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The great American biotic interchange and diversification history in *Dynastes* beetles (Scarabaeidae; Dynastinae)

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Biotic interchange between geographic regions can promote rapid diversification. However, what are the important factors that determine the rate of diversification (e.g., trait-dependent diversification) vary between study systems. The evolutionary history of *Dynastes* beetles, which can be found in both North and South Americas and exhibit two different altitudinal preferences (highland and lowland) is tested for the effects of biotic interchange between continents and different ecological preferences on the rate of species diversification. Additionally, the hypotheses of geological time-dependent and lineage specific diversification rates are also tested. Results from this study indicate that in *Dynastes* beetles a pre-landbridge dispersal hypothesis from South to North America is preferred and that the speciation rates estimated using BAMM are similar between lineages of different geographic origins and different altitudinal preferences (i.e., diversification rate is not trait-dependent). On the other hand, my result from marcoevolutionary cohort analysis based on BAMM outputs suggests that the rate of speciation in *Dynastes* beetles is, instead of trait-dependent (geographic and ecological), lineage specific. Furthermore, a steadily increasing speciation rate can be found in Pliocene and Pleistocene, which implies that geological and climatic events, i.e., colonizing North America, habitat reformation in the Amazonia, and forest contraction in Pleistocene, may have together shaped the current biodiversity pattern in *Dynastes* beetles.

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INTRODUCTION

Biotic interchange between geographic realms creates opportunities for species diversification (Cody *et al.*, 2010; Gillespie *et al.*, 2012); however, the colonization and diversification histories can differ significantly between taxa. Closely related lineages found in both North and South America characterize the Great American Biotic Interchange (GABI), and provide excellent candidates for studying the effects of biotic interchange on generating biodiversity. It is hypothesized that the closure of the Isthmus of Panama around 3.5 million years ago initiated GABI (Marshall, 1988). Many terrestrial lineages expanded their geographic ranges into previously unreachable regions by travelling through the newly emerged land bridge (H_1). Recent studies have revealed additional colonization routes that can also account for, and may have more significant effects on, GABI: travelling (e.g. rafting) across the marine barrier before the closure of the Isthmus of Panama (H_2; Bacon *et al.*, 2015) and island-hopping via the Antilles Archipelago (H_3; e.g. Ali, 2012). It is also possible that a widely distributed ancestor prior to the separation of Laurasia from Gondwana gave rise to similar descendant lineages throughout North and South America (H_4; e.g. Crisci *et al.*, 1991). Currently lineage diversification patterns across different groups of organisms living in both North and South America differ significantly (Cody *et al.*, 2010), which may reflect different historical contingencies resulting from colonization via different routes.

In addition to the colonization history, different species diversification patterns associated with GABI

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have also intrigued generations of evolutionary biologists. For example, multiple mammalian lineages of North American origin experienced radiation after colonizing South America (Simpson, 1950; Marshall, 1988), whereas plant lineages of South American origin diversified after colonizing North America (Cody *et al.*, 2010; Bacon *et al.*, 2015). Colonization into a new geographic area could have resulted in a sudden increase in species number simply because of the newly founded habitats containing multiple open niches. In addition, multiple forest ecoregions have arisen contemporaneously with GABI in South America that could also promote species diversification. Specifically, habitat reformation occurred in Amazonia, where the Andes Mountains extended northwards and distinct forest ecoregions were formed in the Pliocene (Morrone, 2006; Hoorn *et al.*, 2010). Furthermore, forest contraction during the Pleistocene could further accelerate allopatric divergence between forest-dwelling taxa (Garzón-Orduña, Benetti-Longhini & Brower, 2014). The question of whether differences in diversification rate between evolutionary lineages are trait-dependent (geographical and ecological), geological time-dependent (Miocene vs. Pliocene–Pleistocene), or evolutionary lineage-specific (these are not mutually exclusive, and can be analysed under the same framework; Rabosky, 2014; Rabosky *et al.*, 2014) has been tested intensively in macroevolutionary studies, however, and can vary significantly depending on the systems studied.

