UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de doutorado

Estruturação funcional e filogenética das assembleias de borboletas frugívoras ao longo de gradientes florestais na Amazônia brasileira

RICARDO LUÍS SPANIOL

Porto Alegre, maio de 2019

Estruturação funcional e filogenética das assembleias de borboletas frugívoras ao longo de gradientes florestais na Amazônia brasileira

Ricardo Luís Spaniol

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para a obtenção do título de Doutor em Ciências com ênfase em Ecologia.

Orientador: Prof. Dr. Milton de Souza Mendonça Jr.
Coorientador: Prof. Dr. Cristiano Agra Iserhard

Comissão Examinadora
Prof^{a}. Dra. Marina do Vale Beirão (UFOP)
Prof^{a}. Dra. Luciana Regina Podgaiski (UFRGS)
Prof. Dr. Vanderlei Julio Debastiani (UFRGS)

Porto Alegre, maio de 2019

CIP - Catalogação na Publicação

Spaniol, Ricardo Luís
Estruturação funcional e filogenética das assembleias de borboletas frugívoras ao longo de gradientes florestais na Amazônia brasileira / Ricardo Luís Spaniol. -- 2019.
115 f.
Orientador: Milton de Souza Mendonça Jr..

Coorientador: Cristiano Agra Iserhard.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Programa de Pós-Graduação em Ecologia, Porto Alegre, BR-RS, 2019.

1. Borboletas frugívoras. 2. Floresta Amazônica. 3. Ecologia funcional. 4. Ecologia filogenética. 5. Coloração animal. I. de Souza Mendonça Jr., Milton, orient. II. Agra Iserhard, Cristiano, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Os quatro anos de doutorado podem ser definidos para mim como o período de maior amadurecimento pessoal e profissional. Junto a pessoas incríveis e com bastante trabalho, foi possível viabilizar planos que sequer cogitava ao ingressar no PPG em Ecologia/UFRGS. As inesquecíveis expedições na floresta amazônica, do período memorável na Inglaterra, as várias passagens pela Floresta Nacional de São Francisco de Paula... e acabei trilhando um percurso infinitamente melhor às expectativas iniciais e das incertezas que um aluno têm ao ingressar na pós-graduação. Muito disso se deve por escolhas acertadas, orientações inspiradoras, e principalmente pela liberdade em poder desenvolver a tese de um jeito próprio, deixando a minha marca como pesquisador.

-Por isso devo em primeiro lugar, um agradecimento especial aos meus orientadores. Tenho o Prof. Milton como uma referência de pessoa e profissional. Em cada uma de nossas conversas me abastecia de empolgação, e retornava ao laboratório motivado e seguro de que eu estava na direção certa. E esse é um dos papéis mais brilhantes que um grande orientador pode ter! O Prof. Cristiano, por sua vez, foi o primeiro a embarcar nessa experiência única que é conhecer a Amazônia. Esteve presente do começo ao fim, e tenho um grande apreço pela sua parceria e amizade. “Me segue por aqui!” Obrigado Mestres, por essa jornada!

Também fica meu agradecimento especial para várias pessoas e instituições que contribuíram e me acompanharam nos últimos quatro anos:

-Ao Leandro Duarte, por me apresentar a ecologia filogenética.

-À Sandra Hartz, pelo incentivo em levar adiante meu trabalho sobre coloração animal.

-Ao Martin Stevens, que me guiou no fantástico universo da coloração animal.

-À todos os ajudantes de campo que estiveram comigo na Amazônia, e também aos que me auxiliaram no trabalho de laboratório.

-Aos colegas e amigos com quem tive o prazer de conviver no Laboratório de Ecologia de Interações/UFRGS.

-Ao programa de pós-graduação em Ecologia pelas oportunidades e pelo meu crescimento profissional, e à Capes pela bolsa de doutorado.

-Ao Projeto Dinâmica Biológica de Fragmentos Florestais/PDBFF e ao Instituto Nacional de Pesquisas da Amazônia/INPA, através do Programa PDBFF de Auxílio-Pesquisa Thomas Lovejoy pelo auxílio no financiamento e suporte logístico nos trabalhos de campo.

-À Universidade de Exeter e ao Sensory Ecology and Evolution Laboratory por me acolher durante um importante período na Inglaterra.

-Conheci pessoas incríveis em uma cidade agradável que é Porto Alegre. Muitas delas também vindas de longe, e com isso criamos um vínculo muito mais próximo nesta nossa segunda casa. Meu grande abraço ao Gabriel Nakamura, Lucas Porto, Maico Fiedler e Pedro Nitschke. Tenho certeza que todos lembram das pedaladas na orla do Guaíba, dos botecos na Cidade Baixa, do futebol nas quartas e dos incontáveis cafés no laboratórios. E um agradecimento muito especial à Paula Pereyra pela paciência, carinho e parceria nessa reta final do doutorado.

-Meu agradecimento especial aos meus pais, Élio e Lourdes, e às minhas irmãs, Nayara e Graciela, pelo constante incentivo e confiança durante toda minha trajetória acadêmica. Ficam as boas lembranças da minha avó Theresia, grande pessoa com quem pude compartilhar as experiências amazônicas e de um doutorado, mas que infelizmente não pôde ver o resultado final dessa aventura.

Escrever essa tese foi incrível, divertido e prazeroso. É assim que a ciência e a vida acadêmica devem ser!

SUMÁRIO

RESUMO

As diferentes estratégias de adaptação e sobrevivência das borboletas proporcionam um verdadeiro repertório de cores, formas, tamanhos e comportamentos ocorrendo na natureza. Conceitualmente, estas estratégias são definidas como atributos funcionais. A performance dos atributos permite que as espécies possam ocorrer em diferentes combinações de condições ecológicas, ou ainda restringe sua ocupação para habitats específicos. Muitas vezes estes padrões funcionais se misturam com a história evolutiva compartilhada entre as espécies, indo além da relação atributo-ambiente. Por isso, a ecologia funcional e filogenética combinadas são fundamentais para uma compreensão robusta sobre a distribuição das espécies em escalas espaciais e temporais. No capítulo I da tese apresentamos um uma seleção de atributos funcionais em borboletas frugívoras, incentivando sua aplicação em estudos ao longo de gradientes florestais. Detalhamos a importância da avaliação de quatro grandes categorias de atributos: performance de voo, estratégias de defesa, atributos ecofisiológicos e percepção do habitat. Derivamos ainda uma série de hipóteses sobre como seria a relação destes atributos com diferentes cenários ambientais. Já no capítulo II aplicamos o protocolo proposto no capítulo anterior, descrevendo a estruturação funcional de borboletas frugívoras em gradientes de fragmentação e sucessão florestal na Amazônia. Observamos a participação do componente filogenético na composição de espécies e de atributos. A filogenia se mostrou importante ao longo do gradiente de sucessão florestal, e quando controlado seu efeito, identificamos atributos relacionados à performance de voo e estratégias de defesa conduzindo a estruturação das assembleias de borboletas. As diferentes estratégias de defesa manifestadas através das cores motivou no capítulo III, um estudo sobre os efeitos das perturbações florestais na coloração das borboletas. Através da degradação e perda da floresta Amazônica, foram identificadas importantes mudanças na composição das cores nas assembleias. Enquanto a camuflagem se mostra como uma das principais estratégias de defesa, os indivíduos mais coloridos estão entre os primeiros a se extinguir localmente devido a conversão e perda de habitats florestais na Amazônia.

Palavras-chave: Atributos, coloração, filogenia, floresta tropical, fragmentação florestal, habitat, sucessão florestal.

ABSTRACT

The diversity of strategies for butterfly adaptation and survival provides an extraordinary repertoire of colours, shapes, sizes and behaviours present in Nature. Conceptually, these strategies are defined as functional traits. Trait performance allows species to occur under a range of ecological conditions, or even constrain their occupation to specific habitats. These functional patterns are often linked with the evolutionary history shared by species, extending beyond a trait-environment relationship. Therefore, combining both functional and phylogenetic ecology provides a deeper understanding of species distribution from spatial and temporal viewpoints. In chapter I, we review functional traits for fruit-feeding butterflies, encouraging its wide application in studies across forest gradients. This article details the importance of evaluating four major trait categories: flight performance, defense strategies, ecophysiological traits and habitat perception. We also derived expected results for a wide application of these traits in butterfly studies, with emphasis on different environmental scenarios. In Chapter II, we tested several functional traits proposed in the previous study, and from those, we disentangled community structuring of fruit-feeding butterflies along forest fragmentation and succession gradients in the Amazon. We also described the effects of phylogeny in functional and species composition. Phylogeny was important for the successional gradient, and with its effects controlled, we identified traits related to flight performance and defense strategies leading to the structuring of butterfly assemblages. The defense strategies manifested through colours motivated a new study (Chapter III) on the effects of forest disturbances on the colouring of butterflies. Through the degradation and loss of the Amazon rainforest, important changes in colour composition were identified. Whereas camouflage appears as the main anti-predatory strategy, the most colourful butterflies are among the first to be extinguished locally due to the conversion and loss of forest habitats in the Amazon Rainforest.

Keywords: Colouration, forest fragmentation, forest succession, habitat, phylogeny, species traits, tropical forest.

LISTA DE TABELAS

Capítulo 1

Capítulo 2

LISTA DE FIGURAS

Capítulo 2

Capítulo 3

INTRODUÇÃO GERAL

Apresentação

Ao longo do meu doutorado, investiguei sobre a estrutura funcional e filogenética de borboletas frugívoras na Floresta Amazônica. As borboletas são um bom modelo de estudo, associado a um bioma altamente ameaçado pelas rápidas transformações antrópicas que avançam na região centro-oeste e norte do Brasil. A degradação das florestas tropicais revela consequências ecológicas muitas vezes ignoradas do ponto de vista da conservação e restauração ambiental, mas que são fundamentais para acessar a real dimensão dos efeitos históricos pregressos, atuais e futuros sobre um dos maiores refúgios de biodiversidade no planeta. Ao longo dos três capítulos desta tese busquei compreender os atributos funcionais e a história evolutiva compartilhada entre as espécies de borboletas envolvidos nos processos ecológicos em ecossistemas florestais. A tese foi estruturada em três partes: I) no primeiro capítulo é fornecido ao leitor uma visão sobre atributos funcionais em borboletas frugívoras que podem ser acessados para tentar compreender as adaptações das espécies aos ambientes florestais; II) no segundo capítulo estes atributos são testados em cenários reais de gradientes de fragmentação e sucessão, e III) no terceiro capítulo avançamos no estudo da coloração animal, investigando como a degradação da floresta amazônica afeta as cores das borboletas em suas diferentes estratégias ecológicas. A seguir é apresentada uma breve revisão dos principais tópicos que guiam a construção dos três capítulos, e como estes foram motivados ao longo do doutorado.

A floresta amazônica e suas principais ameaças

A floresta amazônica é mundialmente conhecida pela sua biodiversidade singular e incomparável com qualquer outra região do planeta (Silva et al., 2005). Sua extensão continental ocupa aproximadamente 7 milhões de hectares, sendo capaz de regular processos climáticos e ciclos biogeoquímicos em escalas biogeográficas que vão muito além dos limites da floresta (Werth & Avissar, 2002; Nobre et al., 2016). A floresta realiza importantes serviços ecossistêmicos, como por exemplo a emissão e concentração de 20% do oxigênio e da água doce do planeta, do sequestro de carbono com efeitos globais, e por produzir 10% da biomassa terrestre (Malhi et al., 2008). Mesmo com incertezas nas estimativas, a região amazônica parece abrigar cerca de 20% da fauna global, números que devem ser ainda superiores pela própria velocidade com que novas espécies são anualmente descritas (WWF, 2017).

Atualmente mais de 30 milhões de pessoas (incluindo povos nativos) vivem na região, e se abastecem direta ou indiretamente dos recursos florestais disponíveis. Como consequência da presença humana, em torno de 20% da floresta já foi convertida, e está prestes a atingir um limiar a partir do qual a floresta amazônica pode passar por mudanças irreversíveis (Lovejoy & Nobre, 2018). Quando os índices atuais de desmatamento são comparados com dados históricos monitorados desde o final dos anos 80, há uma importante redução da sua dimensão (INPE, 2019). Mas, isso não necessariamente significa que hoje existe um melhor controle através de políticas públicas, uma vez que a perda florestal na região amazônica voltou a crescer em 2018, e tem avançado em Unidades de Conservação e terras indígenas. Atualmente, os maiores índices de desmatamento neste bioma se concentram no arco da fronteira agrícola, principalmente nos estados brasileiros de Mato Grosso e Pará (INPE, 2019). Este panorama demonstra a necessidade de grandes mudanças nas práticas de exploração e uso dos recursos florestais para alcançar níveis satisfatórios, e um esforço ainda maior para conseguir recuperar outros milhares de hectares de áreas degradadas (Chazdon, 2003).

Isso torna a Amazônia um espaço ideal e simultaneamente urgente do ponto de vista da conservação para o estudo das respostas dos organismos às mudanças ambientais (Mesquita et al., 2015). Mudanças de uso do solo na região podem levar a trajetórias distintas da fauna e flora, através da fragmentação e perda de habitats florestais, e do abandono e sucessão da vegetação em áreas degradadas (Bierregaard et al., 1992). Ao mesmo tempo em que o mundo testemunha avanços preocupantes nos índices de desmatamento na Amazônia e de outras importantes florestas tropicais (Souza et al., 2013), está ocorrendo uma extinção em massa de espécies ao redor do planeta (Sánchez-Bayo & Wyckhuys, 2019). As consequências imediatas das transformações florestais nem sempre são evidentes, e demandam um acompanhamento detalhado das respostas ecológicas e evolutivas de diferentes espécies (Caro et al., 2017). Mas, antes disso, precisamos conhecer a origem das mudanças ambientais, e como essas alterações podem atuar sobre os organismos de um lugar ou região.

Com o propósito de investigar as consequências do desmatamento e da fragmentação florestal na Amazônia, foi criado no final dos anos 70 o Projeto Dinâmica Biológica de Fragmentos Florestais – PDBFF (Biereegaard et al., 1992). Uma inédita parceria entre governo brasileiro, pecuaristas e pesquisadores viabilizou nas últimas quatro décadas o mais longevo experimento em atividade no mundo sobre o tema. Inicialmente, foram realizados recortes de fragmentos florestais de diferentes tamanhos, que se mantiveram isolados por uma matriz de pastagens (Laurance et al., 2018). Já nos anos 90, uma crise na economia brasileira levou ao fim dos incentivos governamentais aos fazendeiros, o que provocou o abandono geral de extensas áreas de terra. A partir de então a floresta vem se regenerando nas áreas de matriz, o que tem proporcionado um “laboratório de estudos” sobre a sucessão florestal. É neste cenário que os artigos desta tese são realizados, e tem a Amazônia Central como referência para entender o impacto humano na região.

Por que borboletas frugívoras?

Assim como muitos insetos, as borboletas desempenham papéis ecológicos centrais na natureza (Freitas & Marini-Filho, 2011). Elas são essenciais para a manutenção da integridade de ecossistemas terrestres. Sua afinidade com as comunidades vegetais desde os estágios imaturos levaram a co-evolução de complexas redes de interações, estratégias voltadas à dispersão, e uma grande diversidade de comportamentos de defesa e reprodução (Briolat et al., 2018). Diante disso as borboletas manifestam rápidas respostas às variações nas características dos habitats, tornando-as um excelente modelo de estudo para acompanhar as mudanças ambientais (Brown et al., 2005).

Evolutivamente as borboletas são classificadas em seis grandes famílias, e com base nas características alimentares são separadas em duas guildas: nectarívora e frugívora. As borboletas frugívoras pertencem à família Nymphalidae, e são agrupadas em quatro subfamílias: Charaxinae, Biblidinae, Satyrinae, e algumas poucas tribos dento de Nymphalinae (Wahlberg et al., 2009). A vantagem de trabalhar com essa guilda é seu fácil monitoramento na natureza, que pode ser realizado simultaneamente em diferentes locais ou regiões através de metodologias padronizadas. O uso de armadilhas com iscas atrativas, por exemplo, é um método de coleta de baixo custo e que é amplamente usado para estudar sistemas florestais ao redor do mundo (Freitas et al., 2014). Este método pode ser combinado com capturas ativas através de puçá entomológico, complementando a representatividade de borboletas (Checa et al., 2018).

Usando estes métodos de coleta, mais de 450 espécies de borboletas foram registradas nas áreas de estudo do PDBFF entre os anos de 1980 a 1995 (Brown & Hutchings, 1997). Esses dados dimensionam a grande diversidade de Lepidoptera que é abrigada na Amazônia, e o tamanho do desafio dos pesquisadores para compreender a estruturação destas comunidades tão diversas. Nos diferentes ambientes que formam o mosaico florestal que é a Amazônia, a diversidade fenotípica das borboletas pode ser uma consequência da seleção de atributos que maximizam a performance sob determinada combinação de condições locais (Börschig et al., 2013). Interações ecológicas e estratégias de vida bem integradas a certos habitats podem não mostrar a mesma aptidão em outros ambientes distintos (Caro et al., 2017), sobretudo quando há rápidas transformações. Portanto, é provável que regiões florestais sob fortes pressões antrópicas e aceleradas mudanças em suas características físicas e biológicas estejam sendo predominantemente ocupados por espécies com características funcionais e história filogenética particulares a estes, o que demonstra a importância de avançarmos em estudos de diversidade que abarquem estes aspectos avançados - ecologia funcional e filogenética - para borboletas em ambientes como a Amazônia

A ecologia funcional e filogenética

Uma compreensão precisa das causas que levam à acelerada perda de espécies dificilmente seria alcançada sem uma extensa análise das características individuais (de Bello et al., 2010; Moretti et al., 2017). Enquanto muitos táxons apresentam grande sensibilidade às mudanças ambientais, alguns se mostram pouco afetados e passam a predominar nestes novos cenários do Antropoceno (Dirzo et al., 2014). Essa sensibilidade deve se dar principalmente pelas características funcionais dos organismos. Por isso, para entender estas diferentes performances requer-se a avaliação de atributos que atuam nas estratégias ecológicas e evolutivas (de Bello et al., 2010), através de experimentos e monitoramentos.

A ecologia funcional é uma disciplina que surgiu nos últimos anos e vem complementando trabalhos tradicionais na ecologia. Como ferramenta, ela propõe a avaliação das características (atributos) dos organismos, que refletem sua história evolutiva e sua resposta ao ambiente (Pillar & Duarte, 2010). Em invertebrados, por exemplo, os atributos podem ser relativos à morfologia, fisiologia, comportamento ou o uso do habitat (Violle et al., 2007, Podgaiski et al., 2011), e influenciam a habilidade competitiva, a coexistência, ciclagem de nutrientes e resistência a perturbações (Cianciaruso et al., 2009). Estas características tendem a ser moldadas de acordo com o ambiente que uma espécie ocupa, através da ação de filtros ambientais, e pela interação com outros organismos (e.g. competição, mutualismo), estruturando as comunidades com base na convergência e divergência nos padrões funcionais (McGill et al., 2006).

No entanto, muitos padrões funcionais encontrados na natureza podem ser resultado não somente de processos ecológicos, mas também de eventos evolutivos (Pavoine et al., 2014). Espécies mais próximas filogeneticamente tendem a ter respostas ecológicas mais similares entre si do que entre linhagens evolutivamente mais distantes, podendo também compartilhar de atributos funcionais mais semelhantes (sinal filogenético no atributo) (Losos, 2008; Münkemüller et al., 2012). As comunidades, portanto, podem ser compostas por linhagens que compartilham da mesma história evolutiva (Gerhold et al., 2015). Quando isso acontece (presença de sinal filogenético no atributo e na composição das comunidades), os padrões funcionais se misturam aos efeitos da filogenia, e podem ser interpretados separadamente para descrever a relação de um atributo com o ambiente (de Bello et al., 2015; Duarte et al., 2018). Por isso, a ecologia funcional e filogenética combinadas podem ser considerados ótimas descritoras para explicar a distribuição dos organismos ao longo de gradientes ambientais.

A Coloração animal

A coloração animal é um atributo-chave em diversas situações ecológicas, e se revela através de estratégias variadas capazes de contribuir na sobrevivência e reprodução dos organismos (Stevens, 2016). As cores manifestadas por um indivíduo podem servir tanto como um meio de comunicação ao propagar sinais visuais, ou ainda contribuir na termorregulação mediante absorção ou deflexão do calor radiante (Kingsolver, 1985; Cuthill et al., 2017; Briolat et al., 2018). No caso da comunicação, são identificadas tanto estratégias intraespecíficas que agem principalmente na seleção sexual, quanto sinais interespecíficos envolvendo sobretudo estratégias anti-predatórias (Endler, 1978; Kemp, 2007).

Diferentes estratégias anti-predatórias podem ser identificadas em animais, incluindo as borboletas, que manifestam um amplo repertório em seus padrões de cores junto a uma grande variedade de ambientes. As borboletas são talvez os insetos com mais óbvias funções para as cores visíveis, tendo em vista a estrutura da asa com ampla área e o hábito diurno. Dentre seus padrões de coloração, a camuflagem atua como um meio de dificultar a detecção da presa, através de cores e contrastes que a confundem com seu ambiente (Cuthill et al., 2017). De maneira oposta, o aposematismo é capaz de evitar ataques de predadores com a manifestação de cores conspícuas, geralmente advertindo a presença de alguma qualidade ou característica desvantajosa (Briolat et al., 2018). Padrões aposemáticos também podem levar a formação de padrões miméticos comuns, quando duas ou mais espécies passam a compartilhar características fenotípicas (Mallet & Gilbert, 1995). Além destes, muitas borboletas contam com “ocelos” em suas asas, através de desenhos circulares capazes de simular olhos de vertebrados, intimidando potenciais predadores e reduzindo seus ataques, ou ainda desviando-os para partes não vitais como as extremidades das asas (Stevens, 2005).

