UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de Doutorado

Dispersão, processo chave para modular a dinâmica ecológica e evolutiva de borboletas

Dirleane Ottonelli Rossato

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Federal do Rio Grande do Sul como um dos pré-requisitos para obtenção do título de Doutora em Ciências – ênfase em Ecologia.

Orientador: Prof. Dr. Leandro da Silva Duarte

Co-orientador: Prof. Dr. Cristiano Agra Iserhard

Porto Alegre, Maio de 2018.

Dispersão, processo chave para modular a dinâmica ecológica e evolutiva de borboletas

Dirleane Ottonelli Rossato

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Federal do Rio Grande do Sul como um dos pré-requisitos para obtenção do título de Doutora em Ciências – ênfase em Ecologia.

Orientador: Prof. Dr. Leandro da Silva Duarte

Co-orientador: Prof. Dr. Cristiano Agra Iserhard

Banca examinadora:

Prof. Dra Helena Piccoli Romanowski

Prof. Dr. Thales Renato O. de Freitas

Dr. Lucas Jardim

Data de defesa pública 28 de maio de 2018.

Pés, para que os quero, se tenho asas para voar?

Frida Kahlo

AGRADECIMENTO

Aos orientadores, Professor Leandro Duarte pela orientação, discussão e proposições investigativas tão interessantes envolvendo sistemas ecológicos e evolutivos complexos, Professor Cristiano pela coorientação e suporte na identificação das borboletas.

Aos colaboradores de cada um dos capítulos da presente tese, vocês foram essenciais para que esta tese contemplasse diferentes aspectos das causas e efeitos do processo dispersivo de borboletas. Ao Dr. Kaminski pelos seus instigantes questionamentos e interessantes perspectivas sobre a evolução dos caracteres, além da grande colaboração em diversas discussões teóricas e filosóficas relacionadas ou não com a presente tese. Ao Dr. Vanderlei pela sua destreza em resolver questões metodológicas e analíticas de maneira tão eficiente e colaborativa. Ao Professor André Freitas por possibilitar o acesso à coleção de borboletas do museu de Lepidópteros da UNICAMP, além da sua intensa colaboração e participação nos diferentes níveis de desenvolvimento do trabalho. À Professora Nicola por ter sido sempre muito presente e proativa enquanto supervisora durante o desenvolvimento do meu doutorado sanduíche, principalmente pelo seu suporte nas etapas de desenvolvimento das bibliotecas genômicas e montagem das sequências.

Aos pesquisadores e colegas que direta ou indiretamente contribuíram com esta tese. Ao Dr. Kaminski, Dr. Jorge e Professor Milton pelas importantes contribuições durante a minha qualificação. Ao Naka pelas intensas discussões e questionamentos ecológicos e analíticos. Ao Murilo por viabilizar os meus primeiros contatos com pesquisadores estrangeiros me auxiliando e instigando sempre a sair da minha zona de conforto. Ao Lucas pelas importantes discussões sobre os processos macroevolutivos. À Taís pela ajuda no delineamento das áreas de coleta e dos aspectos paisagísticos a serem considerados nas coletas. Ao Thiago pela parceria na proposição do projeto Universal CNPq e pelas primeiras saídas a campo. Ao Gavin, Raquel e Mel pelo suporte na etapa de preparação da biblioteca genômica. A Ema e Mel pela ajuda nas primeiras etapas da montagem das sequências genômicas. A Carol (UFRGS), Giovanna (UFRGS) e Érico (INPA) pelas importantes discussões a respeito das filtragens e análises de dados genômicos. À Jacque pelas discussões acerca dos aspectos moleculares. À Ariele pela parceria em ministrarmos juntas a disciplina de Ecologia Evolutiva junto ao PPG Ecologia. Ao Laboratório de *Drosophila* em nome da Professora Vera e da Professora Maríndia pela amizade e por abrirem as portas do laboratório para eu realizar a extração de DNA dos espécimes de borboletas. Ao convite feito pelo Professor Richard ffrench-Constant para escrever sobre a variação na forma da asa em anéis miméticos e que tomou dimensões mais amplas onde revisamos pela primeira vez os distintos filtros bióticos e abióticos atuando sobre a forma da asa dentro de um contexto de anéis miméticos.

Aos laboratórios e orientadores que contribuíram para a minha formação durante toda minha trajetória acadêmica e que possibilitaram que eu transitasse entre diferentes perspectivas ecológicas e evolutivas durante o desenvolvimento da presente tese de doutorado. À Dra. Adriana pela aprendizagem sobre as distintas ferramentas moleculares para avaliar a dispersão de fragmentos de DNA dentro do genoma, pela competência e exemplo de pesquisadora, amiga e profissional que és. À Professora Vera por seu espírito maternal, entusiasmo e renomada pesquisadora que és. À Professora Maríndia pelas discussões, contribuições e auxilios quando necessário. Ao Professor Élgion pelos primeiros contatos com a genética e com os elementos de transposição em *Drosophila* durante minha passagem pela UFSM. Ao Professor Ruiz pelas discussões e valiosas contribuições. Ao Professor Gilson pela aprendizagem sobre os aspectos eco-morfológicos e evolutivos de borboletas. À Dra. Gislene pelos primeiros contatos com estudos de genética de populações. Ao Professor Fornel e a Dra. Danessa pela aprendizagem quanto aos aspectos morfológicos da variação na forma da asa em borboletas.

