

Combining functional traits and phylogeny to disentangling Amazonian butterfly assemblages on anthropogenic gradients

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Abstract. Environmental gradients consist of sequential changes in the physical and structural characteristics of a region. These allow us to follow species responses and tolerances under different habitat conditions. Among them, forest fragmentation and succession comprise the most common examples of forest gradients, where organismal responses require distinct morphological, physiological, and behavioral adaptations. However, environmental changes can impose ecological and evolutionary constraints that act on species traits, as well as on local species assemblages through their phylogenetic history. In this study, we evaluated the differences in species distribution and composition on fruit-feeding butterfly assemblages along forest fragmentation and succession gradients. We combine functional and phylogenetic methods for determining butterfly assemblages, and inferred species resistance and resilience according to habitat changes in tropical forests. We used a database of 471 fruit-feeding butterflies of 60 species sampled from different environments in the central Amazon rainforest. A total of 13 functional traits were measured, and a phylogenetic tree was obtained for the sampled species. The trait–environment relationship was analyzed along both forest fragmentation and succession gradients, controlling for phylogenetic signal on species distribution and functional composition when necessary. Several traits presented phylogenetic signal, and phylogeny was also driving butterfly species distribution along the successional gradient. After controlling for phylogeny, individual characteristics related to flight speed (thoracic weight) and anti-predatory strategies (camouflage) increased in early-successional forests, with large butterflies (body length) prevailing in primary forests. No clear functional and phylogenetic pattern was identified for the fragmentation gradient. Our results are consistent with the idea that butterflies may be employing distinct functional strategies to attenuate habitat change effects. Larger butterflies, with lower dispersal ability, are preferentially susceptible to local extinctions in the early-successional environments, mainly when forested habitat and its resources become spatially restricted. In addition, several anti-predatory strategies related to conspicuous colors may be losing their functionality in open areas, where not being distinctive against the background becomes the primary defense against predation.

Key words: Amazon rainforest; color; flight performance; forest fragmentation; forest succession; fruit-feeding butterflies; species traits.

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INTRODUCTION

A major area of community ecology involves research on ecological and evolutionary processes, aiming to understand species performance and diversity through environmental gradients (Keddy 1991, Murphy et al. 2016). Gradients consist of incremental, gradual changes in one or more physical and structural characteristics of habitats, leading to contrasting species co-occurrence patterns and thus community structure across different space and time scales (Tylianakis and Morris 2017). In forest environments, we can identify different gradients with distinct origins and effects, among which the most common are forest fragmentation and succession, both usually induced by human activities (Guariguata and Ostertag 2001, Fahrig 2013). These gradients have been considered as providing valuable information about the effects of disturbances, such as the consequences of habitat loss, species resistance and resilience to deforestation, and changes in habitat structure (Filgueiras et al. 2016, de Andrade et al. 2017).

Forest fragmentation gradients allow disentangling the effects of habitat loss and patch arrangement on species (Shahabuddin and Ponte 2005, Murphy et al. 2016). With increasing habitat fragmentation in natural areas, there are also increases in isolation and pervasive effects of human-transformed habitats (Haddad et al. 2015). Forest patches are generally interspersed within a structurally distinct matrix that may be regenerating from previous disturbances. Thus, a second gradient is formed within the first, that of forest succession (Nyafwono et al. 2014). For both scenarios, changes in the local environmental conditions play fundamental roles in community dynamics, with important effects on local extinctions and species turnover inhabiting tropical forests (Dent and Wright 2009).

This happens because species respond in different ways to environmental changes, which may be related to specific organismal characteristics (functional traits; McGill et al. 2006). Under certain environmental conditions, some species can occur in high abundances, while others are quite rare or even absent (Boukili and Chazdon 2017). Among them, butterflies are a group known to respond rapidly to environmental

changes, and because of their relatively well-known taxonomy represent an ideal study system for assessing the effects of disturbances in forests (Bonebrake et al. 2010). Moreover, standardized functional traits for several insect groups have been proposed as important predictors of community structuring (Moretti et al. 2017). This includes quantifiable morphological, physiological, and behavioral traits, which reflect different adaptation and survival strategies in heterogeneous environments (Violle et al. 2007).

At present, there are no standardized protocols providing functional traits for butterflies and its relationship to the environment. However, based on the natural history knowledge about butterflies, and testable protocols existing for other arthropod groups (Fountain-Jones et al. 2015, Moretti et al. 2017), the understanding of functional composition in butterflies along forest gradients requires an analysis of four major functional categories: flight performance, defense strategies, ecophysiological characteristics, and habitat perception. These characteristics can summarize the response many organisms give to forest area suppression, and to help understand their occurrence in different habitats (Vandewalle et al. 2010). In addition, the trait–environment relationship may not be exclusively explained by current ecological processes but can also reflect the evolutionary history shared by the species in communities (Harvey and Pagel 1991). This is possible when both life traits and species composition across communities are phylogenetically structured (Duarte et al. 2018). Moreover, phylogenetic information complements our understanding of species occurrence, a currently underexploited point of view for tropical regions (Muenchow et al. 2017). This is why combining both functional and phylogenetic methods becomes essential for a more accurate understanding of community assembly along environmental gradients (Xu et al. 2017).

