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Research Article

Tracing the origins and evolution of nymphalid butterflies (Lepidoptera) in the Atlantic Forest

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Understanding the relative roles of diversification and dispersal is key to explaining large-scale biogeographical patterns. Although both processes are known to shape biodiversity, their relative contributions remain understudied for many organisms. Here, we examine how these processes have jointly contributed to the exceptional diversity and endemism of Nymphalidae butterflies in South America's Atlantic Forest, a global biodiversity hotspot. We obtained DNA sequences for 65 Nymphalidae species and integrated them into published time-calibrated phylogenies. We used dispersal–xtinction–ladogenesis models and biogeographical stochastic mapping to infer historical biogeographical patterns over time, and the cladogenetic diversification rate shift (ClaDS) model to estimate region-specific diversification patterns. We further evaluated whether regional patterns of diversification are associated with occurrence in montane environments or across the north–south biogeographical break within the Atlantic Forest. Our results show that nymphalid butterfly diversity in the region was driven primarily by recurrent dispersal from Amazonia and the Andes, rather than by elevated in situ diversification rates, which remained low and stable through time. Although dispersal increased progressively during the Cenozoic, we found no evidence that the diagonal of open formations acted as a major barrier, indicating that forest corridors probably allowed extensive dispersal between the Atlantic Forest and other Neotropical regions. Southern Atlantic Forest lineages exhibited slightly higher diversification rates than northern ones, especially among montane generalist species. However, overall diversification contributed little to the current species diversity patterns compared to

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the sustained input of dispersing lineages from other Neotropical biomes. Together, these findings highlight the central role of biome connectivity and dispersal in shaping Atlantic Forest Nymphalidae diversity, while underscoring the importance of jointly considering diversification and dispersal processes to better understand the macroevolutionary dynamics underlying current biodiversity patterns.

Keywords: biogeography, diagonal of open formations, dispersal, diversification, Neotropics, Nymphalidae

Introduction

Biogeographical patterns are determined by the interplay between rates of diversification (comprising speciation and extinction) and dispersal (Hubbell 2001). Traditionally, macroevolutionary explanations for biotic assembly have emphasised two main ideas: 1) that older clades and larger geographic areas accumulate more species – the *time-* and *area-for-speciation* hypotheses, or together as the *time-integrated species–area effect* (Wallace 1878, Fine and Ree 2006); and 2) that regional differences in species richness result from episodic shifts in within-region speciation and extinction rates – the *rate-of-diversification* hypothesis (Mittelbach et al. 2007). Nevertheless, more recent frameworks also acknowledge the influence of asymmetric dispersal rates between regions (Goldberg et al. 2005, 2011, Kuhnhäuser et al. 2025). This has led to a better characterisation of the relative importance of time, diversification of lineages within a region (hereinafter in situ diversification) and dispersal in explaining extant regional differences in species diversity (Igea and Tanentzap 2019, Carrillo et al. 2020, Kuhnhäuser et al. 2025). In the Neotropics, empirical evidence is still mainly restricted to a few taxonomic groups and regions (Pérez-Escobar et al. 2017, Antonelli et al. 2018a) or is mainly focused on diversification rates (Meseguer et al. 2022), particularly the long-standing Neogene versus Pleistocene debates (Hoorn et al. 2011, Rull 2020), thereby neglecting the joint contribution of dispersal and diversification in explaining large-scale biodiversity patterns in understudied species-rich groups such as insects.

With only a fraction of its original vegetation remaining (8–22%), mostly in small, disconnected fragments (Ribeiro et al. 2009, Joly et al. 2014, Vancine et al. 2024), the Atlantic Forest is considered one of the most threatened biodiversity hotspots on Earth (Myers et al. 2000, Marques and Grelle 2021). This biogeographical domain harbours high levels of endemism across plants (Martini et al. 2007, Fiaschi and Pirani 2009), amphibians (Hadad et al. 2013), birds (Silveira et al. 2003), and butterflies (Brown and Freitas 2000, Santos et al. 2018), among other groups. Throughout its area, congruent patterns of species richness and endemism across animals and plants are thought to be shaped by contemporary and historical climatic changes and topographical variation. For instance, a large turnover in ecological communities exists between the southern and northern regions (Peres et al. 2020), but the relative contribution of species diversification and dispersal in shaping such patterns remains unknown for most species in the Atlantic Forest (Peres et al. 2020, da Silva et al. 2024).

Dispersal dynamics between the Atlantic Forest and other Neotropical regions have been linked to paleoenvironmental changes in the Cenozoic (i.e. the past 66 million years). During the Paleogene (ca 66 to 23 million years ago, Mya), Amazonia and the Atlantic Forest were interconnected (Sobral-Souza and Lima Ribeiro 2017). Yet, the extent of this connectivity remains under debate, and the timing of the Atlantic Forest's origin is still unclear (Jaramillo and Cárdenas 2013, Jaramillo 2023). Global cooling and the major Andean uplift in the Neogene (ca 23 to 2.6 Mya) promoted the expansion of open habitats in the Neotropics, likely reducing the connectivity between the Atlantic Forest and other Neotropical forested regions (Hoorn et al. 2010, Werneck 2011). The evolution of these habitats led to what is known today as the diagonal of open formations, encompassing the Caatinga, Cerrado and Chaco biomes, and has been proposed in several studies as an important biogeographical barrier for rainforest-adapted species (Costa 2003, Batalha-Filho et al. 2013). However, fossil and molecular evidence suggests that intermittent humid corridors maintained connectivity during wet periods of the Pleistocene (Batalha-Filho et al. 2013, Coelho et al. 2022), as well as earlier in the Neogene (Batalha-Filho et al. 2013, Marques-Souza et al. 2022). Under this scenario, the increasing isolation of the Atlantic Forest, combined with intermittent reconnections to other regions, may have led to different dispersal dynamics and biogeographical patterns across lineages (Batalha-Filho et al. 2013, Prates et al. 2016, Sobral-Souza and Lima-Ribeiro 2017, Bocalini et al. 2021).