Diante de todas essas manifestações fenotípicas conhecidas, a mensuração objetiva das cores vem sendo aperfeiçoada e aplicada em estudos ecológicos. Atualmente existem diversos métodos e ferramentas que permitem acessar diferentes variáveis da coloração a partir do uso de fotografias digitais (Stevens et al., 2007, Troscianko & Stevens, 2015). Com isso fascinantes estudos ecológicos, como a famosa espécie de mariposa de Manchester (*Biston betularia*) pós revolução industrial na Inglaterra (Walton & Stevens, 2018), e a diversidade de cores de animais e plantas descritas por importantes naturalistas (Dalrymple et al., 2015) tem sido resgatados e testados a partir de métodos atuais.

Mediante as transformações antrópicas recentes das características dos habitats nos trópicos, o sucesso adaptativo das borboletas através dos seus padrões de cores é colocado à prova. Estudos históricos e contemporâneos apontam que padrões fenotípicos menos integrados às novas condições locais tem sua frequência reduzida, enquanto que outros aumentam nos novos ambientes antropizados (Endler, 1993; Walton & Stevens, 2018). O mesmo pode ser estendido para a perda de habitats e regeneração florestal - dois gradientes ambientais cada vez mais frequentes e que se espalham ao longo da paisagem amazônica (Arroyo-Rodríguez et al. 2017). Os efeitos de ambos podem ser quantificados a partir da resposta das borboletas através de suas cores, o que significa uma perspectiva inédita do ponto de vista da conservação biológica.

Objetivos da tese

Esta tese teve como objetivo principal descrever as diferentes adaptações morfológicas, fisiológicas e comportamentais de borboletas frugívoras ao longo de gradientes de fragmentação e sucessão florestal na Amazônia brasileira. Os três manuscritos apresentados fazem parte de uma construção sequencial de ideias, que criaram forma na medida em que novos resultados foram alcançados, e estimulavam novas observações e perguntas. Por isso, nos objetivos específicos buscamos: i) avaliar quais atributos funcionais poderiam estar atuando diretamente na estruturação das assembleias de borboletas frugívoras ao longo de gradientes florestais; ii) verificar qual a participação do componente filogenético nos padrões funcionais e na composição de espécies durante a regeneração natural da floresta, e em relação ao tamanho de fragmentos florestais remanescentes e de floresta contínua; e iii) analisar se atributos relacionados à coloração podem ser bons preditores da resposta das borboletas à degradação florestal na Amazônia.

Para responder a estes objetivos foi necessária, primeiro, uma ampla busca de trabalhos sobre a história natural de borboletas realizados nas últimas décadas. A compilação destas informações permitiu reunir uma lista de atributos funcionais que pudessem ser facilmente avaliados na natureza, e respondessem objetivamente para ajudar a entender a distribuição das espécies sob diferentes condições ambientais. Essa questão conduziu o estudo seguinte, onde além de apontar quais atributos em borboletas maximizaram a relação espécie-ambiente, foi visto se estes estão realmente associados com os diferentes cenários ecológicos (gradientes florestais) ou simplesmente com as relações de parentesco entre as espécies. Baseado nestes resultados, e principalmente na relação dos atributos de cores com os ambientes, foi conduzida uma investigação mais aprofundada sobre a coloração das borboletas e a performance das várias estratégias anti-predatórias que derivam dos padrões fenotípicos. Este último capítulo reuniu uma série de abordagens incluindo diferentes categorias de cores em nível de indivíduo. Somados estes esforços, apresentamos uma nova visão sobre a relação das borboletas e sistemas florestais, incluindo a presença humana nas paisagens atuais.

Referências

Arroyo-Rodríguez, V., F.P.L. Melo, M. Martínez-Ramos, F. Bongers, R.L. Chazdon, J.A. Meave, N. Norden, B.A. Santos, I.R. Leal, & M. Tabarelli. (2017) Multiple successional pathways in human-modified tropical landscapes: new insights from forest succession, forest fragmentation and landscape ecology research. *Biological Reviews*, **92**, 326–340.

Börschig, C., A.M. Klein, H. von Wehrden, & J. Krauss. (2013) Traits of butterfly communities change from specialist to generalist characteristics with increasing land-use intensity. *Basic and Applied Ecology*, **14**, 547–554.

Briolat, E.S., E.R. Burdfield-steel, S.C. Paul, H.R. Katja, B.M. Seymoure, T. Stankowich, & A.M.M. Stuckert. (2018) Diversity in warning coloration: selective paradox or the norm? *Biological Reviews,* 1–27.

Brown, K.S. (2005) Geologic, evolutionary, and ecological bases of the diversification of neotropical butterflies: implications for conservation, pp. 166–201. *In* C.W. Dick and G.

Moritz (eds.), Tropical rainforest: past, present, and future. The University of Chicago Press, Chicago, LA.

Caro, T., M.C. Stoddard, D. Stuart-fox, (2017) Animal coloration research : why it matters. *Philosophical Transactions of the Royal Society B*, **372**: 20170047.

Checa, M.F., D.A. Donoso, J. Rodriguez, E. Levy, A. Warren, & K. Willmott. (2018) Combining sampling techniques aids monitoring of tropical butterflies. *Insect Conservation and Diversity,* doi: 10.1111/icad.12328

Cianciaruso, M.V., I.A. Silva, & M. A. Batalha. (2010) Diversidades filogenética e funcional: novas abordagens para a Ecologia de comunidades. *Biota Neotropica*, **9**, 93–103.

Cuthill, I.C., W.L. Allen, K. Arbuckle, B. Caspers, G. Chaplin, M.E. Hauber, G.E. Hill, N.G. Jablonski, C.D. Jiggins, A. Kelber, J. Mappes, J. Marshall, R. Merrill, D. Osorio, R. Prum, N.W. Roberts, A. Roulin, H.M. Rowland, T.N. Sherratt, J. Skelhorn, M.P. Speed, M. Stevens, M.C. Stoddard, D. Stuart-Fox, L. Talas, E. Tibbetts, & T. Caro. (2017) The biology of color. *Science*, **357**: eaan0221.

Dalrymple, R.L., D.J. Kemp, H. Flores-Moreno, S.W. Laffan, T.E. White, F.A. Hemmings, M. L. Tindall, & A.T. Moles. (2015) Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. *Global Ecology and Biogeography*, **24**, 1424–1432.

de Bello, F., M.P. Berg, A.T.C. Dias, J.A.F. Diniz-Filho, L. Götzenberger, J. Hortal, R.J. Ladle, & J. Lepš. (2015) On the need for phylogenetic 'corrections' in functional trait-based approaches. *Folia Geobotanica*, **50**, 349–357.

de Bello, F., S. Lavorel, S. Díaz, R. Harrington, J.H.C. Cornelissen, R.D. Bardgett, M.P. Berg, P. Cipriotti, C.K. Feld, D. Hering, P.M. da Silva, S.G. Potts, L. Sandin, J.P. Sousa, J. Storkey, D.A. Wardle, & P.A. Harrison. (2010) Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity and Conservation*, **19**, 2873–2893.

Duarte, L.D.S., V.J. Debastiani, M.B. Carlucci, & J.A.F. Diniz-Filho. (2018) Analyzing community-weighted trait means across environmental gradients: should phylogeny stay or should it go? *Ecology*, **99**, 385–398.

Endler, J.A. (1978) A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319-364.

Endler, J.A. (1993) The color of light in forests and its implications. *Ecological Monographs*, **63**, 1–27.

Freitas, A.V.L. & Marini-Filho, O.J. (2011) Plano de ação nacional para a conservação dos lepidópteros ameaçados de extinção. ICMBio & MMA, Brasília.

Gerhold, P., J.F.C. Jr, M. Winter, I.V. Bartish, & A. Prinzing. (2015) Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). *Functional Ecology*, **29**, 600–614.

Instituto Nacional de Pesquisas Espaciais - INPE (2018) Disponível em: <www.obt.inpe.br/prodes> Acessado em: 18/03/2019.

Jennings, E., R. Dirzo, H.S. Young, M. Galetti, N.J.B. Isaac, & B. Collen. (2005) Defaunation in the Anthropocene. *Science*, **345**, 401–406.

Kemp, D.J. (2007) Female butterflies prefer males bearing bright iridescent ornamentation. *Proceedings-Biological sciences / The Royal Society*, **274**, 1043–1047.

Laurance, W.F., J.L.C. Camargo, P.M. Fearnside, T.E. Lovejoy, G.B. Williamson, R.C.G. Mesquita, C.F.J. Meyer, P.E.D. Bobrowiec, & S.G.W. Laurance. (2018) An Amazonian rainforest and its fragments as a laboratory of global change. *Biological Reviews*, **93**, 223–247.

Losos, J.B. (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology* *letters*, **11**, 995–1003.

Lovejoy, T.E., & C. Nobre. 2018. Amazon Tipping Point. *Science Advances*, **4**:eaat2340.

Malhi, Y., J.T. Roberts, R.A. Betts, T.J. Killeen, W. Li, & C.A. Nobre. (2008) Climate change, deforestation, and the fate of the Amazon. *Science*, **319**, 169–172.

Mallet, J. & Gilbert, L.E. (1995) Why are there so many mimicry rings? Correlations between habitat, behavior and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean Society*, **55**, 159–180.

McGill, B.J., B.J. Enquist, E. Weiher, & M. Westoby. (2006) Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, **21**, 178–185.

Mesquita, R.D.C.G., P.E.D.S. Massoca, C.C. Jakovac, T.V. Bentos, & G.B. Williamson. (2015) Amazon Rain Forest Succession: Stochasticity or Land-Use Legacy? *BioScience*, **65**, 849–861.

Moretti, M., A.T.C. Dias, F. de Bello, F. Altermatt, S.L. Chown, F. M. Azcárate, J.R. Bell, B. Fournier, M. Hedde, J. Hortal, S. Ibanez, E. Öckinger, J.P. Sousa, J. Ellers, & M.P. Berg. (2017) Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, **31**, 558–567.

Münkemüller, T., S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schiffers, & W. Thuiller. (2012) How to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, **3**, 743–756.

Nobre, C.A., G. Sampaio, L.S. Borma, J.C. Castilla-Rubio, J.S. Silva, & M. Cardoso. (2016) Land-use and climate change risks in the Amazon and the need of a novel sustainable development paradigm. *Proceedings of the National Academy of Sciences*, **113**, 10759–10768.

Pavoine, S., M. Baguette, V.M. Stevens, M.A. Leibold, C. Turlure, & M.B. Bonsall. (2014) Life history traits, but not phylogeny, drive compositional patterns in a butterfly metacommunity. *Ecology*, **95**, 3304–3313.

Pillar, V.D., & L.D.S. Duarte. (2010) A framework for metacommunity analysis of phylogenetic structure. *Ecology Letters*, **13**, 587–596.

Podgaiski, L.R., M.S. Mendonça Jr., & V.D. Pillar. (2011) O uso de atributos funcionais de invertebrados terrestres na ecologia: O que, como e por quê? *Oecologia Australis*, **15**, 835–853.

Chazdon, R.L. (2003) Tropical forest recovery: legacies of human impact and natural disturbances. *Perspectives in Plant Ecology, Evolution and Systematics*, **6**, 51–71.

Sánchez-Bayo, F., & K.A.G. Wyckhuys. (2019) Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, **232**, 8–27.

Silva, J.M.C., Rylands, A.B. & Fonseca, G.A.B. (2005) The fate of the Amazonian areas of endemism. *Conservation Biology*, **19**, 689–694.

Souza, C.M., J.V. Siqueira, M.H. Sales, A.V. Fonseca, J.G. Ribeiro, I. Numata, M.A. Cochrane, C.P. Barber, D.A. Roberts, & J. Barlow. (2013) Ten-year landsat classification of deforestation and forest degradation in the brazilian amazon. *Remote Sensing*, **5**, 5493–5513.

Stevens, M. (2005) The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biological Reviews*, **80**, 573–588.

Stevens, M., C. Parraga, I.C. Cuthill, J.C. Partridge, & T.S. Troscianko. (2007) Using digital photography to study animal coloration. *Biological Journal of the Linnean Society*, **90**, 211–237.

Troscianko, J., & M. Stevens. (2015) Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution*, **6**, 1320–1331.

Violle, C., M.L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, & E. Garnier. (2007) Let the concept of trait be functional! *Oikos*, **116**, 882–892.

Wahlberg, N., J. Leneveu, U. Kodandaramaiah, C. Pena, S. Nylin, A.V.L. Freitas, & A.V.Z. Brower. (2009) Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 4295–4302.

Walton, O.C. & Stevens, M. (2018) Avian vision models and field experiments determine the survival value of peppered moth camouflage. *Communication Biology*, **1**, 118.

Werth, D. & Avissar, R. (2002) The local and global effects of Amazon deforestation. *Journal of Geophysical Research*, **107**, 8087-8095.

World Wide Fund for Nature - WWF. (2017) Disponível em: <https://www.wwf.org.br/> Acessado em: 18/03/2019.

PARTE I

OS ATRIBUTOS FUNCIONAIS EM BORBOLETAS FRUGÍVORAS

Capítulo 1

Manuscrito formatado nas normas da revista Environmental Entomology

Reviewing functional traits for fruit-feeding butterflies: applications to the study of community assembly across forest gradients

R. L. Spaniol[1]*, A. Richter[2], M. S. Mendonça Jr.[1] and C. A. Iserhard[2]

[1]Departamento de Ecologia, Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

[2]Instituto de Biologia, Programa de Pós-graduação em Biologia Animal, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul, Brazil

*Corresponding author: ricardospaniol@yahoo.com.br

Abstract

In addition to providing a deeper understanding of community dynamics and structure, functional ecology allows the study of mechanistic consequences of habitat changes, and their implications for the conservation of species, species interactions, and ecosystem services. However, standardization of trait selection and measurement is still poorly solved even for groups that are widely studied, such as butterflies. We suggest that the understanding of functional organization in butterflies requires an analysis of four major trait categories: flight performance, defense strategies, ecophysiological characteristics, and habitat perception. The first concerns flight aspects and mobility. The second is related to interactions with biotic environmental factors, acting mainly through defense against predation. The third describes organismal energy allocation and efficiency for both survival and reproduction, while the fourth category provides butterflies with the ability to recognize an environment and access its available resources. Based on this protocol, we have derived expected results for a wide application of the suggested traits in butterfly studies, with emphasis on forest gradients. We draw a parallel on how community functional organization would appear in different environments. The functional approach adopted here can result, for example, in a detailed diagnosis of the relationship between fruit-feeding butterflies and their environment. We encourage a wide use of this approach for both basic and applied butterfly studies, in order to test hypotheses regarding the functional community structuring in response to environmental gradients.

Keywords: butterfly behavior, butterfly ecophysiology, forest fragmentation, forest stratification, functional morphology.

Introduction

In order to lead us toward a deeper understanding of how environmental variation shapes biodiversity patterns and ecosystems services, it is crucial to use analytical approaches and methods to adequately measure changes in biological diversity (Flynn et al. 2009). Species richness, abundance, equability, and composition are traditional variables used to assess biodiversity (Magurran 2004), but these parameters fail to consider the main requirements and ecological functions of organisms in their habitats (Gerisch et al. 2012). Thus, complementary measures such as functional traits have been introduced to understand, at a finer scale, the biological community structure (De Bello et al. 2010, Podgaiski et al. 2011).

Functional traits are organismal aspects that may act like responses to the abiotic and biotic features of habitats. Traits are expressed through morphology, physiology, behavior or habitat use, which may in its turn be related to evolutionary history (De Bello et al. 2010, Pillar and Duarte 2010). Therefore, they may influence ecological interactions (like competitive ability), leading to coexistence among species, as well as resistance and resilience to perturbations (Cianciaruso, Silva and Batalha 2009). This way, functional ecology might be applied to the study of environmental changes associated with anthropogenic pressures, threats to biodiversity, and species conservation (Vandewalle et al. 2010, Fountain-Jones et al. 2015, Moretti et al. 2017).

Organisms have different limits to their distribution due to differing ecophysiological tolerances and habitat requirements (Verberk 2011). However, not all species are equally affected by disturbance (Steffan-Dewenter and Tscharntke 2000), because they have distinct responses derived from the possession of a given set of functional traits. While many taxa are very sensitive to direct perturbations and its secondary effects, and can even disappear from large areas, others remain stable or achieve high dominance in modified habitats (Uehara-Prado, Brown and Freitas 2007). Bearing this in mind, it is important to adopt methods using species traits that could fully characterize ecological processes under disturbances through individual characteristics, which would allow us to more completely evaluate community structures and their changes in space and time.

Environmental gradients and functional ecology: a new approach to an old question

Environmental gradients consist of gradual changes in the physical environment and/or in abiotic components, and can affect ecosystems and the distribution of organisms given their distinct ecological tolerance (Zelazny et al. 2007). These environmental changes are thus responsible for shaping diversity patterns and community assembly across space and time. In forested habitats, for example, there are several forms of influence that generate distinct gradients through anthropogenic actions or natural perturbations (Fahrig 2003; Ries et al. 2017). These forest gradients occur both in the vertical and horizontal dimensions, leading to distinct effects on species. The former consists of differences in spatial distribution of species from the ground to the canopy (Basset et al. 2003), related to variation in abiotic conditions in the vertical layering of a habitat (DeVries 1988, Schulze, Linsenmaier and Fiedler 2001). The latter constitutes environmental variation near the ground, related to landscape heterogeneity including habitat structure and dynamics, and edaphic features (Basset et al. 2015).

Forest gradients can be accessed through its effects on forest fragments and its surrounding matrix, edge effects (Leidner, Haddad and Lovejoy 2010; Meehan, Glassberg and Gratton 2013), ecological succession (Nyafwono et al. 2014), forest gap dynamics (Murphy et al. 2016), and stratification between the understory and canopy (DeVries et al. 2012). Currently, there are several studies disentangling the consequences of fragmentation on forest habitats, its changes in vegetation between understory and canopy and gaps inside the forest (Hill et al. 2001; Basset et al. 2015). These approaches are interesting to better comprehend species responses and occurrence from continuous areas to small ones; isolated forest patches within an inhospitable matrix; or how intrinsic habitat characteristics can modify the distribution, dynamics and persistence of populations in the landscape (Murphy et al. 2016). However, we are suggesting an important next step, which is to combine other biological components (e.g. functional traits, evolutionary history) to complement the current knowledge on the ecological processes responsible for driving species diversity and community assembly across forest gradients.

In general, successful survival on forest gradients require a set of morphological, physiological and behavioral adaptations in animals (Börschig et al. 2013; Basset et al. 2003). This happens because differences in forest vegetation modify the availability and quality of forest habitats across vertical and horizontal dimensions (Basset et al. 2015; Laurance, Sayer and Cassman 2014). Due to distinct environmental tolerances, some intervals across gradients can be characterized as extreme for some species, tolerable to others, or can lead to coexistence when conditions became more “agreeable” from the point of view of these species. This way, the inclusion of functional ecology may be crucial for a better understanding of which selective pressures drive the adaptations of different organisms, as expressed in their traits (Flynn et al. 2009). However, bearing in mind the conceptual basis of functional ecology, it is necessary to first provide measurement protocols for functional traits of widely studied organisms, and to encourage their application in potentially new and revealing approaches.

Traits of fruit-feeding butterflies for the study of forest gradients

Fruit-feeding butterflies are considered an interesting model for ecological studies since this group has high sensibility to environmental changes due to their strong association to vegetation, microhabitats, and the landscape (Brown 1991). This guild comprises about 50-75% of all the species belonging to the family Nymphalidae found in the Neotropics and is represented by Charaxinae, Biblidinae, Satyrinae, and also a few tribes within Nymphalinae (Brown 2005). The fruit-feeding butterflies feed on fermented fruits, carrion, feces, and plant exudates (DeVries 1988), and because of this habit they can be easily captured in the field by standardized methodology, with bait traps, which can be carried out simultaneously at different localities (Freitas et al. 2014).

In the Neotropics, there has been an increase in butterfly inventories in the last 20 years, enhancing our knowledge on occurrence and distribution of these insects in different ecosystems and biomes. Some key characteristics put the butterflies in a good position as tools to evaluate functional ecology. These include evident morphological structures that are easy to measure, ease in determining and employing behavioral and habitat use aspects, revealing life habits or ecological interactions. On the other hand, there are also limitations, mainly in the assessment of immature stages and the specific relationship with their host plants. Linked to this, there is a shortage of studies describing associations of distribution and diversity between immatures and imagos, since habits, resource use, and niche occupancy are very distinct between them. Nevertheless, the same is true for several taxa apart from Lepidoptera, in which functional approaches are still poorly understood, unstandardized, and remain sparse in the literature.

