



# Revisiting the Great American Biotic Interchange through analyses of amphitropical bees

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The Great American Biotic Interchange (GABI) is zoogeographic event characterized by the exchange of taxa between North and South America, typically associated with the rise of the Isthmus of Panama in the late Pliocene. Recent geologic evidence suggests the connections between North and South America may be much older, and that the interchange of organisms between the two continents could have therefore happened much earlier than 3 Ma. Most of the research investigating the GABI has come from tropical vertebrate taxa; little work has been done on invertebrates or on non-tropical species. To investigate how the GABI shaped the distribution of arid-adapted species, particularly those with amphitropical distributions (i.e. taxa found in South and North American xeric regions yet absent from the tropics), we examine the historical biogeography of the bee genus *Diadasia* using a hypothesis of *Diadasia* phylogenetic relationships. Nuclear and mitochondrial genetic loci are used to reconstruct a phylogeny of *Diadasia*, which is then used to estimate divergence dates and reconstruct ancestral area relationships. Our analyses suggest the divergence between North and South American *Diadasia* species occurred between 20.5 and 15 Ma, long before the formation of the Isthmus of Panama. This study is the first to show a Miocene connection for an amphitropically-distributed insect group. It suggests that the biotic connection between continents is more complicated than previously thought and may have initiated long before the late Pliocene.

The connection of North America to South America via the Isthmus of Panama facilitated one of the most celebrated biogeographical events in the Western Hemisphere, the Great American Biotic Interchange (GABI) (Marshall et al. 1982, Stehli and Webb 1985, Marshall 1988). The GABI, which is most often characterized by the movement of land mammals between North and South America (Webb 2006), enabled the movement of multiple taxa between continents, re-shaping the biotic composition of both regions. While the rise of the Isthmus of Panama in the late Pliocene (3.5–3.1 Ma) (Coates and Obando 1996) has often been cited as the event that facilitated the GABI (Webb 2006, Weir et al. 2009, reviewed by Woodburne 2010), most of the support for this event comes from indirect geochemical or biotic studies (Montes et al. 2012).

Several recent analyses have suggested that there were intercontinental exchanges that preceded the Pliocene connection between North and South America (reviewed by Cody et al. 2010, Pinto-Sánchez et al. 2011). These movements between continents could have resulted from long-distance dispersal (Kirby et al. 2008, Cody et al. 2010), or through island hopping along the paleo-Antillean islands that were situated between North and South America during the Miocene (23–25 Ma, Iturralde-Vinent and MacPhee 1999, Sturge et al. 2009). Recent geological

evidence suggests that most of the landmass comprising the Isthmus of Panama may have been above sea level from the late Eocene (~40 Ma) and that only a narrow strait separated southern Central America and South America (Montes et al. 2012). Although multiple lines of biotic and geologic evidence clearly show that the GABI is more complicated than previously thought (Iturralde-Vinent and MacPhee 1999, Webb 2006, Sturge et al. 2009, Montes et al. 2012), more research is needed on non-mammalian taxa (Pinto-Sánchez et al. 2011), particularly for invertebrate taxa, in order to better understand the history of biotic connections between new world land masses. Furthermore, most of the research investigating the GABI has been done on tropical taxa inhabiting northern South America and Central America (Weir et al. 2009, Pinto-Sánchez et al. 2011). It remains unclear how temperate and arid-adapted groups, which inhabited areas farther from the actual land bridge, were influenced by the GABI.

While the formation of the Isthmus of Panama clearly facilitated movement of tropical taxa from the Central American tropics to the South American tropics and vice versa, these tropical environments are often viewed as a barrier to arid-adapted organisms (Raven 1963, Michener 1979). Despite this barrier, several taxa exhibit an amphitropical distribution, where they are found in the North and

South American temperate or desert regions but are rare in the tropics (Raven 1963). These include bees and other insects (Michener 1979, Michelette and Camargo 2000, Roig-Junent and Cicchino 2001), lichen (Tibell 1994), fish (Lindberg 1991), some mammal species (Rietz 1940), and many plants including *Sphaeralcea* (Bray 1898, Kearney 1935, Krapovickas 1949, Fryxell 1997) and *Opuntia* (Raven 1963). Organisms with this distribution are often thought to be of recent origin (Raven 1963) and may have dispersed during the late Pleistocene (126 000–11 000 yr ago) when much of the new world tropics resembled savanna or grassland (Woodburne 2010). However, the timing of divergence and the role of the GABI remains untested for most amphitropically distributed organisms.