Pleistocene climatic fluctuations have also been major determinants of diversification and community assembly within the Atlantic Forest – as initially proposed in the *Pleistocene refugia* hypothesis (Haffer 1969, Brown 1976) and later expanded to include divergent selection in the *Vanishing refuge model* (Damasceno et al. 2014). Both paleoclimatic (Carnaval and Moritz 2008) and phylogenetic data (Carnaval et al. 2009, De Mello Martins 2011, Silva et al. 2012) support distinct regional evolutionary trajectories during climatic fluctuations in the Atlantic Forest: while the region north of the Doce River (central Bahia) has remained paleoclimatically stable, the southeast underwent dramatic environmental reconfigurations during cool phases, prompting many lineages to retreat to mountain refuges (Carnaval et al. 2009, Peres et al. 2020). These regional differences may have led to different diversification rates through time: constant in the north and increasing in the unstable south during climatic fluctuations (Paz et al. 2021).

To offer further insights into the Atlantic Forest's macroevolutionary history, we focus on the species-rich butterfly family Nymphalidae, with nearly 500 species across the region (Brown and Freitas 2000, Santos et al. 2018). Their distribution patterns in the Atlantic Forest are well documented (Santos et al. 2018, Shirai et al. 2019) and, similar to other taxa, show the highest species richness in the southeastern montane regions (Iserhard et al. 2017, Santos et al. 2018). We build upon the most comprehensive global phylogeny of Nymphalidae to date (Chazot et al. 2021) and sequenced 65 species, including 43 species previously absent from global time-calibrated butterfly phylogenies (Chazot et al. 2021, Kawahara et al. 2023). Based on compiled distribution data for 1377 species, we explored whether the diversity of Atlantic Forest Nymphalidae results from 1) a long history of complete biotic isolation, reflected in early dispersal events (i.e. during the Paleogene) followed by in situ diversification (i.e. within the Atlantic Forest); 2) gradual isolation, leading to decreasing interchange with Amazonia and a stronger role of local speciation; or 3) continuous connectivity with other Neotropical biomes, where recurrent dispersal dominates and local diversification plays a minor role. Furthermore, we evaluated whether occurrence in montane regions and along the north–south biogeographical break may explain the extant diversification rates within the Atlantic Forest nymphalids.

Material and methods

Molecular data

We targeted Nymphalidae species from the Atlantic Forest that were not previously sequenced in phylogenetic studies. We sampled 65 adult specimens across eastern Brazil, including 43 species newly sequenced for time-calibrated phylogenies (Supporting information). We extracted DNA from legs using DNeasy Blood and Tissue Kits (QIAGEN), and libraries were prepared and sequenced on an Illumina NovaSeq 6000 platform by the company Novogene (UK). We checked the reads quality (FastQC ver. 0.12.1; Andrews 2010), trimmed the adapters (fastp; Chen 2023) and assembled de novo contigs (SPAdes ver. 3.15.4; Prjibelski et al. 2020). Following the BUTTERFLY1.0 probe set (Espeland et al. 2018, Kawahara et al. 2023), we retained 391 loci, including 11 legacy genes traditionally used in butterfly systematics (Wahlberg and Wheat 2008, Kawahara et al. 2018). We used the SECAPR ver. 2.2.3 environment (Andermann et al. 2018, Ribeiro et al. 2021) to process data from de novo contig assembly to multiple sequence alignments.

We focused on monophyletic groups from the Nymphalidae phylogeny of Chazot et al. (2021), with more than half of the species diversity occurring in the Neotropics. The function *CladeByTrait()* of the R package 'speciesgeocodeR' (Töpel et al. 2016) found nine Neotropical groups ('Clades for phylogenetic inference' in Table 1, Supporting information), representing the clades Danainae, Heliconiinae, Limenitidini, Biblidini, a clade containing Cyrestinae and Nymphalinae, Charaxinae, a Satyrinae subclade (comprising the tribes Morphini, Brassolini, Amathusiini, Haeterini and Melanitini), Euptychiina and Pronophilina. We merged our data with the most comprehensive phylogenies of Nymphalidae (Chazot et al. 2021) and butterflies in general (Kawahara et al. 2023), and conducted multi-species alignments for each of the nine clades using MAFFT ver. 7.520 (Katoh and Standley 2013), only retaining loci present in $\geq$ 10% of the species in that clade. Final alignments were checked in Geneious Prime ver. 2023.1.2 and are archived in the Dryad repository (see Data Accessibility Statement).

Time-calibrated phylogenetic inference

We infer phylogenetic relationships for each of the nine clades using IQ-TREE ver. 2.2.0 (Minh et al. 2020) under a concatenated, codon-partitioned scheme (Chernomor et al.

Table 1. Number of samples per clade used for phylogenetic inference and biogeographical and diversification analyses. Nine clades were extracted for phylogenetic inference and calibration. The nine clades were then subdivided into fourteen subclades for biogeographical and diversification analyses, in order to minimise the number of species outside the Neotropics.