This study evaluates the effects of both forest fragmentation and succession on fruit-feeding butterfly assemblages structure, in view of the functional composition associated with phylogeny. We here aim to answer the following questions: (1) Is functional trait distribution in fruit-feeding butterfly assemblages influenced by the different forest gradients? (2) How does

trait composition change relative to fragment size and succession stages? (3) Is phylogeny acting on functional composition of fruit-feeding butterflies along these gradients? We expected that the effect of time after disturbance, as well as the disturbance intensity reflected in forest fragment size, could lead to differences in species distribution, and consequently in functional and phylogenetic structuring of butterfly assemblages.

MATERIALS AND METHODS

Study area

Butterflies were sampled in areas belonging to the Biological Dynamics of Forest Fragments Project (BDFFP), of the National Institute of Amazonian Research (INPA), 90 km north of Manaus/AM, Brazil. Within a 1000-km² area, three farms (named Dimona, Porto Alegre, and Colosso) were defined as sample areas (Fig. 1). Each sample area was composed of forest fragments of 1, 10, and 100 ha, early-successional and secondary forest areas surrounding the fragments, and continuous Amazonian Rainforest (Laurance et al. 2002). The early-successional sites consist of open areas with dense and short shrubs after three years of regeneration, which isolate the forest fragments from other environments. The secondary forest covers areas previously occupied by cattle pastures, which with time were abandoned and after 30 yr of regeneration already form a great forested structure. The fragments are isolated patches of primary forest, structurally similar to the continuous forest (Laurance and Vasconcelos 2009). These continuous forest areas occupy extensive adjacent regions and are used as a control in this study. In these landscapes, two distinct gradients coexist: forest fragmentation and succession. We considered early-successional sites, secondary forest, and continuous forest as forming the succession gradient; forest fragments of 1, 10, and 100 ha as forming the fragmentation gradient, having the continuous forest as a control (Fig. 1).

Butterfly sampling

Two field expeditions were performed at the beginning of the dry season in August and September of 2015 and 2016. Each one of the three sample areas (Dimona, Porto Alegre, and

Colosso farms) was compound by the following environments design: three forest fragments with area size of 1, 10, and 100 h; three adjacent areas in early-successional (ES) stage surrounding the forest fragments and isolating each of them 100 meters from other environments in the landscape; three areas in the secondary forest (SF) which extends immediately around the ES; and three areas in the continuous forest (CF) as reference areas (Fig. 1) separated 500 m to each other inside the forest. Overall, 12 independent sampling units (SUs) were performed within each sample area, totaling 36 SUs including Dimona, Porto Alegre, and Colosso farms. Each SU had a set of five portable traps containing attractive bait, made with banana plata variety fermented in sugar cane juice for 48 h before the samplings (Freitas et al. 2014). The traps were arranged linearly, and the distance between neighboring traps within the SU was 20 m, placed at a height between 100 and 130 cm tall above the ground (Freitas et al. 2014).

On each sampling occasion, the traps remained exposed for eight consecutive days in each farm (a total of 24 trapping days per field expedition), being reviewed in intervals of 48 h to renew the bait, collect, mark, and identify captured butterflies. According to Graça et al. (2017a), those efforts are enough to meet both the taxonomic and ecological responses of fruit-feeding butterflies in the central Amazon. During the review process, two experienced collectors with entomological hand nets (diameter of 40 cm made with a soft wire bag) also performed active samples of fruit-feeding butterflies around the traps, as long as they were visibly attracted by the bait. These combined methods allow complementing the representation of the fruit-feeding guild, including mainly those species that were generally attracted but not necessarily caught (Checa et al. 2018). Individuals of each species were collected for subsequent identification and functional trait measurement in the laboratory. Butterfly species identification was made through a specialized catalogue (Garwood and Lehman 2009) and by experts at the entomological collections of two institutions: DZUP/UFPR and ZUEC/UNICAMP. This material is deposited in the Laboratório de Ecologia de Interações, of Universidade Federal do Rio Grande do Sul, Brazil.

