Brownian motion (BM) model.

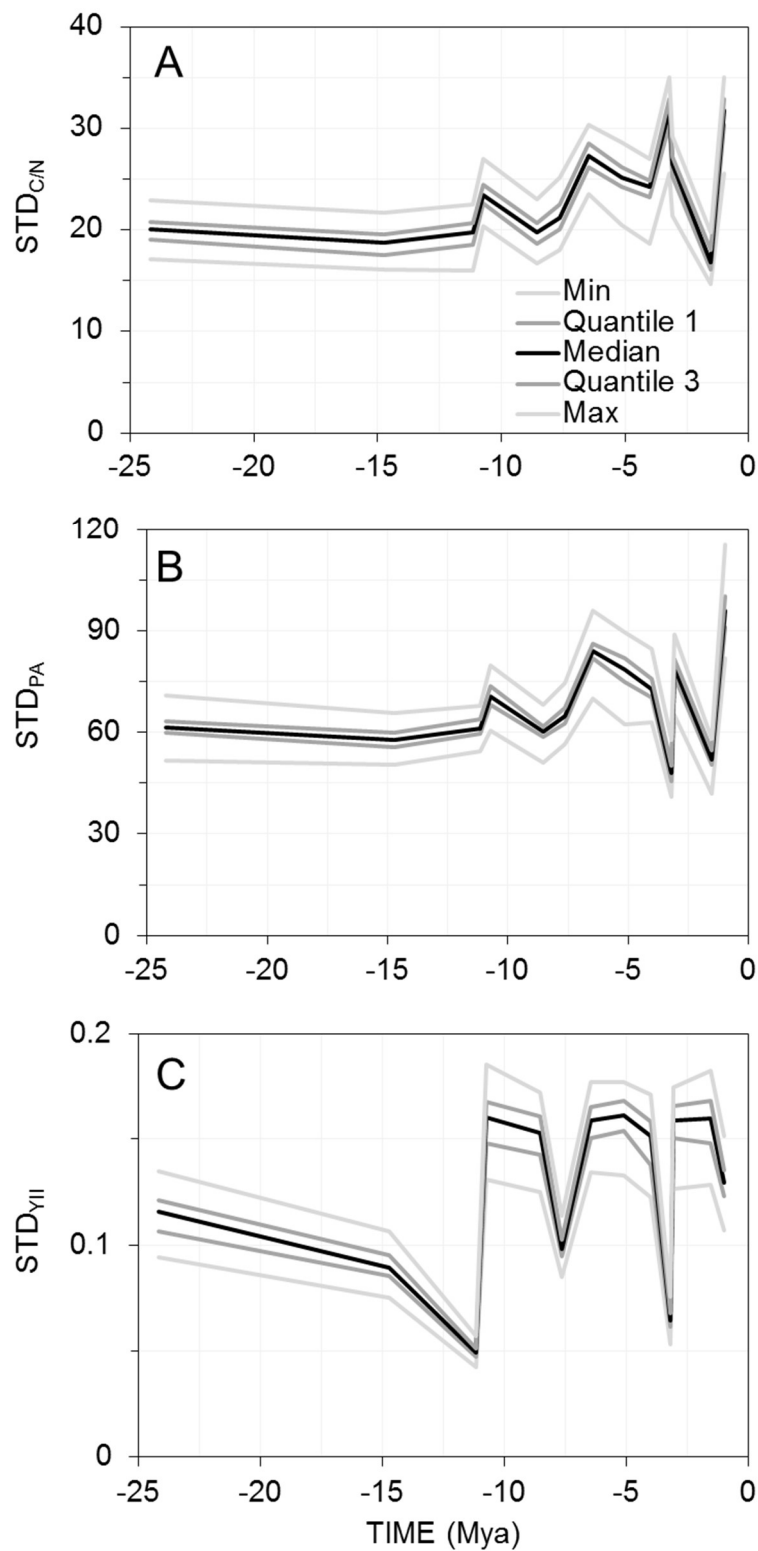


Fig. S2 Standard deviation (SD) of trait differences in (A) C/N, (B) PA, and (C) YII estimated under the Brownian motion (BM) model along the divergence time. The simulations of these trait divergences revealed a higher fluctuation trend in more recently diverged taxa; by contrast, the changes of SD were relatively stable in old lineages due to longer coalescent intervals. The SD distribution is computed from 200 datasets, and each dataset comprises of 500-times trait simulations under BM model.