Clades for phylogenetic inference	Total samples (phylogenetic inference)	Species from Chazot et al. (2021)	Species from Kawahara et al. (2023)	Species from this study	Subclades for biogeographical analyses	Species number (biogeographical analyses)
Danainae	436	376	60	0	Ithomiini	331
Heliconiinae	192	148	42	2	*Actinote*	37
					Heliconiini	69
Limenitidini	107	88	19	0	*Adelpha*	67
Biblidini	210	154	42	14	Biblidini	170
Cyrestinae and Nymphalinae	447	380	67	0	*Hypanartia*	9
					Marpesia	13
					Melitaeini	174
Charaxinae	265	228	29	8	*Memphis*	54
					Prepona	23
Satyrinae subclade	242	173	59	10	Haeterini	17
					Morphini and Brassolini	108
Euptychiina (Satyrini, Satyrinae)	318	201	89	28	Euptychiina subclade	194
Pronophilina (Satyrini, Satyrinae)	144	96	45	3	Pronophilina	111
Total	**2361**	**1844**	**452**	**65**		**1377**

2016), with model selection performed by ModelFinder (Kalyaanamoorthy et al. 2017). Branch support was calculated using 1000 ultrafast bootstrap replicates (Minh et al. 2013), Shimodaira–Hasegawa approximate likelihood ratio tests, and approximate Bayes test (Anisimova et al. 2011). For each clade, we included outgroups representing sister subfamilies for the phylogenetic inferences (Supporting information).

To time calibrate each of the nine tree topologies, we used the Bayesian approach implemented in MCMCTree from 'PAML' package ver. 4.10.6 (Yang 2007). We used an independent-rates relaxed molecular clock model (Drummond et al. 2006) and the most complex substitution model available in MCMCTree, the HKY85 (Hasegawa et al. 1985). Due to the limited availability of butterfly fossils, we used nineteen secondary calibration points, encompassing major relationships among subfamilies and crown ages of the main tribes at every clade (Supporting information), which were extracted from the fossil-calibrated phylogeny of Chazot et al. (2021). To ensure convergence, the analysis was run two times independently using random seeds, and similar results were observed (Supporting information).

Inference of biogeographical history

We estimated range evolution using the dispersal–xtinction–ladogenesis (DEC) model in the R package 'BioGeoBEARS' ver. 1.1.3 (Matzke 2018). For this, we subdivided the nine time-calibrated trees into fourteen Neotropical subclades to reduce noise coming from lineages outside the Neotropics as much as possible, and to improve computational performance (Table 1, Supporting information). We subdivided the Neotropics into eight biogeographical regions, largely following the regionalisation proposed by Morrone (2014): 1) Mesoamerica and northwestern lowland Andean slopes, 2) northern Andes (Ecuador, Colombia, Venezuela), 3) central Andes (Peru, Bolivia), 4) Amazonia, 5) diagonal of open formations encompassing the Caatinga, Cerrado and Chaco biomes, 6) northern Atlantic Forest and 7) southern Atlantic Forest, both delimited by the Doce river which reflects a strong community turnover of animal and plant diversity, and 8) outside the defined Neotropical areas (Fig. 1). To avoid unrealistic biogeographical scenarios, we disallowed dispersal events between Mesoamerica and the diagonal of open formations and the Atlantic Forest. This constraint was applied globally across the trees and not stratified temporally. In addition to the constrained analysis, we also performed an unconstrained analysis for comparison. Species ranges were compiled from the literature, including original descriptions, published checklists (Penz et al. 2017, Shirai et al. 2019, Vila-Verde and Paluch 2020, Garwood and Jaramillo 2022, Garwood et al. 2022), systematic revisions (see Willmott 2003 for *Adelpha*) and biogeographical studies (Matos-Maraví et al. 2013, 2021), databases of approximate range maps (Lepidoptera and some other life forms; Savela, M. unpubl.), geo-referenced occurrences (GBIF 2020 and iNaturalist), and experts' knowledge (distributional data reported in the Supporting information; Fig. 1a shows the number of species sampled per region).

To account for divergence time uncertainty, we utilised 50 randomly selected trees from the MCMCtree posterior distribution for each study subclade, and to address ancestral geographical state uncertainty, we performed 50 biogeographical stochastic mappings per tree (BSM; Dupin et al. 2017). Dispersal rates were extracted in 1 million-year (Myr) intervals and scaled by total branch length per bin, following the approach described in Antonelli et al. (2018a) and the R scripts from Matos-Maraví et al. (2021).

Within-region species diversification analysis

We used CLaDS (Maliet et al. 2019, Maliet and Morlon 2022) to estimate branch-specific diversification rates, assuming a constant turnover across the entire phylogeny (one for each of the 14 subclades), and accounting for incomplete taxon sampling with a sampling probability vector (Supporting information). To calculate diversification rates within geographic regions, we combined the CLaDS outputs with 'BioGeoBEARS' ancestral range estimates from BSM. For each branch section, we identified the region(s) it occupied, weighted its diversification rate by the proportion of its length in that region ('occupancy'), and further adjusted by the frequency of occurrence across all BSM replicates. This approach ensured that branches only partially present in a region, or with uncertain regional assignment, contributed proportionally less to the regional diversification estimates. This was done for every 1 Myr time bin, allowing us to extract the diversification rate through time at each region (R scripts in the Dryad repository, see Data accessibility statement).

Effect of altitudinal variation on the diversification of Atlantic Forest Nymphalidae

We assessed whether the altitudinal range of nymphalids in the Atlantic Forest was associated with extant speciation rate heterogeneity among clades (i.e. tip rates). Based on literature, expert knowledge and regional checklists (Santos et al. 2018, Shirai et al. 2019), we categorised species into lowland (distribution mainly up to 700 m a.s.l.), highland (above 700 m a.s.l.), or altitude generalist, and into northern, southern, or widespread distributions. Speciation rate for each tip was estimated using either the ClaDS estimates or the tip-specific DR-statistic (Redding and Mooers 2006), a non-model-based approach that approximates speciation rates (Jetz et al. 2012), calculated with the R package 'epm' (Title et al. 2022). To test whether geographical occurrence predicted tip speciation rates, we used phylogenetic generalised least-squares regression (PGLS; Martins and Hansen 1997), as implemented in the R package 'caper' (Orme et al. 2012). We ran the analyses for every subclade separately, using either CLaDS rate or DR as response variables.

An extended version of the 'Material and methods' section is available in the Supporting information, and the R scripts used here are available in the Dryad repository (Data accessibility statement).