Ao Centro Ecológico de Dom Pedro de Alcântara, Rio Grande do Sul, no nome do Professor André Gonçalves, do Técnico Cristiano e da Dona Marlene, que viabilizaram o contato com os agricultores e a estadia da equipe para desenvolver um dos capítulos da presente tese. Aos agricultores, suas esposas e família que gentilmente cederam suas áreas para realizarmos o trabalho, em nome de: Valdeci, Jorge, Lucas, Toninho, Adroaldo, Jacó, Arlindo, Zeca, Tiríva e Dirceu. Ao Honório que sempre foi muito prestativo para viabilizar minha estadia na Cachoeira dos Borges e tornou-se um grande amigo. Aos ajudantes de campo que foram essenciais para as coletas e possibilitaram as coletas com bom humor e eficiência: Lucilene, Naka, Andressa, Taína, Ane, Nicolas, Júnia, Marina, Luan, Lucas, Wilian e Nádia.

Ao pessoal da UNICAMP pela acolhida e auxílio para fotografar os espécimes do museu de Lepidópteros da UNICAMP em nome do Professor André Freitas, Júnia, Tamara, Jêssie e Tina.

Às pessoas queridas que o doutorado sanduíche me presenteou, Sabrina, Lídia, Matheus, André, Anusha, Srajan e Sônia.

Às queridas amizades que conheci a o ingressar no PPG Ecologia/UFRGS ainda durante o Mestrado e que são para mim muito especiais, Anaclara, Fernando, Lucilene, Elise e Ane.

Ao Pessoal do LEFF, desde os antigos até os atuais, Vander, Thiago, Gabriel, Luquita, Jaque, Berga, Arieli, Alina, Aline, Elisa e Jorge.

Ao PPG Ecologia por todo o suporte e auxílio, em nome das Professoras Sandras e das Secretárias Paula e Silvana. A Dani pela disponibilidade e auxílios para acessar o laboratório de ecologia molecular do PPG Ecologia.

A bolsa de doutorado e doutorado-sanduíche concedida pela CAPES, assim como ao recurso obtido pelo projeto CNPq Universal e recurso proveniente do NERC (Natural Environment Research Council) da Universidade de Sheffield, os quais viabilizaram as saídas a campo e sequenciamentos de amostras moleculares durante o período do meu doutorado sanduíche.

Aos amigos de fora da academia sempre tão queridos e presentes, em nome da Anelise e da Miche. Ao BIOLAR sempre tão cheio de mulheres maravilhosas, Ana Paula, Fernanda (mana), Rosângela, Crisla, Taína, Mariana, Fernanda, Itxaso, Giordana e Luty.

À minha família, vocês são minhas raízes mais profundas e minhas asas mais livres e coloridas, obrigada por todo o suporte de sempre, ele foi fundamental para que esse processo de quatro anos fosse agradável e bem vivido. Aos agregados, vocês tornam a nossa família melhor, completa e divertida.

RESUMO

A dispersão é o resultado da interação de um conjunto de atributos do indivíduo com o meio o qual pode ser moldado ao longo do tempo recente ou mais profundo. A partir disso, diferentes cenários ecoevolutivos podem ser inferidos. Adicionalmente, a maneira como as espécies dispersoras interagem com o meio pode afetar as dinâmicas ecológicas e evolutivas da própria espécie e as interações com outras espécies. Portanto, a dispersão pode ser causa, mas também consequência de processos ecoevolutivos. Sendo assim, a presente tese se propõe investigar como distintas dinâmicas ecológicas e evolutivas relacionadas ao processo de dispersão de borboletas afeta e é afetada por diferentes processos. Para avaliar isso, atributos morfológicos e sequências genômicas foram utilizadas para avaliar a dispersão a nível micro e macroevolutivo. Quanto às estruturas morfológicas, a asa é o principal atributo morfológico que pode ser utilizado em associação com outras estruturas para predizer a capacidade dispersora. Somado a morfologia, a informação genética também prediz dispersão através do compartilhamento de sequências genéticas semelhantes entre distintas populações. Neste sentido, a presente tese se propõe a avaliar os efeitos e as consequências de diferentes processos que atuam sobre os atributos morfológicos dispersivos, mas também as consequências da capacidade dispersora sobre os processos de diversificação de Nymphalidae. Para isso, estruturamos a tese da seguinte forma: (i) Propomos um arcabouço teórico para investigar as principais etapas e filtros que atuam e moldam a forma da asa de borboletas, apontando os estágios de desenvolvimento mais propícios para as modificações, desde os níveis micro até macroevolutivos (Capítulo 1); (ii) Testamos empiricamente o efeito de diferentes filtros ambientais numa escala local e microevolutiva. Distintas dimensões ecológicas foram consideradas, desde o nível de indivíduos e de populações (genômica) como o de comunidades (taxonômica) (Capítulo 2); e, (iii) Testamos empiricamente diferentes modelos evolutivos que poderiam explicar a evolução dos atributos morfológicos relacionados à dispersão. Somado a isso, avaliamos como estes atributos estão afetando processos de especiação e extinção de Nymphalidade (Capítulo 3). Como principais achados desta tese sugerimos o primeiro arcabouço teórico para o entendimento das principais etapas que moldam a forma da asa, sendo o período entre o estágio larval e pré-pupal o mais importante. Empiricamente, encontramos uma simplificação genômica em locais com cultivo convencional de banana. Já num tempo mais profundo de tempo, encontramos que atributos relacionados à dispersão evoluíram por deriva ao longo da história de Nymphalidade e distintos filtros, bióticos e abióticos, podem ter atuando sobre a evolução dos atributos relacionados à capacidade de dispersão, conferindo a eles uma interação diferente com processos de especiação e extinção. Formas da asa mais extremas, alongadas ou arredondadas, estiveram mais relacionadas à maior taxa de especiação, enquanto que a proporção entre volume do tórax e área da asa apresentou uma relação linear inversa à taxa de especiação. Neste sentido, a presente tese contribui para o entendimento da dispersão desde o nível micro até macroevolutivo sugerindo e testando de maneira inédita um arcabouço teórico para o entendimento da variação na forma da asa de borboletas.