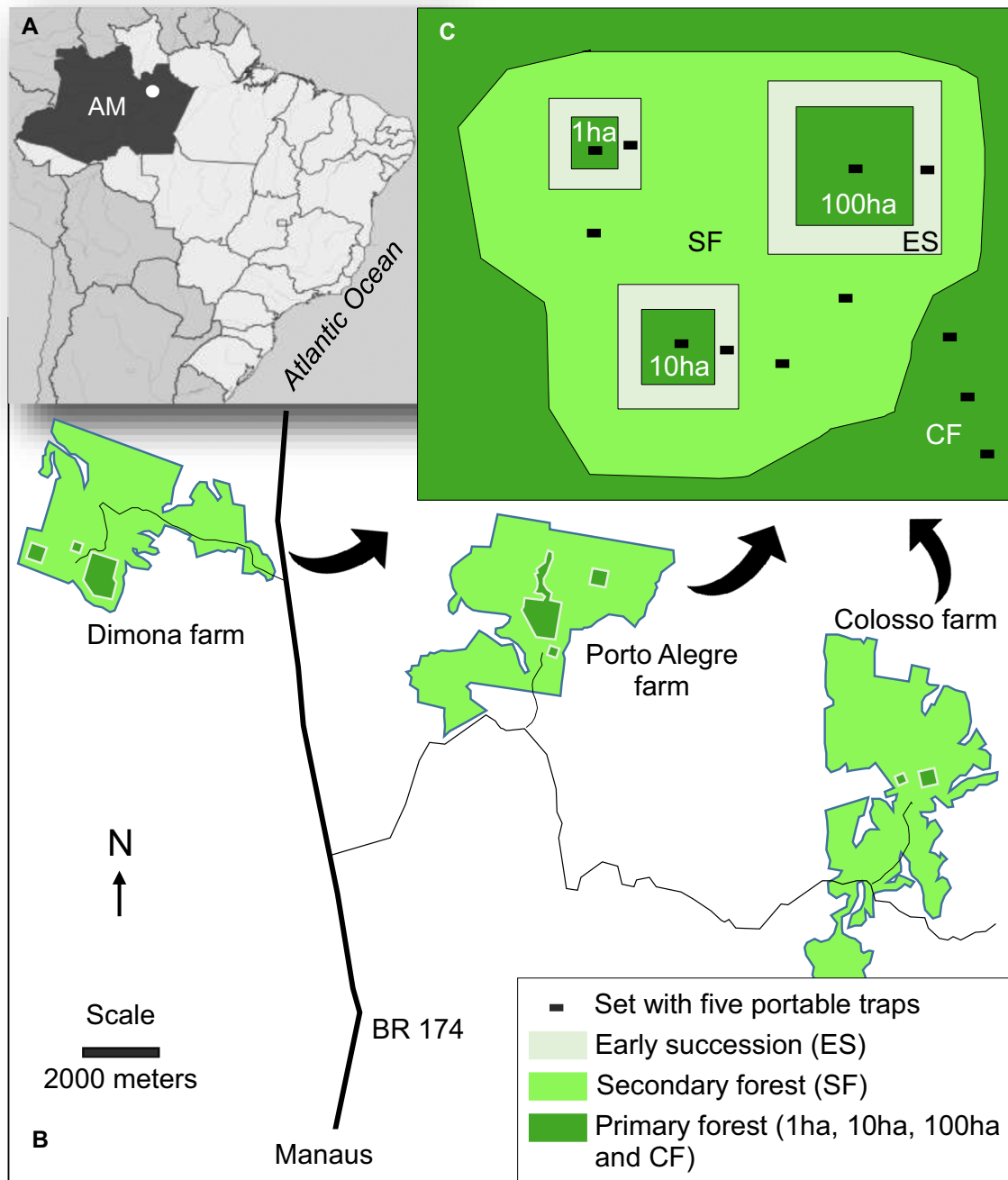


Fig. 1. Images showing the study area and sampling design location in each sample area, three in total: Dimona, Porto Alegre, and Colosso farms. (A) Location of study area (white dot) in Amazon State (AM), north Brazil. (B) Image showing the placement of the three studied farms with the disposition of each sampled environment. (C) Scheme presenting the sampling design carried out in each sample area, in which black rectangles represent a set of five fruit-feeding butterfly portable traps (sample unit), placed in forest fragments (each dark green square with primary forest) of 1 hectare (1 ha), 10 hectares (10 ha), 100 hectares (100 ha), early-succession (gray square surrounding each forest fragment), secondary forest (light green), and continuous forest areas (primary forest surrounding secondary forest regions) of the central Amazon (BDFFP).

Functional trait measurement

We selected up to 10 individuals per species from our reference collection to measure 13 functional traits, observing whenever possible the same number of males and females, to control for sexual dimorphism in any of the traits. All measured characteristics were previously defined including four categories: flight performance (forewing length, forewing area, hindwing area, body length, body mass, thoracic width, wing load as continuous data), defense strategies (iridescence, camouflage, mimetic rings, and wing eyespots as binary data), ecophysiological aspects (melanism as categorical data), and habitat perception (eye size as continuous data).