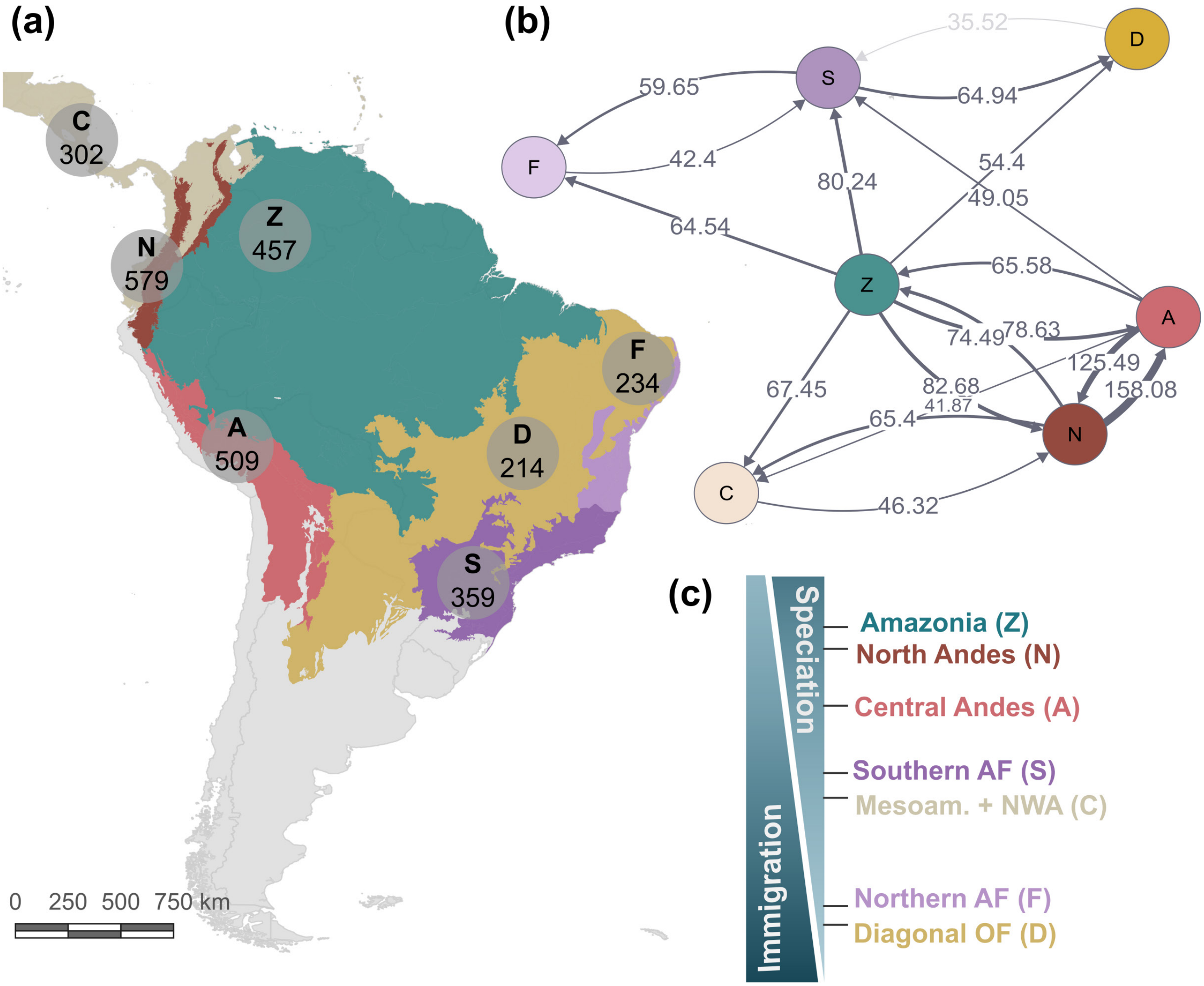

Figure 1. Nymphalidae interchange (dispersal events) across Neotropical regions. The analyses are based on 1377 Nymphalidae species. (a) Map of Neotropical biogeographical regions considered in this study following Morrone (2014), together with the species number per area included in our biogeographical analyses. Letters correspond to: F = northern Atlantic Forest; S = southern Atlantic Forest; D = diagonal of open vegetation formations; Z = Amazonia; C = Mesoamerica + NW slopes of Andes; N = northern Andes; A = central Andes. (b) Nymphalidae dispersal events from the biogeographical stochastic mapping results. Arrows indicate the direction and number of events; line thickness is proportional to the number of events, with a minimum of 30. (c) Proportion of within-speciation and immigration events among regions. Proportions were estimated by dividing the number of immigration events (sum of dispersal events into each region) by the number of extant species included in the analysis per region. Within-speciation events were calculated as the rest of the extant number of species minus the immigration events.

Results

Molecular data and time-calibrated phylogenetic inference

The size of our molecular datasets varied between 159 716 bp (Charaxinae clade) and 162 060 bp (Satyrinae subclade), representing 387 and 391 loci, respectively. The maximum likelihood tree topologies inferred in IQ-TREE 2.2.0 (Minh et al. 2020) were congruent with other recent published phylogenies focused on specific taxa (Silva-Brandão et al. 2008, Yang and Zhang 2015, Chazot et al. 2021, Barbosa et al. 2022, Cicconardi et al. 2023, Espeland et al. 2023, Kawahara et al. 2023, Yan et al. 2023). The few differences found were either at the species level or for relationships among lineages that have been previously difficult to resolve: e.g. the genera *Brassolis* (Brassolini), *Methona, Hypothyris,* and *Hyalyris* (Ithomiini), which were paraphyletic in our inferred phylogenies but were also reported as having low phylogenetic support in previous studies (Chazot et al. 2019, Matos-Maraví et al. 2021). Regardless, because such incongruences were very few and mostly at shallow phylogenetic levels, we do not consider them to have biased our macroevolutionary and biogeographical inferences. The posterior estimates of node ages for the principal tribes examined here were consistent with those reported in previous studies (Supporting information).