Sumário

INTRODUÇÃO GERAL 10

Referências Bibliográficas 18

CAPÍTULO 1 21

More Than Colours: An Eco-Evolutionary Framework for Wing Shape Diversity in Butterflies 21

Abstract 22

Introduction 23

Wing shape and function 26

Evaluating the Wing Shape 27

Ontogenetic drivers 33

Biotic filters 37

Intraspecific Interaction 37

Interspecific Interaction 39

Abiotic filters 43

Macroevolutionary processes 45

Concluding remarks 48

Acknowledgements 52

References 52

CAPÍTULO 2 63

The impact of agroforestry and conventional banana plantations on genomic and species diversity of butterfly 63

Abstract 64

Introduction 65

Material and Methods 68

Sample collection 68

Bioinformatics analysis 71

Effects on genomic individuals information 72

Population structure analysis 73

The genomic and species diversity 74

Results 75

Individual and population genomic level 75

Effects at genomic site information and diversity 77

Discussion 80

Acknowledgements 81

Reference 82
Supplementary material 86
CAPÍTULO 3 102
Macroevolutionary patterns of eco morphological traits related to dispersal in Nymphalidae butterflies 102
Abstract 103
Introduction 104
Material and Methods 106
Species sample 106
Dispersal traits measurements 106
Nymphalidae phylogeny 111
Estimating evolutionary model for dispersal-related traits 111
Diversification in Nymphalidae by dispersal traits 112
Results 113
Morphological dispersal trait relations and evolution 113
Speciation and extinction rate 117
Discussion 120
Conclusions 122
Competing interests 123
Authors' contributions 123
Acknowledgements 123
Funding 124
Reference 124
Supplementary material 130
CONSIDERAÇÕES FINAIS 138

INTRODUÇÃO GERAL

A dispersão é um processo que possibilita o entendimento da ocorrência e da persistência de indivíduos, população e comunidade ao longo do tempo e do espaço. O movimento de um indivíduo a partir de um local de nascimento até um ou sucessivos locais de reprodução (Ronce, 2007) é resultado da integração de fatores genéticos, morfológicos, fisiológicos e, algumas vezes, comportamentais de um indivíduo com o ambiente (Clobert et al., 2009). Essa complexa relação combina características individuais aos efeitos ambientais e pode afetar diferentes escalas espaciais e temporais, desde local até regional, assim como, desde o nível micro até macroevolutivo. Entretanto, além da capacidade de modificar diferentes aspectos ecológicos e evolutivos a dispersão também está sujeita aos efeitos de diferentes pressões seletivas, as quais podem moldar os seus atributos e, por conseguinte, afetar a sua interação com o meio. Sendo assim, a dispersão pode ser causa, mas também consequência dos processos ecológicos e evolutivos (Clobert et al., 2012). Neste sentido, a presente tese propõe um arcabouço teórico a fim de identificar as principais etapas e impactos de diferentes pressões seletivas (bióticas e abióticas) sobre a morfologia da asa de borboletas (Capítulo 1) e posteriormente, testa empiricamente os efeitos de diferentes filtros sobre os processos ecoevolutivos relacionados a dispersão, tanto numa escala micro (Capítulo 2) como macroevolutiva (Capítulo 3).

A caracterização da dispersão frequentemente emprega marcadores genéticos, morfológicos e até mesmo a coocorrência de táxons em lugares distintos, podendo ser especificamente inferida por meio da (i) ocorrência de variação alélica em algum gene associado à dispersão; (ii) alta similaridade genética entre indivíduos pertencente a distintas populações, predizendo alta intensidade de fluxo gênico; (iii) utilização de medidas morfológicas relacionadas a intensidade de movimento; e (iv) coocorrência das mesmas espécies em diferentes comunidades. Dentre estas, a morfologia é a maneira mais utilizada para inferir dispersão devido à facilidade de obtenção dos dados e baixo custo. No entanto, o surgimento recente do sequenciamento de Nova Geração (*Next Generation Sequencing – NGS*) revolucionou as investigações evolutivas possibilitando genotipar marcadores SNPs (*Single Nucleotide Polymorphism*) em larga escala para organismos que não possuem genoma de referência. Estas particularidades tornam esse método atraente para estudos de genética de população a nível genômico (Baird et al., 2008) inclusive permite avaliar o fluxo gênico de forma mais refinada. Neste sentido, estudos avaliando a dispersão a nível molecular têm se popularizado. Dentre os diferentes NGS, sequenciamentos de representação reduzida como o RADseq (*Restriction site Associated DNA Sequencing*) tem sido um dos mais frequentemente utilizados, por seu baixo custo e obtenção de um grande banco de dados genômicos que possibilita avaliar de forma mais refinada, eventos de fragmentação e seus impactos sobre a formação das comunidades locais. Considerando que o processo dispersivo pode ser uma característica herdável ou ser moldado pelo ambiente, o entendimento dos aspectos genéticos relacionados a este processo possibilita entender o quanto a variação da dispersão pode estar relacionada aos processos genéticos aditivos ou pode ser explicada por variações ambientais, como, por exemplo, efeitos transgeracionais. Quando moldada pelas condições ambientais a dispersão pode assumir um caráter bastante plástico, e a variação genética pode estar associada ao ambiente (Saastamoinen et al., 2018). Neste sentido, os elementos moleculares seriam sensíveis ao ambiente e estudos epigenéticos, bastante escassos até o momento, poderiam esclarecer o quanto uma característica é herdável ou moldada pelo ambiente (Saastamoinen et al., 2018).