The forewing length is a measure of the longest straight-line distance from the wing base (insertion point in the thorax) to the wing tip, using a digital caliper for high accuracy (Hook et al. 2012). The wing area was obtained from dorsal (forewing area) and ventral (hindwing area) photographs, and through an image processing program, ImageJ (version v 1.50i, U.S. National Institutes of Health, Bethesda, Maryland, USA), the wing area was manually selected and measured. Body length corresponds to the sum of both thorax and abdomen length (Chai and Srygley 1990). For body mass, we considered the dry mass of the whole individual, measured through an analytical balance (Shimadzu model AY-220, Shimadzu Corporation, Kyoto, Japan). Wing load was calculated as the ratio between body mass and forewing area (Berwaerts et al. 2002), which express the amount of body mass sustained by wing area unit. Defense strategies were visually categorized of the main type of anti-predatory strategies and then binary-catalogued (presence/absence) for each species. Wing eyespots can be easily identified on the ventral face of Nymphalid wings (Stevens 2005). Mimetic rings and iridescence traits are manifested mainly in the dorsal part because they are usually recognized during flight (Mallet and Gilbert 1995). Camouflage colors (especially browns) and wing shapes are in close association with their background (Stevens and Ruxton 2019), resembling environmental features (leaves, trunks, stones). Regarding melanism, we categorized this trait into four classes observing its intensity (low, 0–25%; medium, 26–50%; high 51–75%; and black color wings, more than 75%). We calculated eye size by the eye surface area,

following a previous methodology that uses a set of linear measurements (see Rutowski 2000).

Data analysis

For each SU, the mean species traits values were weighted by community-weighted means (CWM; Lavorel et al. 2008) and used in the next step to evaluate the differences in species distribution and composition on fruit-feeding butterfly assemblages along forest gradients (Fig. 2). CWM is an expression of which species/lineages are distributed in which assemblages. Firstly, we tested the effect both forest fragmentation and succession in view of the functional responses (all fragments as a single category vs. continuous forest; all succession stages as a single category vs. continuous forest). Considering only traits that showed a significant relationship with forest gradients, we tested for the importance of the succession stages and the fragment sizes separately, performing a CWM.sig analysis (Duarte et al. 2018).

Before performing the functional composition analysis, we tested the phylogenetic component along forest gradients based on a phylogeny for Nymphalidae (Wahlberg et al. 2009). We overlapped our butterfly species list on that phylogeny and generated a phylogenetic tree for our data (Fig. 3). Species and genera absent in the original phylogeny were inserted as polytomies in the terminal branches according to the taxonomic affinity (species within genera and genera within families) using the information available at Tree of Life Web Project. Before performing the statistical analyses, we make sure that the built tree was ultrametric (Webb et al. 2008). We analyzed the phylogenetic signal in species functional traits using *K*-statistic (continuous data; Blomberg et al. 2003) and *D*-statistic (binary data; Fritz and Purvis 2010). We also verified whether species distribution in the communities resulted from phylogeny through a principal coordinates of phylogenetic structure (PCPS; Duarte et al. 2016), via ADONIS function implemented in the vegan package of R (Dixon 2003). When phylogenetic signal in both trait (*K*- and *D*-statistic ≥ 0.5) and community composition (PCPS.sig with *P*-value ≤ 0.05) was identified, it was then deemed necessary to discount its effect on CWM to reduce chances of type I error in a CWM ~ Environment model. When phylogenetic