Based on an extensive literature search, the present study selected papers including those on the natural history and ecology of butterflies to help elucidate functional aspects. A compilation of key functional traits for fruit-feeding butterflies is presented as a list (Table 1). This aims to contain all relevant functions, but explicitly those associated with forest gradients in tropical and subtropical sites, trying to link this information with community assembly. Assuming that individuals have distinct responses across forest gradients, this should result in the expression of different phenotypes on which natural selection has acted or currently acts.

Four trait categories were selected aiming to include crucial factors in the functional organization of fruit-feeding butterfly assemblages: 1) flight performance, 2) defense strategies, 3) ecophysiology; and 4) habitat perception. *Flight performance* is associated with locomotion, movement, and flight capacity reflecting the performance of the individual while exploring the environment. These traits are obtained through morphological measurements of body and wings. *Defense strategies* are related to the immediate response of butterfly individuals to abiotic and biotic factors. This category includes color patterns related to interactions, like camouflage, aposematism, iridescence, presence of wing eyespots and participation on mimetic rings. *Ecophysiological traits* include mechanisms associated to individual allocation of energy and its efficiency, like thermoregulation, longevity, reproductive effort and voltinism. *Habitat perception* refers to visual ability and habitat use, including eye measurements, habitat specificity (generalist/specialist species), home range, and host plant use during the immature phase.

Table 1. List of the fruit-feeding butterfly functional traits and functional responses suggested for forest gradients studies.

Functional categories / Traits	**Functional response**	**References**
Flight performance		
Aspect ratio	Dispersion capacity	(Berwaerts et al. 2002; DeVries et al. 2010)
Wing load	Dispersion capacity	(Berwaerts et al. 2002; Shahabuddin and Ponte 2005)
Body length	Flight speed	(Chai and Srygley 1990; Dudley and Srygley 1994)
Thoracic mass	Flight speed	(Thomas et al. 1998; Berwaerts et al. 2002)
Wing size	Flight speed	(Dudley 2000; Kemp et al. 2006)
Wing shape	Flight speed / Maneuverability	(Betts and Wootton 1988; Thomas et al. 1998)
Wing centroid	Flight aerodynamics	(Dudley 2000; Betts and Wootton 1988)
Defense strategies		
Warning coloration	Avoiding attack	(Stevens and Ruxton 2012; Briolat et al. 2018)
Camouflage	Avoiding detection	(Ruxton et al. 2004; Stevens and Merilaita 2009)
Mimetic rings	Fooling predators	(Mallet and Gilbert 1995; Hegedus et al. 2008)
Wing eyespots	Fooling predators	(Stevens 2005; Olofsson et al. 2010)
Ecophysiologycal traits		
Longevity	Life history strategy	(Braby and Jones 1995)
Thermoregulation	Activity / Metabolic rate	(Kingsolver 1985; Ellers and Boggs 2004)
Voltinism	Reproductive strategy	(Cizek et al. 2006; Altermatt 2010)
Reproductive effort	Reproductive strategy	(Kingsolver and Huey 2008)

Habitat perception		
Eyes size	Habitat perception	(Rutowski 2000; Rutowski et al. 2009)
Habitat specialist	Environment recognition and use	(Krauss et al. 2003; Brückmann et al. 2010)
Home range	Environment recognition and use	(Brändle et al. 2002; Rhodes et al. 2005)
Immature food specificity	Food specialization	(Poÿry et al. 2009; Kitching et al. 2013)

Flight performance traits

This category includes traits related to speed, maneuverability, wing beat frequency, flight form, and flight duration, presenting trade-offs associated to mass allocation and allometry (Marden and Chai 1991).

Body measurements: These are represented by wing measurements and body weight, either in its entirety or for specific body parts (Kemp, Wiklund and Van Dyck 2006). Thorax measures can be considered traits associated with displacement, because the thorax houses the flight muscles (Thomas, Hill and Lewis 1998). Wing size, both in length or total area, seems to be an important descriptor related to organismal mobility and to flight strategies (Thomas et al. 1998; Dudley 2000). Forewings provide greater force during flight, while hindwings are necessary to allow maneuvers (Jantzen and Eisner 2008). Thoracic mass and width, forewing length, and wingspan are cited as measures directly correlated to flight speed (Chai and Srygley 1990; Dudley and Srygley 1994).

Wing loading: This trait is related to the ratio between body mass and wing area (Berwaerts, Van Dyck and Aerts 2002), which indicates flight performance through the amount of corporal mass sustained by wing area unit. Wing loading is, in general, high in insects with large corporal mass in a proportion that increases linearly (Dudley 2000), being directly proportional to flight speed (Shahabuddin and Ponte 2005).

Aspect ratio: This trait is used to express wing shape through the ratio between length and area of the forewing (Dudley 2000; Berwaerts et al. 2002). Higher values of aspect ratio are found in butterflies with long and narrow wings, having longer and more energy-efficient flights (Betts and Wootton 1988; DeVries, Penz and Hill 2010).

Wing centroid: This expresses mass distribution over the wings, which is associated with flight aerodynamics (Betts and Wootton 1988). When the centroid is distally displaced (i.e. higher values), air might be moved at higher speeds providing stronger aerodynamic forces per unit area of wing (Dudley 2000, Berwaerts et al. 2002).

Defense strategy traits

Prey species have several morphological, physiological, and behavior traits adapted to reduce chances of predation (Marden and Chai 1991). Animal coloration plays an important role in interactions with the background and other organisms (Stevens and Ruxton 2018). For many butterflies, color patterns act to maximize some abilities or features as signals to visually-oriented predators, aiming to avoid predation, thereby increasing fitness (Briolat et al. 2018; Pinheiro et al. 2016).

Avoiding detection: Camouflage, including many ways to look indistinguishable from the environment, is the most well-known strategy to avoid predation (Ruxton, Sherratt and Speed 2004). The *background matching* strategy (crypsis) includes the imitation of environmental colors, brightness, and forms like leaves and plant shoots (Merilaita and Lind 2005). *Disruptive coloration* prevents the detection of body shape of an individual by the predator through high contrast colors, creating false outlines of their bodies (Stevens and Merilaita 2009). *Transparency* is commonly manifested to avoid detection linked to changes in wavelength and light polarization, as well as greater camouflage in sites with low light intensity (Ruxton et al. 2004).

Avoiding attack: This category contains traits related to conspicuous color patterns that makes the predator aware of aggressive behavior, escape movement, defense structures, and chemical compounds useful when detected by a predator (Briolat et al. 2018). Such traits provide gains to prey, increasing fitness when predators learn to recognize these (Ruxton et al. 2004). The most well-known of these is *aposematism*, using *warning coloration* as a strategy in the communication between predator and prey (Stevens and Ruxton 2012, Dell'Aglio, Stevens and

Jiggins 2016). Besides signaling the presence of chemical defenses, conspicuous color patterns in prey wings are able to warn predators about how difficult it would be to catch them (Pinheiro and Freitas 2014), mainly through active predation escaping abilities.

Fooling predators: Even after prey recognition by the predator, there are strategies to avoid predation (Arenas and Stevens 2017). *Mimetism* is the imitation of other unprofitable prey organisms through appropriate colors and forms (Hegedus, DeVries and Penz 2018), generally displaying conspicuous color - in the case of Müllerian and Batesian mimetisms. *Mimetic rings* can be formed, in which animals co-occur in a region displaying similar color patterns aiming to maximize unprofitable prey patterns recollection (Mallet and Gilbert 1995). This strategy may also occur between organisms signaling escape ability (Pinheiro and Freitas 2014). Moreover, large central *wing-eyespots* (mimicking owl eyes) and small peripheral wing-eyespots contrast with the butterfly wing background. These may have both mimetic and deflective functions, leading to avoidance or deviation of the attack to non-vital body parts (Stevens 2005; Olofsson et al. 2010).

Ecophysiological traits

This set of traits addresses energy allocation for vital functions and reproductive features in butterflies. Here we consider traits related to environmental factors regulating metabolic activity.

Thermoregulation: This refers to the ability of an organism to keep its vital activities functional in environments outside the ideal temperature range. A greater melanization increases radiant heat absorption, and under cool environmental conditions allows reaching an optimal body temperature more rapidly. On the other hand, bright colors can work most efficiently in places where overheating is more likely, avoiding desiccation (Ellers and Boggs 2004; Kingsolver 1985). If body temperature falls outside the optimal range, homeostasis is unbalanced and the performance (e.g., foraging, reproduction abilities) of individuals can decline, or even basic life functions capable of keeping them active can cease (Huey and Kingsolver 1989).

Longevity: This trait denotes the flight period or lifespan for adults (Braby and Jones 1995). During this time, individuals actively participate in reproduction/oviposition and are able to disperse. Longevity can be estimated by mark–release–recapture studies in the field, although there is a limitation in data usually considering a few or only the current generation (Poÿry et al. 2009).

Voltinism: Refers to the number of generations butterflies are able to complete during a given time (usually a year). In addition to physiological conditioning, voltinism is affected by environmental fluctuations in temperature (Altermatt 2010) and hostplant phenology (Cizek, Fric and Konvicka 2006). Therefore, different reproductive strategies (univoltine or multivoltine) may be adopted by butterflies to overcome unfavorable seasons.

Reproductive effort: comprises the size and number of eggs (clutch size), being a proxy for reproductive effort, and hence for offspring fitness. Female fecundity might be affected by environmental features including present or past resources and conditions (Braby and Jones 1995). For ectothermic insects like butterflies, smaller eggs and larger clutch sizes are frequently associated with maximal performance at higher environment temperatures (Kingsolver and Huey 2008).

Habitat perception traits

This set of traits allow us to evaluate how butterfly habitat perception acts on species distribution, and can help explain spatio-temporal patterns.

Eye size: This trait represents the visual ability and environment perception of adult individuals (Rutowski 2000). The surface area of compound eyes can affect the visual field through changes in eye structure, leading to a distinct performance in sensitivity and visual acuity (see Rutowski, Gislén and Warrant 2009). Higher sensitivity is important when luminosity is low, while a greater visual acuity increases recognition capacity of conspecific and vegetation features (Turlure et al. 2016).

Habitat specialists: This concerns the specialization level of butterflies for a particular habitat or condition. Forests can be perceived as environmental mosaics, where specialization to particular habitats may restrict species occurrence. This can lead to a higher species turnover when butterflies have stricter ecological requirements (Krauss, Steffan-Dewenter and Tscharntke 2003). Thus, habitat specialists respond to characteristics of the environment (e.g. habitat size, structural complexity, connectivity), and both natural variations and human impacts can have direct ecological effects (Brückmann, Krauss and Steffan-Dewenter 2010).

Home-range: This trait predicts the spatial range that an individual needs to use during his lifetime. Home-range definition goes beyond random habitat choices by the individual (Morris 2003). There are several properties that may impact butterflies in terms of habitat selection and predict the effective geographic range explored. These include habitat extension, host plant distribution, population size, individual traits related to dispersion and interactions with competitors/predators (Brändle, Öhlschläger and Brandl 2002; Rhodes et al. 2005).

Immature food specificity: It refers to the diversity of host-plants caterpillars can use as a food resource. Butterflies that have a single host plant species can be classified as specialists (monophagous). Generalists would be able to use two (oligophagous) or several (polyphagous) host-plant species (Poÿry et al. 2009). Therefore, locally available larval host plants will drive both butterfly species richness and community assembly, influencing the habitat specialization for adults (Beccaloni 1997; Kitching et al. 2013).

Functional traits and butterfly community assembly

Several traits addressed in this review show an individual-based approach or have had their association with certain environments tested. Others are brand new suggestions and need to be observed directly in nature or tested in field experiments. Traits related to flight performance are widely used in community assembly research. Since body measurements are relatively easy to obtain in the field and from scientific collections, they are often used to try and understand their association with habitat use (DeVries et al. 2010; Turlure et al. 2016). Butterfly color has been treated quantitatively ever more widely in recent years. However, how color patterns and diversity respond to ecological patterns like forest gradients is still poorly understood (Xing et al. 2016; Briolat et al. 2018). Ecophysiological traits are mostly unsolved in butterflies, requiring a description of the relationship between caterpillars and host plants (Bagchi et al. 2018), as well as aspects of adult survival and reproduction under different environments. Details about butterfly visual systems and how to measure them have been successfully proposed and used (Rutowski et al. 2009). It is still necessary to address how visual information guides butterfly behavior in nature, how human impact can change these contexts, and how to extend these to the community level.

Based on existing research, we hypothesized that butterfly community in forest interior habitats (compared to open/disturbed environments, dealt with in the next paragraph) can be characterized by a combination of the following functional traits: larger body and wing size, accompanied by less thoracic mass. Given the wide range in background offered, color diversity encompassing anti-predatory strategies should be higher, with some strategies probably unique to those environments. In a variety of butterflies, large central wing-eyespots resembling a vertebrate eye (predator mimicry) could prevail. Lower light in the forest interior may require a higher visual sensibility (larger eyes and facets) for visual recognition of signals and threats. A lower reproductive effort (less but larger eggs) accompanied by univoltine or bivoltine strategies could be common given the constancy in conditions and resources for the growth of immatures across a great period of time, and no more than one or two reproductive windows. Due to environmental constancy, we predict that species would have a specialized diet during their larval phase.

In the same way, our hypothesis for community structure in open/disturbed habitats may show the following functional patterns: short/stouter bodies with larger thoracic muscles, associated with smaller wings, which permits quick beating and greater acceleration. Color diversity (including mimetic rings) may be decreased since fewer background opportunities should be available, and different defense strategies may have distinct performances. Those related to conspicuity may not have the same effectiveness in open environments since it would leave the butterfly more exposed. Therefore, camouflage with brown/cryptic wing colors and small peripheral eyespots should prevail. High longevity may be unlikely in environments with greater physical stress and resource restrictions. Butterflies would change reproductive effort strategy to compensate for a shorter expected adult lifetime (more but smaller egg), supported by multivoltine cycles in time available. Generalist species regarding to habitat use and food resources in their larval phase should be more common. Butterflies provided with better visual acuity would also be able to successfully explore open/disturbed habitats, in order to reduce their unnecessary exposure against predators.

Concluding remarks

All four functional categories chosen for this study have key traits capable of determining the structure of fruit-feeding butterfly communities along forest gradients. Most of the suggested traits should be relatively easy to measure and/or have reliable information available in the literature. Some other traits, like reproductive ones and the relation between host plant and immature stages, are harder to evaluate for entire communities. This information needs more effort to become reliable and available, but will probably result in a heuristic approach to functional studies of butterflies. In retrospect, we would like to recommend that some of the suggested traits are highly likely to result in detection of important effects, such as the use of (i) wing loading and aspect ratio (flight performance traits); (ii) camouflage, warning coloration, mainly by mimetic rings and presence of wing eyespots (defense traits); (iii) thermoregulation (ecophysiological trait); (iv) eye size and feeding specialization (habitat perception traits).

We aimed to review and propose fruit-feeding butterfly traits that could be used to understand ecological structure and dynamics of these insects in any forested habitats across the world. This can also be considered as a coordination of various hypotheses, usually thought of in terms of single, isolated traits, suggested here to explain how butterflies occupy forests in terms of vertical and horizontal space. We hope for a wide application of the suggested traits in butterfly studies to verify whether structuring of fruit-feeding butterfly communities follows the described patterns highlighted in this study. Finally, it is hoped that this handbook can help increase our understanding of butterfly diversity and distribution as a response to constant pressures to survive and reproduce, so as to maintain a dynamic structure of communities in space and time in an ever-changing environment such as forests.

Acknowledgements

We are grateful for many very helpful comments on the manuscript from S. M. Murphy. M. S. Mendonca, Jr. thanks CNPq for a productivity scholarship (309616/2015-8). Furthermore, R. L. Spaniol is most grateful to Capes-Brasil for granting a Doctorate fellowship.

References

Altermatt, F. 2010. Climatic warming increases voltinism in European butterflies and moths. Proc. R. Soc. Lond. *B* Biol. Sci. 277: 1281–1287.

Arenas, L. M. and M. Stevens. 2017. Diversity in warning coloration is easily recognised by avian predators. J. Evol. Biol. 30: 1288–1302.

Bagchi, R., L. M. Brown, C. S. Elphick, D. L. Wagner, and M. S. Singer. **2018**. Anthropogenic fragmentation of landscapes: mechanisms for eroding the specificity of plant–herbivore interactions. Oecologia. 187: 521–533.

Basset, Y., P. M. Hammond, H. Barrios, J. D. Holloway, and S. E. Miller. 2003. Vertical stratification of arthropod assemblages, pp.17–27. *In* Y. Basset, V. Novotny, S.E. Miller, and R.L. Kitching (eds.), Arthropods of tropical forests: spatiotemporal dynamics and resource use in the canopy. Cambridge University Press, Cambridge, UK.

Basset, Y., L. Cizek, P. Cuénoud, R. K. Didham, V. Novotny, F. Ødegaard, T. Roslin, A. K. Tishechkin, J. Schmidl, N. N. Winchester, D. W. Roubik, H. P. Aberlenc, J. Bail, H. Barrios, J. R. Bridle, G. Castaño-Meneses, B. Corbara, G. Curletti, W. D. Rocha, D. Bakker, J. H. C. Delabie, A. Dejean, L. L. Fagan, A. Floren, R. L. Kitching, E. Medianero, E. G. Oliveira, J. Orivel, M. Pollet, M. Rapp, S. P. Ribeiro, Y. Roisin, J. B. Schmidt, L. Sørensen, T. M. Lewinsohn, and M. Leponce. 2015. Arthropod distribution in a tropical rainforest: Tackling a four dimensional puzzle. PLoS One 10: e0144110.

Beccaloni, G. 1997. Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host–plant height. Biol. J. Linn. Soc. 62: 313–341.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Funct. Ecol. 16: 484–491.

Betts, C.R., and R. J. Wootton. 1988. Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): A preliminary analysis. J. Exp. Biol. 138: 271–288.

Börschig, C., A. M. Klein, H. von Wehrden, and J. Krauss. 2013. Traits of butterfly communities change from specialist to generalist characteristics with increasing land-use intensity. Basic. Appl. Ecol. 14: 547–554.

Braby, M. F., and R. E. Jones. 1995. Reproductive patterns and resource allocation in tropical butterflies: influence of adult diet and seasonal phenotype on fecundity, longevity and egg size. Oikos 72: 189–204.

Brändle, M., S. Öhlschläger, and R. Brandl 2002. Range sizes in butterflies: Correlation across scales. Evol. Ecol. Res. 4: 993–1004.

Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Rönkä, K. H., Seymoure, B. M., Stankowich, T. and A. M. M. Stuckert. 2018. Diversity in warning coloration: selective paradox or the norm? Biol. Rev. 1–27.

Brown Jr, K. S. 1991. Conservation of Neotropical Environments: Insects as Indicators, pp. 349–404. *In* N.M. Collins and J.A. Thomas (eds.), The Conservation of Insects and their Habitats. Academic Press, London, UK.

Brown, K. S. 2005. Geologic, evolutionary, and ecological bases of the diversification of neotropical butterflies: implications for conservation, pp. 166–201. *In* C.W. Dick and G. Moritz (eds.), Tropical rainforest: past, present, and future. The University of Chicago Press, Chicago, LA.

Brückmann, S. V., J. Krauss, and I. Steffan-Dewenter. 2010. Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. J. Appl. Ecol. 47: 799–809.

Cianciaruso, M. V., I. A. Silva, and M. A. Batalha. 2009. Diversidades filogenética e funcional: novas abordagens para a ecologia de comunidades. Biota Neotrop. 9: 93–103.

Cizek, L., Z. Fric, and M. Konvicka. **2006**. Host plant defences and voltinism in European butterflies. Ecol. Entomol. 31: 337–344.

Chai, P., and R. B. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. Am. Nat. 135: 748–765.

de Bello, F., S. Lavorel, S. Díaz, R. Harrington, J. H. C. Cornelissen, R. D. Bardgett, M. P. Berg, P. Cipriotti, C. K. Feld, D. Hering, P. M. da Silva, S. G. Potts, L. Sandin, J. P. Sousa, J. Storkey, D. A. Wardle, and P. A. Harrison. 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodivers. Conserv. 19: 2873–2893.

Dell'aglio, D. D., M. Stevens, and C. D. Jiggins. 2016. Avoidance of an aposematically coloured butterfly by wild birds in a tropical forest. Ecol. Entomol. 41: 627–632.

DeVries, P. J. 1988. Stratification of fruit-feeding Nymphalid butterflies in a Costa Rican rainforest. J. Res. Lepid. 26: 98–108.

DeVries, P. J., C. M. Penz, and R. I. Hill. **2010**. Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies. J. Anim. Ecol. 79: 1077–1085.

DeVries, P. J., L. G. Alexander, I. A. Chacon, and J. A. Fordyce. 2012. Similarity and difference among rainforest fruit-feeding butterfly communities in Central and South America. J. Anim. Ecol. 81: 472–482.

Dudley, R. 2000. The Biomechanics of Insect Flight: Form, Function, Evolution. University Press, Princeton, NJ.

Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies: allometry of airspeeds during natural free flight. J. Exp. Biol. 191: 125–39.

Ellers, J., and C. L. Boggs. 2004. Functional ecological implications of intraspecific differences in wing melanization in Colias butterflies. Biol. J. Linn. Soc. 82: 79–87.

Endler, J. A. 1978. A predator's view of animal color patterns. Evol. Biol. 11: 319–364.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34: 487–515.