The evolutionary history of a group of giant beetles (genus *Dynastes* MacLeay, 1819; Table 1) with a hypothesized South American origin (Dutrillaux & Dutrillaux, 2013), from the Americas, is used in this study to investigate the biogeographic history of GABI and to test the effects of different macorevolutionary factors that may drive species diversification. There are two major lineages in this genus. (1) Subgenus *Theogenes* Burmeister, 1847, which includes *Dynastes neptunus* (Quensel, 1805) and *Dynastes satanas* Moser, 1909. These two species are restricted to the highland Andes of South America, and can be distinguished from species of the other subgenus by completely black elytral coloration and distinct tarsal morphology (Hwang, 2011). (2) Subgenus *Dynastes*, which is distributed throughout North and South America, and is composed of two major groups that can be found in both highland and lowland forest habitats: (2.1) the Giant Hercules group, which includes at least ten evolutionarily independent lineages, and can be found in the Neotropics and the Lesser Antilles islands (Chalumeau & Reid, 2002; Huang & Knowles, 2015; Silvestre, 1996); (2.2) the White Hercules group, which includes five evolutionarily independent lineages, and can be found in forested habitats of North and Central America (Morón, 2009; Huang & Knowles, 2015).

The difference in preference for lowland or highland habitats is important, because intercontinental dispersal was most likely achieved via the newly emerged lowland land bridge or via rafting between lowland coastal regions, whereas highland distribution can be associated with restricted geographic distribution and local endemism (Hoorn *et al.*, 2010).

Table 1. Species included in this study

Subgenus	Taxa	Abbr.	States*	Geographic distribution
Dynastes	*D. granti*	Dg	0,1	Highland of the southern edge of the Rockies
	D. hyllus	Dhy	1,1	Sierra Madre of Mexico
	D. moroni	Dmo	0,1	Sierra de Los Tuxtlas
	D. maya	Dma	1,1	Central American rainforest
	D. tityus	Dty	1,1	Southeast of North America
	D. h. hercules	Dhh	1,0	Guadeloupe and Dominique
	D. h. reidi	Dhr	1,0	Saint Lucia and Martinique
	D. h. paschoali	Dhp	1,0	Northern Atlantic Forest of Brazil
	D. h. occidentalis	Dho	0,0	The Chocó-Darién
	D. h. septentrionalis	Dhs	0,1	Cloud forests of Central America
	D. h. lichyi	Dhl	0,0	Highland cloud forest of the Andes
	D. h. bleuzeni	Dhb	1,0	Orinoco delta
	D. h. trinidadensis	Dht	1,0	Trinidad and Tobago
	D. h. morishimai	Dhm	0,0	The Yungas of Bolivia
	D. h. ecuatorianus	Dhe	1,0	Lowland Amazonian rain forest
Theogenes	*D. neptunus*	Dn	0,0	Highland cloud forest of the Andes
	D. satanas	Ds	0,0	The Yungas of Bolivia

*Altitudinal (before comma) and geographic (after comma) states of each taxon are represented by 0 (highland or South America) or 1 (lowland or North America).