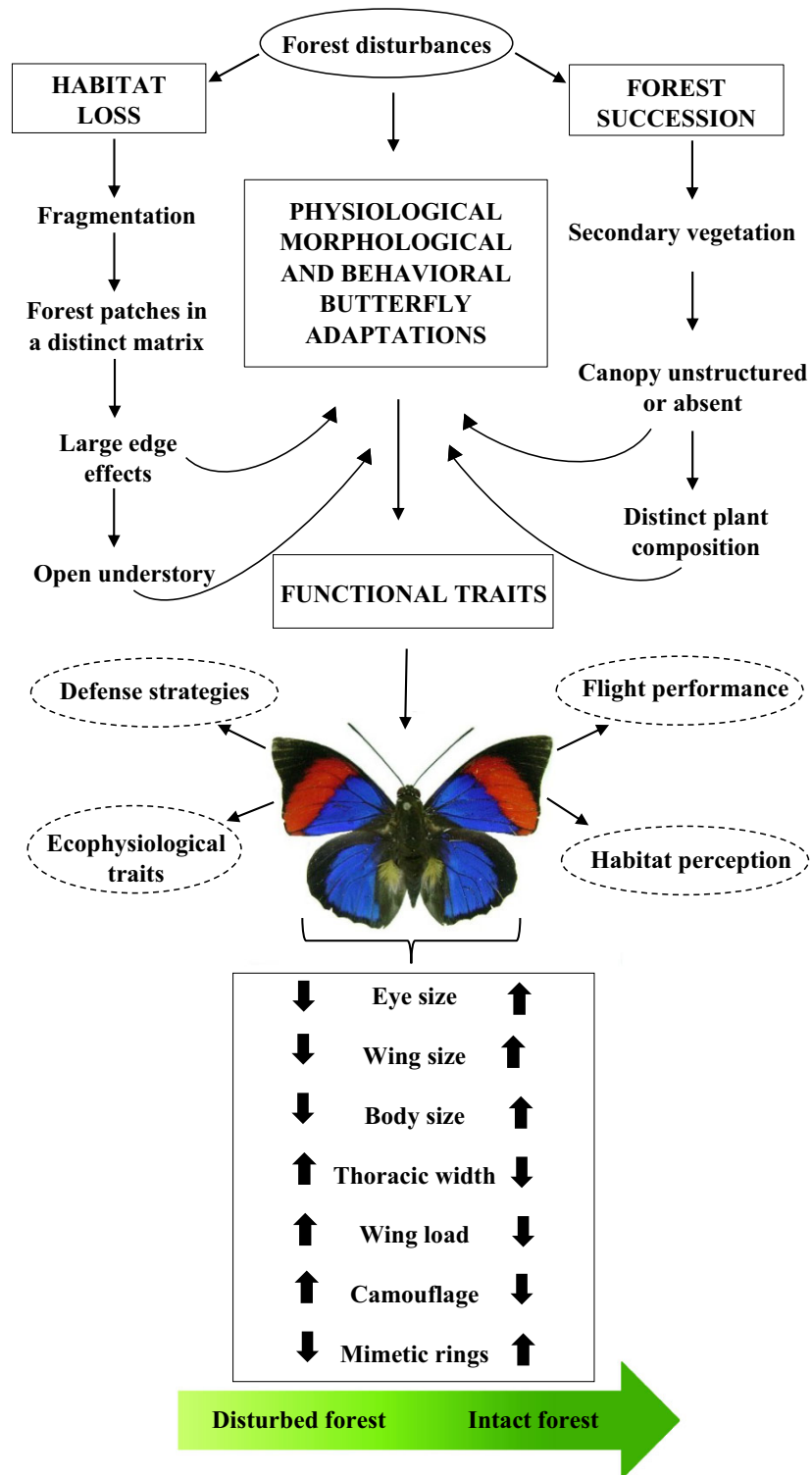


Fig. 2. Conceptual framework and hypotheses on trait–environment patterns for fruit-feeding butterflies. We

RESULTS

After 48 d of sampling effort, 471 individuals were recorded in 60 species belonging to all the fruit-feeding butterfly subfamilies of Nymphalidae (Fig. 3). A total of 10 species traits showed phylogenetic signal (statistic value ≥ 0.5 ; Table 1), indicating that phylogenetically closer species presented more similar characteristics to each other than expected by chance. Phylogeny was also driving butterfly species distribution along the succession gradient (PCPS Mean $F = 10.52$; $P = 0.01$). This gradient affects the distribution of species across the community (site shuffle $P = 0.01$), and the influence of the succession gradient on species distribution across communities depends on the phylogenetic relatedness among them (taxa shuffle $P = 0.03$). This means that evolutionarily closer lineages responded

similarly to environmental changes along this gradient, thus occurring in structurally similar areas. Analysis of the butterfly assemblages showed that species composition and functional traits were not mediated by phylogeny along the fragmentation gradient.

Community structuring by phylogeny in the succession gradient can be shown through the PCPS diagram (Fig. 4). Charaxinae, in the first axis, is separated from other butterfly lineages, in which its species are associated mainly with secondary and continuous forest. The second axis includes Biblidinae and Nymphalinae associated with the secondary forest, while Satyrinae species, in their turn, were predominantly found in both early-successional and continuous forest environments.

After controlling for phylogenetic relatedness on the functional and community composition

Table 1. Effects of forest succession and fragmentation gradients on the fruit-feeding butterfly species community composition (site shuffle) and functional trait composition (trait shuffle), after controlling for phylogeny effects identified on the succession gradient.

Phylogenetic signal	Traits	Succession gradient						Fragmentation gradient		
		Successional stages \times continuous forest			Successional stages			Forest fragments \times continuous forest		
		F model	Site shuffle	Trait shuffle	F model	Site shuffle	Trait shuffle	F model	Site shuffle	Trait shuffle
0.9173	Eye size	1.827	0.196	0.489	2.234	0.136	0.137
0.9220	Body mass	3.162	0.082	0.347	3.456	0.076	0.072
0.6331	Body length	3.976	0.05†	0.311	0.516	0.481	0.739	0.518	0.495	0.502
13.739	Forewing length	0.401	0.533	0.763	0.276	0.591	0.590
13.378	Forewing area	0.235	0.635	0.786	2.455	0.149	0.111
13.410	Hindwing area	0.183	0.680	0.821	3.335	0.065	0.089
12.688	Thoracic width	13.790	0.002†	0.05†	2.055	0.152	0.447	0.129	0.702	0.713
0.3350	Wing load	0.166	0.764	0.816	0.855	0.356	0.390
0.4220	Iridescence	0.125	0.739	0.859	0.003	0.956	0.948
0.9680	Wing eyespots	1.020	0.318	0.601	0.080	0.780	0.756
0.7586	Melanism	0.776	0.396	0.670	0.052	0.822	0.809
0.8820	Camouflage	7.559	0.01†	0.180	10.259	0.003†	0.104	2.038	0.170	0.140
0.4340	Mimetic rings	2.000	0.183	0.477	0.450	0.519	0.472

Note: Relationship between fruit-feeding butterfly functional traits and forest fragmentation and succession gradients is such that forest fragments \times continuous forest compares traits found in all forest fragments with continuous forest; successional stages \times continuous forest compares traits found in all successional environments with continuous forest; and successional stages compares the early succession with secondary forest, considering only traits that showed a significant relationship with the successional gradient. Ellipses indicate functional traits without relationship with the forest gradient.