Flynn, D. F. B., M. Gogol-Prokurat, T. Nogeire, N. Molinari, B. T. Richers, B. B. Lin, N. Simpson, M. M. Mayfield, and F. DeClerck. 2009. Loss of functional diversity under land use intensification across multiple taxa. Ecol. Lett. 12: 22–33.

Fountain-Jones, N. M., S. C. Baker, and G. J. Jordan. 2015. Moving beyond the guild concept: Developing a practical functional trait framework for terrestrial beetles. Ecol. Entomol. 40: 1–13.

Freitas, A. V. L., C. A. Iserhard, J. P. Santos, J. Y. O. Carreira, D. B. Ribeiro, D. H. A. Melo, A. H. B. Rosa, O. J. Marini-Filho, G. M. Accacio, and M. Uehara-Prado. 2014. Studies with butterfly bait traps: an overview. Rev. Colomb. Entomol. 40: 203–212.

Gerisch, M., V. Agostinelli, K. Henle, and F. Dziock. 2012. More species, but all do the same: Contrasting effects of flood disturbance on ground beetle functional and species diversity. Oikos 121: 508–515.

Hegedus, M., DeVries, P. and C. M. Penz. 2018. The Influence of Mimicry on Wing Shape Evolution in the Butterfly *Papilio dardanus* (Lepidoptera: Papilionidae). Ann. Entomol. Soc. Am. https://doi.org/10.1093/aesa/say045

Hill, J., K. Hamer, J. Tangah, and M. Dawood. 2001. Ecology of tropical butterflies in rainforest gaps. Oecologia 128: 294–302.

Huey, R. B., and J. G. Kingsolver. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends Ecol. Evol. 4: 131–135.

Jantzen, B., and T. Eisner. 2008. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. Proc. Natl. Acad. Sci. 105: 16636–16640.

Kemp, D. J., C. Wiklund, and H. Van Dyck. 2006. Contest behaviour in the speckled wood butterfly (*Pararge aegeria*): Seasonal phenotypic plasticity and the functional significance of flight performance. *Behav. Ecol. Sociobiol.* 59: 403–411.

Kingsolver, J. G. 1985. Thermoregulatory significance of wing melanization in *Pieris* butterflies (Lepidoptera: Pieridae): physics, posture, and pattern. Oecologia 66: 546–553.

Kingsolver, J. G., and R. B. Huey. **2008**. Size, temperature, and fitness: Three rules. Evol. Ecol. Res. 10: 251–268.

Kitching, R. L., L. A. Ashton, A. Nakamura, T. Whitaker, and C.V. Khen. 2013. Distance-driven species turnover in Bornean rainforests: Homogeneity and heterogeneity in primary and post-logging forests. Ecography 36: 675–682.

Krauss, J., I. Steffan-Dewenter, and T. Tscharntke. 2003. Local species immigration, extinction, and turnover of butterflies in relation to habitat area and habitat isolation. Oecologia 137**:** 591–602.

Laurance, W. F., J. Sayer, and K. G. Cassman. 2014. Agricultural expansion and its impacts on tropical nature. Trends Ecol. Evol. 29: 107-116.

Leidner, A. K., N. M. Haddad, and T. E. Lovejoy. 2010. Does tropical forest fragmentation increase long-term variability of butterfly communities? PLoS One 5: e9534.

Magurran, A. E. 2004. Measuring biological diversity. Blackwell Science, Oxford, UK.

Mallet, J., and L. E. Gilbert. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. Biol. J. Linn. Soc. 55: 159–180.

Marden, J. H., and P. Chai. 1991. Aerial predation and butterfly design: how palatability, mimicry, and the need for evasive flight constrain mass allocation. Am. Nat. 138: 15–36.

Meehan, T. D., J. Glassberg, and C. Gratton. 2013. Butterfly community structure and landscape composition in agricultural landscapes of the central United States. J. Insect Conserv. 17: 411–419.

Merilaita, S. and J. Lind. 2005. Background-matching and disruptive coloration, and the evolution of cryptic coloration. **Proc. R. Soc. B**. 272: 665–670.

Moretti, M., A. T. C. Dias, F. de Bello, F. Altermatt, S. L. Chown, F. M. Azcárate, J. R. Bell, B. Fournier, M. Hedde, J. Hortal, S. Ibanez, E. Öckinger, J. P. Sousa, J. Ellers, and M. P. Berg. 2017. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. Funct. Ecol. 31: 558–567.

Morris, D. W. 2003. Toward an ecological synthesis: a case for habitat selection. Oecologia, 136: 1–13.

Murphy, S. M., Battocletti, A. H., Tinghitella, R. M., Wimp, G. M., and L. Ries. 2016. Complex community and evolutionary responses to habitat fragmentation and habitat edges: What can we learn from insect science? Curr. Opin. Insect Sci. 14: 61–65.

Nyafwono, M., A. Valtonen, P. Nyeko, and H. Roininen. 2014. Butterfly community composition across a successional gradient in a human-disturbed afro-tropical rain forest. Biotropica 46: 210–218.

Olofsson, M., A. Vallin, S. Jakobsson, and C. Wiklund. 2010. Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. PLoS One 5: e10798.

Pillar, V. D., and L. S. Duarte. 2010. A framework for metacommunity analysis of phylogenetic structure. Ecol. Lett. 13: 587–596.

Pinheiro, C. E. G., and A. V. L. Freitas. 2014. Some Possible Cases of Escape Mimicry in Neotropical Butterflies. Neotrop. Entomol. 43: 393–398.

Pinheiro, C. E. G., A. V. L. Freitas, V. C. Campos, P. J. DeVries, and C. M. Penz. 2016. Both palatable and unpalatable butterflies use bright colors to signal difficulty of capture to predators. Neotrop. Entomol. 45: 107–113.

Podgaiski, L. R., M. S. Mendonça Jr., and V. D. Pillar. 2011. O uso de atributos funcionais de invertebrados terrestres na ecologia: O que, como e por quê? Oecologia Australis 15: 835–853.

Pöyry, J., M. Luoto, R. K. Heikkinen, M. Kuussaari, and K. Saarinen. 2009. Species traits explain recent range shifts of Finnish butterflies. Glob. Chang. Biol. 15: 732–743.

Ries, L., S. M. Murphy, G. M. Wimp and R. J. Fletcher Jr. 2017. Closing persistent gaps in knowledge about edge ecology. Curr. Landscape Ecol. Rep. 2:30–41

Rhodes, J. R., C. A. Mcalpine, D. Lunney, and H. P. Possingham. 2005. A spatially explicit habitat selection model incorporating home range behavior. Ecology 86: 1199–1205.

Rowland, H. M. 2007. Countershading enhances cryptic protection: an experiment with wild birds and artificial prey. Anim. Behav. 74: 1249–1258.

Rutowski, R. L. 2000. Variation of eye size in butterflies: inter- and intraspecific patterns. J. Zool. 252: 187–195.

Rutowski, R. L., L. Gislén, and E. J. Warrant. 2009. Visual acuity and sensitivity increase allometrically with body size in butterflies. *Arthropod* Struct. Dev. 38: 91–100.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding attack: The evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press, Oxford, UK.

Schulze, C. H., K. E. Linsenmaier, and K. Fiedler. 2001. Understory versus canopy: patterns of vertical stratification and diversity among Lepidoptera in a Bornean rain forest. J. Plant Ecol. 153: 133–152.

Shahabuddin, G., and C. A. Ponte. 2005. Frugivorous butterfly species in tropical forest fragments: Correlates of vulnerability to extinction. Biodivers. Conserv. 14: 1137–1152.

Steffan-Dewenter, I., and T. Tscharntke. 2000. Butterfly community structure in fragmented habitats. Ecol. Lett. 3: 449–456.

Stevens, M. 2005. The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. Biol. Rev. 80: 573–588.

Stevens, M., and S. Merilaita. 2009. Defining disruptive coloration and distinguishing its functions. Philos. Trans. R. Soc. B. 364: 481-488.

Stevens, M., and G. D. Ruxton. 2012. Linking the evolution and form of warning coloration in nature. Proc. R. Soc. Lond. *B* Biol. Sci. 279: 417–426.

Stevens, M. and G. D. Ruxton. 2018. The key role of behaviour in animal camouflage. Biol. Rev. https://doi.org/10.1111/brv.12438

Thomas, C. D., J. K. Hill, and O. T. Lewis. 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. J. Anim. Ecol. 67: 485–497.

Turlure, C., N. Schtickzelle, H. Van Dyck, B. Seymoure, and R. Rutowski. 2016. Flight morphology, compound eye structure and dispersal in the bog and the cranberry fritillary butterflies: an inter- and intraspecific comparison. PLoS One 11: e0158073.

Uehara-Prado, M., K. S. Brown, and A. V. L. Freitas. 2007. Species richness, composition and abundance of fruit-feeding butterflies in the Brazilian Atlantic Forest: Comparison between a fragmented and a continuous landscape. Global Ecol. Biogeogr. 16: 43–54.

Vandewalle, M., F. de Bello, M. P. Berg, T. Bolger, S. Dolédec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. M. da Silva, M. Moretti, J. Niemellä, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodivers. Conserv. 19: 2921–2947.

Verberk, W. 2011. Explaining General Patterns in Species Abundance and Distributions. Nat. Educ. Knowl. 3(10):38.

Xing, S., T. C. Bonebrake, C. C. Tang, E. J. Pickett, W. Cheng, S. E. Greenspan, S. E. Williams, B. R. Scheffers. 2016. Cool habitats support darker and bigger butterflies in Australian tropical forests. Ecol. Evol. 6: 8062–8074.

Zelazny, V. F., G. L. Martin, M. Toner, M. Gorman, M. Colpitts, H. Veen, B. Godin, B. McInnis, C. Steeves, L. Wuest, and M. R. Roberts. 2007. Our landscape heritage: the story of ecological land classification in New Brunswick. Department of Natural Resources, Fredericton, NB.

PARTE II

TESTANDO OS ATRIBUTOS FUNCIONAIS EM DIFERENTES CENÁRIOS FLORESTAIS NA AMAZÔNIA

Capítulo 2

Manuscrito submetido na revista Ecosphere

The multiple dimensions of diversity in butterfly assemblages across a successional gradient in the Amazon Rainforest

Ricardo Luís Spaniol[a]*, Cristiano Agra Iserhard[b], Leandro da Silva Duarte[a], Milton de Souza Mendonça, Jr.[a]

[a]Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil

[b]Programa de Pós-graduação em Biologia Animal, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul, Brasil

*Corresponding author (ricardospaniol@yahoo.com.br)

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, were 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 assemblies through their phylogenetic history. In this study, we evaluated the differences in species distribution and composition on fruit-feeding butterfly communities along forest fragmentation and succession gradients. We combine functional and phylogenetic methods for determining butterfly community structure, and inferred community 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 analysed 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-succession 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 changes 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: Coloration, flight performance, forest fragmentation, forest succession, fruit-feeding butterflies, species traits, tropical forest.

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 (Fahrig 2013, Guariguata and Ostertag 2001). These gradients have been considered as providing valuable information about the effects of disturbances on species resistance and resilience in forest systems (de Andrade et al. 2017, Filgueiras et al. 2016).

Forest fragmentation gradients allow disentangling the effects of habitat loss and patch arrangement on species (Murphy et al. 2016, Shahabuddin and Ponte 2005). 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).

Based on extensive literature search, we suggest that the understanding of functional organization 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 summarise 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 under-exploited point of view for tropical regions (Muenchow et al. 2018). 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 community structure, in view of the functional responses associated with phylogeny. We will try to answer the following questions: i) Is functional trait distribution in fruit-feeding butterfly communities influenced by the different forest gradients? ii) How do trait composition change relative to fragment size and succession stages? iii) Is phylogeny acting on functional composition of fruit-feeding butterflies along these gradients? We expected that the effect of time after disturbance (resilience), as well as the disturbance intensity reflected in forest fragment size (resistance), could lead to differences in species distribution, and consequently in the structuring of butterfly communities.

Material 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 hectares, early successional and secondary forest areas surrounding the fragments, and continuous Amazonian Rainforest (Laurance et al. 2002). The early succession sites consist of open areas with dense and short shrubs with about 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 years 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 hectares as forming the fragmentation gradient, having the continuous forest as a control (Fig. 1).

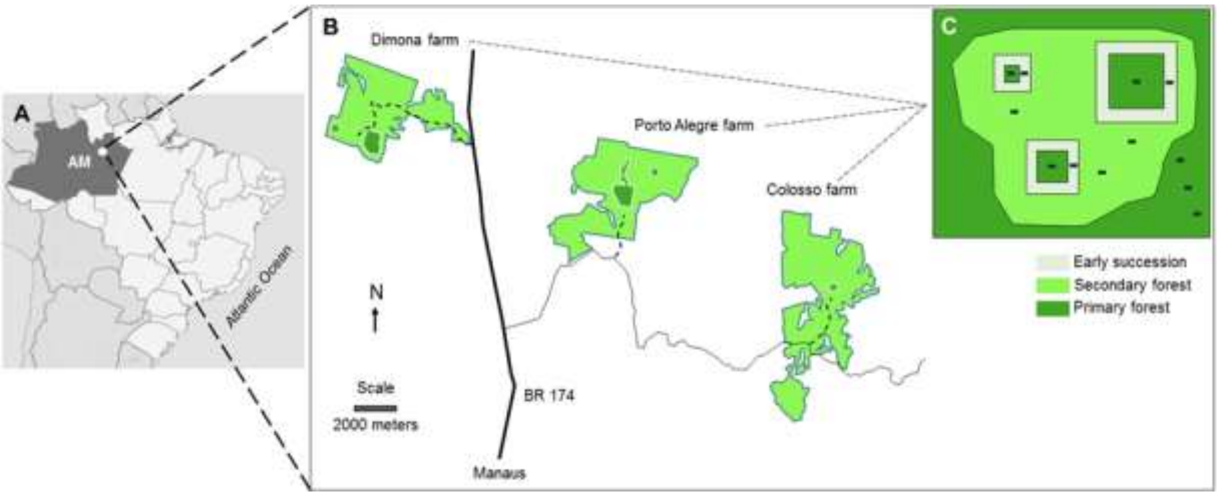


Figure 1. Images showing the study area and sampling design location in each sample area (Dimona, Porto Alegre and Colosso farms). A) Location of study area (white dot) in Amazon State (AM), north Brazil; B) Image showing the placement of the three studied farms with the disposition of each sampled environment;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

Butterfly sampling

Two field expeditions were performed at the beginning of the dry season in August and September of 2015 and 2016. Sample units (SUs) were installed in each sampled area, with three SUs in early successional sites, three in secondary forest sites, one in each forest fragment size (1, 10 and 100 hectares) and three in the adjacent continuous forest (Fig. 1). This same sampling design was then repeated for each sampling area (Dimona, Porto Alegre and Colosso). Each SU received a set of five portable traps containing attractive bait, made with bananas fermented in sugar cane juice for 48 hours before the samplings (Freitas et al. 2014). The distance between neighboring traps within the SU was at least 20 m, placed at a maximum height of 1.5 m above the ground. On each sampling occasion, the traps remained exposed for eight consecutive days in each farm (a total of 24 days of sampling per field expedition), being reviewed in intervals of 48 hours to renew the bait, collect, mark and identify captured butterflies. During the review process, two samplers with an entomological net 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. Up to 10 individuals of each species were collected for subsequent identification and functional trait measurement in the laboratory. This material is deposited in the Laboratório de Ecologia de Interações, of Universidade Federal do Rio Grande do Sul, Brazil.

Data analysis

We selected up to 10 individuals per species from our reference collection to measure 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 (wing area and length, body length, body mass and 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). 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 communities along forest gradients (DATA S1). CWM is an expression of which species/lineages are distributed in which assemblies. 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, using for both the CWM.sig analysis (Duarte et al. 2018).

Before performing the functional structure 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. 2). 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). We analysed 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), via ADONIS (Duarte et al. 2016). 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 signal was not detected, we only controlled the effects of species composition, randomizing species within the communities. For both we used a CWM.sig analysis, analysing each trait independently. This allowed us to understand if the forest gradients act on species distribution among communities (Site shuffle); if species distribution across communities depends on trait similarity among species (Trait shuffle); or both processes (for an overview, **see** Duarte et al. 2018). Before running the CWM.sig analysis, we tested if trait values had a normal distribution, and a logarithmic transformation was been done for data normalization when necessary. All analyses were performed in R - version 3.4.4 (R Development Core Team 2018).

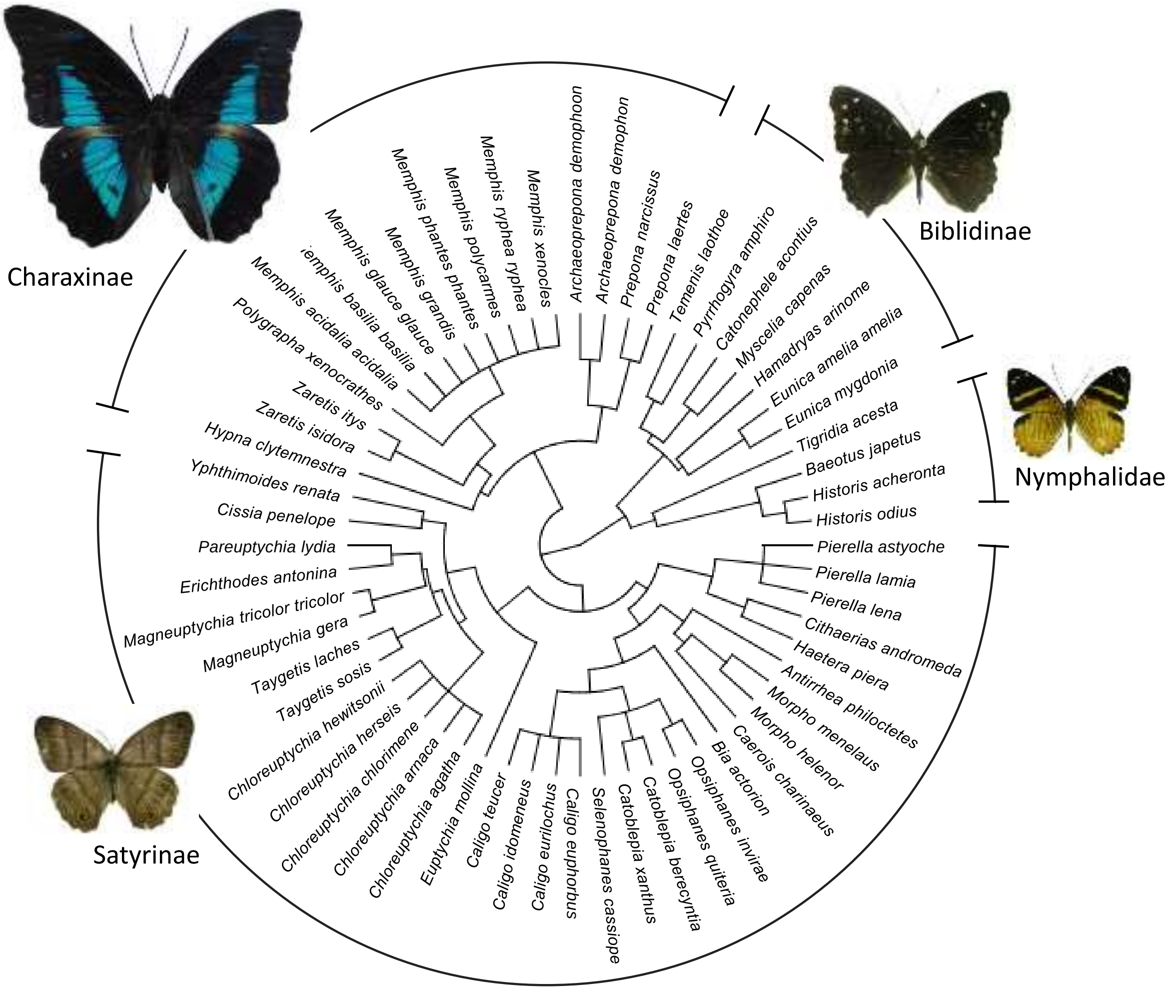


Figure 2. Phylogenetic tree constructed considering all 60 sampled fruit-feeding butterfly species.

Results

After 48 days of sampling effort, 471 individuals were recorded in 60 species belonging to all the fruit-feeding butterfly subfamilies of Nymphalidae (Fig. 2). Several species traits showed phylogenetic signal (Table 1), indicating that phylogenetically closer species presented more similar characteristics to each other than expected by chance. Phylogeny was also important for butterfly distribution along the succession gradient (PCPS Mean F = 10.52; p=0.01). This gradient affects the distribution of species across the communities (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. No evidence was found on community structuring by phylogeny along the fragmentation gradient.

Community structuring by phylogeny in the succession gradient can be shown through the PCPS diagram (Fig. 3). Charaxinae, in the first axis, is separated from other butterfly lineages, in which its species are associated mainly to 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.