Furthermore, drastic changes in lowland and highland forest habitats are recorded in the recent geological history (Hoorn *et al.*, 2010; Garzón-Orduña *et al.*, 2014), which may have affected the associated diversification rates differently. Therefore, the geographic distribution and the difference in altitudinal preference make *Dynastes* beetles an excellent system to study how species diversification proceeds when intercontinental biotic interchange occurred. Additionally, rafting (H_2), island-hopping (H_3), and walking across the Isthmus of Panama (H_1) are all possible explanations for the current distribution of *Dynastes* beetles. For example, the ability to raft across oceans on drifting wood has been demonstrated in arthropods (Coulson *et al.*, 2002). In addition to having a mobile adult stage, Hercules beetles have larval periods where they are constrained to rotten wood, which may serve as overwater dispersal vessels for *Dynastes* beetles. Nevertheless, Hercules beetles have the highest species diversity in Central America (Morón, 2009), which implies that Central America might have been the first region to be colonized, and this favours H_1 and H_2 over H_3 as being the most likely routes. Additionally, the time to the common ancestor of the *Dynastes* beetles is estimated around 11 Mya (95% probability density ranges from 6.5 to 17.5 Mya; Huang & Knowles, 2015), which precludes the effect on species diversification from H_4 (which expects a common ancestor emerged prior to the Triassic, 200 Mya). This study hence focuses on testing whether the complete formation of the Isthmus of Panama had promoted the colonization of North America (H_1), or whether Hercules beetles travelled across the narrow oceanic strait before 3.5 Mya (H_2).

The reconstructed species tree of *Dynastes* beetles proposed by Huang & Knowles (2015), where the evolutionary independence of each tip taxon is quantitatively tested using multiple data types, is used here to study the biogeographic and diversification history. Ancestral area reconstruction and macroevolutionary comparative methods are used to answer the following questions: (1) does the closure of the Isthmus of Panama promote the colonization of North America; (2) do North American taxa have a higher diversification rate; (3) does speciation rate differ according to different altitudinal preferences; and (4) what are the major factors affecting the diversification history in *Dynastes* beetles?

MATERIAL AND METHODS

Species trees

A reconstructed species tree, the majority clade credibility tree, generated by the *BEAST analysis proposed by Huang & Knowles (2015), was obtained from the Dryad data repository (doi: 10.5061/dryad.8p6m0). Taxa included in this study and their associated geographic areas and ecoregions are summarized in Table 1. Note that species and subspecies in *Dynastes* beetles can be statistically equivalent based on molecular, morphological, and ecological data (Huang & Knowles, 2015). In this study, species and subspecies are all treated as different species (they all merit different species status, following the general lineage concept; Huang & Knowles, 2015).

Reconstructing biogeographic history

A geographic state, North or South America, was assigned to each taxon (Table 1). Central American taxa were assigned into the North America category, whereas taxa from the Lesser Antilles Islands were assigned to the South America category. The dispersal, extinction, and cladogenesis model (DEC; Ree & Dmith, 2008) was used to reconstruct the biogeographic history for *Dynastes* beetles. The unconstrained dispersal rate was first used to reconstruct the biogeographic history. I further tested two additional models to investigate whether dispersal happened after the closure of the Isthmus of Panama. The first alternative model assumed that the dispersal rate between North and South America was 50% less before the closure (3.5 Mya) than after; the second alternative model assumed that there was no dispersal prior to the completion of the land bridge. To account for uncertainty in molecular dating, two additional sets of analyses that applied different times for the rate switch (specifically, 4 and 5 Mya) were also performed. Differences in fitting these models to explain the diversification pattern of the species tree were directly compared using the estimated likelihood values between models.

Estimating speciation/diversification rate

The net diversification, speciation, and extinction rates based on the *Dynastes* species tree were estimated using the program BAMM (Rabosky, 2014; Rabosky *et al.*, 2014). Specifically, a total of 2×10^8 generations of reversible-jump Markov chain Monte Carlo (rjMCMC) searches, with samples stored every 1×10^5 generations, was performed using the speciation–extinction analyses via BAMM. A total of 1000 post burn-in samples (50%) were retained. Note that a separate BAMM analysis that incorporated the information about possible missing taxa, specifically one in the *Theogenes* group and three in the Giant Hercules beetles [i.e. *Dynastes neptunus rouchei* Nagai, 2005, *Dynastes hercules takakuwai* Nagai, 2002, and *Dynastes hercules tuxtlaensis* Moron, 1993 (Hwang, 2011), and a genetically distinct *Dynastes* *hercules reidi* Chalumeau, 1977 lineage from the island of Martinique (Huang, 2014)], resulted in a similar pattern of diversification rate through time (Fig. S1); therefore, only the results that assume complete taxon sampling from the current species tree were shown. The estimated speciation rate of the *Dynastes* species tree was plotted using the plot.bammdata function from the R package BAMMtools (Rabosky *et al.*, 2014). Additionally, the number of post burn-in MCMC samples that support a significant rate shift on the species tree was calculated using a Bayes factor threshold of 3. The estimated net diversification and speciation rates through time were then plotted using the plotRateThroughTime function. Furthermore, a macroevolutionary cohort analysis (Rabosky *et al.*, 2014) was used to test if the estimated speciation rate is highly correlated between closely related lineages using the function getCohortMatrix and cohorts from BAMMtools. Although the extinction rate was estimated as a model component in BAMM, the extinction rate was not reported in this study, and the interpretation of changes in extinction rate was avoided because of controversies regarding estimating extinction rates from molecular phylogenies (Rabosky, 2010).