The amphitropical bee genus *Diadasia* contains 48 species, 30 found in the arid parts of North America and 18 found in the xeric portions of South America (Sipes and Wolf 2001). The tribe Emphorini, to which *Diadasia* belongs, likely originated in South America sometime after the breakup of Gondwanaland, as evidenced by the fact that all emphorine genera are restricted to North and South America, with many exclusively in South America (Sipes and Wolf 2001). Only *Diadasia* is well-represented in North America. While some Emphorini, including a few *Diadasia* species are found in tropical Central America, the group as a whole is predominantly arid-adapted, which suggests that the few mesic-adapted species represent derived states rather than ancestral habitat preferences. This genus provides an ideal system to study the potential role of the GABI in present-day distributions of non-tropical taxa. Here, we investigate the function of the GABI in creating the extant amphitropical distribution of *Diadasia* by estimating the timing of diversification events, and by reconstructing the ancestral distributions of the genus. Through these analyses we specifically test whether Pleistocene savannas allowed for movement between continents, if the Pliocene connection of the Isthmus of Panama was responsible for this movement, or if older events played a role.

## Material and methods

### Taxon and molecular sampling

A total of 27 *Diadasia* species were included in this analysis (22 from North America and five from South America), as well as specimens representing five outgroup genera. We include one species from the *Dasiapis* subgenus of *Diadasia* (*D. ochracea*). This subgenus includes three cryptic taxa that may represent geographic varieties of one species (Snelling 1994). The *Dasiapis* subgenus is unique in that it includes *D. tropicalis*, the only *Diadasia* species to span tropical Central and South America.

Specimens used in this study represent a subset of those specimens analyzed by Sipes and Wolf (2001). Specifically, we only included a single representative of each of the species treated by Sipes and Wolf (2001). Species level sampling was greater in North American species than in the South American species due in part to the increased availability of fresh specimens from North America, which yielded higher quality DNA for molecular analyses. Two genetic regions

were amplified from each specimen, the nuclear gene elongation factor 1-alpha (EF1-alpha: ~1 kb) and the mitochondrial region including cytochrome oxidase I (COI), the tRNA leucine, and part of cytochrome oxidase II (COII) ~2 kb in total (for details see Sipes and Wolf 2001). All DNA sequences used in this study were obtained from the authors that generated them (Sipes and Wolf 2001) and are also available from Genbank (COI/II: Accession Nos. AF300521–AF300575; EF1-alpha: Accession Nos. AF300474–AF300520). Because of some apparent heteroplasmy in the mitochondrial regions, all variable sites were coded as polymorphisms (Sipes and Wolf 2001). In addition to *Diadasia*, five of the eight other genera belonging to the tribe Emphorini (*Alepidoscelis*, *Meliphilopsis*, *Melitoma*, *Ptilothrix*, and *Toromelissa*) were included as outgroups.

### Phylogeny reconstruction and molecular dating

The relationships and divergence dates among *Diadasia* species were estimated through a Bayesian Markov Chain Monte Carlo (MCMC) averaging approach to rate smoothing using the program BEAST ver. 1.4.8 (Drummond and Rambaut 2007) using a combined molecular dataset. Because single terminals represent individual species, a Yule process speciation prior for branching rates was implemented and the general time-reversible model with invariant sites and gamma-distributed rate variation across sites (GTR + I +  $\Gamma$ ) was applied with base frequencies estimated during the analysis. An uncorrelated lognormal model was applied to estimate the relaxed molecular clock. The analysis was run using the default MCMC parameters with the MCMC chains being set for 10 000 000 generations and sampled every 1000 generations with a 10% burnin removed from the analysis. Convergence and burn-in were assessed using Tracer ver. 1.4.1 (Rambaut and Drummond 2007).

While there are no known fossils of *Diadasia*, we calibrated our divergence dates based on an estimated age of the crown group of Emphorini (~38 Ma: Cardinal et al. 2010). This estimated age is based on a dated phylogeny that was constructed using seven genetic regions and calibrated with 10 paleontologically based calibration points (Cardinal et al. 2010). Because of the uncertainty in the age of calibration points, we applied a normally distributed prior to the root node, with a mean age of 38 Ma and a standard deviation of 5 Ma.

### Ancestral area reconstructions

The historical biogeography of Emphorini was estimated using RASP ver. 2.0b (reconstruct ancestral state in phylogenies: Yu et al. 2010, 2011) based on the tree that resulted from the BEAST analysis. In this analysis we assigned terminal taxa as either inhabitants of North America or South America. For the outgroup genera, area was assigned at the generic level (i.e. while *Ptilothrix bombiformis* is a North American species, most other members of the genus *Ptilothrix* inhabit South America so the taxon was coded as inhabiting both areas). We used the F81 model for the Bayesian MCMC analyses, allowing for different rates of change among ancestral areas.