Somado a isso, a busca por sequências moleculares que possibilitem a inferência da capacidade dispersora a nível genômico é importante para entender as dinâmicas ecológicas e evolutivas relacionadas (Saastamoinen et al., 2018). Neste sentido, poderia ser predito a velocidade de evolução de um fenótipo num cenário de mudanças ambientais (Hoffman & Willi, 2008). O qual, por conseguinte, possibilita avaliar os efeitos das mudanças climáticas sobre a demografia dos organismos uma vez que poderia ser inferido o efeito sobre o processo dispersivo (Saastamoinen et al., 2018). Assim sendo, as buscas por sequências genéticas relacionadas à capacidade de dispersão em borboletas têm sido amplamente estudada em “Glanville fritillary” (*Melitaea cinxia*) e a variações alélicas no gene *Pgi* responsável pela enzima celular (Mattila & Hanski, 2014) é o caso mais amplamente descrito na literatura (revisado por Niitepold & Saastamoinen, 2017). Neste sentido, a ocorrência de um alelo específico no gene *Pgi* esteve mais frequente em indivíduos que se estabeleceram recentemente como populações isoladas (Haag et al., 2005). Neste mesmo sentido, Hanski et al. (2004), encontraram que indivíduos que dispersam muito foram encontrados em ambientes mais isolados sugerindo que a dispersão estaria associada com a capacidade de estabelecer novas populações. Analisando os dados obtidos por NGS em *Drosophila melanogaster*, Jordan et al. (2012) encontrou que de 2,5 milhões SNPs apenas 220 estiveram associados a 192 genes que apresentam alguma relação com a capacidade dispersora. Por outro lado, destes, apenas 12 SNPs explicaram cerca de 60% da variação observada em ambos os sexos (Jordan et al. 2012).

Somado a isso, a informação genética possibilita acessar o parentesco entre indivíduos e predizer a ocorrência de reprodução entre indivíduos, que é um processo pré-requisito para a inferência da dispersão. Neste sentido, o compartilhamento de sequências genéticas entre indivíduos que ocorrem em diferentes locais formando distintas populações representam a ocorrência de fluxo gênico. Além disso, a informação genética também pode ser utilizada para inferir a diversidade genética dentro e entre populações de uma determinada espécie. A diversidade genética possibilita avaliar o “status” de conservação das espécies e é, portanto, um fator fundamental para a resposta a diferentes distúrbios, pois, quanto maior a diversidade maior será a possibilidade do organismo responder de maneiras distintas a diferentes impactos, por exemplo. Uma vez que a variação alélica pode resultar em diferenças funcionais no organismo e com isso possibilitar o surgimento de novas saídas frente a eventos catastróficos. Portanto, a informação genética, possibilita a inferência da capacidade de dispersão através da ocorrência de um alelo específico, do compartilhamento de sequências genética entre distintas populações (fluxo gênico), mas também a inferência de diversidade genética.

Comparativamente à informação genética, os atributos morfológicos são bons preditos de mobilidade para borboletas, os quais já são amplamente utilizados. Para insetos os principais atributos utilizados para inferência da dispersão é a relação entre o tórax e a área da asa (carga alar), a forma da asa (acessado mais frequentemente pelo índice *aspect ratio*) e a força muscular do tórax (Van Dyck, Matthysen & Wiklund, 1998; Turlure et al., 2010; Zera & Brisson, 2012). Considerando o nível populacional a capacidade de grande deslocamento através de processos migratórios resulta em diferenças morfológicas quando as populações migratórias são comparadas com as sedentárias. Por exemplo, indivíduos de uma população de *Danaus plexippus* com comportamento migratório apresentaram asas mais alongadas e maiores do que indivíduos de populações residentes (Satterfield & Davis, 2014).

Por outro lado, considerando os aspectos da dispersão numa perspectiva de comunidade, estudos que consideram a relação entre a informação genômica aos aspectos morfológicos da assembleia são mais escassos. Neste sentido, as comunidades são frequentemente preditas por uma medida morfológica média para a comunidade. Neste sentido, a formação de comunidades locais é resultado da interação de diferentes dimensões ecológicas entre elas a dispersão, a heterogeneidade ambiental e a equivalência das espécies. Entretanto, o papel do processo dispersivo para a formação das comunidades locais ainda permanece pouco compreendido (Logue et al., 2011) evidenciando a importância em compreender esse processo numa perspectiva de ecologia de comunidade integrada a estudos de outros níveis ecológicos e evolutivos. Neste sentido, diferentes escalas evolutivas e espaciais podem ser utilizadas para entender melhor os aspectos que modulam a dispersão. Levando em consideração escalas locais, as alterações de habitats gerada pelo uso e ocupação da terra são uma das principais forças que afetam a dispersão de borboletas as quais funcionando como barreiras (Enfjäll & Leimar, 2009) e influenciam a sobrevivência dos indivíduos. Sendo assim, podemos avaliar os impactos tanto a nível populacional quanto de comunidade através das informações morfológicas e evolutivas nestes níveis, os quais podem estar associados entre si ou não.