TESTING THE EFFECTS OF DIFFERENT GEOGRAPHIC DISTRIBUTIONS AND ALTITUDINAL PREFERENCES ON SPECIES DIVERSIFICATION

Because speciation rate can not only change through time, but also correlated with phylogenetic relatedness, trait-dependent evolutionary analyses that do not take these factors into consideration can result in erroneous inferences (Rabosky & Goldberg, 2015). The structured rate permutations on phylogenies (STRAPP) analysis was developed to cope with such problems by comparing the observed difference in speciation rate between species that exhibit different trait states to a background speciation rate through randomizing the estimated tip speciation rates from the BAMM outputs (Rabosky & Huang, 2015). STRAPP analyses for testing trait-dependent speciation rate in this study were performed using the traitDependentBAMM function from BAMMtools. Specifically, the speciation rate in *Dynastes* beetles correlates with different altitudinal preferences or with different geographic origins assessed by 1×10^4 permutations.

RESULTS

BIOGEOGRAPHIC HISTORY RECONSTRUCTION

The model with no dispersal constraint performs best among all three models ($-\ln L = 9.453$; alternative models, $-\ln L = 9.545$ and 9.868 for 50% and 100% less dispersal prior to 3.5 Mya, respectively), implying that the closure of the Isthmus of Panama may not have had a significant effect on the colonization of North America in *Dynastes* beetles. Analyses that assumed different times of rate switching lead to the same results. The maximum-likelihood reconstructed ancestral area for each branch is shown based on results from the best (unconstrained) model (Fig. 1). South America is inferred as the ancestral state. The ancestral state for the lineage leading to Hercules beetles (subgenus *Dynastes*) is either South America ($\ln L = -10.18$, $P = 0.4837$) or widespread ($\ln L = -10.35$, $P = 0.4076$) (Fig. 1). Two inferred dispersal events can be found on branches leading to the White Hercules taxa and to a Giant Hercules taxon, Dhs (Fig. 1). The estimated global dispersal and extinction rates are 0.03285 and 4.285×10^{-9}, respectively.