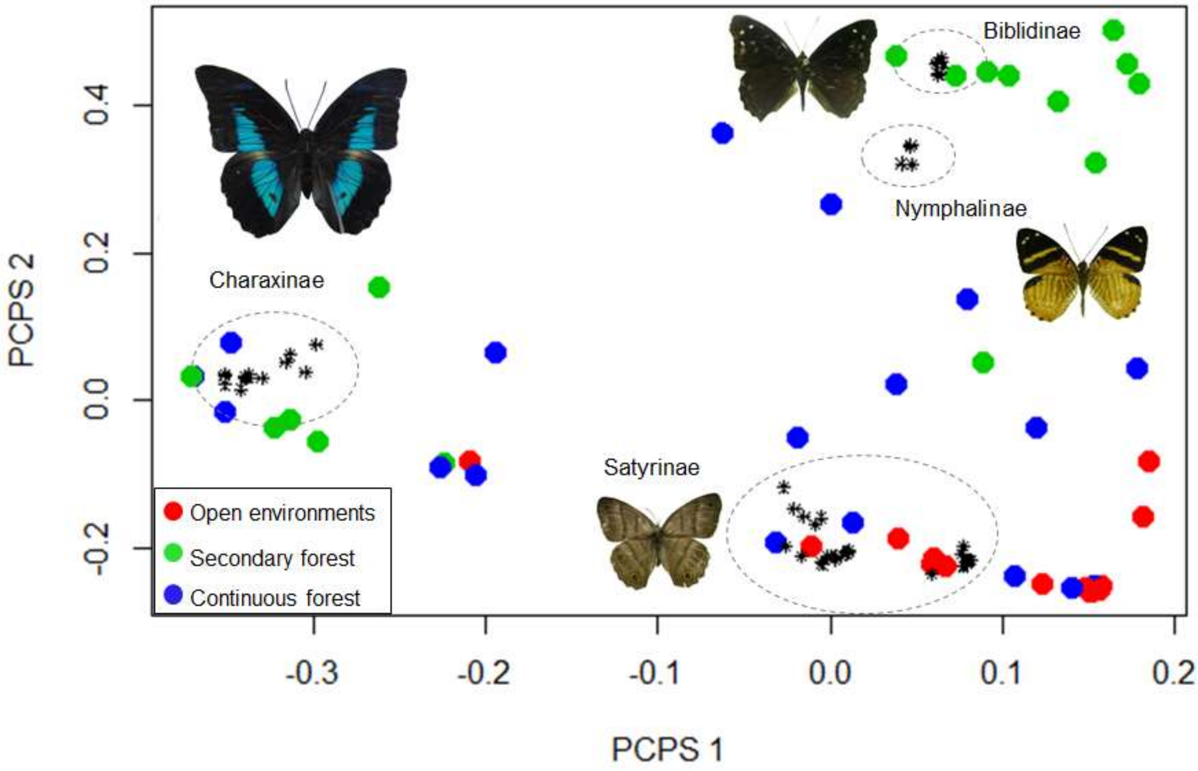


Figure 3. Scatter diagram showing the PCPS scores for communities and fruit-feeding butterfly species related to the forest succession gradient. Asterisks represent species and dotted lines enclose the four butterfly subfamilies. Small circles colour-code communities in the different environments that compound the succession gradient.

After controlling for *phylogenetic* relatedness on the functional and community composition across the successional gradient, flight performance and defense strategies traits still varied in response to forest regeneration stage (Fig. 4). 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 functional 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).

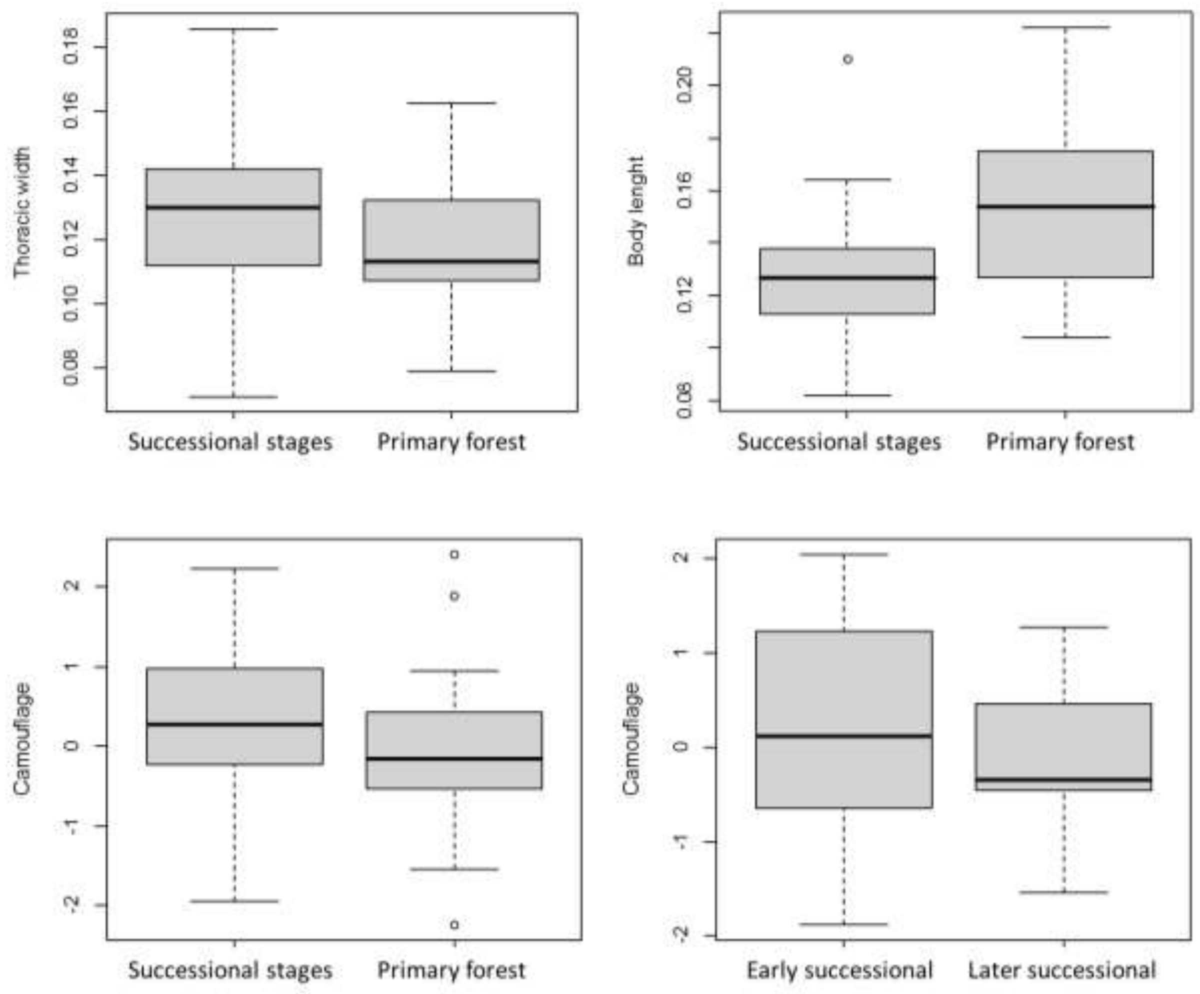


Figure 4. Fruit-feeding butterfly functional traits along the forest succession gradient, including comparisons between successional stages *vs.* continuous forest, and between early *vs.* late successional stages in the Central Amazon – Biological Dynamics of Forest Fragments Project (BDFFP). The y-axes contain normalized values measured for each related trait.

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. Relationship between fruit-feeding butterfly functional traits and forest fragmentation and succession gradients are such that 'Forest Fragments x Continuous Forest' compares traits found in all forest fragments with continuous forest; 'Successional Stages x 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.

		Succession gradient						**Fragmentation gradient**		
		Successional Stages X Continuous Forest			**Successional Stages**			**Forest Fragments X Continuous Forest**		
Phylogenetic signal	**Traits**	**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 lenght	3.976	0.05*	0.311	0.516	0.481	0.739	0.518	0.495	0.502
1.3739	Forewing length	0.401	0.533	0.763	-	-	-	0.276	0.591	0.590

1.3378	Forewing area	0.235	0.635	0.786	-	-	-	2.455	0.149	0.111
1.3410	Hindwing area	0.183	0.680	0.821	-	-	-	3.335	0.065	0.089
1.2688	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

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

Discussion

Functional structure of fruit-feeding butterflies

Environmental changes have important effects on the structuring of fruit-feeding butterfly communities. However, species occurrence along forest fragmentation and succession gradients are 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 responses. 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 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 (Pellissier et al. 2018, Stevens et al. 2013). Looking as imperceptible as possible in environments with greater exposure appear to be another necessary condition for survival in early succession 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 structuring 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 towards 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 (Duarte et al. 2018). In other words, evolutionary closer lineages in butterflies show similar functional responses to environmental variation also due to their shared evolutionary history. Our findings are consistent with this idea when traits were separately analysed, 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 the communities along the fragmentation gradient, as we had initially expected. The mutual influence of phylogeny on the functional traits and community 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, as already observed for plant communities (Ding et al. 2012) and aquatic environments (Helmus et al. 2010).

On the other hand, communities integrating the primary forest mosaic along the fragmentation gradient are composed of the same butterfly 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 (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 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.

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, and the larvae will obtain their food resources to complete its life cycle. In this aspect, different lineages can be equally distributed among fragments, leading to an absence of phylogenetic structuring for communities 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. 2018). This may lead to a high turnover of lineages in the butterfly community, with species compositional changes and evident phylogenetic patterns.

Understanding butterfly communities 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). 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 (Tulure 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 communities 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) has been recently incorporated to disentangle butterfly assemblies along *environmental gradients* in the tropics (Graça et al. 2017). Nevertheless, this is the first study that also combines phylogeny performance with species distribution in the community ecology approach. 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.

Acknowledgements

We thank the Biological Dynamics of Forest Fragments Project (BDFFP) through the Thomas Lovejoy research fellowship program for providing financial and logistical support in the execution of fieldwork. We also thanks S. M. Hartz and R. Maestri for providing useful comments that improved on the manuscript. R. L. Spaniol is grateful to Capes for a PhD fellowship, M. Mendonça thanks CNPq for a Research Productivity grant.

Authors’ contributions

Spaniol R. L., Mendonça Jr. M. S., Iserhard C. A. and Duarte L. S. conceived the ideas and designed methodology. Spaniol R. L. and Iserhard C. A. collected the data. Spaniol R. L. and Duarte L. S. analysed the data. All authors led the writing of the manuscript and contributed critically to the drafts and gave final approval for publication.

Literature Cited

Arroyo-Rodríguez, V., J. Cavender-Bares, F. Escobar, F. P. L. Melo, M. Tabarelli, and B. A. Santos. 2012. Maintenance of tree phylogenetic diversity in a highly fragmented rain forest. Journal of Ecology 100:702–711.

Bierregaard, R. O., T. E. Lovejoy, V. Kapos, A. A. Santos, and R. W. Hutchings. 1992. The biological dynamics of tropical rain forest fragments. Bioscience, 42(11), 859–866. doi: 10.2307/1312085

Blomberg, S. P., T. Jr. Gallard, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. Evolution, 57(4), 717–745.

Bonebrake, T. C., L. C. Ponisio, C. L. Boggs, and P. R. Ehrlich. 2010. More than just indicators: A review of tropical butterfly ecology and conservation. Biological Conservation 143:1831–1841.

Boukili, V. K., and R. L. Chazdon. 2017. Perspectives in Plant Ecology , Evolution and Systematics Environmental filtering , local site factors and landscape context drive changes in functional trait composition during tropical forest succession. Journal of PPEES Sources 24:37–47.

de Andrade, R. B., J. K. Balch, J. Y. O. Carreira, P. M. Brando, and A. V. L. Freitas. 2017. The impacts of recurrent fires on diversity of fruit-feeding butterflies in a south-eastern Amazon forest. Journal of Tropical Ecology 33:22–32.

Delhey, K., and A. Peters. 2016. Implications for conservation of anthropogenic impacts on visual communication and camouflage. Conservation Biology 31:1–36.

Dent, D. H., and S. J. Wright. 2009. The future of tropical species in secondary forests : A quantitative review. Biological Conservation 142:2833–2843.

Ding, Y., R. Zang, S. G. Letcher, S. Liu, and F. He. 2012. Disturbance regime changes the trait distribution, phylogenetic structure and community assembly of tropical rain forests. Oikos 121:1263–1270.

Duarte, L. D. S., V. J. Debastiani, A. V. L. Freitas, and V. D. Pillar. 2016. Dissecting phylogenetic fuzzy weighting: theory and application in metacommunity phylogenetics. Methods in Ecology and Evolution 7:937–946.

Duarte, L. D. S., V. J. Debastiani, M. B. Carlucci, and J. A. F. Diniz-Filho. 2018. Analyzing community-weighted trait means across environmental gradients: should phylogeny stay or should it go? Ecology 99:385–398.

Fahrig, L. 2013. Rethinking patch size and isolation effects: The habitat amount hypothesis. Journal of Biogeography 40:1649–1663.

Filgueiras, B. K. C., D. H. A. Melo, I. R. Leal, M. Tabarelli, A. V. L. Freitas, and L. Iannuzzi. 2016. Fruit-feeding butterflies in edge-dominated habitats: community structure, species persistence and cascade effect. Journal of Insect Conservation 20:539–548.

Freitas, A. V. L., C. Agra Iserhard, J. Pereira Santos, J. Y. Oliveira CarreiraI, D. Bandini Ribeiro, D. H. Alves Melo, A. H. Batista Rosa, O. J. Marini-filho, G. Mattos Accacio, M. Uehara-prado, A. Victor, L. Freitas, C. A. Iserhard, J. P. Santos, J. Yasmin, O. Carreira, D. B. Ribeiro, D. Henrique, A. Melo, A. Henrique, B. Rosa, O. J. Marini-filho, and G. M. Accacio. 2014. Studies with butterfly bait traps: an overview. Revista Colombiana de Entomología 40:203–212.

Fritz, S. A., and A. Purvis. 2010. Selectivity in mammalian extinction risk and threat types: A new measure of phylogenetic signal strength in binary traits. Conservation Biology 24:1042–1051.

Graça, M. B., P. A. C. L. Pequeno, E. Franklin, and J. W. Morais. 2017. Coevolution between flight morphology, vertical stratification and sexual dimorphism: what can we learn from tropical butterflies? Journal of Evolutionary Biology 30:1862–1871.

Guariguata, M. R., and R. Ostertag. 2001. Neotropical secondary forest succession: Changes in structural and functional characteristics. Forest Ecology and Management 148:185–206.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, C. D. Collins, W. M. Cook, E. I. Damschen, R. M. Ewers, B. L. Foster, C. N. Jenkins, A. J. King, W. F. Laurance, D. J. Levey, C. R. Margules, B. A. Melbourne, A. O. Nicholls, J. L. Orrock, D. Song, and J. R. Townshend. 2015. Habitat fragmentation and its lasting impact on Earth ' s ecosystems:1–9.

Harvey, P. H. and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford.

Helmus, M. R., W. Keller, M. J. Paterson, N. D. Yan, C. H. Cannon, and J. A. Rusak. 2010. Communities contain closely related species during ecosystem disturbance. Ecology Letters 13:162–174.

Hill, J., K. Hamer, J. Tangah, and M. Dawood. 2001. Ecology of tropical butterflies in rainforest gaps. Oecologia 128:294–302.

Keddy, P. A. 1991. Working with heterogeneity: An operator’s guide to environment gradients. In: Ecological Heterogeneity. Kolasa J. and Pickett S. T. A., eds. Springer, New York, NY, pp. 181-201.

Laurance, W. F., T. E. Lovejoy, H. L. Vasconcelos, E. M. Bruna, R. K. Didham, P. C. Stouffer, C. Gascon, R. O. Bierregaard, S. G. Laurance, and E. Sampaio. 2002. Ecosystem decay of Amazonian forest fragments: A 22-year investigation. Conservation Biology 16:605–618.

Laurance, W. F., and H. L. Vasconcelos. 2009. Consequências ecológicas da fragmentação florestal na amazônia. Oecologia brasiliensis 13:434–451.

Lavorel, S., K. Grigulis, S. McIntyre, N. S. G. Williams, D. Garden, J. Dorrough, S. Berman, F. Quétier, A. Thébault, and A. Bonis. 2008. Assessing functional diversity in the field - Methodology matters! Functional Ecology 22:134–147.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, *21*(4), 178–185.

Moretti, M., A. T. C. Dias, F. de Bello, F. Altermatt, S. L. Chown, F. M. Azcárate, J. R. Bell, B. Fournier, M. Hedde, J. Hortal, S. Ibanez, E. Öckinger, J. P. Sousa, J. Ellers, and M. P. Berg. 2017. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. Functional Ecology 31:558–567.

Muenchow, J., P. Dieker, J. Kluge, M. Kessler, and H. von Wehrden. 2017. A review of ecological gradient research in the Tropics: Identifying research gaps, future directions, and conservation priorities. Biodiversity and Conservation, 27(2), 273-285.

Murphy, S. M., A. H. Battocletti, R. M. Tinghitella, G. M. Wimp, and L. Ries. 2016. ScienceDirect Complex community and evolutionary responses to habitat fragmentation and habitat edges : what can we learn from insect science ? Current Opinion in Insect Science 14:61–65.

Nyafwono, M., A. Valtonen, P. Nyeko, and H. Roininen. 2014. Butterfly community composition across a successional gradient in a human-disturbed afro-tropical rain forest. Biotropica 46:210–218.

Pellissier, L., C. Albouy, J. Bascompte, N. Farwig, C. Graham, M. Loreau, M. A. Maglianesi, C. J. Melián, C. Pitteloud, T. Roslin, R. Rohr, S. Saavedra, W. Thuiller, G. Woodward, N. E. Zimmermann, and D. Gravel. 2018. Comparing species interaction networks along environmental gradients. Biological Reviews 93:785–800.

Pinotti, B. T., C. P. Pagotto, and R. Pardini. 2012. Forest Ecology and Management Habitat structure and food resources for wildlife across successional stages in a tropical forest. Forest Ecology and Management 283:119–127.

R Development Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Schtickzelle, N., A. Joiris, H. Van Dyck, and M. Baguette. 2007. Quantitative analysis of changes in movement behaviour within and outside habitat in a specialist butterfly. BMC Evolutionary Biology 7:1–15.

Shahabuddin, G., and C. A. Ponte. 2005. Frugivorous butterfly species in tropical forest fragments: Correlates of vulnerability to extinction. Biodiversity and Conservation 14:1137–1152.

Stevens, M. 2007. Predator perception and the interrelation between different forms of protective coloration. Proceedings of the Royal Society B: Biological Sciences 274:1457–1464.

Stevens, M., and S. Merilaita. 2009. Animal camouflage: current issues and new perspectives. Philosophical Transactions of the Royal Society B: Biological Sciences 364:423–427.

Stevens, V. M., A. Trochet, S. Blanchet, S. Moulherat, J. Clobert, and M. Baguette. 2013. Dispersal syndromes and the use of life-histories to predict dispersal. Evolutionary Applications 6:630–642.

Thomas, C. D. 2000. Dispersal and extinction in fragmentted landscapes. Proceedings of the Royal Society B: Biological Sciences, 267(1439), 139–145.

Turlure, C., N. Schtickzelle, H. Van Dyck, B. Seymoure, and R. Rutowski. 2016. Flight morphology, compound eye structure and dispersal in the bog and the cranberry fritillary butterflies: An inter- And intraspecific comparison. PLoS ONE 11:1–17.

Tylianakis, J. M., and R. J. Morris. 2017. Ecological Networks Across Environmental Gradients. Annual Review of Ecology, Evolution, and Systematics 48:annurev-ecolsys-110316-022821.

Wahlberg, N., J. Leneveu, U. Kodandaramaiah, C. Pena, S. Nylin, A. V. L. Freitas, and A. V. Z. Brower. 2009. Nymphalid butterflies diversify following near demise at the

Cretaceous/Tertiary boundary. Proceedings of the Royal Society B: Biological Sciences 276:4295–4302.

Watling, J. I., A. J. Nowakowski, M. A. Donnelly, and J. L. Orrock. 2011. Meta-analysis reveals the importance of matrix composition for animals in fragmented habitat. Global Ecology and Biogeography 20:209–217.

Valtonen, A., G. M. Malinga, M. Nyafwono, P. Nyeko, A. Owiny, and H. Roininen. 2017. The successional pathway of the tree community and how it shapes the fruit-feeding butterfly community in an Afrotropical forest. Journal of Tropical Ecology 33:12–21.

Vandewalle, M., F. de Bello, M. P. Berg, T. Bolger, S. Dolédec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. M. da Silva, M. Moretti, J. Niemelä, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation 19:2921–2947.