## Results

The Bayesian phylogenetic analysis of the combined molecular dataset resulted in a well-supported tree (Fig. 1), with most nodes supported with a posterior probability of 0.95 or greater. This phylogenetic hypothesis suggested the same relationships among *Diadasia* species as previous analyses that used the same dataset (Sipes and Wolf 2001). The molecular dating analysis suggests an early Miocene (~25 Ma) age of *Diadasia*, with most of the species-level diversification in the North American species occurring before the Pleistocene (2.5 Ma), primarily taking place between 15 and 5 Ma (Fig. 1).

Ancestral area reconstructions for each node were recovered with a posterior probability of 0.85 or greater, and show

that ancestral Emphorini most likely inhabited, and originated in, South America (Fig. 1). Furthermore, there were at least four dispersal events between continents, two in outgroup Emphorini genera (*Ptilothrix* and *Melitoma*) and two within *Diadasia*. While our analysis does not include enough species-level diversity in outgroup genera to measure the precise age of their intercontinental exchanges, the age of dispersal events in *Ptilothrix* and *Melitoma* could have occurred as early as 19 Ma (Fig. 1). There appear to be two migratory events within *Diadasia*. In one, the common ancestor of North American *Diadasia* is dated between 20.5 and 15 Ma (depending on if the dispersal occurred in the stem group or the crown group). The other *Diadasia* dispersal event occurred in *D. ochracea*, and could have taken place as early as 13.5 Ma (Fig. 1) but our data do not allow us to speculate