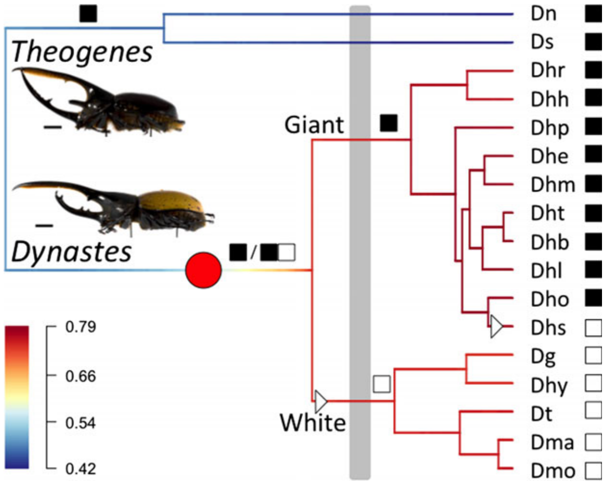

Figure 1. Results from BAMM and LAGRANGE analyses. Branch colour represents estimated speciation rate, where a warmer colour indicates a faster rate. A red dot on the branch leading to subgenus *Dynastes* indicates a speciation rate shift event. Black and white squares denote geographic states of South and North America, respectively. The black and white squares located on branches denote the reconstructed ancestral geographic area, whereas the black and white squares next to the taxon abbreviations indicate current geographic states (note that the reconstructed ancestral state for the common ancestor of subgenus *Dynastes* can be either South America or widespread). White triangles indicate inferred dispersal events into North America. A grey shaded area indicates the time frame when the Isthmus of Panama was completely formed (3.4–3.6 Mya). Representative samples of *Dynastes neptunus* (subgenus *Theogenes*) and *Dynastes hercules ecuatorianus* (subgenus *Dynastes*) are shown with a scale bar of 1 cm.

CHANGES IN DIVERSIFICATION RATES

The rjMCMC searches in the BAMM analysis reached plateau soon after the first 1000 generations. By using a Bayes factor of 3 as the threshold, 33% of the post burn-in samples indicate a significant rate shift, and this rate shift, which is an increase in diversification rate, is located on the branch leading to subgenus *Dynastes* (Fig. 1). The rate through time (RTT) plots unveil steadily increasing speciation and net-diversification rates through time, where a sudden increase in rates can be found around 4 Mya (Fig. 2). The results from macroevolutionary cohort analysis reveal that the phylogenetic distance between taxa is highly correlated with the estimated speciation rate. For example, species from the White Hercules beetles share a highly similar speciation rate, whereas the estimated speciation rates between species from White and Giant Hercules beetles are less similar (Fig. 3).

THE EFFECTS OF DIFFERENT GEOGRAPHIC DISTRIBUTIONS AND ALTITUDINAL PREFERENCES

The estimated speciation rates for North and South American taxa are 0.75 ± 0.006 (SE) and 0.71 ± 0.043, respectively (Fig. 4). A Student's t-test assuming unequal variance shows insignificant difference in estimated speciation rates ($t = -0.9226$, d.f. = 10.403, $P = 0.3771$). In addition, the STRAPP result also indicates that the estimated speciation

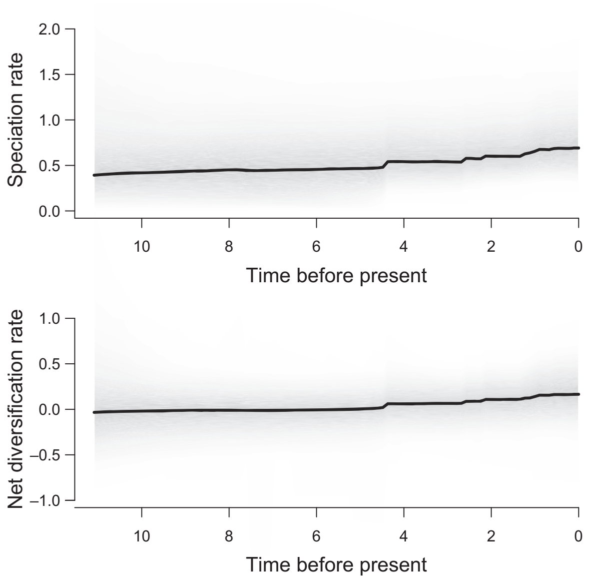

Figure 2. Results from rate-through-time plots. The scale of the x-axis is in million years. Solid lines indicate mean rates, whereas grey areas represent the 5–95% Bayesian credible regions for the distributions of the rates.