Neste sentido, um dos biomas brasileiros propícios para a melhor compreensão empírica do papel e do efeito da dispersão e dinâmica ecoevolutivas em borboletas é a Mata Atlântica, pois, é uma área altamente impactada pelo uso e ocupação do solo (Ribeiro et al., 2009). A expansão da agricultura e da pecuária figuram entre os principais fatores para a perda da biodiversidade nestes locais, apresentando alto nível de fragmentação (Ranta et al., 1998; Costa & Futemma, 2006). Dentre os principais cultivos a plantação de banana é a maior influência agrícola no nordeste do Rio Grande do Sul. Os bananais foram introduzidos durante os anos de 1960 através de um sistema convencional (produção de banana com pesticidas). Entretanto, desde 1991, vários produtores de banana se adaptaram a não utilização de pesticidas e passaram a cultivar banana orgânica (sem utilização de químicos). Posteriormente, associado a esse sistema, um sistema mais complexo de cultivo agrícola foi incorporado nas áreas, onde a produção de banana foi realizada conjuntamente com a plantação de outras espécies da fauna. Esse sistema que visa uma maior aproximação estrutural e de biodiversidade com a área nativa original, recebe o nome de sistema agroflorestal.

Além da fragmentação ambiental, que pode afetar a dispersão de diversas espécies, a incorporação de estudos que avaliam o cultivo agrícola com utilização de agrotóxico ou plantio feito através de um sistema agroflorestal, possibilita acessar os efeitos dos químicos ou do cultivo agrícola sobre a biodiversidade, mais especificamente sobre a dispersão e a composição genética de borboletas. Portanto, um conjunto de áreas formadas por cultivo convencional e sistema agroflorestal podem ser comparados a áreas nativas, as quais possibilitam avaliar o impacto dos cultivos e dos agrotóxicos sobre a biodiversidade. Somado a isso, a área de estudo possibilita testar diversas questões teóricas e aplicadas sobre as dinâmicas ecológicas e evolutivas. Principalmente, considerando que o consumo de agrotóxicos no mundo aumentou consideravelmente nos últimos anos e o Brasil está entre os cinco maiores consumidor de inseticidas (dos Santos et al., 2018). Neste sentido, estudos que avaliam os efeitos da utilização de agrotóxicos em cultivos agrícolas atuando sobre a biodiversidade são extremamente importantes levando em conta o crescente estimulo para liberação de mais agrotóxicos no Brasil a partir de proposições de projeto de leis (PL), como a número 6299/02, chamada de PL do veneno que podem comprometer a biodiversidades e diversos serviços ecossistêmicos.

Adicionalmente a isso, e considerando uma perspectiva em escala temporal maior, podemos investigar como a dispersão, acessada por atributos morfológicos, evoluiu e afetou processos de diversificação. A utilização de atributos associados a informações filogenéticas, através dos métodos comparativos, possibilita a inferência de fatores que podem ter moldado a evolução do atributo ao longo do tempo. Considerando que distintas forças evolutivas atuam nesta escala maior de tempo, diferentes cenários ecoevolutivos podem ser inferidos para explicar a evolução do atributo. Dentre os distintos cenários que podem ser preditos está a estocasticidade, a deriva, a radiação adaptativa ou a seleção direcional. Áreas que incorporam estudos macroevolutivos têm crescido bastante nos últimos anos devido as novas e mais robustas inferências filogenéticas que estão mais refinadas e próximas da realidade. Embora a inferência dos fatores causais ainda seja mais difícil de serem previstos, uma vez que não temos acesso à atuação deste no tempo profundo, algumas possibilidades podem ser previstas.

Ainda numa escala macroevolutiva, a dispersão também pode atuar como causa, afetando distintos processos ecológicos e evolutivos (Clobert et al., 2012). Estudos macroevolutivos possibilitam entender, por exemplo, como a dispersão pode ter influenciado os processos de diversificação de espécies, seja da própria espécie dispersora como de espécies relacionadas. Neste contexto, considerando que a ocorrência da dispersão a nível populacional esteja acompanhada por reduzido fluxo gênico entre distintas populações e que processos de seleção natural afetem a taxa de especiação (Garcia & Trewick, 2014), encontraremos um aumento na taxa de especiação quando as populações permanecem separadas por um longo tempo (Butlin et al., 2009). Um modelo bastante interessante é o da dispersão intermediária, o qual prediz que indivíduos com alta e baixa capacidade de dispersão apresentam uma reduzida taxa de especiação, enquanto que indivíduos com dispersão intermediária apresentaria maior taxa de especiação (Mayr, 1963). Embora, poucos trabalhos empíricos tenham encontrado esse padrão, o uso de dados contínuos para inferência da intensidade de dispersão pode ser utilizado para avaliar essa hipótese. Tentando explorar essa perspectiva, alguns trabalhos avaliaram essa hipótese, mas acabaram por encontrar uma relação linear inversa entre capacidade de dispersão e taxa de especiação (Claramount et al., 2011). Claramount et al. (2011) não corroboram a dispersão intermediária relacionada a alta taxa de especiação, mas encontraram uma relação inversa do valor da dispersão com a taxa de especiação, estudos nesta linha possibilitam esclarecer muitos aspectos ecoevolutivos ao longo do tempo mais antigo. Estudos que avaliam a dispersão considerando uma perspectiva macroevolutiva possibilitam inferir os efeitos da dispersão sobre os processos de diversificação, os quais podem ser usados em associação as estratégias de conservação e manutenção das espécies e da biodiversidade de borboletas.

