Title: The Great American Biotic Interchange and diversification history in *Dynastes* beetles (Scarabaeidae; Dynastinae)

Running head: The biogeography of *Dynastes* beetles

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Abstract:

Biotic interchange between geographic regions can promote rapid diversification. However, what are the important factors that determine the rate of 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 rate 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 are similar between lineages of different geographic origins and different altitudinal preferences. On the other hand, the result from marcoevolutionary cohort analysis suggests that the rate of speciation in Dynastes beetles is, instead of trait (geographic and ecological) dependent, 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.

Keywords: Amazonia, BAMM, Pleistocene, Pliocene, trait dependent diversification

Introduction:

Biotic interchange between geographic realms creates opportunities for species
diversification (Cody et al. 2010, Gillespie et al. 2012). However, how did intercontinental biotic interchange occur differ significantly between taxa. Closely related lineages found in both North and South Americas characterize the Great American Biotic Interchange (GABI), which 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 region by traveling through the newly emerged landbridge (H$_1$). However, recent studies have revealed additional events that can also account for, and may have more significant effects on, GABI – they are traveling (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 Americas differ significantly (Cody et al. 2010), which may reflect different historical contingencies resulted from colonization via different routes.

In addition to the colonization history, different species diversification patterns associated with GABI also intrigue generations of evolutionary biologists. For example, multiple mammalian lineages of North American origin experienced radiation after colonizing South America (Simpson 1950, Marshall 1988), while plant lineages of South American origin diversified after colonizing North America (Cody 2010, Bacon et al. 2015). The 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 Andean mountain extent northward and distinct forest ecoregions were formed in Pliocene (Morrone 2006, Hoorn et al. 2010). Furthermore, forest contraction during Pleistocene could further accelerate allopatric divergence between forest dwelling taxa (Garzón-Orduña et al. 2014). However, the answer to if species diversification is trait dependent (geographical and ecological), geological time dependent (Miocene vs. Pliocene-Pleistocene), or evolutionary lineage specific (which are not mutually exclusive and can be analyzed under the same
framework; Rabosky 2014, Rabosky et al. 2014), although has been tested intensively in macroevolutionary studies, can vary significantly depending on the studied systems.

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 utilized in this study to investigate the biogeographic history of GABI and to test the effects of different macroevolutionary factors that may drive species diversification. There are two major lineages in this genus: (1) Subgenus Theogenes, Burmeister 1847, which includes D. neptunus, and D. satanas. 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 Americas and 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). (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 preferring lowland or highland habitats is important because intercontinental dispersal is most likely via the newly emerged lowland landbridge or rafting between lowland coastal regions, while highland distribution can be associated with restricted geographic distribution and local endemism (Hoorn et al. 2010). Furthermore, changes in lowland and highland forest habitats are found drastically in the recent geological history (Hoorn et al. 2010, Garzón-Orduña et al. 2014), which may have impacted 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 (H2), island hopping (H3), and walking across the Isthmus of Panama (H1) 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. The Hercules beetles nevertheless have the highest species diversity in Central America (Morón 2009), which implies
that Central America might be the first colonized region and favors $H_1$ and $H_2$ over $H_3$.

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 $H_4$ (a common ancestor emerged prior to 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 did Hercules beetles travel across narrow oceanic strait before 3.5 MYA ($H_2$).

The reconstructed species tree of Dynastes beetles proposed by Huang and 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 utilized 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?

Materials and methods:

Species trees

A reconstructed species tree, the majority clade credibility tree, generated by *BEAST analysis proposed by Huang and Knowles (2015) were 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 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, while taxa from the Lesser Antilles Islands were assigned with the South America category. The dispersal, extinction, and cladogenesis model (DEC, Ree & Smith 2008) was utilized to reconstruct the
biogeographic history for Dynastes beetles. Unconstrained dispersal rate was first used to
reconstruct the biogeographic history. I further tested two additional models to investigate if
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 landbridge. To account for uncertainty in molecular dating, two
additional sets of analyses that applied different times of 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 $2 \times 10^8$ generations of rjMCMC searches with samples stored every $1 \times 10^5$ generations
was performed using the speciation-extinction analyses via BAMM. A total of 1000 post burnin
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., D. neptunos rouchei, D. hercules takakuwai, and D. hercules
tuxtlaensis [Hwang 2011] and an genetically distinct D. h. reidi lineage from the island of
Martinique [Huang 2015]), 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 burnin 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 utilized to test if the
estimated speciation rate is highly correlated between closely related lineages using the function
cohortMatrix and cohorts from BAMMtools. Although the extinction rate was estimated as a
model component in BAMM, extinction rate was not reported in this study and the interpretation

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of changes in extinction rate was avoided due to 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, if speciation rate in Dynastes beetles is correlated with different altitudinal preferences or with different geographic origins was accessed by $1 \times 10^4$ permutations.

Results:

Biogeographic history reconstruction

The model without setting any dispersal constraint performs best among all three models (-lnL = 9.453; alternative models: −lnLs = 9.545 & 9.868 for 50% & 100% less dispersal prior to 3.5 MYA, respectively), implying that the closure of the Isthmus of Panama may not have significant effect on the colonization of North America in Dynastes beetles. Analyses that assumed different times of rate switch lead to the same results. The ML 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) can either be South America (lnL = -10.18, P = 0.4837) or widespread (lnL = -10.35, P = 0.4076) (Fig. 1). Two inferred dispersal events can be found on branches leading to the White Hercules taxa and to Dhs (Fig. 1). The estimated global dispersal and extinction rates are 0.03285 and $4.285 \times 10^{-9}$, respectively.
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 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 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 t-test assuming unequal variance shows insignificant difference in estimated speciation rates (t = -0.9226, df = 10.403, P = 0.3771). In addition, the STRAPP result also indicates that the estimated speciation 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, df = 7.146, P = 0.1849). The STRAPP result also reveals insignificant support for altitudinal preference dependent speciation rate (P = 0.9105).