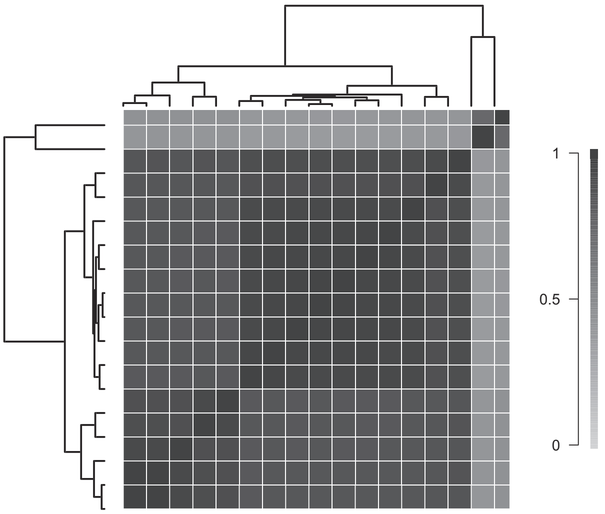

Figure 3. Results from macroevolutionary cohort analysis. A correlation matrix based on speciation rates between tip lineages of the phylogeny is plotted, where each correlation is a posterior frequency that the two compared species are found in the same macroevolutionary rate regime. A darker colour represents a higher correlation than a lighter colour. The correlation between any two species can be found by locating their intersection in the matrix.

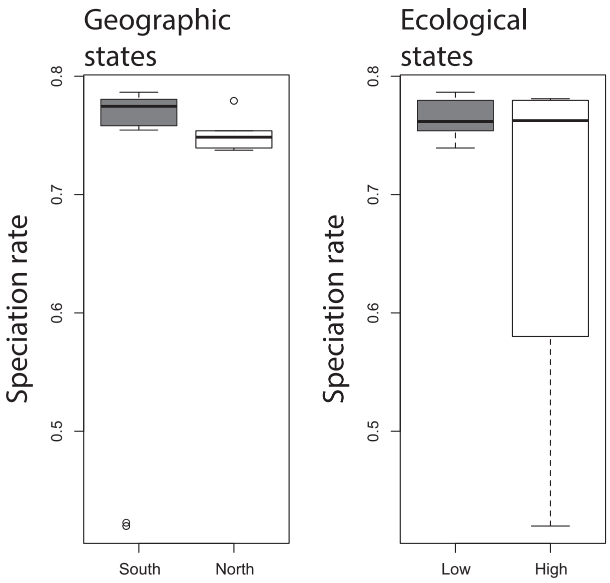

Figure 4. Comparisons of estimated speciation rates between trait states. Left panel: the estimated rates between South (black) and North (white) American *Dynastes* beetles. Right panel: the estimated tip rates between lowland living (black) and highland living (white) species.

rate is not significantly dependent on the geographic states ($P = 0.919$). Similarly, the estimated speciation rates between taxa of different altitudinal preferences (0.76 ± 0.006 and 0.68 ± 0.057 for lowland and highland species, respectively; Fig. 4) are not statistically different ($t = 1.4672$, d.f. = 7.146, $P = 0.1849$). The STRAPP result also reveals insignificant support for an altitudinal preference-dependent speciation rate ($P = 0.9105$).

DISCUSSION

A recently reconstructed *Dynastes* phylogeny, where all taxa are statistically supported as evolutionarily independent lineages, is used in this study to test biogeographic hypotheses about processes that may promote species diversification. It has been shown that a failure to recognize true biological entities in a macroevolution study can severely affect the interpretation of mechanisms that lead to the current biodiversity (Smith *et al.*, 2013). Given a fairly complete sampling of taxa that are statistically delimited as different species (Huang & Knowles, 2015), results and inferences made from this study should be robust. It is revealed here that *Dynastes* beetles have a South American origin, and that the GABI in Hercules beetles pre-dates the closure of the Isthmus of Panama. The estimated speciation rate is highly lineage specific and a rate increase can be identified on the branch leading to Hercules beetles. Results from RTT plots further indicate an increase, although slight, in the diversification rate in the Pliocene and the Pleistocene, which corresponds to a slowing down of geological activity and an increased frequency in climatic fluctuation, and the reformation of ecoregions in the Amazonia (Hoorn *et al.*, 2010; Garzón-Orduña *et al.*, 2014). The geographic states of North and South America have a similar effect on species diversification; additionally, different ecological states of preferring lowland and highland habitats also have similar effects on the speciation rate. The biogeographic history and factors affecting the diversification of *Dynastes* beetles are discussed in the following sections.