Inference of biogeographical history

We recovered distribution data of 1377 species (Fig. 1a for species number per region), of which 375 occur in the Atlantic Forest, 234 in the northern region and 359 in the southern. These numbers correspond to approximately 80% of the species richness estimated for each region (Brown 1992, Brown and Freitas 2000). Regarding endemism, our dataset includes a higher proportion of endemic species from the southern Atlantic Forest than for the northern Atlantic Forest (~ 70 versus ~ 20%). Our biogeographical stochastic mapping for the whole Neotropical region showed that the highest number of dispersal events were into and out of Amazonia (231 events into and 427 events out), followed by the Andes (336 events into and 423 events out) (Fig. 1). Regarding the Atlantic Forest, Amazonia was also the major source (145 events from Amazonia to southern and northern Atlantic Forest), compared to the 131 events from the Andes to the Atlantic Forest.

Dispersal into the Atlantic Forest from other Neotropical regions occurred constantly through time until about 10 Mya, when it accelerated toward the present (Fig. 2). Overall, Amazonia has been the primary source of lineages dispersing into the Atlantic Forest relatively constant throughout the Neogene, only surpassed by the Andean dispersal during the Eocene (prior to 33 Mya) and around 15 Mya, during the mid Miocene (Fig. 2). Dispersal from the diagonal of open formations into the Atlantic Forest intensified from 11 Mya toward the present (Fig. 2). When the northern and southern regions of the Atlantic Forest were analysed separately, connectivity with other Neotropical regions was higher for the southern region (Supporting information). The unconstrained analysis showed a higher connectivity between northern Andes and the Atlantic Forest, rather than central Andes (Supporting information).

The first extant lineages to disperse to the Atlantic Forest were Morphini (Satyrinae) which occurred around 47 Mya.

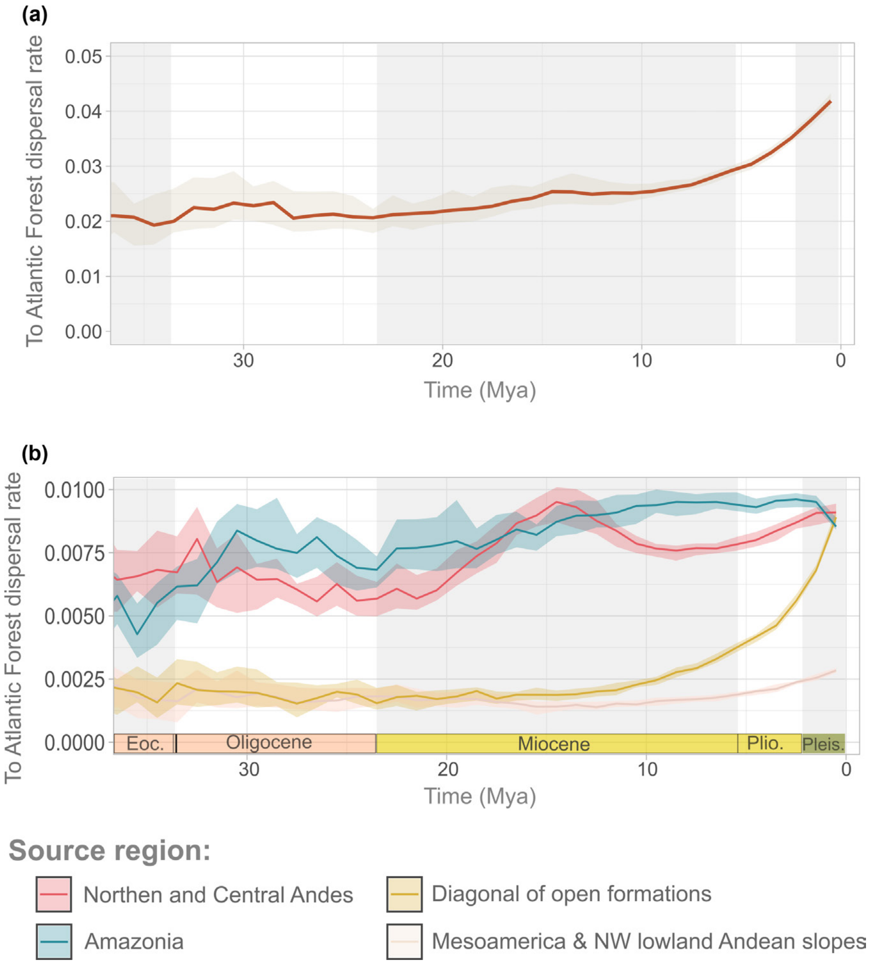

Figure 2. Dispersal rates through time to the Atlantic Forest overall (a) and (b) from different Neotropical regions. Andes = red; Amazonia = blue; diagonal of open formations = dark yellow; Mesoamerica and NW slopes of Andes = light sand-coloured. Continuous lines are the median values, and coloured ribbons are the lower and upper quantiles (0.40 and 0.60). Abbreviations: Eoc: Eocene, Plio: Pliocene, Plei: Pleistocene, Mya: million years ago. The plots were generated with 'ggplot2' (Wickham 2016) in R and edited with Inkscape (https://inkscape.org).

This was followed by lineages of Charaxinae (*Memphis* and *Prepona*) and Biblidini (Biblidinae), during the Eocene, around 43 Mya, and Euptychiina (Satyrinae) around 37 Mya. During the Oligocene (33.9–23 Mya), extant lineages from the Haeterini (Satyrinae) and Melitaeini (Nymphalinae) clades dispersed to the Atlantic Forest. The remaining Nymphalidae clades dispersed to the Atlantic Forest during the Miocene. Within Charaxinae, two genera (*Memphis* and *Prepona*) showed the highest dispersal rates into the Atlantic Forest, with these rates increasing exponentially since the late Miocene. Another notable clade was the Heliconiini (Heliconiinae), which showed high dispersal rates from Amazonia to the Atlantic Forest around 5 Mya (Supporting information).