† Traits with a significant association with the forest gradients for site shuffle and trait shuffle.

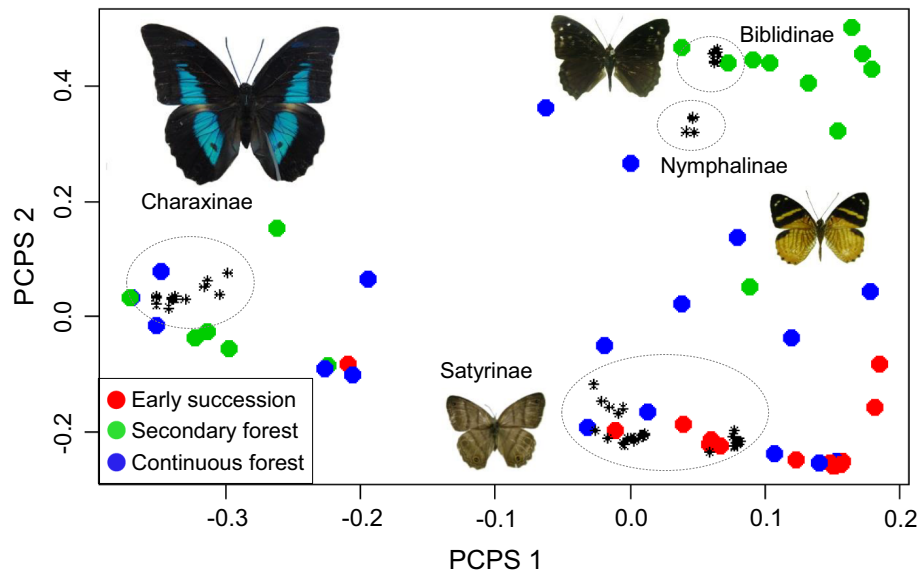


Fig. 4. Scatter diagram showing the principal coordinates of phylogenetic structure (PCPS) scores for communities and fruit-feeding butterfly species related to the forest succession gradient. Asterisks represent species, and dotted lines enclose the four butterfly subfamilies. Small circles color-code communities in the different environments that compound the succession gradient.

across the successional gradient, flight performance and defense strategies traits still varied in response to forest regeneration stage (Fig. 5). Camouflage was the main anti-predation strategy related to the early forest succession. This trait was identified comparing between all succession environments (as a single category) against the continuous forest, but also among separate successional stages. Thoracic width also increased toward to the early succession, due to both community composition (site shuffle) and through the similarity among species for thoracic width trait (trait shuffle). With less muscular mass in the thoracic region, body length in butterflies tends to increase toward the continuous forest. The forest fragmentation gradient had no significant effect on the functional composition, without differences between fragments and continuous forest (Table 1).

DISCUSSION

Functional structure of fruit-feeding butterflies

In agreement with our predictions, environmental changes along forest gradients have important effects on the structuring of fruit-feeding butterfly

assemblages. However, species occurrence along forest fragmentation and succession gradients is not mediated by the same adaptations in functional terms. The origin and nature of the disturbances seem to differentiate the two gradients according to the environmental pressures that act on the morphological, ecological, and behavioral characteristics, configuring distinct functional composition. While the main limitation in fragmented areas is thought to be the amount of habitat and their connectivity in the landscape (Watling et al. 2011), forest succession starts from the regeneration of often decharacterized communities, with extreme and restrictive environmental conditions for many species (Guariguata and Ostertag 2001).

Body size measurements reveal butterfly flight and dispersal characteristics in a landscape (Hill et al. 2001). As was found, species that occur in successional areas have traits that provide flight acceleration and speed, expressed by smaller wings associated with a greater muscular robustness (thicker thoraces), allowing fast flights over longer distances. In the early-successional sites, where environmental conditions may be more restrictive in terms of resources and exposure to