Neste sentido, insetos são bons organismos para responder às alterações e pressões seletivas devido à forte relação com o meio, portanto, representam um grupo importante para avaliar as causas e efeitos da dispersão sobre diferentes escalas evolutivas (Dikötter et al., 2007). A presente tese foca-se no estudo de borboletas como organismo escolhido para responder diversas questões ecoevolutivas. De modo especial as borboletas, frequentemente utilizadas como organismo de estudo, refletem algumas características peculiares incluindo a sua íntima associação com o ambiente onde vivem, através de uma relação estreita com recursos e fatores bióticos e abióticos em diversas escalas, desde micro hábitats até grandes paisagens (Machado et al., 2008; Freitas & Marini-Filho, 2011). Dentre os diferentes grupos de borboletas, Nymphalidade é uma família de ampla distribuição, é a mais diversificada em termos de hábito e morfologia, e apresenta o maior número de espécies conhecidas (Duarte et al., 2012).

A presente tese foca principalmente no processo de dispersão, como causa e consequência de dinâmicas ecológicas e evolutivas, tanto a escala micro até macro evolutiva foi investigada. Embora, a dispersão seja o cerne central outras informações biológicas como a diversidade genômica e de espécies também foram incorporadas, assim como efeito de diferente uso e ocupação do solo. A dispersão na presente tese foi acessada através de medidas morfológicas, filogenéticas e genéticas. Finalmente, estruturamos a tese em três capítulos, os quais tentam explorar aspectos micro e macroevolutivos da dispersão. Primeiramente, realizamos uma ampla revisão e propomos um arcabouço teórico e depois com dois estudos empíricos, um numa escala micro e outro macroevolutivo.

Os capítulos são:

- CAPITULO 1. Mais do que cores: uma abordagem ecológica evolutiva para a diversidade de formas de asa em borboletas

(Capítulo em fase de impressão, volume 54 do livro Advances in Insect Physiology)

A asa é uma estrutura fundamental para o movimento de organismos alados. Considerando isso, realizamos uma ampla revisão sobre os principais fatores (bióticos e abióticos) que afetam a forma da asa de borboletas. O impacto destes fatores foi avaliado levando em conta as principais etapas de desenvolvimento das borboletas, onde a variação morfológica estaria mais suscetível às pressões ecológicas e evolutivas. Para isso diferentes níveis evolutivos foram considerados desde o nível micro até macroevolutivo. A ocorrência repetida de alguns processos microevolutivos ao nível de indivíduo, população e comunidade possibilita identificar padrões macroevolutivos, se considerarmos estudos num tempo maior, macroevolução. Neste nível, é possível inferirmos a ocorrência de pressões seletivas que moldaram a morfologia ao longo da evolução. Dentre os fatores bióticos que estariam moldando a forma da asa, a formação de anéis miméticos através da convergência morfológica para um determinado padrão, foi uma das pressões seletivas consideradas.

- CAPÍTULO 2. Impactos de sistema agroflorestal e convencional de cultivo de banana sobre diversidade genômica e de espécies de borboletas

(A ser submetido à *Journal of Applied Ecology*)

Adentrando os aspectos microevolutivos, avaliamos de maneira empírica como os diferentes tipos de cultivo de banana estariam moldando a informação genômica dentro de uma espécie e a diversidade taxonômica de espécies de borboletas. Para isso, acessamos a informação genômica e dispersiva de uma espécie amplamente distribuída dentre as áreas amostradas*, Heliconius ethilla narcaea*. Utilizamos a informação molecular para inferir fluxo gênico e diversidade, a fim de avaliar se os indivíduos estão estruturados em populações genéticas e acessar se a diversidade genômica das populações é afetada pelos diferentes tipos de cultivos agrícolas. Investigamos também se a diversidade taxonômica de espécies responde aos diferentes cultivos e se está correlacionada à diversidade genômica. Como principal achado, encontramos uma simplificação genômica em locais de cultivo convencional em comparação as borboletas de cultivo agroflorestal e área nativa.

- CAPÍTULO 3. Processos macro evolutivos de dispersão sobre a diversificação em Nymphalidae

(A ser submetido à *Proceedings of the Royal Society B: Biological Sciences*)

Avaliamos as causas e as consequências do movimento dispersivo na família Nymphalidae. Para isso a capacidade de dispersão foi inferida através de diferentes medidas morfológica e avaliada numa escala macroevolutiva. A dispersão como consequência, ou seja, sendo moldada ao longo do tempo profundo, foi acessada por modelos evolutivos. Por outro lado, considerando a dispersão como fator causal, ou seja, a capacidade de moldar processos ecoevolutivos, foi avaliada através de dois métodos distintos. Os atributos morfológicos evoluíram principalmente por deriva, os quais apresentaram uma relação diferente com processos de especiação e extinção, provavelmente relacionadas às distintas características do processo dispersivo, como a velocidade e a desempenho de vôo.

Referências Bibliográficas

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers, *PLoS One* 3:e3376.

Butlin RK, Galindo J, Grahame JW (2008) Sympatric, parapatric or allopatric: the most important way to classify speciation? Philosophical *Transactions of the Royal Society B: Biological Sciences*, **363**, 2997–3007.