BIOGEOGRAPHY

A South American origin with subsequent dispersal events into North America is inferred in this study for the *Dynastes* beetles using the DEC model. This finding is in congruence with a recent study that investigated karyotypes (Dutrillaux & Dutrillaux, 2013). In that study, ancestral and derived chromosomal types were found in *Dynastes* taxa from South America, but the ancestral type was absent in North American taxa. It is also true that both subgenus *Dynastes* and *Theogenes* are distributed in South America, whereas there is no *Theogenes* taxa in North America. A South American origin of the genus *Dynastes* is thus a favoured hypothesis, and is supported by multiple lines of evidence.

Although the reconstructed time-calibrated species tree of *Dynastes* beetles reveals that lineages leading to North American taxa originated after the closure of the Isthmus of Panama (Fig. 1), the DEC model results indicate that assuming a constant dispersal rate between the Americas through time fits the species tree better. The results presented here may suffer from the effect of low statistical power to discriminate between models because of the small sampling size (only 17 tip taxa), but the clear trend of decreasing likelihood value for models assuming constrained dispersals before 3.5 Mya implies that the completion of the Isthmus of Panama may not be a major driving force for GABI in *Dynastes* beetles. Two Giant Hercules taxa (Dhh and Dhr) have successfully colonized the islands of the Lesser Antilles, i.e. Saint Lucia, Martinique, Dominique, and Guadeloupe (Chalumeau & Reid, 2002). A historical record indicates that they might have made it to Hispaniola as well (Wetherbee, 1985). The mobile adult stage, which could fly for a decent geographic distance, and a potential dispersing larval stage via drifting wood could have enabled Hercules beetles to travel across the narrow oceanic strait before the closure of the Isthmus of Panama (for a map of the Isthmus between 12 and 6 Mya, see Leigh, O'Dea & Vermeij, 2014). Such inference has also been reported in many terrestrial organisms, where specifically biotic introduction from South to North America became apparent around 6 Mya (Bacon *et al.*, 2015). Given the fact that Central America has the highest species diversity from the Hercules beetle lineage and a pre-land bridge dispersal model is favoured, the most likely historical scenario explaining the biogeography of *Dynastes* is H_2: i.e. pre-land bridge dispersal through rafting across Central America.

Conventional molecular biogeographic studies focusing on a similar question tend to infer a predominate role of the land bridge in intercontinental biotic dispersal if the estimated age of the common ancestor between North and South American lineages is found to be generally younger than or close to 3.5 Myr, based on a dated phylogeny. Although a pre-land bridge dispersal is inferred if the estimated common ancestor between lineages of North and South America significantly pre-dates 3.5 Mya, few studies have tried fitting different models on the reconstructed phylogeny and compared the goodness of fits between models before making inferences (Bacon *et al.*, 2013). The results from studying the *Dynastes* beetles presented here indicate that interpreting the deterministic process by observing molecular phylogenetic patterns in a biogeographic study without applying statistical tests between alternative explanations should be undertaken with caution.