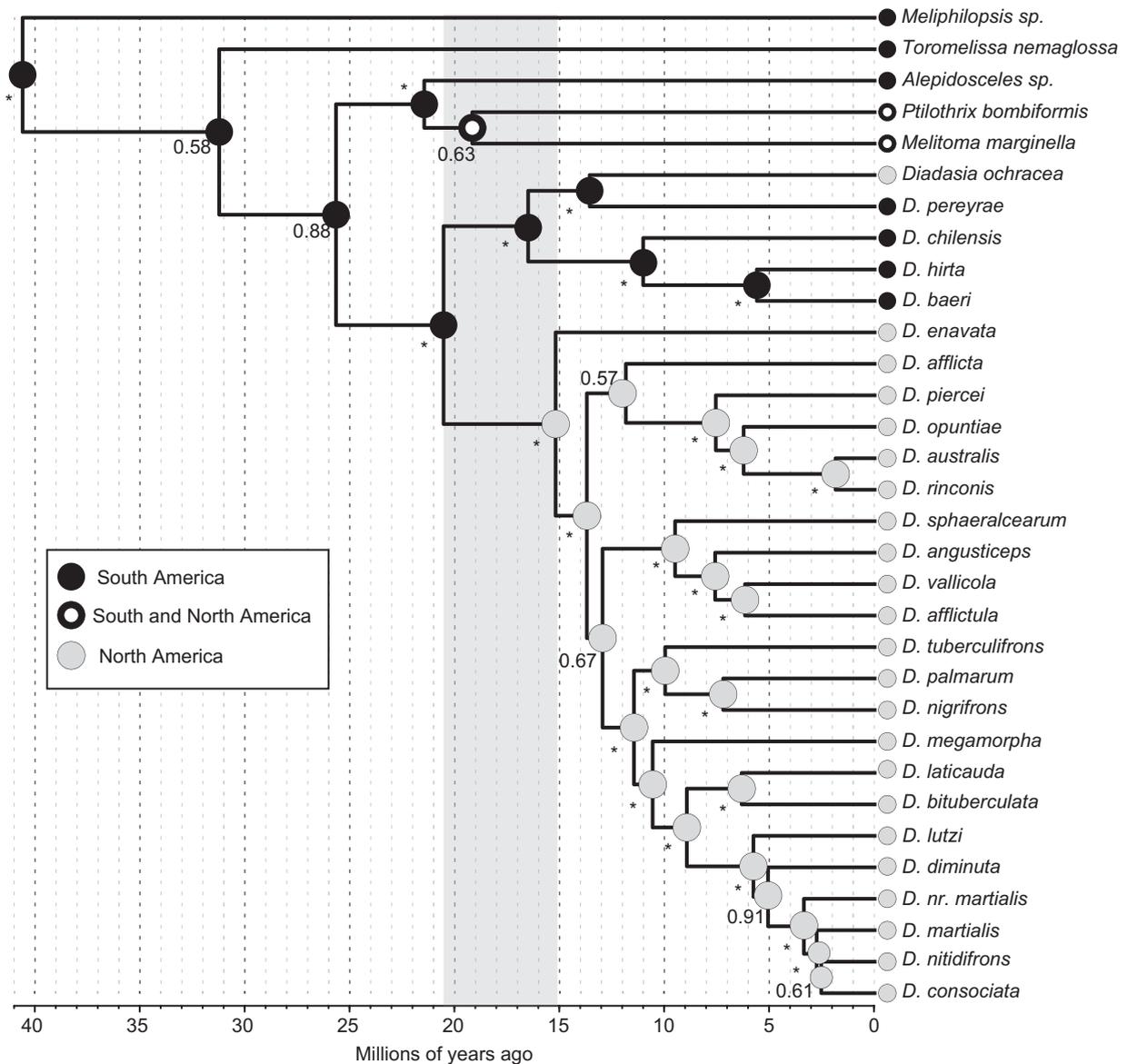


Figure 1. Time-calibrated phylogeny of *Diadasia* based on COI and EF1-alpha. Posterior probabilities are given for each node with \*representing a posterior probability of 0.95 or greater. Symbols next to each terminal taxon represent the area in which they are found, with outgroup taxa being coded at the genus-level. Each internal node is marked with the predicted distribution of that ancestor based on a Bayesian ancestral reconstruction implemented in RASP. All ancestral area reconstructions are supported by a posterior probability of 0.85 or greater. The grey bar between 20.5 Ma and 15 Ma represents the time *Diadasia* likely migrated between continents.

about the actual age of a northward migration for this group. Only two speciation events within *Diadasia* are estimated to have occurred during the Pleistocene (Fig. 1).

## Discussion

South American species of *Diadasia* diverged from North American species between 15 and 20.5 Ma. Our study demonstrates the possibility of transcontinental movement of *Diadasia* before the Pliocene, which is when North and South America appear to have initially become contiguous via the Isthmus of Panama. Specifically, we find evidence of a Miocene biotic connection between North and South America (Fig. 1). Similar results have been found in other taxa, including tropical fish (Bermingham and Martin 1998), frogs (Pinto-Sánchez et al. 2011), and many plants (reviewed by Cody et al. 2010). To our knowledge however, our study is the first to show such a Miocene connection of an amphitropically-distributed insect group using molecular dating techniques, though older connections have been implied in various cladistic bee classifications (Michener 1979, Simpson and Neff 1985). Through our analyses and others, it is becoming clear that the Great American Biotic Interchange took place over a longer period of time than previously assumed.

The biology of *Diadasia* obliges us to consider the plausibility of an early Pleistocene/late Miocene arrival of *Diadasia* to North America. First, *Diadasia* are medium to large bees that nest exclusively in the ground; they likely require contiguous (or nearly so) land masses when moving. Long-distance dispersal (i.e. via birds, or, as in some other bees that nest in wood, via driftwood) has been used as a mechanism to explain the distribution of some amphitropical bee groups (Michener 1979, 2000). However, long-distance dispersal is unlikely for *Diadasia* because of their nesting requirements. Geologic evidence has suggested that southern Central America arose as a series of volcanic islands (an archipelago), resulting eventually in the isthmus (Iturralde-Vinent and MacPhee 1999, Sturge et al. 2009). If the islands were close enough together, island hopping may have occurred. Alternatively, recent studies have demonstrated that southern Central America was a peninsula, separated from South America by one narrow body of water that connected the Pacific and Atlantic oceans (Kirby et al. 2008, Montes et al. 2012). There may have, therefore, been a near continuous path for transcontinental dispersal well before the Isthmus of Panama was fully formed.

A second consideration for the northward dispersal of *Diadasia* is that all *Diadasia*, and in fact the Emphorini tribe as a whole, are pollen specialists. Rather than collecting pollen from any plant that may be in bloom, they limit themselves to a subset of available floral resources, usually plants within the same genus or family. Their floral choices are consistent between non-overlapping generations, and persist throughout the geographic range of the bee. *Diadasia* species specialize on one of five plant families: six species visit only Cactaceae, one species each visits only Onagraceae (*Clarkia*), Asteraceae (*Helianthus*), and Convolvulaceae (*Calystegia*), the rest specialize to various degrees on Malvaceae, with some species collecting pollen from only