Clobert J, Le Galliard JF, Cote J, Meylan S, Massot M (2009) Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters* **12**: 197–209.

Clobert J, Baguette M, Benton TG, Bullock JM (2012) Dispersal Ecology and Evolution, 2012.

Costa RC, Futemma CRT (2006) Racionalidade com Compromisso: Os Assentados do Ribeirão Bonito (Teodoro Sampaio-SP) e o Projeto de Conservação Ambiental. *Ambiente & Sociedade*, v. 9, p. 128-148.

Diekötter T, Haynes KJ,Mazeffa D, Crist TO (2007) Direct and indirect effects of habitat area and matrix composition on species interactions among flower-visiting insects. *Oikos*, **119**: 1588-1598.

dos Santos CF, Otesbelgue A, Blochtein B (2018) The dilemma of agricultural pollination in Brazil: Beekeeping growth and insecticide use. *PLOS ONE*, **13**: e0200286.

Duarte M, Marconato G, Specht A, Casagrande MM (2012) Lepidoptera. In: Rafael JÁ, Melo GAR, Carvalho CJB de, Casari AS, Constantino R(Ed.). Insetos do Brasil: diversidade e taxonomia. Ribeirão Preto: Holos. 625-682.

Enfjäll K, Leimar O (2009) The evolution of dispersal – the importance of information about population density and habitat characteristics. *Oikos*, **118**: 291-299.

Freitas AVL, Marini-Filho OJ (2011) Plano de Ação Nacional para a Conservação dos Lepidópteros Ameaçados de Extinção. Brasília*: Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio).124 pg.

Garcia-R, JC, Trewick, SA (2015). Dispersal and speciation in purple swamphens (Rallidae: Porphyrio). *The Auk*, **132**: 140–155.

Haag CR, Riek M, Hottinger JW, Pajunen VI, Ebert D (2005) Genetic diversity and genetic differentiation in *Daphnia* metapopulations with subpopulations of known age. *Genetics* **170:** 1809–1820.

Hanski I, Erlahti C, Kankare M, Ovaskainen O, Sirén H (2004) Variation in migration rate among individuals maintained by landscape structure. *Ecology Letters* **7**: 958–966.

Jordan KW, Carbone MA, Yamamoto A, Morgan TJ, Mackay TFC (2007) Quantitative genomics of locomotor behavior in Drosophila melanogaster. *Genome Biology*, **8:** (8).

Logue JB, Mouquet N, Peter H, Hillebrand H, The Metacommunity Working Group (2011) Empirical approaches to metacommunities: a review and comparison with theory. *Trends in Ecology & Evolution*, **26**: 482-491.

Machado AB, Drummond GM, Paglia AP (2008) Livro vermelho da fauna brasileira ameaçada de extinção. Brasília: *Ministério do Meio Ambiente*. 1420 pg.

Mattila ALK (2015). Thermal biology of flight in a butterfly: genotype, flight metabolism, and environmental conditions. *Ecology and Evolution* **5:** 5539–5551.

Mayr E (1963) Animal species and evolution. Cambridge, MA: Belknap Press.

Niitepõld K, Saastamoinen M (2017). A candidate gene in an ecological model species: phosphoglucose isomerase (*Pgi*) in the Glanville fritillary butterfly (*Melitaea cinxia*). *Annales Zoologici Fennici* **54**: 259–273.

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, ***142*:** 1141–1153.

Ranta P, Blom T, Niemelae J, Joensuu E, Siitonen M (1998) The fragmented Atlantic rain forest of Brazil: size, shape and distribution of forest fragments. *Biodiversity and Conservation*, v. 7, p. 385-403.

Ronce O (2007) How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology Evolution and Systematics*, **38**: 231–253.

Saastamoinen M, Bocedi G, Cote J, Legrand D, Guillaume F, Wheat CW, Fronhofer EA, Garcia C, Henry R, Husby A, Baguette M, Bonte D, Coulon A, Kokko H, Matthysen E, Niitepõld K, Nonaka E, Stevens VM, Travis JMJ, Donohue K, Bullock JM, del Mar Delgado M (2018), Genetics of dispersal. *Biological Review*, **93**: 574–599.

Satterfield DA, Davis AK (2014). Variation in wing characteristics of monarch butterflies during migration: Earlier migrants have redder and more elongated wings. Animal Migrator, **2**: 1–7

Singer MC, Thomas CD (1996) Evolutionary responses of a butterfly metapopulation to human- and climate-caused environmental variation. Am Nat, 148:S9–S39.

Turlure C, Schtickzelle N, Baguette M (2010). Resource grain scales mobility and adult morphology in butterflies. *Landscape Ecology*, 25: 95–108.

CAPÍTULO 1

More Than Colours: An Eco-Evolutionary Framework for Wing Shape Diversity in Butterflies

Dirleane O. Rossato[1], Lucas A. Kaminski[1], Cristiano A. Iserhard[2], Leandro Duarte[1]