Discussion:

A recently reconstructed Dynastes phylogeny, where all taxa are statistically supported as evolutionary independent lineages, is used in this study to test biogeographic hypotheses about processes that may promote species diversification. It has been shown that fail 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 unravelled here that Dynastes beetles have
a South American origin and that the GABI in Hercules beetles predates 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 slightly, in diversification rate in Pliocene and Pleistocene, which corresponds to a slowing down in geological activity and an increased frequency in climatic fluctuation and reformation of ecoregions in the Amazonia (Hoorn et al. 2010, Garzón-Orduña et al. 2014). The geographic states of North and South America have similar effect on species diversification; additionally, different ecological states of preferring lowland and highland habitats also have similar effect on speciation rate. The biogeographic history and factors affecting the diversification in 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 done by investigating karyotypes (Dutrillaux & Dutrillaux 2013). In their study, ancestral and derived chromosomal types are found in Dynastes taxa from South America, but the ancestral type is absent in North American taxa. It is also true that both subgenus Dynastes and Theogenes are distributed in South America, while there is no Theogenes taxa in North America. A South American origin of the genus Dynastes is thus a favored hypothesis and 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 results indicate that a model assuming constant dispersal rate between the Americas through time fits the species tree better. The results presented here may suffer from the effect of low statistic power to discriminate between models because of a 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 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 (see Leigh et al. 2014 for a map of the Isthmus between 12 and 6
MYA). Such inference has also been reported in many terrestrial organisms, where specifically a
South to North America biotic introduction 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-landbridge dispersal model is favored, the most likely historical scenario
explaining the biogeography of Dynastes is $H_2$: pre-landbridge dispersal through rafting across
Central America.

Conventional molecular biogeographic studies focusing on similar question tend to infer
a predominate role of landbridge in inter-continental biotic dispersal if the estimated age of the
common ancestor between North and South American lineages are found generally less than or
close to 3.5 MYA based on a dated phylogeny. While pre-landbridge dispersal are inferred if the
estimated common ancestor between lineages of North and South America significantly predates
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 Dynastes beetles presented here indicate that interpreting deterministic
process by observing molecular phylogenetic patterns in a biogeographic study without applying
statistic tests between alternative explanations should be taken with cautions.

Diversification process

It is revealed in this study that 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 having a South
American origin tend to have limit success to diversify in North America (Simpson 1950,
Marshall 1988, Webb 1991). The rain forest habitat, for example, only covers a small proportion
of North America, allowing only a small area for species of South American origin to
successfully diversify assuming phylogenetic niche conservatism. Additionally, repeated
 glaciations during Pleistocene in North America may have exterminated decedents from lineages
of tropical South American origin. It is also intriguing to point out that the evolution of different
ecological preferences does not correlate to differences in speciation rate. Although lowland living may have facilitated the colonization into previously isolated continents, speciation rate can be dependent on other factors, which may not be associated with the specific trait state that facilitate dispersal. Specifically, the number of available niches that may promote speciation in Dynastes beetles can be highly correlated to the number of allopatrically/parapatrically distributed forest ecoregion (speciation predominated by allopatric process; Huang & Knowles 2015), which can be independent from being a 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 are generated within 3 million years), 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, can not fully explain the result of similar speciation rates between Dynastes lineages from North and South Americas. Speciation rate in White Hercules beetles is not comparable to that of its South American counterpart, Giant Hercules beetles (i.e., at least 10 species are generated within 3 million years; Fig. 1). Whereas speciation rate in White Hercules beetles is faster than that in the subgenus Theogenes (Fig. 1). Because South American lineages are composed of fast diverging Giant Hercules beetles and slow diverging Theogenes taxa (Fig. 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), which 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 subsequently the forest contraction because of a drier climate condition in 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 Pliocene and Pleistocene. Additionally, the results from macreovolutionary 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 [see Huang & Knowles 2015 for geographic distributions]; table 1; Fig. 1 & 3), where the habitats changed most drastically in the recent history. Comparing
to geographical and ecological explanations of different diversification patterns found between biological systems in the Americas, the importance of lineage specific properties and the formation of ecoregions in Pliocene and forest contraction during Pleistocene (Garzón-Orduña et al. 2014) have received less attention. However, it is clear 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 is studied and a potential problem of inferring historical process by observing divergence patterns and times from a reconstructed phylogeny is unravelled in this study. Although all North American lineages are formed after 3.5 MYA, dispersals between 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 fits better the reconstructed Dynastes species tree. Speciation rate in Dynastes beetles may not be dependent on specific geographic or ecological trait states. Instead, it is shown in this study that speciation rate in Dynastes beetles changes in a lineage specific manner, which can be due to different lineage specific historical processes. Specifically, the determinant of diversification in Dynastes beetles is likely the availability of different forest ecoregions, which is the result of changes in climatic condition and geological activity and varies across geological times.

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References:


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Table 1.

<table>
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<th>Subgenus</th>
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<th>Abbr.</th>
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*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).

**Figure legends:**

1. Results from Bamm and LAGRANGE analyses. Branch color represents estimated speciation rate, where a warmer color 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. Black and white squares located on branches denote reconstructed ancestral geographic area, while those that next to the taxon abbreviations 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).

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

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 color represents a higher correlation than a lighter color. The correlation between any two
species can be found by locating their intersection in the matrix.

Fig. 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.