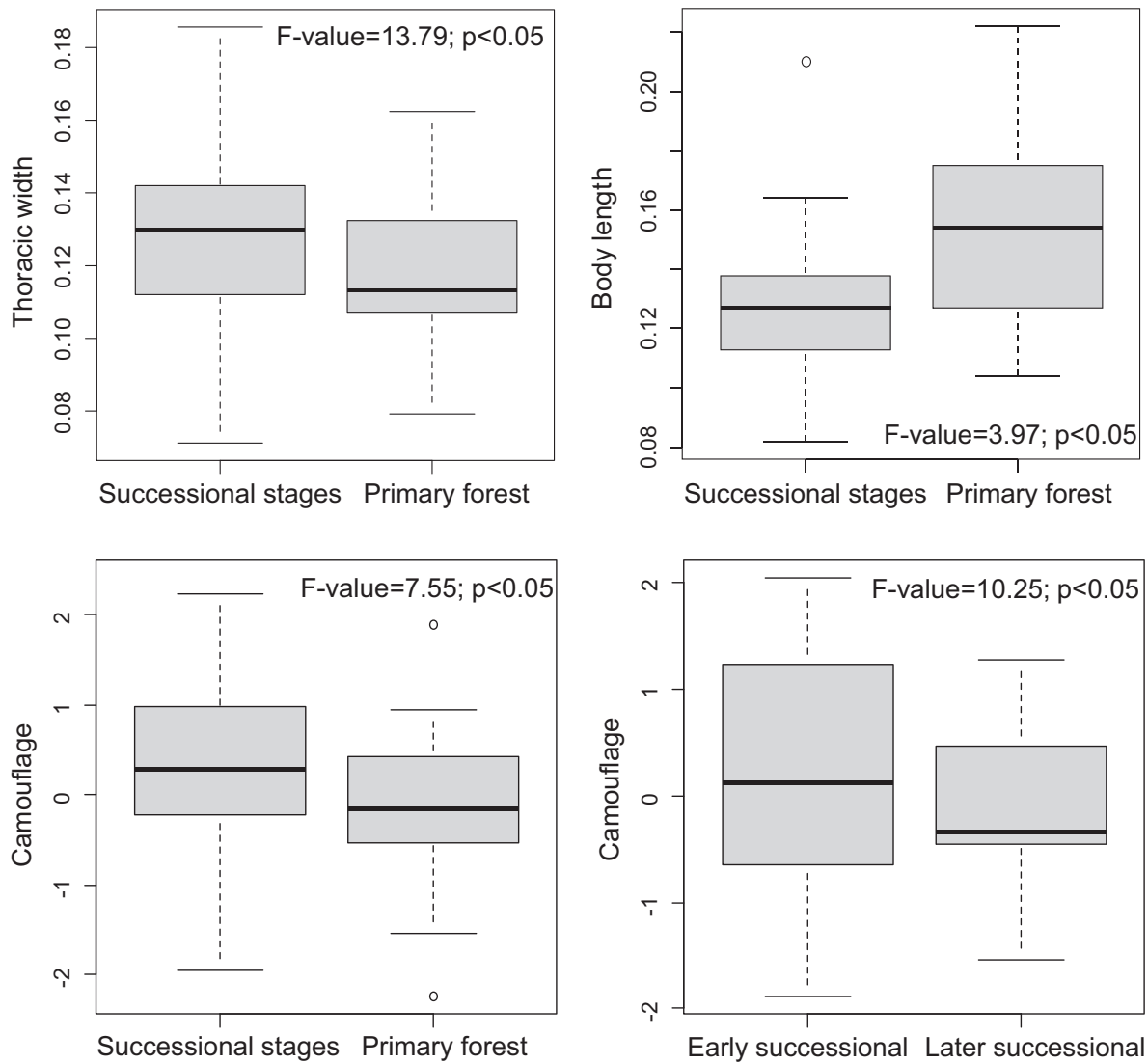


Fig. 5. Fruit-feeding butterfly functional traits along the forest succession gradient, including comparisons between successional stages vs. continuous forest and between early vs. late successional stages in the Central Amazon—Biological Dynamics of Forest Fragments Project. The y -axes contain normalized values measured for each related trait, in a total of 51 independent samples used in each analysis and figure.

predators, flight performance may reflect a larger home-range exploitation and avoid predation. This may help minimize the effects of antagonistic behaviors (territoriality and competition) and facilitate access to environmental resources (Stevens et al. 2013, Pellissier et al. 2018). Looking as imperceptible as possible in environments with greater exposure appears to be another necessary condition for survival in early-successional stages. Thus, camouflage appears as a crucial anti-predatory

strategy, reducing detectability by visually oriented predators in open areas. This includes both similar shapes and colors to the environment, such as contours on the wings that draw false edges with wing eyespots in some species, making it difficult to perceive body boundaries, avoiding or diverting attacks to non-lethal parts of the body (Stevens and Merilaita 2009).

There is no evidence of functional patterns for the fragmentation gradient that can be detected

from the set of evaluated traits. One explanation could stem from the matrix structure permeating primary vegetation patches (Schtickzelle et al. 2007), which can be attenuated in its harshness by the progress in forest succession. The secondary forest prevailing in the matrix may make these environments more easily transposable to different butterfly lineages, even for those with lower flight capacity. This is supported by the absence of phylogenetic structuring for species composition in this gradient. One of the initial aims of the BDFFP, to study the ecological consequences of deforestation and forest fragmentation (Bierregaard et al. 1992), may have important applications also toward understanding forest succession nowadays. This scheme of different types of development of vegetation may be enough to well connect isolated forest fragments of primary vegetation to continuous areas, allowing the use by several species of fruit-feeding butterflies, and facilitating the transposition in this more suitable matrix.