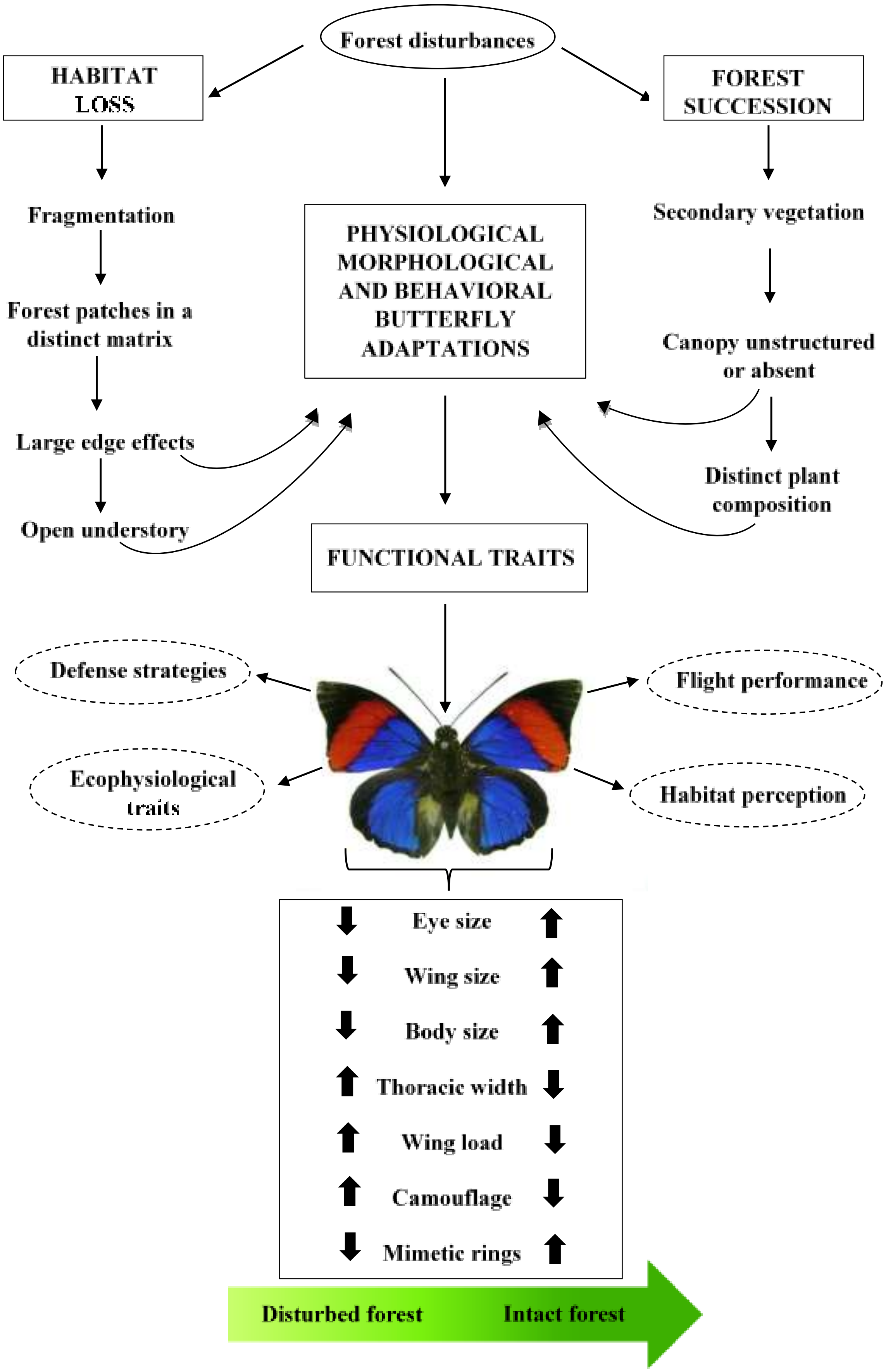
Vasconcelos, H. L. and E. M. Bruna. 2012. Arthropod responses to the experimental isolation of Amazonian forest fragments. Zoologia 29:515–530.

Violle, C., M. L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos 116:882–892.

Xu, J., Y. Chen, L. Zhang, Y. Chai, M. Wang, Y. Guo, T. Li, and M. Yue. 2017. Using phylogeny and functional traits for assessing community assembly along environmental gradients: A deterministic process driven by elevation. Ecology and Evolution, 7(14), 5056–5069.

SUPPORTING INFORMATION

DATA S1. Conceptual framework and hypotheses on trait-environment patterns for fruit-feeding butterflies. We expect that butterfly species will have distinct adaptations for each type of environment along forest disturbance gradients. According to our predictions, butterflies can manifest key-traits providing greater flight acceleration and speed (robust muscles associated with smaller wings) to move between patches of habitat, and defense strategies (camouflage) to avoid unnecessary exposure to predators in disturbed environments. Forest specialists should manifest erratic flights amidst vegetation (larger body and wings but fewer muscles), better visual sensibility (larger eyes), and more diverse colors providing different defense strategies.


Forest disturbances
HABITAT LOSS
FOREST SUCCESSION
Fragmentation
Forest patches in a distinct matrix
Large edge effects
Open understory
PHYSIOLOGICAL MORPHOLOGICAL AND BEHAVIORAL BUTTERFLY ADAPTATIONS
Secondary vegetation
Canopy unstructured or absent
Distinct plant composition
FUNCTIONAL TRAITS
Defense strategies
Ecophysiological traits
Flight performance
Habitat perception
⬇ Eye size ⬆
⬇ Wing size ⬆
⬇ Body size ⬆
⬆ Thoracic width ⬇
⬆ Wing load ⬇
⬆ Camouflage ⬇
⬇ Mimetic rings ⬆
Disturbed forest
Intact forest

PARTE III

A COLORAÇÃO DAS BORBOLETAS NA FLORESTA AMAZÔNICA

Capítulo 3

Manuscrito submetido na revista Biological Conservation

Discolouring the Amazon Rainforest: How do forest gradients affect butterfly coloration?

Ricardo Luís Spaniol[1]*, Milton de Souza Mendonça Jr.[1], Sandra Maria Hartz[1], Cristiano Agra Iserhard[2], Martin Stevens[3]

[1]Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

[2]Programa de Pós-graduação em Biologia Animal, Universidade Federal de Pelotas, Capão do Leão, Rio Grande do Sul, Brazil

[3]Centre for Ecology & Conservation, College of Life & Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall, TR10 9FE, U.K.

*Corresponding author (ricardospaniol@yahoo.com.br)

Abstract

Butterflies are among the most colourful organisms in the world and colour plays a central role in many butterfly life-history strategies. However, the efficacy of coloration strategies can be affected by sudden environmental changes, including anthropogenic ones such as habitat loss and fragmentation. Here we investigate the effect of forest disturbance gradients on fruit-feeding butterfly colours in different environments (continuous primary forest, as opposed to both secondary forest and forest fragments) in the Amazon Rainforest. We obtained measures of colour patches corresponding to hue, saturation, brightness, plus contrast between patches, and wing-colour diversity for 220 individuals, belonging to 60 species. Butterflies in the secondary forest and continuous primary forest are more colourful than those in the early succession environments. These individuals show higher average values for hue and saturation, but lower ones for brightness. Accompanying changes in colour composition, wing-colour diversity among species decreases in human-disturbed environments, such as recently deforested areas. These results provide evidence that deforestation has effects on behavioural strategies related to coloration, a negative effect that has to date been poorly explored and or demonstrated for butterflies. Conspicuous individuals are among the first to disappear from recently disturbed areas, suggesting an accelerated loss of local fauna and a "discolouration" of the Amazonian butterflies.

Key words: Fruit-feeding butterflies, camouflage, deforestation, colour diversity, tropical forest, forest succession.

Introduction

Research on animal coloration is a classical issue that has fascinated naturalists since the great naturalist expeditions around the world (Bates, 1863). This interest is partly due to the relevance of colours for intra- and interspecific interactions, links to the abiotic environment, and the importance of such traits in illuminating our understanding of ecological and evolutionary processes (Stevens, 2016; Endler and Mappes, 2017). Through coloration, animals display broad repertoires in anti-predatory and mating strategies, aiming to maximize survival and reproductive success (Cuthill et al., 2017).

Regardless of the strategies (e.g. anti-predatory or reproductive) employed by an organism, environmental characteristics work as key aspects selecting phenotypic patterns (Roslin et al., 2017). For example, recent research continues a long tradition in testing how visual signal transmission is related to the habitat in which animals occur and their contrast with the visual environment (e.g. Xing et al., 2016, Willmott et al., 2017; Walton and Stevens, 2018). Therefore, environmental changes may affect the adaptive value of individual coloration (Delhey and Peters, 2017), in the same way that species assemblages from different habitats may house individuals with specific functional features and adaptations (Valtonen et al., 2017).

In butterflies, the evolutionary history and current selection pressures on phenotypic variations are printed on their wings, ranging from conspicuous colours, camouflage, wing-eyespots, to mimetic complexes (rings), which together are responsible for colour diversity in nature (Joshi et al., 2018). Environmental changes can affect colour patterns on butterfly wings in different ways. One of these is through the supply of host plants as food resource to the immatures (Talloen et al., 2004). Caterpillar diet is essential for chemical compound synthesis (e.g. flavonoids) which leads to diverse phenotypic expression, including of body/wing colours (Johnson et al., 2014). Once the species composition of plant communities is modified, more specialized butterflies (in terms of food resources) may disappear (Soga et al., 2015), or differ in colour pattern due to the nature and amount of available resources. A second way to understand the relationship between colours and habitat features is in the way visual signals are transmitted to other organisms. Different communication strategies, including those used to mediate prey-predator interactions, potential mates, or with other biotic components, may be affected by structural changes in the environment (Briolat et al., 2018; Walton and Stevens, 2018). This means that some strategies in protective coloration and signalling may successfully work under specific combinations of environmental conditions, but cease to be effective under accelerated environmental changes. Specifically, the value of a range of colour patterns will be affected by their contrast and interaction with the visual environment.

The Amazon Rainforest is an ideal region to test the above issues because it is still largely preserved, yet is under great threat and suffering extensive deforestation, affording the potential to study organismal responses to human-induced environmental disturbance (Mesquita et al., 2015). In recent decades, this region has been widely transformed with high deforestation rates driving the advance of agricultural frontiers, with concerning consequences for tropical biodiversity (Souza et al., 2013). At present, public policies and interventions adopted are sparse and have promoted insufficient results for controlling the loss of huge areas of forest every year (Arima et al., 2014), which is accompanied by high species extinction rates (Stork, 2010; Barlow et al., 2016). The immediate consequences of forest transformation are often unpredictable and require careful monitoring of ecological and evolutionary responses from different species (Caro et al., 2017).

From the perspective of different coloration properties (hue, saturation, brightness, wing-colour diversity), our study aims to advance knowledge on how deforestation and habitat-size decrease may influence the colour patterns observed by the species that remain, and its potential effects on their anti-predation strategies. We hypothesized that fruit-feeding butterflies along forest gradients would be distinct with regards to their colour patterns, and that prevalent phenotypic characteristics should change according to habitat size and regeneration stage after a disturbance, including changes in the visual environment (light conditions, background contrast) (Fig. 1). From this viewpoint, we predicted that: (i) with a decline of vegetation structure and changes in abiotic characteristics, homogenization due to human activities will result in conspicuous butterflies being removed first, while cryptic individuals (mainly brown winged ones) may persist by avoiding detection against general brown substrates. (ii) Those generally cryptic butterflies should cope well and persist not only in disturbed habitats, but along the gradient. (iii) Colourful butterflies may be those most affected by forest disturbances, being the first to disappear due to increased exposure in more open habitats. This way, colour diversity and richness should be lower in disturbed environments when compared to the preserved primary forest. As a counter-prediction, some types of intrinsically conspicuous coloration may be effective to a certain extent regardless of the visual environment (e.g. aposematism) and therefore persist even in degraded habitats. In contrast, species with specialist camouflage to a limited range of backgrounds may decline if those backgrounds are removed.

Methods

Study area

Butterflies were sampled in areas of the Biological Dynamics of Forest Fragments Project (PDBFF) (2°21'36.14"S, 59°57'45.60" W), belonging to the National Amazon Research Institute (INPA). These are spread over 1000 km², 90 km north of Manaus/AM, in a Brazilian Federal Protected Area created in the late 1970s to investigate the consequences of deforestation and forest fragmentation in the Amazon (Biereegaard et al., 1992). Three farms (Dimona, Porto Alegre, and Esteio) were defined as study areas. Each study area includes the following environments: forest fragments of 1, 10 and 100 hectares, environments in an initial stage of succession around the previous fragments, secondary forests, and large extensions of primary continuous forest (Fig. 2). The initial succession comprised vegetation with approximately three years of regeneration, and isolated fragments from the other environments. The secondary forest covers areas formerly used as cattle pasture, and which now have forests with 30 years of regeneration. Forest fragments are isolated patches of primary forest, structurally similar to the large areas of continuous forest (Laurance and Vasconcelos, 2009). The primary continuous forest occupies extensive adjacent regions, and are used in this study as a control. In these landscapes, two distinct environmental gradients can be identified and monitored to understand the response of organisms to habitat variations: habitat-size and forest succession. We refer to the forest succession gradient as including the areas in initial succession, secondary forest and continuous forest; the habitat-size gradient, in turn, includes fragments of 1, 10 and 100 hectares, and the continuous forest (CF) as a control.

Study organisms

Butterflies represent ideal study models for environmental assessments, as they provide a rapid response to habitat changes, and allow relatively easy monitoring in nature (Freitas et al., 2014). These insects manifest a large repertoire of colours, which may be associated with the different habitats they occupy and different strategies for distinct functions (Endler, 1993; Dalrymple et al., 2015). Their responses can be extended to several other groups of animals that share similar requirements. The fruit-feeding guild in particular comprises approximately 50-75% of all butterfly species belonging to the family Nymphalidae found in the Neotropics (Brown, 2005). This group is represented by four subfamilies: Charaxinae, Biblidinae, Satyrinae, and also a few tribes within Nymphalinae (Freitas et al., 2014). The fruit-feeding butterflies have preferences for fermented fruits, carrion, feces and plant exudates, and because of this feeding habit they can be easily captured in the field using standardized methodology with bait traps, and simultaneously at different localities (DeVries et al., 2012). According to butterfly responses to anthropic-changes in the Amazon Rainforest, we provide the following conceptual framework and predictions on colour-environment patterns (Fig. 1).

Data sampling

Two field expeditions were performed between August and September 2015 and 2016, at the beginning of the dry season in the Amazon region. Each sampled area received a sample unit (SU) with five portable traps, containing attractive bait made with bananas fermented in sugar cane juice for 48 hours (Freitas et al., 2014). Sample units were installed on each farm, with three SUs in early successional sites, three in secondary forest sites, one for each forest fragment size (1, 10 and 100 hectares) and three points in the adjacent continuous forest, totalling 60 traps per farm. A minimum distance of 20 meters between neighbouring traps was observed, placed between 100 – 130 cm above the ground (Freitas et al., 2014). SUs were kept at least 500 meters from each other within each farm.

At each sampling occasion, traps remained exposed for eight consecutive days in each farm, being reviewed at 48h intervals to renew the bait, collect, identify, mark and release the captured butterflies. During the review process, two samplers equipped with entomological nets performed active samples of fruit-feeding butterflies around the traps, as long as they were visibly attracted by the bait. These combined methods allow us to complement the representation of butterfly species, including especially those species attracted but not caught by the traps (Checa et al., 2018). At last 10 individuals of each species were collected for subsequent lab measurement and analysis (Fig. 3).

Colour measurements

The collected butterflies were fixed and deposited in entomological collection (Laboratório de Ecologia de Interações - UFRGS) for gathering individual information. From 60 fruit-feeding butterfly species, we selected 220 individuals with well-preserved colour characteristics. Under natural light conditions, each individual was photographed in a ventral position. We took all pictures using a Nikon D5300 camera with 18-55mm lens, accompanied with a scale bar and a grey card (18%) to correct variations in light conditions between photographs (Stevens et al., 2007). The photographs were taken in RAW format and selected for appropriate exposure in RawTherapee software (version 5.3). All images were imported into the ImageJ program and through the MICA Toolbox add-on (version 1.22), and we generated multispectral images ".mspec" calibrated from the grey card (Troscianko and Stevens, 2015). For every image, we drew “regions of interest” (ROI’s) in the wing areas, based on the principle colours found on the wings. ROI's were chosen on colour spots that could transmit visual information, such as wing areas with strong colour contrast in the wings as a whole. All photographed butterflies had their colour patterns quantified following a previous approach that made use of calibrated digital images (Stevens et al., 2007).

We standardized images and corrected for differences in light conditions with regards to the grey card, and obtained colour data corresponding to reflectance in the three colour channels: red, green, and blue (RGB) on a scale of 0–255 for each colour channel. From these values, we were able to calculate several colour variables for each butterfly colour patch: hue (colour type), saturation (colour 'richness' compared to white light), brightness, maximum contrast between patches, and wing-colour diversity (see Stevens et al., 2007). Finally, we present the frequency of four main putative anti-predatory strategies using colour patterns: camouflage, transparency, warning colours, and wing-eyespots. Each species and its type of defence was categorically classified by their general comparison with the visual environment, observing the predominance of brown/cryptic colours, translucent wings, highly contrasting colours (for example, yellow, red, orange combined with black or white), and circular features on the wing resembling an eye. We appreciate that this is to a certain extent subjective but full categorisation of the type of defence each species primarily relies on was beyond the scope of this study (requiring, for example, toxicity analysis and behavioural experiments).

Data analysis

We converted standardized RGB reflectance values to XY coordinates in a triangular colour space and calculated saturation as the Euclidian distance from the centre of the colour space (Endler and Mielke, 2005; Stevens et al., 2014). To calculate hue, we followed a range of past approaches in describing hue in the form of colour channels that describe the variation in colours present in the dataset (Komdeur et al., 2005; Spottiswoode and Stevens, 2012; Stevens et al., 2014). To determine the most appropriate channels, a principal component analysis (PCA) was used to summarize the importance of each colour channel in butterfly phenotype variation. To eliminate achromatic differences and to analyse only colour differences, we removed brightness from the dataset by standardizing the colour values to proportions (Cuthill et al., 1999; Spottiswoode and Stevens, 2012), and then from the resulting PCA derived colour channels that best described the variation in colour that existed among species and colour patches (Spottiswoode and Stevens, 2012; Stevens et al., 2014). To quantify wing-colour diversity, we use the Mean Euclidean Distance between *x*, *y* colour space coordinates for all individuals within each treatment. Through a resampling analysis (N = 999) we corrected the differences in sample size between environments in each gradient. The number of resampled butterflies was equal to the smallest group in the analysis (see Spottiswoode and Stevens, 2012). For brightness, we calculated the average brightness in reflectance across the RGB channels.

All colour variables (hue, brightness, saturation, differences between patches and colour diversity) were then individually analysed along the habitat-size and forest succession gradients, using generalized linear mixed models. From these analyses, we control for differences in abundance between species. In order to make the coloration results independent of the butterflies assemblages in each habitat, we also controlled for the effects of species composition. All computations were performed with R, using PAVO and lmer4 statistical packages (R Core Team, 2018).

Results

First, we checked if there is structure in colour composition of the fruit-feeding butterfly assemblages along both habitat-size and forest succession gradients. From the achromatic central point (grey) in the colour space triangles, we can observe a trend for LW presence in all the environments, and the colour values cross through the centre of the colour space from LW to SW and MW parts (Fig. 4). This was confirmed by Principal Component Analysis (PCA) using a covariance matrix with standardized values for each colour channel.

For the forest succession gradient, the first two axes of the PCA expressed 99% of all variation in butterfly colour. The first axis separated LW from the other colour channels, explaining 79% of the phenotypic patterns. The second axis contains 20% of the variation, separating SW from LW and MW (PC1 coefficients: SW: 0.554; MW: 0.525; LW: -0.645. PC2: SW: 0.660; MW: -0.749; LW: -0.042). The first two axes of the PCA also expressed 99% of the phenotypic variation of the butterflies to the habitat-size gradient. In a similar pattern, the first axis separated LW from MW and SW, explaining 72% of the phenotypic patterns observed. The second axis contains 27% of the variation, separating SW from LW and MW (PC1 coefficients: SW: 0.553; MW: 0.483; LW: -0.677. PC2: SW: 0.634; MW: -0.771; LW: -0.031).

Significant changes were found along the forest succession gradient for the following colour variables: hue, saturation, brightness and wing-colour diversity. Higher hue values were observed in primary forest regions (F-value = 6.60, P = 0.01). In other words, longer wavelength rich colours (LW) such as brown/red prevail in older forested areas regarding succession. For saturation, we detected lower values in the early succession (F-value = 4.48, P = 0.03). This means that butterflies are less colourful in recently disturbed environments when compared with continuous forest. Similarly, brightness values are smaller in the early succession (F-value = 3.93, $P = 0.05$) (Fig. 5), in which butterflies with lighter colours are more common. The forest interior may be housing melanic patterns, phenotypically darker individuals. We found a larger wing-colour diversity for the continuous forest ($P < 0.05$ for 75.6% of resamples with $N = 40$) when compared to the treatments under succession. A similar pattern is also observed for forest patches ($P < 0.05$ for 67.1% of resamples with $N = 7$), which show a lower colour diversity than continuous forest (Fig. 5). Apparently, larger forest habitats can house butterfly assemblages with more diverse phenotypic features and colours.

Among putative anti-predatory strategies using colour patterns (Fig. 6), camouflage appears as a predominant feature in all studied environments. As the forest succession progresses, wing-eyespots become more important and appear frequently. Transparency seems to be an exclusive strategy for the well-preserved continuous forest. Thus, the co-occurrence of all different phenotypic manifestations is observed exclusively under areas of intact vegetation, and reinforce our results on colour diversity.

Discussion

In agreement with our predictions, colour composition of butterfly assemblages are in close association with habitat features. Several colour variables changed among environments, especially in those along the forest succession gradient. Butterflies utilising conspicuous patterns seems to be the most affected by human-activities, appearing among the first to disappear from recently disturbed forest regions in the Amazon. The worldwide decline of the insects and the increasing number of endangered butterfly species (Jansen and

Hallwachs, 2019; Sánchez-Bayo and Wyckhuys, 2019) might be one of the most imminent consequences of anthropic pressures on colour patterns. On the other hand, this study also demonstrates the positive effect of natural regeneration in recovering colour composition and ecological structure in modified tropical forests (Crouzeilles et al., 2017). The maintenance of both primary and secondary forests in human-modified landscapes might be helpful for re-colonization of plant species and interaction networks (Pellissier et al., 2018; Rozendaal et al., 2019), in the same way that regeneration may also assist for the maintenance of diversity in protective coloration and signalling in butterflies.