Within-region species diversification analysis

The estimates of regional diversification rates obtained by ClaDS and 'BioGeoBEARS' BSM suggested that Atlantic Forest lineages had a steady and low diversification rate through time (Fig. 3). This trend was observed in both the northern and southern Atlantic Forest lineages, although the latter exhibited a higher rate of diversification. A notable peak in diversification was estimated to have occurred around 15–17 Mya in the southern Atlantic Forest, alongside a decline in diversification rates between 20 and 25 Mya for both the southern and northern Atlantic Forest (Fig. 3). This diversification increase in the southern Atlantic Forest coincides with high global speciation rates of Ithomiini and *Adelpha* clades during this period (Supporting information). In addition, the speciation rates of Euptychiina also stand out from the rest during the Paleogene, ca 35–30 Mya (Supporting information).

Effect of altitudinal variation on the diversification of Atlantic Forest Nymphalidae

We did not find any phylogenetic correlation between tip-specific speciation rates and the current distributional patterns of nymphalid butterflies in the Atlantic Forest, except for the tribe Ithomiini and the genus *Actinote* (Heliconiinae: Acraeini) (Supporting information). For Ithomiini, we found that altitudinal generalists (species that are widespread along elevational gradients) that occur in the southern Atlantic Forest had higher speciation rates compared to other lineages across the Atlantic Forest. Both ClaDS estimates (estimate = 0.0593, p-value = 0.0065) and DR metrics (estimate = 0.1768, p-value = 0.0216) produced the same results. A similar correlation was found for *Actinote*; however, it was statistically significant only when using ClaDS' speciation rates (estimate = 0.0155, p-value = 0.0418) but not with DR metrics estimates (estimate = −0.0155, p-value = 0.6624). Although a significant positive effect on speciation by the lowland and widespread state was found using the DR metrics in Haeterini (estimate = 0.102, p-value = 7.306×10^{-5}), the effect was found to be non-significant using ClaDS' speciation rates (estimate = 0.0046, p-value = 0.1304).

Discussion

The Atlantic Forest is regarded as one of the most threatened biodiversity hotspots in the world (Myers et al. 2000). However, the macroevolutionary processes explaining extant biodiversity patterns of many species-rich groups remain understudied. In this study, we inferred the evolutionary history of neotropical nymphalid butterflies and

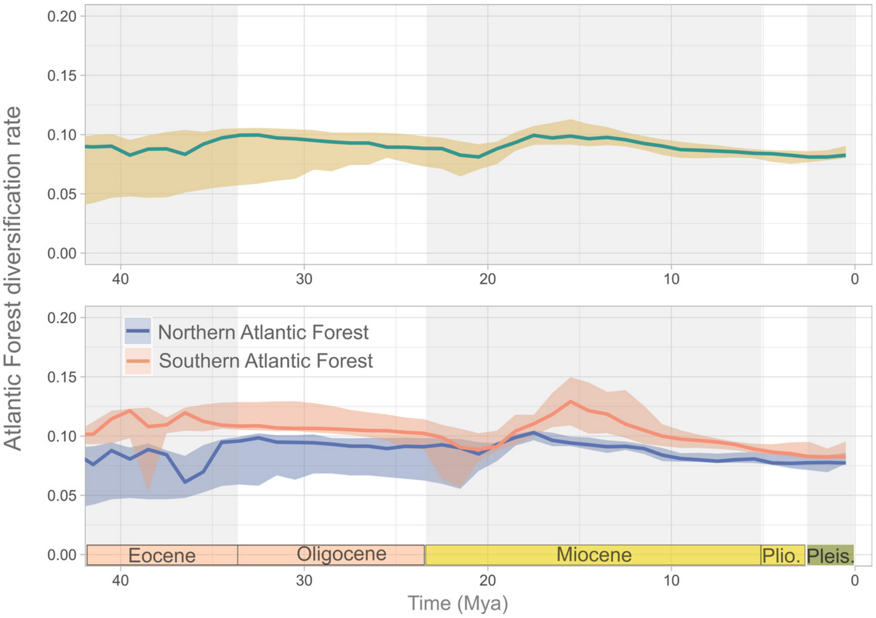

Figure 3. Diversification rates from ClaDS results for the entire Atlantic Forest (green), southern Atlantic Forest (orange) and northern Atlantic Forest (blue). Continuous lines are the median values, and coloured ribbons are the lower and upper quantiles (0.40 and 0.60). Abbreviations: Plio: Pliocene, Plei: Pleistocene, Mya: million years ago.

found that the extant species diversity in the Atlantic Forest resulted primarily from a continuous biotic influx from other Neotropical regions, followed by a consistently low and steady net diversification rate over time. Notably, dispersal rates into the Atlantic Forest intensified around 15 Mya (Fig. 2a), suggesting that regional nymphalid diversity was primarily shaped by dispersal from other regions – fundamentally from Amazonia and the Andes – rather than in situ diversification (i.e. the third scenario outlined in the Introduction).

Atlantic Forest dispersal dynamics

We observed an overall increase in species dispersal to the Atlantic Forest since the mid-Miocene, around 15 Mya, with an acceleration in the last ~ 7 million years (Fig. 2a). The sources of dispersal into the Atlantic Forest varied over time (Fig. 2b), which may be partially attributed to major environmental and geological changes. During the Neogene, the major Andean uplift and the global cooling trend drastically reconfigured the landscape, precipitation patterns, and climate across the Neotropical region, resulting in the expansion of dry and open formations in northern and central South America (Hoorn et al. 2010, Werneck 2011). Yet, the precise timing of the establishment of the diagonal of open formations remains under debate. While molecular phylogenies suggest an early to mid-Miocene split between forest biomes, fossil and molecular evidence from C4 grasses and woody savanna flora indicate that their dominance began in the late Miocene (Azevedo et al. 2020). Nevertheless, there is evidence of open vegetation formations as far back as the Paleocene (Aguiar et al. 2020).