Phylogenetic structure of fruit-feeding butterflies

Some trait–environment relationships across environmental gradients can result from phylogenetic influence (Pavoine et al. 2014, Duarte et al. 2018). In other words, evolutionary closer lineages in butterflies show similar functional traits to environmental variation also due to their shared evolutionary history. Our findings are consistent with this idea when traits were separately analyzed, controlling for the existing phylogenetic component. While phylogeny has an important role along the forest succession gradient, we did not detect clear phylogenetic structure on butterfly assemblages along the fragmentation gradient, as we had initially expected. The mutual influence of phylogeny on the functional traits and assemblage composition may be more intense along succession because the latter is formed by quite distinct environments, both in their habitat characteristics and in the disturbances that lead to their origin. Thus, this gradient may be more rigorous in terms of lineage selection, restricting its occupancy to phylogenetically close and functionally more similar groups in each of the successional stages. Similar results were also observed for plant communities in both old-growth and secondary forest across a lowland and montane rain forest in

China (Ding et al. 2012), and for zooplankton communities in lakes under distinct disturbance type, in the north-central United States and southern Canada (Helmus et al. 2010).

According to the results obtained, butterfly assemblages integrating the primary forest mosaic along the fragmentation gradient are composed of the same lineages, that is, they are not phylogenetically structured. Habitat size itself is not determinant for the distribution of certain groups, neither on the formation of phylogenetic patterns, a trend already observed in plant communities on the northern limit of tropical rain forest for the Neotropical region, in Mexico (Arroyo-Rodríguez et al. 2012). This does not mean that soon after the fragmentation process, community trajectories do not change over time and that species in those communities do not converge in some functional traits acting on their ecological performance (e.g., eye and wing size, body mass). Especially, those traits were related to habitat perception and individual dispersion between habitat patches (Thomas 2000, Turlure et al. 2016), as manifested in the surrounding matrix during the successional process, driving a resilience process. Overall, these patterns were not detected in our fragmented landscapes. The main conclusion from this is that phylogeny may not be the most important driver for functional composition and evolutionary responses to fragmentation, a tendency also observed for butterfly assemblages in fragmented grasslands in southern Belgium (Pavoine et al. 2014).

Despite the limitation in habitat area size, forest fragmentation seems to have a reduced impact in terms of vegetation diversity and composition when compared to forest succession. Even in a recently fragmented primary forest, an adult butterfly will likely continue to find its host plant for oviposition, the larvae will obtain their food resources to complete its life cycle—despite a reduced dietary specialization as the most recent surveys show (Bagchi et al. 2018), and habitat area size seem did not have uniform consequences on all species (Cagnolo et al. 2009). In this aspect, different lineages can be equally distributed among fragments, leading to an absence of phylogenetic structuring for butterfly assemblages along fragmentation gradients. Regarding forest regeneration, the entire floristic species composition tends to change according to

the successional stage (Guariguata and Ostertag 2001). Thus, the supply of host plants to butterfly caterpillars and resources to adults may be quite specific for each stage of this gradient (Pinotti et al. 2012, Valtonen et al. 2017). This may lead to a high turnover of lineages in the butterfly assemblage, with species compositional changes and evident phylogenetic patterns.

Understanding butterfly assemblages assembly in tropical forests

In an overview, we can predict that larger fruit-feeding butterflies with lower dispersal abilities (e.g., as *Morpho*, *Caligo*, *Catoblepia*, and *Pierella* genus) are more vulnerable to disturbances in forest systems. These species are probably the first to disappear because of their greater dependence on the forest interior and more stable environments. These conclusions are in agreement with Shahabuddin and Ponte (2005) studying fruit-feeding butterflies in tropical forest fragments in Venezuela. Even if these species have a greater sensitivity or visual acuity to better perceive the environment, a low dispersal ability becomes a limiting factor, especially for the use and transposition of frequently inhospitable matrices (Turlure et al. 2016).

In addition to the negative pressures on larger butterflies that changes in vegetation cover may promote, there is strong evidence for a discoloration in butterfly assemblages in the Amazon rainforest. Animal coloration has implications for a broad range of ecological interactions and manifests itself in different ways, one of them transmitting information for protection from predation purposes (Stevens 2007). Bearing this in mind, the predominance of the camouflage strategy especially in those environments under forest succession signals a loss of color-related functions. It has been suggested that butterflies exhibiting conspicuous colors, including aposematic and iridescent contrasts, may be declining in their abundance or even disappearing in sites with recent disturbances (Delhey and Peters 2016). The interactions in which these species are involved may have low functional effectiveness in these environments.

The evaluation of phylogenetic signal strength in butterfly traits (mainly those related to flight characteristics and habitat use) has been recently incorporated to disentangle butterfly assemblages along environmental gradients (Pavoine et al.

2014), including studies in the Amazon rainforest (Graça et al. 2017b). Nevertheless, this is the first study that also combines phylogeny with butterfly species distribution along forest gradients, controlling for its effects on functional composition. We learned from this that many of the trait–environment associations and community composition changes we usually describe are also related to evolutionary events along with current ecological processes. Linking evolutionary and ecological issues can be especially interesting for megadiverse regions such as Amazon, given the vast biodiversity under threat. This allows for a more accurate understanding of current patterns of diversity and offers new perspectives for studies that seek to take a step further to elucidate community assembly processes along environmental gradients.

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