In tropical forests, high biological diversity can lead to the coevolution of several adaptive strategies, including butterfly interactions with predators, competitors, mutualists, or even potential mates. This makes the tropical region not only rich in species but also in their phenotypic and behavioural diversity, including colour related strategies (Adams et al., 2014). Whatever the kind of interaction, environmental features can produce effects on species assemblages through individual colour composition, and this was observed for butterfly response to the forest degradation and habitats loss in the Amazon. Firstly, camouflage appears as a dominant feature against predation in several animal groups across the tropics, including birds and butterflies (Dalrymple et al., 2015). With a predominance of individuals with cryptic colours (especially browns) throughout the forest succession gradient, and even in continuous forest, this study supports that idea, suggesting that camouflage is one of the main defence strategies for Lepidoptera and is a valuable defence even as the environment composition is altered. This also suggests that generalist dull cryptic colour patterns may enable concealment in a range of habitats. Moreover, a lower colour saturation in butterflies occurring in recently disturbed sites reveals that conspicuous individuals may be receiving greater predatory pressures, especially in open environments.

While one may expect that conspicuous markings may operate across a range of visual backgrounds, it is possible that these conspicuous species may become too exposed when contrasting with new backgrounds created by disturbances. Consequently, more colourful butterflies may be among the first to disappear locally soon after the deforestation process. In addition, habitat degradation may alter the composition of predators and allow a greater number of naïve predators into the forest area. This may result in greater attack rates on butterflies that normally can rely to a great extent on the learnt avoidance by predators of their conspicuous warning, startle, or mimicry signals. From a high diversity regional pool of species, selection may therefore lead to lower diversity in butterfly colours, as well as in their protective and signalling strategies, in degraded habitats.

Furthermore, we note that conspicuity is not necessarily an exclusive feature of butterflies living in primary continuous forests. There is a remarkable variance for the colour properties we analysed (hue, brightness, saturation and colour diversity), showing that conspicuous and cryptic individuals even with different performances in their communication roles can make use of the same habitats. In addition, habitats in a preserved forest are capable of harbouring greater colour diversity and subsequently of anti-predatory strategies. This may be possible because of the large supply of substrates such as leaves, trunks, branches, stones, with which the individuals can "interact" in terms of their phenotypic appearance (Pinheiro et al., 2016). Such environmental resources are not always readily available after a severe disturbance, where abrupt and intense changes can lead to changes in the habitat structure and vegetation resilience trajectories (Jakovac et al., 2015). Differences may also relate to changes in predator communities (Mappes et al., 2014).

Analysing the habitat-size gradient, we discovered that forest patches with different sizes might have smaller effects on butterfly assemblage colour composition than the disturbance gradient. Our study areas comprise an already well-developed forest matrix, which may be permeable to many species, including forest specialists (Debinski and Ries, 2001; Schtickzelle et al., 2007). Reduced forest patches do not necessarily lead to strong changes in vegetation composition or structural features (Brown and Hutchings, 1997), which however are easily identified along the forest succession (Mesquita et al., 2015). Therefore, the supply of immature host plants, adult food and substrates may remain. Complementarily, the way visual signals are propagated may remain mostly unchanged since forest characteristics are substantially maintained. This means butterflies with distinct phenotypes may be able to move between forest patches with a relatively reduced risk of predation. Environmental conditions and resources such as leaves, twigs, trunks and light entry through the forest canopy may also remain available and relatively constant, helping colour patterns remain the same within forest habitats patches.

Conclusions

Our study shows that butterflies from a regional species pool are under selection for lower diversity in their colours as well as in their defence strategies due to human interference. Certainly, coloration is only one of several organismal traits offering quick responses to environmental changes, but this has been seldom applied in our quest to understand threats to biodiversity and processes that lead to species extinctions (Hook et al., 1997; Caro et al., 2017). Therefore, our study is novel and highlights the study of animal coloration as an excellent basis for the evaluation of environmental health and in the planning for management and conservation of forest ecosystems. Colour shifts are important markers of several anthropogenic changes (Caro et al., 2017), and by observing phenotypic aspects in animals, we can monitor the performance of protective colouration and signalling under different environmental conditions.

In a moment when up-to-date deforestation rates are increasing again in the largest tropical forest in the world (INPE, 2018), this study draws attention to the importance of maintaining well-preserved forest remains in the Amazon. In addition, 30 year-recovering forested habitats (secondary forest) show increasing in colour diversity, allowing the movement and permanence of organisms near the primary forests, maintaining ecosystem services. Thus, the maintenance of legal reserves inside private properties and restoration programs in degraded areas with higher biological importance (Lovejoy and Nobre, 2018; Vieira et al., 2019) should be encouraged, being key factors aiming conservation of biodiversity in tropical forests. We also suggest the presented results and actions should be incorporated into public policy, reinforcing that information based on scientific research is crucial to the decision-making process.

Once unplanned forest conversion leads to constant uncertainties about the compliance with Brazilian environmental legislation, we provide strong evidence that a significant portion of the butterfly fauna may be facing extinction in the near future. This is just the visible part of the Amazonian fauna - in the literal sense of the word, where the most colourful butterflies are the first to be locally extinguished by removing the native vegetation. It is necessary to use the protagonism this insect group has in the public eye to warn about the limits of forest exploitation. Otherwise, the largest rainforest in the world will be less colourful without some of its main inhabitants.

Acknowledgments

We thank the Biological Dynamics of Forest Fragments Project (BDFFP) through the Thomas Lovejoy research fellowship program for providing financial and logistical support for fieldwork. We also thank Yang Niu, Anna Hughes and Gabriel Nakamura for useful comments that improved on previous versions of the manuscript. Ricardo Spaniol thanks Capes for his PhD fellowship. Sandra Hartz (process 304820/2014-8) and Milton Mendonça (process 309616/2015-8) are CNPq researchers. Chico Mendes Institute for Biodiversity Conservation - ICMBio conceded licenses and permissions for the research activities (License numbers 48786-1 and 48786-2).

References

Adams, J.M., Kang, C., June-Wells, M., 2014. Are tropical butterflies more colorful? Ecol. Res. 29, 685–691.

Arima, E.Y., Barreto, P., Araújo, E., Soares-Filho, B., 2014. Public policies can reduce tropical deforestation: Lessons and challenges from Brazil. Land use policy 41, 465–473.

Barlow, J., Lennox, G.D., Ferreira, J., Berenguer, E., Lees, A.C., Nally, R. Mac, Thomson, J.R., Ferraz, S.F.D.B., Louzada, J., Oliveira, V.H.F., Parry, L., Ribeiro De Castro Solar, R., Vieira, I.C.G., Aragaõ, L.E.O.C., Begotti, R.A., Braga, R.F., Cardoso, T.M., Jr, R.C.D.O., Souza, C.M., Moura, N.G., Nunes, S.S., Siqueira, J.V., Pardini, R., Silveira, J.M., Vaz-De-Mello, F.Z., Veiga, R.C.S., Venturieri, A., Gardner, T.A., 2016. Anthropogenic disturbance in tropical forests can double biodiversity loss from deforestation. Nature 535, 144–147.

Bates, H.W., 1862. Contributions to an insect fauna of the Amazon valley (Lepidoptera: Heliconidae). Transactions of the Linnean Society of London 23, 495-566.

Bierregaard, R.O., Lovejoy, T.E., Kapos, V., Santos, A.A., Hutchings, R.W., 1992. The Biological Dynamics of Tropical Rainforest Fragments: A prospective comparisonof fragments and continuous forest. BioScience 11, 859-866.

Briolat, E.S., Burdfield-steel, E.R., Paul, S.C., Katja, H.R., Seymoure, B.M., Stankowich, T., Stuckert, A.M.M., 2018. Diversity in warning coloration: selective paradox or the norm? Biol. Rev. https://doi.org/10.1111/brv.12460

Brown Jr, K.S., Hutchings, R.W. (1997) Disturbance, fragmentation, and the dynamics of diversity in Amazonian forest butterflies. In: Laurance, W.F., Bierregaard Jr., R.O. (Eds.), Tropical Forest Remnants - Ecology, Management and Conservation of Fragmented Communities. The University of Chicago Press: Chicago, pp. 91-110.

Caro, T., Stoddard, M.C., Stuart-fox, D., 2017. Animal coloration research: why it matters. Trans. R. Soc. B 372, 20160333.

Checa, M.F., Donoso, D.A., Rodriguez, J., Levy, E., Warren, A., Willmott, K., 2018. Combining sampling techniques aids monitoring of tropical butterflies. Insect Conserv. Divers. https://doi.org/10.1111/icad.12328

Cuthill, I.C., Bennett, A.T.D., Partridge, J.C., Maier, E.J., 1999. Plumage reflectance and the objective assessment of avian sexual dichromatism. Am Nat., 153, 183–200.

Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E., Hill, G.E., Jablonski, N.G., Jiggins, C.D., Kelber, A., Mappes, J., Marshall, J., Merrill, R., Osorio, D., Prum, R., Roberts, N.W., Roulin, A., Rowland, H.M., Sherratt, T.N., Skelhorn, J., Speed, M.P., Stevens, M., Stoddard, M.C., Stuart-Fox, D., Talas, L., Tibbetts, E., Caro, T., 2017. The biology of color. Science, 80, 357.

Dalrymple, R.L., Kemp, D.J., Flores-Moreno, H., Laffan, S.W., White, T.E., Hemmings, F.A., Tindall, M.L., Moles, A.T., 2015. Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. Glob. Ecol. Biogeogr. 24, 1424–1432.

Debinski, D.M., Ries, L., 2001. Butterfly responses to habitat edges in the highly fragmented prairies of Central Iowa. J. Anim. Ecol. 70, 840–852.

Delhey, K., Peters, A., 2016. Implications for conservation of anthropogenic impacts on visual communication and camouflage. Conserv. Biol. 31, 1–36.

Devries, P.J., Alexander, L.G., Chacon, I.A., Fordyce, J.A., 2012. Similarity and difference among rainforest fruit-feeding butterfly communities in Central and South America. J. Anim. Ecol. 81, 472–482.

Endler, J.A., 1993. The Color of Light in Forests and Its Implications. Ecol. Monogr., 63, 1–27.

Endler, J.A., Mielke, P.W., 2005. Comparing entire colour patterns as birds see them. Biol. J. Linn. Soc., 86, 405–431.

Freitas, A.V.L., Iserhard, C.A., Santos, J.P., Yasmin, J., Carreira, O., Ribeiro, D.B., Henrique, D., Melo, A., Henrique, A., Rosa, B., Marini-filho, O.J., Accacio, G.M., 2014. Studies with butterfly bait traps: an overview. Rev. Colomb. Entomol. 40, 203–212.

Hook, T.V., 1997. Insect coloration and implications for conservation. The Florida Entomologist 80, 193–210.

Instituto Nacional de Pesquisas Espaciais - INPE, 2018. Projeto Prodes – Monitoramento da floresta Amazônica brasileira por satélite. http://www.obt.inpe.br/prodes/ (accessed 18 March 2019).

Jakovac, C.C., Peña-Claros, M., Kuyper, T.W., Bongers, F., 2015. Loss of secondary-forest resilience by land-use intensification in the Amazon. J. Ecol. 103, 67–77.

Janzen, D.H., Hallwachs, W., 2019. Where might be many tropical insects? Biol. Conserv. 233, 102–108.

Johnson, H., Solensky, M.J., Satterfield, D.A., Davis, A.K., 2014. Does skipping a meal matter to a butterfly's appearance? Effects of larval food stress on wing morphology and color in monarch butterflies. PLoS One 9, 1–9.

Joshi, J., Prakash, A., Kunte, K., 2017. Evolutionary Assembly of Communities in Butterfly Mimicry Rings. Am. Nat. 189, 58–76.

Komdeur, J.M., Oorebeek, M., Van Overveld, T., Cuthill, I. C., 2005. Mutual ornamentation, age, and reproductive performance in the European starling. Behav. Ecol., 16, 805–817.

Laurance, W.F., Vasconcelos, H.L., 2009. Consequências ecológicas da fragmentação florestal na amazônia. Oecologia Bras. 13, 434–451.

Lovejoy, T.E., Nobre, C., 2018. Amazon Tipping Point. Sci. Adv. 4, eaat2340.

Mappes, J., Kokko, H., Ojala K., Lindström, L., 2014. Seasonal changes in predator community switch the direction of selection for prey defences. Nat. Commun., 5, 5016.

Merilaita, S., Scott-Samuel, N.E., Cuthill, I.C., 2017. How camouflage works. Philos. Trans. R. Soc. B Biol. Sci. 372.

Mesquita, R.D.C.G., Massoca, P.E.D.S., Jakovac, C.C., Bentos, T.V., Williamson, G.B., 2015. Amazon Rain Forest Succession: Stochasticity or Land-Use Legacy? Bioscience 65, 849–861.

Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., Maglianesi, M.A., Melián, C.J., Pitteloud, C., Roslin, T., Rohr, R., Saavedra, S., Thuiller, W., Woodward, G., Zimmermann, N.E., Gravel, D., 2018. Comparing species interaction networks along environmental gradients. Biol. Rev. 93, 785–800.

Pinheiro, C.E.G., Freitas, A.V.L., Campos, V.C., DeVries, P.J., Penz, C.M., 2016. Both Palatable and Unpalatable Butterflies Use Bright Colors to Signal Difficulty of Capture to Predators. Neotrop. Entomol. 45, 107–113.

R Core Team., 2018. R:A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

Roslin, T., Andrew, N.R., Asmus, A., Barrio, I.C., Basset, Y., Al., E., 2017. Higher predation risk for insect prey at low latitudes and elevations. Science. 356, 742–744.

Rozendaal, D.M.A., Bongers, F., Aide, T.M., Alvarez-Dávila, E., Ascarrunz, N., Balvanera, P., Becknell, J.M., Bentos, T. V, Brancalion, P.H.S., Cabral, G.A.L., Calvo-Rodriguez, S., Chave, J., César, R.G., Chazdon, R.L., Condit, R., Dallinga, J.S., de Almeida-Cortez, J.S., de Jong, B., de Oliveira, A., Denslow, J.S., Dent, D.H., DeWalt, S.J., Dupuy, J.M., Durán, S.M., Dutrieux, L.P., Espírito-Santo, M.M., Fandino, M.C., Fernandes, G.W., Finegan, B., García, H., Gonzalez, N., Moser, V.G., Hall, J.S., Hernández-Stefanoni, J.L., Hubbell, S., Jakovac, C.C., Hernández, A.J., Junqueira, A.B., Kennard, D., Larpin, D., Letcher, S.G., Licona, J., Lebrija-Trejos, E., Marín-Spiotta, E., Martínez-Ramos, M., Massoca, P.E.S., Meave, J.A., Mesquita, R.C.G., Mora, F., Müller, S.C., Muñoz, R., de Oliveira Neto, S.N., Norden, N., Nunes, Y.R.F., Ochoa-Gaona, S., Ortiz-Malavassi, E., Ostertag, R., Peña-Claros, M., Pérez-García, E.A., Piotto, D., Powers, J.S., Aguilar-Cano, J., Rodriguez-Buritica, S., Rodríguez-Velázquez, J., Romero-Romero, M.A., Ruíz, J., Sanchez-Azofeifa, A., de Almeida, A.S., Silver, W.L., Schwartz, N.B., Thomas, W.W., Toledo, M., Uriarte, M., de Sá Sampaio, E.V., van Breugel, M., van der Wal, H., Martins, S.V., Veloso, M.D.M., Vester, H.F.M., Vicentini, A., Vieira, I.C.G., Villa, P., Williamson, G.B., Zanini, K.J., Zimmerman, J., Poorter, L., 2019. Biodiversity recovery of Neotropical secondary forests. Sci. Adv. 5, eaau3114.

Sánchez-Bayo, F., Wyckhuys, K.A.G., 2019. Worldwide decline of the entomofauna: A review of its drivers. Biol. Conserv. 232, 8–27.

Sansevero, J.B.B., Chazdon, R.L., Crouzeilles, R., Monteiro, L., Iribarrem, A., Lindenmayer, D.B., Ferreira, M.S., Latawiec, A.E., Strassburg, B.B.N., 2017. Ecological restoration success is higher for natural regeneration than for active restoration in tropical forests. Sci. Adv. 3, e1701345.

Schtickzelle, N., Joiris, A., Van Dyck, H., Baguette, M., 2007. Quantitative analysis of changes in movement behaviour within and outside habitat in a specialist butterfly. BMC Evol. Biol. 7, 1–15.

Soga, M., Kawahara, T., Fukuyama, K., Sayama, K., Kato, T., Shimomura, M., Itoh, T., Yoshida, T., Ozaki, K., 2015. Landscape versus local factors shaping butterfly communities in fragmented landscapes: Does host plant diversity matter? J. Insect Conserv. 19, 781–790.

Souza, C.M., Siqueira, J. V., Sales, M.H., Fonseca, A. V., Ribeiro, J.G., Numata, I., Cochrane, M.A., Barber, C.P., Roberts, D.A., Barlow, J., 2013. Ten-year landsat classification of deforestation and forest degradation in the brazilian amazon. Remote Sens. 5, 5493–5513.

Spottiswoode, C.N., Stevens, M., 2012. Host-Parasite Arms Races and Rapid Changes in Bird Egg Appearance. Am. Nat. 179, 633–648.

Stevens, M., 2016. Cheats and deceits: how animals and plants exploit and mislead. Oxford University Press, Oxford, U.K.

Stevens, M., Parraga, C. a, Cuthill, I.C., Partridge, J.C., Troscianko, T.S., 2007. Using digital photography to study animal coloration. Biol. J. Linn. Soc. 90, 211–237.

Stevens, M., Lown, A.E., Wood, L.E., 2014. Colour change and camouflage in juvenileshore crabs *Carcinus maenas*. Frontiers in Ecology and Evolution, 2, 14.

Stork, N.E., 2010. Re-assessing current extinction rates. Biodivers. Conserv. 19, 357–371.

Talloen, W., Van Dyck, H., Lens, L., 2004. The cost of melanization: Butterfly wing coloration under environmental stress. Evolution, 58, 360–366.

Troscianko, J., Stevens, M., 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. Methods Ecol. Evol. 6, 1320–1331.

Valtonen, A., Malinga, G.M., Nyafwono, M., Nyeko, P., Owiny, A., Roininen, H., 2017. The successional pathway of the tree community and how it shapes the fruit-feeding butterfly community in an Afrotropical forest. J. Trop. Ecol. 33, 12–21.

Vieira, R.R.S., Pressey, R.L., Loyola, R., 2019. The residual nature of protected areas in Brazil. Biol. Conserv. 233, 152–161.

Walton, O.C., Stevens, M., 2018. Avian vision models and field experiments determine the survival value of peppered moth camouflage. Commun. Biol. 1, 118.

Willmott, K.R., Willmott, J.R.C., Elias, M., Jiggins, C. D., 2017. Maintaining mimicry diversity: optimal warning colour patterns differ among microhabitats in Amazonian clearwing butterflies. Proc. R. Soc. B, 284: 20170744.

Xing, S., Bonebrake, T.C., Tang, C.C., Pickett, E.J., Cheng, W., Greenspan, S.E., Williams, S.E., Scheffers, B.R., 2016. Cool habitats support darker and bigger butterflies in Australian tropical forests. Ecol. Evol. 6, 8062–8074.