Our findings reveal an exponential-like increase in dispersal from the diagonal of open formations into the Atlantic Forest around 10 Mya (Fig. 2). Even though the underlying drivers of such an increase remain elusive, they support the idea of a mid-to-late Miocene reconfiguration of environmental conditions, including the establishment of suitable dispersal corridors for nymphalid lineages with different ecological characteristics. This is evidenced by lineages that arrived during that period, such as the clade Pronophilina during the mid-Miocene (~ 16–11 Mya) (Supporting information). This mountain lineage probably dispersed from the central Andes to the southern Atlantic Forest through a south–central corridor. In addition, Heliconiini butterflies dispersed during the early Miocene (~ 18 Mya), but showed their highest dispersal rate at ~ 5 Mya, when the current flora assembly of the Cerrado was already established (Simon et al. 2009). Considering the high degree of host specialization and low dispersal capacity of these butterflies (Jiggins 2017), this result might support the presence of corridors of forest habitats along the diagonal of open formations. The overall dispersal increase may also reflect a larger source pool of lineages from northwestern South America, triggered by the rapid speciation of many taxa during that period in response to orogenic processes and regional climate change (Antonelli et al. 2018c).

Amazonia as a source of Atlantic Forest diversity

As revealed by Antonelli et al. (2018b), Amazonia played a major role as a source of Neotropical diversity (Fig. 1b), including for the Atlantic Forest. Here we also recovered Amazonia as the main source of Atlantic Forest diversity, with a high and constant dispersal rate through time (Fig. 2b). Despite the ecological barrier posed by the diagonal of open formations, biotic corridors – likely formed by episodic wet forests (Auler et al. 2004, Wang et al. 2004) or continuous wet vegetation along rivers (Por 1992, Oliveira-Filho and Ratter 1995, Costa 2003) – might have enabled the exchange of fauna and flora between Neotropical forest domains. For instance, Prates et al. (2017) suggested that throughout the Miocene, patches of suitable humid habitats may have connected the Atlantic Forest with western South American forests, facilitating species dispersal. Those studies are consistent with our findings and indicate that the expansion of the diagonal of open formations did not significantly reduce dispersal between Amazonia and Atlantic Forest nymphalids.

We speculate that the Neogene forest corridors allowed for continuous dispersal among forest-adapted nymphalid butterflies. These connections likely varied regionally, with stronger links to Amazonia through central and northern routes, and weaker connections through southern corridors, including the Andes. Similar connectivity patterns have been reported previously, although most indicate that the interchange was frequent due to Pleistocene paleoclimatic fluctuations (Costa 2003, Sobral-Souza and Lima-Ribeiro 2017, Peres et al. 2020, Coelho et al. 2022). In contrast, only a few studies have documented older biotic connections during the Neogene (Marques-Souza et al. 2022), possibly mediated by riparian forests or remnant humid corridors (Pirani et al. 2020, Trujillo-Arias et al. 2020). In line with these studies, our results recover continuous connectivity between the Atlantic Forest and other Neotropical biomes since the early Neogene (Fig. 2), likely facilitated by historical forest corridors linking these regions.

We acknowledge that long-distance dispersal events, facilitated, for instance, by extreme winds, could also explain certain biotic interchanges without the necessity of fully connected corridors. Seasonal movements of individual butterflies have been documented over distances of several kilometres in the Atlantic Forest. Additionally, migrations of certain populations and communities can be inferred to have occurred over many hundreds of kilometres (Brown and Freitas 2000). However, we consider that such events are largely random and only possible for a few species, rather than being responsible for the general dispersal patterns we found. Consistent with this view, Penz et al. (2014) showed that the large-sized butterflies *Morpho* and *Bia* can disperse rapidly through continuous forest, but their movement is limited when the landscape is dominated by unsuitable habitats (i.e. savanna-like Cerrado). Thus, we consider that dispersal events between the Atlantic Forest and other forested regions most likely reflect past conditions of a more permeable vegetation matrix over which animal movements could take place.

The role of the Andes on Atlantic Forest diversity

The central Andes are the second largest source of Nymphalidae diversity for the Atlantic Forest, as has also been reported for other taxonomic groups, such as rodents (Vallejos-Garrido et al. 2023), bats (Velazco and Patterson 2013), birds (Fjeldså and Rahbek 2006) and plants (Antonelli and Sanmartín 2011). The Andes have played an important role in generating species diversity in the Neotropics through high diversification and emigration rates (Antonelli et al. 2009, Rangel et al. 2018). For instance, the ithomiine genus *Pteronymia* mainly diversified in the northern Andes, with much of its non-Andean diversity resulting from independent dispersal events out of the region (De-Silva et al. 2017). Similarly, tanagers and hummingbirds seem to have radiated and dispersed out of the northern Andes, which might have acted as cradles and museums of species diversity (Beckman and Witt 2015, Sonne et al. 2022). Indeed, high connectivity between the northern Andes, rather than the central Andes, and the Atlantic Forest was recovered in our unconstrained biogeographical analyses (Supporting information), a pattern also recovered in a synthesis of Andean flora biogeography (Pérez-Escobar et al. 2022).