DIVERSIFICATION PROCESS

It is revealed in this study that the speciation rate in *Dynastes* beetles is not trait dependent. An interesting finding here is that the geographic state of living in North America is not correlated with a higher speciation rate. Although rapid diversification is commonly observed after successful biotic introduction, it has been shown that mammal species with a South American origin tend to have limited success in diversifying in North America (Simpson, 1950; Marshall, 1988; Webb, 1991). The rainforest habitat, for example, only covers a small proportion of North America, allowing just a small area for species of South American origin to successfully diversify, assuming phylogenetic niche conservatism. Additionally, repeated glaciations during the Pleistocene in North America may have exterminated descendants from lineages of tropical South American origin. It is also intriguing to point out that the evolution of different ecological preferences does not correlate with differences in speciation rate. Although lowland living may have facilitated the colonization into previously isolated continents, speciation rate can depend on other factors, which may not be associated with the specific trait state that facilitates dispersal. Specifically, the number of available niches that may promote speciation in *Dynastes* beetles can be highly correlated with the number of allopatrically/parapatrically distributed forest ecoregions (speciation predominated by allopatric process; Huang & Knowles, 2015), which can be independent from the highland or lowland geographic state. That is, both highland and lowland regions have many distinct ecoregions.

Colonizing North America, however, did result in biological diversification in White Hercules beetles (i.e. five species were generated within 3 Myr), and thus the lack of opportunity, which is often invoked to explain the lack of successful diversification in mammals of South American origin after GABI, cannot fully explain the result of similar speciation rates between *Dynastes* lineages from North and South America. The speciation rate in White Hercules beetles is not comparable with that of its South American counterpart, Giant Hercules beetles (i.e. at least ten species are generated within 3 Myr; Fig. 1), whereas the speciation rate in White Hercules beetles is faster than that in the subgenus *Theogenes* (Fig. 1). Because South American lineages are composed of quickly diverging Giant Hercules beetles and slowly diverging *Theogenes* taxa (Figs 1, 3), the estimated speciation rate for all South American lineages as a whole can be misleading. Colonization into a new continent indeed resulted in species diversification in Hercules beetles; however, there were contemporaneous events occurring in South America (Hoorn *et al.*, 2010; Garzón-Orduña *et al.*, 2014) that could have resulted in an even faster speciation rate. Specifically, the recent formation of a variety of ecoregions in Amazonia (Hoorn *et al.*, 2010) and the subsequent contraction of forest because of drier climatic conditions in the Pleistocene (Garzón-Orduña *et al.*, 2014) could together lead to an increase in speciation rate in the South American Giant Hercules lineage. This inference can be further supported by the results from RTT plots, where an increased species diversification rate can be found in the Pliocene and the Pleistocene. Additionally, the results from macorevolutionary cohort analysis clearly suggest that speciation rate is highly lineage specific, and that the fastest diverging lineage in *Dynastes* is composed of taxa that live in ecoregions that are geographically very close to the northern Andes (Dhl, Dhb, Dhm, Dhe, Dho, and Dhs, for geographic distributions, see Huang & Knowles, 2015; Figs 1, 3; Table 1), where the habitats changed most drastically in the recent history. Compared with geographical and ecological explanations for the different diversification patterns found between biological systems in the Americas, the importance of lineage-specific properties and the formation of ecoregions in the Pliocene, and forest contraction during the Pleistocene (Garzón-Orduña *et al.*, 2014), have received less attention. It is clear, however, that geological and climatic events play important roles in shaping different diversity patterns in different *Dynastes* beetle lineages.

CONCLUSION

The biogeographic and diversification history in a giant beetle genus *Dynastes* was studied, and a potential problem of inferring historical processes by observing divergence patterns and times from a reconstructed phylogeny was revealed in this study. Although all North American lineages were formed after 3.5 Mya, dispersals between the Americas do not necessarily have to occur after 3.5 Mya. In fact, a model assuming a constant dispersal rate before and after the closure of the Isthmus of Panama better fits the reconstructed *Dynastes* species tree. The speciation rate in *Dynastes* beetles may not be dependent on specific geographic or ecological trait states. Instead, it is shown in this study that the speciation rate in *Dynastes* beetles changes in a lineage-specific manner, which can result from different lineage-specific historical processes. Specifically, the determinant of diversification in *Dynastes* beetles is likely to be the availability of different forest ecoregions, which is the result of changes in climatic conditions and geological activity, and varies across geological times.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. BAMM results from analysis incorporating information about possible missing taxa in the data.