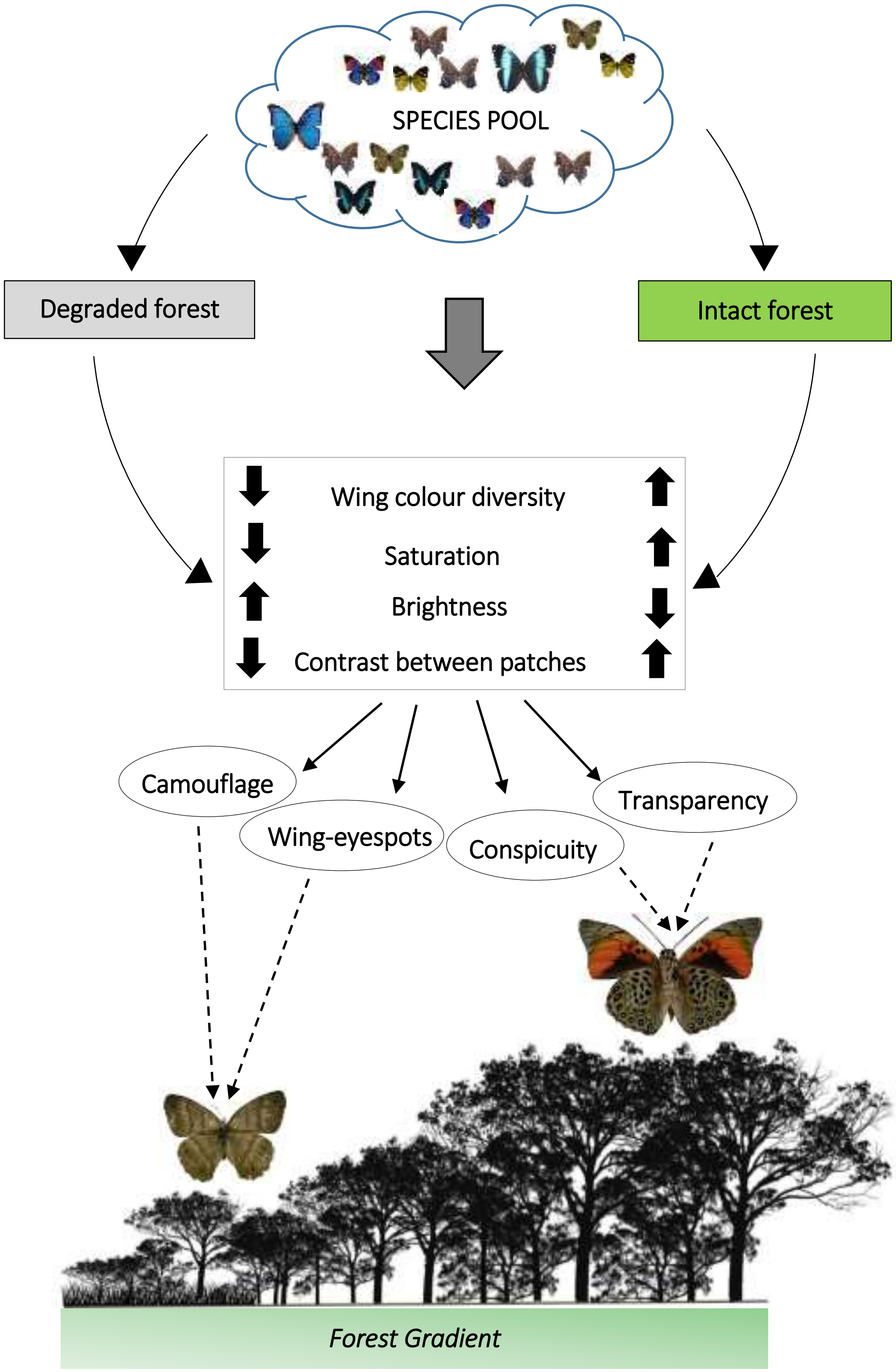


Fig. 1. Conceptual framework and predictions on colour-environment patterns for fruit-feeding butterfly communities in the Amazon Rainforest. From the regional species pool, we

expect that butterfly assemblages under similar habitats in the intact forest can have a remarkable colour composition regarding degraded habitats, where selection pressures may act on phenotype aspects and select for a few colour patterns. Under human-induced changes, anti-predatory strategies may display different performances, leading to differences in some colour properties. Colour richness and intensity (saturation), differences between colour patches and wing colour diversity may decrease with a greater homogenization in resources and abiotic characteristics. In those open and lighted habitats, conspicuous colours highly contrasting with the new habitat may not have the same performance due to increased exposure of those butterflies. Camouflage resembling the remaining substrate after a disturbance combined with the presence of wing-eyespots may increase the survival chance in butterflies, avoiding detection or predator attacks to vital parts of the body. Consequently, we expect a shortage of colourful butterflies due to deforestation in the Amazon Rainforest, sustaining our predictions that the Amazonian fauna is currently undergoing a process of discolouration.

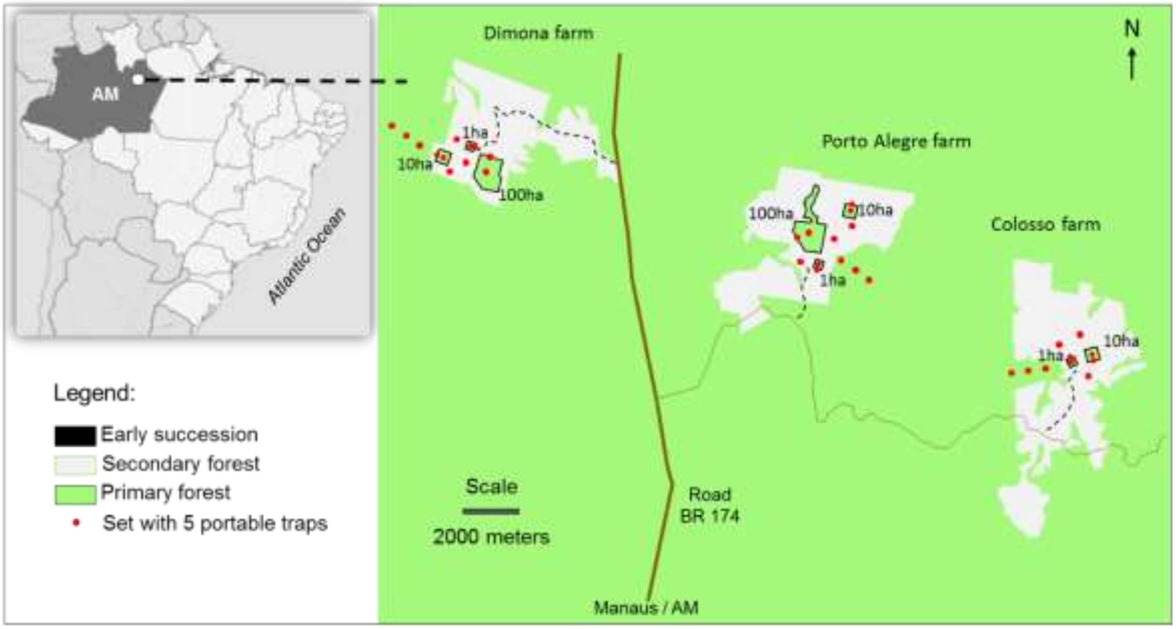


Fig. 2. Map with the three study areas at the Biological Dynamics of Forest Fragments Project (PDBFF), indicating the location of Dimona, Porto Alegre and Colosso farms. White patches represent deforested areas initially used for grazing purposes. The green portion corresponds to primary forest areas, including all the forest fragments used for the experiments in each study area (1, 10 and 100 hectares). The inset reveals the environment disposition on each farm: green is for primary forest (either continuous or in fragments), white for secondary forest (30 years abandonment) and black for early succession patches (around fragments).

Fig. 3. We measured the colours of 220 individuals belonging to 60 species of fruit-feeding butterflies. (a) Transparent butterfly (*Cithaeria andromeda*). Photograph by Iserhard. C.A. (b) Owl butterfly (*Caligo teucer*) with typical wing eyespot. Photograph by Spaniol. R.L. (c) The colorful *Prepona narcissus*. Photograph by Spaniol, R.L.

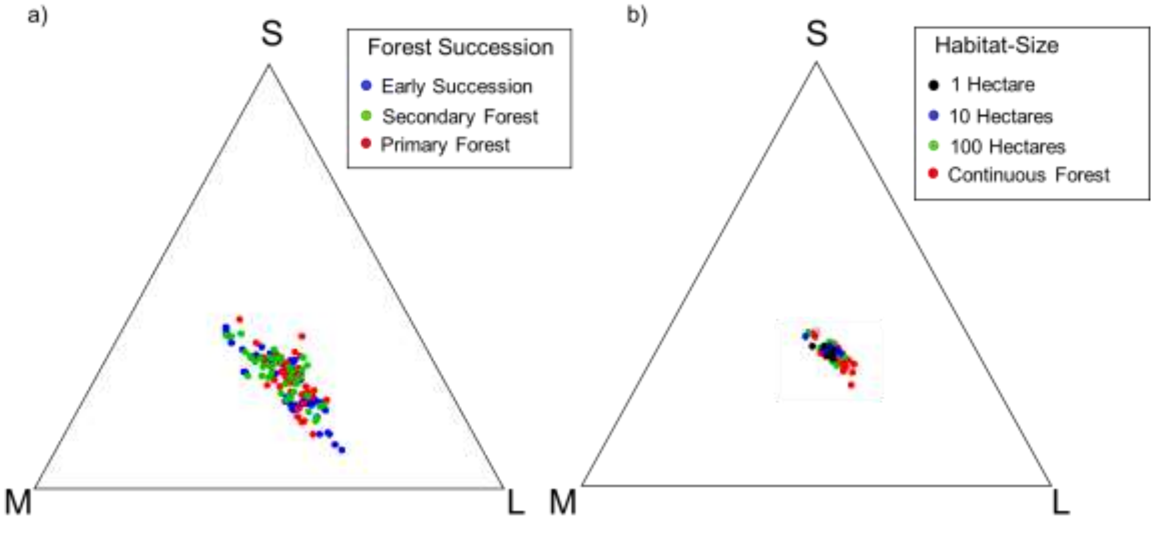


Fig. 4. Butterfly reflectance in a colour space triangle. Each dot represents the reflectance values for each individual observed along the forest gradients in the Biological Dynamics of Forest Fragments Project - PDBFF areas: a) Forest Succession; b) habitat-size. All dots were coloured according to the treatments identified through the legend. S = short wave; M

= medium wave; and L = long wave refers to the wavelength according to the RGB reflectance values.

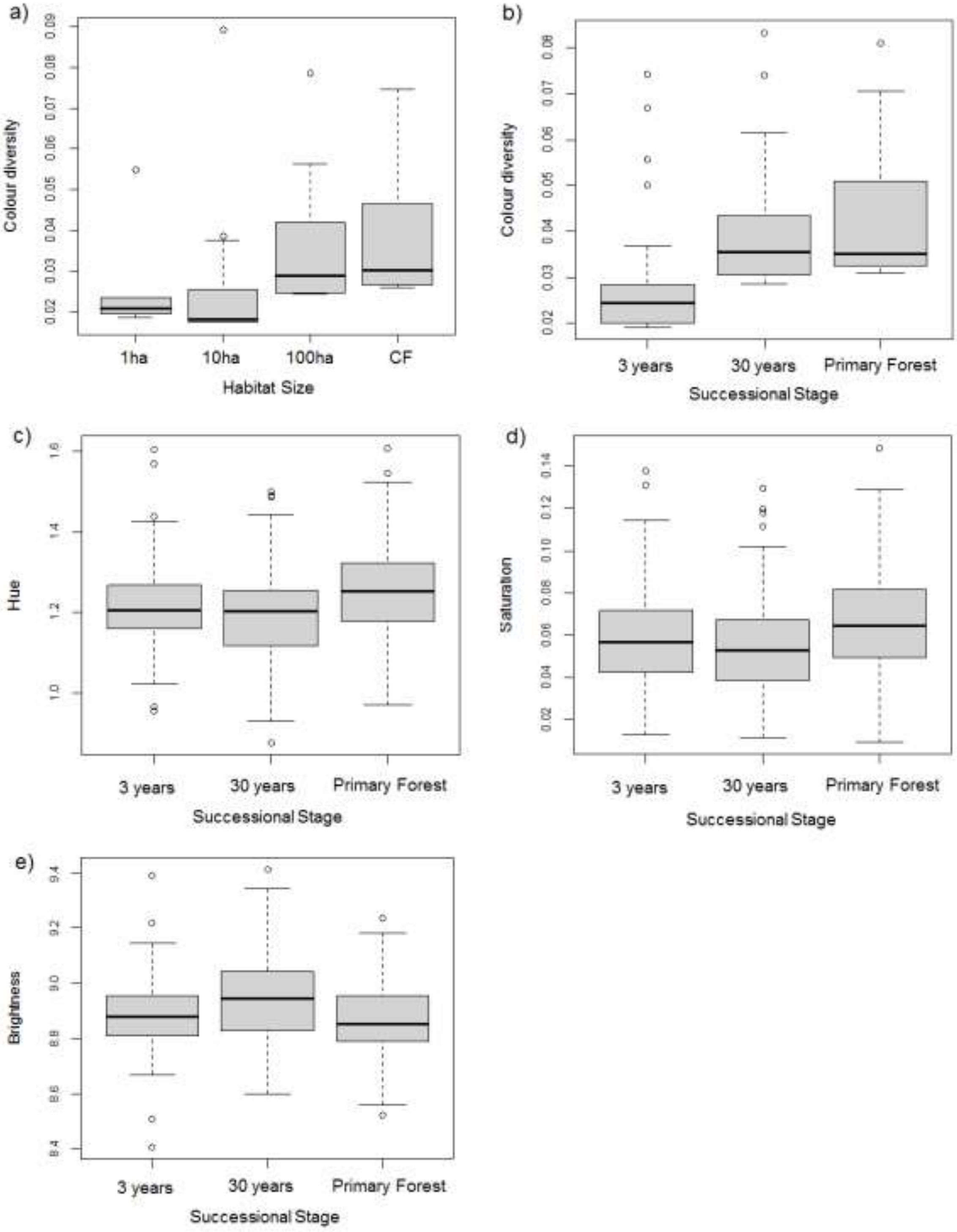


Fig. 5. Boxplots of colour variables for fruit-feeding butterflies. Wing-colour diversity in the different environments of the habitat-size (a) and forest succession (b) gradients. c) Hue; d)

Saturation and e) Brightness in environments of the forest succession gradient (Early Succession – 3 years after the last disturbance, Secondary Forest – 30 years after the last disturbance and Primary Continuous Forest), in areas bellowing to the Biological Dynamic of Forest Fragmentation Project - BDPFF, in the Amazon Rainforest.

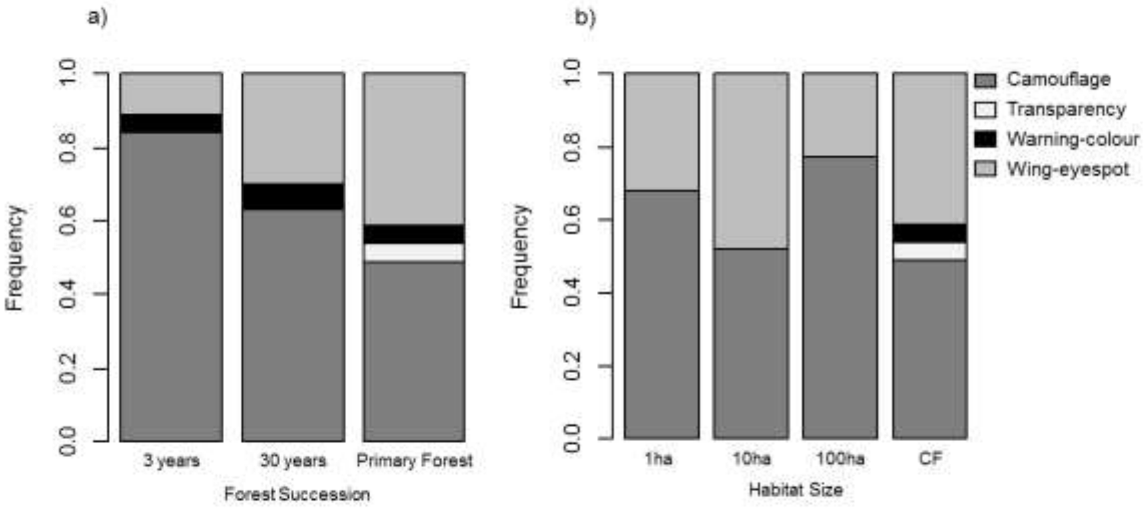


Fig. 6. Main defence strategies manifested by butterflies (camouflage, transparency, warning-colour and wing-eyespots) for different environments that make up the succession (a) and habitat-size (b) gradients in the Amazon Rainforest (for details see text).

CONCLUSÕES

O primeiro capítulo da tese permite uma maior padronização no estudo da ecologia funcional em borboletas frugívoras junto a sistemas florestais. A compilação de atributos-chave (e.g. Moretti et al. 2017) e seu acompanhamento na natureza é um requisito básico para compreender as diferentes adaptações e estratégias de vida das espécies, e com isso a distribuição dos organismos através do espaço e do tempo. Além de propor uma lista de atributos, o manuscrito deriva uma série de hipóteses ecológicas testáveis sob o ponto de vista da relação atributo-ambiente em diferentes cenários florestais.

Essa é a questão abordada no segundo capítulo, onde atributos de dispersão (comprimento do corpo e largura torácica) e de defesa (camuflagem) aparecem entre as principais estratégias das borboletas para contornar os efeitos das mudanças ambientais na Amazônia. Borboletas providas de um corpo menor e com musculatura torácica robusta seriam capazes de realizar voos acelerados e por distâncias maiores, características predominantes em áreas que estão em fase de sucessão inicial. A associação dos indivíduos maiores ao interior da floresta contínua os coloca entre os potencialmente mais afetados pelas consequências ecológicas do desmatamento (Shahabuddin & Ponte, 2005). A camuflagem, outra estratégia que parece predominar em áreas recentemente perturbadas, pode ser capaz de minimizar a exposição dos indivíduos em locais abertos. A conspicuidade parece não ser uma estratégia eficiente em ambientes florestais modificados pela atividade humana, talvez porque a propagação dos sinais visuais e a cadeia de predadores também se altera. Indivíduos maiores e providos de cores que contrastam com o substrato em que vivem, portanto, estão entre os primeiros a se extinguir localmente mediante o desmatamento na Amazônia.

Assim como os atributos funcionais, o componente filogenético indica que a estruturação das comunidades de borboletas está relacionada com a história evolutiva das linhagens. Ao longo da sucessão florestal, espécies evolutivamente mais próximas têm respostas mais similares aos ambientes, tanto em termos de composição de espécies nas comunidades como na similaridade funcional entre elas (Duarte et al., 2018). Cada uma das linhagens de borboletas se restringe a um ou poucos tipos de ambientes, gerando os padrões filogenéticos. O gradiente de fragmentação florestal através do tamanho de habitat têm efeitos reduzidos sobre a estrutura filogenética de borboletas (Pavoine et al., 2015). Isso parece acontecer, sobretudo, quando há uma matriz florestal bem desenvolvida conectando as manchas florestais. Nestas situações pode haver uma maior movimentação de indivíduos entre fragmentos, incluindo diferentes espécies e com atributos diversos. Assim, o tamanho do habitat florestal deixa de ser uma característica limitante para a performance de voo, requisitos reprodutivos ou interações bióticas e abióticas, e os efeitos negativos da fragmentação são amenizados.

Estimulado pelos resultados sobre o atributo de camuflagem, o terceiro capítulo aprofundou a compreensão das consequências da degradação florestal e do desmatamento sobre a coloração das borboletas (e.g. Delhey & Peters, 2016). Indivíduos com padrões crípticos/melânicos parecem não ser exclusivos de áreas em sucessão inicial, mas também estão presentes em grande número em regiões cobertas por florestas secundárias e contínua. No entanto, a intensidade das cores (saturação) é perdida mediante a remoção da cobertura florestal, o que explica uma assembleia de borboletas visualmente menos conspícua. A própria diversidade de cores também é reduzida, em um processo que denominamos aqui de “descoloração” da fauna. A boa notícia em termos de conservação é que estes cenários podem ser revertidos quando se permite a regeneração da floresta, sobretudo com o abastecimento de espécies animais e vegetais a partir de regiões preservadas no entorno.

Este trabalho trouxe um panorama atual dos desafios e ameaças que as florestas tropicais no mundo todo, e em especial a Amazônia, podem estar enfrentando. Através das múltiplas dimensões da diversidade que foram estudadas, compreendemos em vários aspectos sobre como as borboletas estão respondendo às pressões antrópicas em sistemas florestais. Essa visão de um momento recente, por sua vez, deixa de considerar os efeitos históricos de décadas atrás e que podem estar contribuindo para os padrões funcionais e filogenéticos que enxergamos. Considerando que a região de estudo integra o mais longevo experimento no mundo sobre fragmentação e degradação florestal, um passo seguinte seria integrar todas as informações históricas sobre borboletas dos últimos 40 anos do PDBFF – e existentes em coleções científicas e registros nos principais acervos de pesquisa do Brasil. Este resgate ampliaria nossa leitura para uma escala temporal nunca antes vista para a região amazônica, e nos contaria em detalhes sobre a capacidade de resistência e resiliência dos artrópodes à degradação de seus habitats. Estes novos desdobramentos também reforçariam nosso alerta global para o desaparecimento de espécies com características funcionais específicas, além de direcionar uma grande atenção para o maior remanescente de floresta tropical que ainda resiste, a Amazônia.

Referências

Delhey, K. & Peters, A. 2016. Conservation implications of anthropogenic impacts on visual communication and camouflage. *Conservation Biology*, **31**, 30–39.

Duarte, L. D. S., V. J. Debastiani, M. B. Carlucci, and J. A. F. Diniz-Filho. 2018. Analyzing community-weighted trait means across environmental gradients: should phylogeny stay or should it go? *Ecology*, **99**, 385–398.

Moretti, M., A. T. C. Dias, F. de Bello, F. Altermatt, S. L. Chown, F. M. Azcárate, J. R. Bell, B. Fournier, M. Hedde, J. Hortal, S. Ibanez, E. Öckinger, J. P. Sousa, J. Ellers, and M. P. Berg. 2017. Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, **31**, 558–567.

Pavoine, S., M. Baguette, V.M. Stevens, M.A. Leibold, C. Turlure, and M.B. Bonsall. 2015. Life history traits, but not phylogeny, drive compositional patterns in a butterfly metacommunity. *Ecology*, **95**, 3304–3313.

Shahabuddin, G., and C. A. Ponte. 2005. Frugivorous butterfly species in tropical forest fragments: Correlates of vulnerability to extinction. *Biodiversity and Conservation*, **14**, 1137–1152.