Unlike Amazonian dispersal, which has been more or less constant, the Andean dispersal rate has fluctuated over time (Fig. 2b). During the early Miocene, the connectivity between the Andes and the Atlantic Forest increased, reaching its peak around 15 Mya, when it even surpassed dispersal from Amazonia. This pattern may have been enabled by biotic corridors linking the central Andes to the Atlantic Forest through the Cerrado and Chaco transition zones, as reported for several lineages (Matos-Maraví et al. 2013, Prates et al. 2017, Trujillo-Arias et al. 2018, 2020, Moreira-Muñoz et al. 2020, Pirani et al. 2020). In addition, Brown (1987) identified a high similarity in butterfly lineage composition between the Andes and southeastern Brazil, and Safford (2007) reported a similar biogeographical pattern based on floristic composition analyses. Such biotic affinities suggest suitable conditions for the settlement and diversification of Andean lineages in the montane environments of the Atlantic Forest. For instance, the montane genera *Actinote* diversified in the Andes but, once it colonized the southern Atlantic Forest, it achieved its greatest species richness in the mountain forests of this region, with some lineages recolonizing the Andes from the Atlantic Forest (Gueratto 2023, Magaldi et al. 2024). Therefore, the high rates of Andean diversification, together with early Miocene dispersal corridors, might have promoted an interchange of lineages with the Atlantic Forest.

Atlantic Forest diversification

Within the Neotropical region, a gradual accumulation of Nymphalidae lineages is explained by steady speciation rates and low extinction rates (Chazot et al. 2021), a common pattern found in Neotropical butterflies (Matos-Maraví 2016) and plants (Meseguer et al. 2022). We found that net diversification rates within the Atlantic Forest have remained low and constant (Fig. 3), which contrasts with other global tropical biodiversity hotspots characterized by high diversification rates (Igea and Tanentzap 2019). This slow and gradual accumulation of lineages is consistent with previous reports that considered the Atlantic Forest as a 'museum of diversity' for butterflies (Matos-Maraví et al. 2021).

Despite the constant overall diversification rate, we observed regional differences when studying the southern and northern Atlantic Forest individually (Fig. 3, lower panel). In particular, during the Paleogene, the southern region experienced a slight increase in the net diversification rate, likely related to the arrival of rapidly diversifying nymphalid groups across the Neotropics, such as *Adelpha* and Ithomiini (two clades known by their remarkably high species richness in the Andes) (Ebel et al. 2015, Chazot et al. 2016a). Moreover, the complex topography of the south may have further contributed to these regional disparities.

We hypothesise that the Atlantic Forest mountain ranges played an important role in the diversification of such lineages. Different mechanisms are known to promote species diversification in tropical mountains (De-Silva et al. 2017, Rahbek et al. 2019, Perrigo et al. 2020). On the one hand, mountain tops can act as isolated islands promoting allopatric diversification between scattered populations with restricted elevational ranges (Brown 1987, Chaves et al. 2014, Magaldi et al. 2024). On the other hand, habitat complexity of montane environments can act as a species attractors, with higher rates of colonization towards the mountains, as found for the ithomiine subtribe Godyridina in the Andes (Chazot et al. 2016b). Interestingly, a positive correlation emerged between wide altitudinal distribution (i.e. generalist species occurring in highlands and lowlands) and increased speciation rates in ithomiine butterflies of the southern Atlantic Forest. Highland specialists, however, showed no such pattern, contrary to expectations. This pattern may be explained by habitat heterogeneity along mountain ranges that could facilitate population differentiation in widespread lineages and eventually speciation. Indeed, habitat fragmentation driven by Pleistocene climatic fluctuations might have pushed lineages toward mountain climatic refuges and facilitated allopatric diversification (Brown 1987, Carnaval and Moritz 2008, Flantua et al. 2019).

Furthermore, the southern Atlantic Forest has higher levels of species richness than the northern region, which is explained not only by higher dispersal rates but also by higher speciation rates, particularly involving mountain lineages in both the Atlantic Forest and the Andes. Nevertheless, these differences should be interpreted with caution, as the unequal sampling of endemic species among regions may have influenced our results. Despite this, the overall patterns we observed are robust and align with existing hypotheses suggesting higher diversification in the more heterogeneous southern Atlantic Forest (Nery et al. 2023). Both high speciation and dispersal explain the current species richness in mountain ranges but are driven by different processes. For nymphalids, it seems that Pleistocene climatic fluctuations did not significantly increase the overall species diversification, but Nymphalidae species diversity accumulated gradually in the Atlantic Forest mainly through dispersal.

Conclusions

Our study shows that the diversity of Atlantic Forest nymphalids has been shaped by a complex scenario of varying biotic connectivity through time with different biogeographical regions in the Neotropics, in particular Amazonia and the central Andes. Despite the diagonal of open formations being hypothesised as an important biogeographical barrier for wet forest lineages, the increased late Miocene to Pleistocene dispersal detected into the Atlantic Forest suggests that corridors with suitable habitats for nymphalids likely maintained connectivity through time, especially between Amazonia and the Atlantic Forest. Overall, our findings underscore the importance of jointly studying diversification and dispersal rates to understand the macroevolutionary processes shaping extant biodiversity patterns.

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Author contributions

Mar Repullés: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal). **Nicolas Chazot**: Data curation (equal); Methodology (equal); Writing – review and editing (equal). **Leidys Murillo-Ramos**: Data curation (equal); Writing – review and editing (equal). **Marianne Espeland**: Data curation (equal); Writing – review and editing (equal). **Karina Lucas Silva-Brandão**: Conceptualization (supporting); Writing – review and editing (supporting). **Alexandre Antonelli**: Conceptualization (equal); Funding acquisition (equal); Writing – review and editing (equal). **André Victor Lucci Freitas**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Project administration (equal); Writing – review and editing (equal). **Pável Matos-Maraví**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal).

Transparent peer review

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Data availability statement

The raw data sequences generated in this study are available in NCBI assigned to the BioProject PRJNA1297423. Alignments, selected partitions, tree files and R scripts used for the biogeographical analyses and plots, are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.wm37pvn11 (Repullés et al. 2026).

Supporting information

The Supporting information associated with this article is available with the online version.

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