one Malvaceous genus and others collecting from many Malvaceous genera. Therefore, the plant families on which *Diadasia* specialize must have already been present in new areas colonized by bees as they spread north, or the two moved in tandem, with *Diadasia* following their host plants as they migrated north during favorable climatic periods. The most parsimonious assumption for a floral host for the common ancestor of North American *Diadasia* is Malvaceae (Sipes and Tepedino 2005). While the historical distribution of *Sphaeralcea* is unknown, the fossil record indicates that Malvaceae was present in northern South America during the Cretaceous at least 70 Ma. This is well before the arrival of *Diadasia*. The host-switch of some *Diadasia* to Cactaceae appears to have happened around 7 Ma. Interestingly, this coincides with major radiation events among several clades of Cactaceae, including *Opuntia*, one of the primary pollen hosts of cactus-feeding *Diadasia* (Arakaki et al. 2011, Majure 2012). This time period is generally associated with an expansion of arid regions in North America as a result of increased volcanic activity and the formation of the Gulf of Mexico (Axelrod 1979, Wilson and Pitts 2010a). Multiple other desert-adapted organisms also show diversification during this time including rattlesnakes (Douglas et al. 2006), lyresnakes (Devitt 2006), toads (Jaeger et al. 2005), and velvet ants (Pitts et al. 2010, Wilson and Pitts 2010b, Wilson et al. 2012). These Miocene and Pliocene speciation events have been associated with several orogenic (mountain building) and other geologic events (reviewed by Wilson and Pitts 2010a), which may have also supported the diversification of *Diadasia*.

While our findings suggest that *Diadasia* arrived in North America prior to the Pliocene, it is also possible that the common ancestor to North American *Diadasia* originated via allopatric speciation by migrating north of present-day tropics in South America during the long warming period associated with the Miocene (Behrensmeier et al. 1992). The South American *Diadasia* included in this phylogeny are currently distributed south of the Amazon basin (Jørgensen 1912, Janvier 1955, Hazeldine 1997). The northern part of South America could have acted as a holding area for *Diadasia* until the land bridge between North and South America was fully formed. However, considering that the land bridge formed three million years ago, many of the species that occur in North America, including some endemic to California, would have had to migrate north at the same time. This seems unlikely. It is more plausible that speciation events happened after *Diadasia* arrived in North America.

It appears that there were two separate northward migrations of *Diadasia*. The majority of North American *Diadasia* stem from one common ancestor, but *D. ochracea* appears to have arrived in North America from a separate South American progenitor. *Diadasia ochracea* is one taxon in a complex of three which comprise the subgenus *Dasiapis*. The three are difficult to distinguish and may in fact represent geographical varieties (Snelling 1994). *Dasiapis* are unique among *Diadasia* in that they are the best represented in Central and South America, with one species (*D. tropicalis*) spanning the tropical areas from northern South America, through the southern United States. The tropical regions of Central American in particular are

otherwise fairly devoid of *Diadasia*. It is possible that *Dasiapis* arrived in North America at a later date than the ancestor to other North American *Diadasia*, and may have migrated through the humid tropics rather than arid microclimates within, but our limited data precludes us from saying so with any certainty. The unique tropical habitat preference of these species, however, is likely derived.

Finally, our analysis sheds light on the origin of amphitropical distributions. Amphitropical distributions have often been attributed to the Pliocene/Pleistocene formation of the Isthmus of Panama. It had a dramatic effect on the global climate as it created a barrier between the Pacific and Caribbean. The resulting change in water temperatures is hypothesized to be the catalyst for the major glaciations that occurred during the Pleistocene (Bartoli et al. 2005). These glaciations, in conjunction with the continued orogenesis of the Andes and Mesoamerican mountain ranges created a cooler but drier climate in North America, bringing about the movement of numerous species to more southern regions (Axelrod 1985, Bush and Colinvaux 1990), and creating arid pockets on the eastern side of the Andes that facilitated the northward movement of desert-adapted species in South America (Stuart 1954). The fact that *Diadasia* arrived in North America prior to these climatic shifts suggests that other environmental factors may have contributed to these northward dispersal events.

## Summary

Although evidence based on fossil mammal assemblages suggests the GABI did not occur until Pliocene/Pleistocene times (Marshall 1988), we find evidence of an older biotic connection between North and South America. This connection could have taken place through island hopping along the paleo-Antillean islands (Sturge et al. 2009), or along the Isthmus of Panama, which was recently found to have been above sea level during the Miocene (Montes et al. 2012). This research suggests that the interchange of species between North and South America manifested in unique ways for different taxa and that the biotic connection between continents is more complicated than previously thought. Furthermore, this is the first evidence that amphitropically-distributed taxa, those inhabiting temperate zones on either side of the tropics, migrated between continents much earlier than Pleistocene times.

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