[1] Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

[2] Universidade Federal de Pelotas (UFPEL), Pelotas, Rio Grande do Sul, Brazil

---

Capítulo do livro Advances in Insect Physiology

Abstract

Wings are key innovations that have revolutionized the life of winged organisms and have moulded terrestrial ecosystems through new interactions (e.g. pollination). Among flying organisms, butterflies draw attention for their astonishing diversity of colour patterns, but their wings are much more than colours. The wing morphology may vary for different reasons, including communication. Thus, the different factors, acting together or under conflicting ways, moulded the wing shape. Mimicry rings are promising model systems for evaluating the evolution of wing shapes since trait convergence for mimicry is an identifiable force among others selective pressures. An eco-evolutionary framework can help us disentangle the relative role of each step in the evolution of wing shapes. Biotic and abiotic filters can affect wing morphology through pathway changes during the ontogeny, including sexual dimorphism, developmental trade-offs, nutritional food quality and environmental conditions. During the adult stage, both sexual and natural selection determine the combination of genes that are selected and will persist for future generations. All these selective pressures acting repeatedly over the time must result in different macroevolutionary scenarios of traits, like as genetic drift, vicariance, adaptive radiation and speciation. Finally, we propose that comparative studies on mimicry rings, with closely related species presenting changes in wing morphology according to both environmental and sexual behaviour, including sex-limited dimorphism, can be useful systems to understand the role of selective pressures driving the evolution of wing shapes.

Introduction

Currently, the ability to fly is present only in insects, birds and bats. It is an extraordinary trait that has revolutionized terrestrial ecosystems, encouraging new, wondrous interactions, including, for example, specialized pollination systems (Regal, 1977). The wing is the key innovation for winged organisms that allows for travel through open-air offering solutions for many organismal demands (Alexander, 2015). Movement through the air favoured the exploration within and among different habitats, the search and exploration of new adaptive zones, the expansion of the distribution range across large geographical regions and the transposition of high elevations and to fly through the long distance between seasons (e.g. DeVries et al., 2010; Greenslade et al., 1999; Johnson, 1969). Flight-related traits should be important in determining fitness, as the ability to take flight improved foraging efficiency, territorial defence, mate search, escape of predation and avoidance of inadequate conditions (Grimaldi and Engel, 2005; Norberg, 1990). Besides the primary flying function, the wing can operate with communication signals, acting in sexual and territorial displays, in cryptic (camouflage) or warning defence (Batesian and Müllerian mimicry), in thermoregulation and other behaviours (e.g. Baker, 1972; Betts and Wootton, 1988; Davies, 1978; De Keyser et al., 2015; Kingsolver, 1985; Papageorgis, 1975). Studies underlying different functional aspects in wings have allowed for a greater understanding of both the history and the selective pressures throughout the evolution of winged animals. In this review, we will use the concept of functional traits from Violle et al. (2007) in which the trait could be what impacts fitness indirectly on growth, reproduction and survival. Wing size and shape, for example, result from interactions between adaptive process, phylogenetic history, developmental constraints, as well as on the macroevolution scale (Bai et al., 2012; Chazot et al., 2016; Grimaldi and Engel, 2005). Thus, studies that look for the relationships between trait functionality and adaptation under micro- and macroevolutionary scales may allow us to understand different selective pressures and constraints on ecomorphological traits (Fig. 1). Simultaneously, we could come closer to learning the true history of wing shape variation as a result of different processes across time.

One promising winged group that could help to evaluate micro- and macroevolutionary process are the butterflies. As noted by Bates (1864), the relevance of these insects for Biological Science would one day be recognized by the adaptive messages “written” on the expanded wing surfaces of butterflies. Surely, Bates wrote this in the context of his recent discoveries on the adaptive significance of wing colour pattern convergence—Batesian mimicry (Bates, 1862). Today, butterflies are model organisms in evolutionary biology, including genetics, physiology, development and ecology (Beldade and Brakefield, 2002; Kronforst and Papa, 2015). This group is closely related with the environment where it lives, having a high association with biotic and abiotic resources and factors at different scales, from micro-habitats to large landscapes (Bonebrake et al., 2010; Brown, 1997; Freitas and Marini-Filho, 2011). Therefore, butterflies could respond to natural or anthropogenic changes in a short period of time and thereby help us to understand eco-evolutionary processes, such as different spatial scales, time, intensity and frequency of these influences. The selective pressure on butterflies can affect different aspects. Thus, they also can be used to explain the effects of these changes on growth, reproduction and survival. Wing shape is one of these remarkable traits that can uncover the history of the sexual selection, dispersion, flight type, speciation and diversification. All of these processes can evolve in conflict with morphological convergence to mimicry rings (Srygley, 1994). In this case, the similarity to one pattern is dependent on the place where species distantly related occur. Therefore, distinct convergent patterns occur in others place. The mimicry ring act strongly shaping the morphological trait, therefore, its power could compete with other forces evolutionary.

The micro- and macroevolutionary processes could be understood by looking for DNA sequences; however, in microevolution the change occurs with gene frequency within populations and over short periods of time. On the other hand, for macroevolution, the objective is at the species level, and in order to understand this, we need look to the tree of the related species accessing the diversity of the clade (Fig. 1). Using phylogenetic methods, we can investigate how wing traits change over time considering how geographic or other ecological factors (microhabitat use and sexual selection) and macroevolutionary processes (shared ancestry) act on wing evolution.

Recently, there is an increasing in studies on macroevolution approach due to the advent of robust and almost complete phylogenetic hypothesis based on several marks and molecular clocks, calibrated with fossils for dating with confidence (Cadotte and Davies, 2016). These advances have made it possible to understand the tempo and mode of evolution for several living species/lineages. This emergence of analytic tools makes it possible to evaluate how morphological traits evolved and propose some selective pressure that could have acted under it. In this view, it is important to consider the different functions associated with both the traits, the historic and developmental constraints. Integration between the functional and macroevolution approaches can clarify how forces operate upon the evolution of traits. Seeing how selective pressures affect the wing shape variation on the mimicry ring contexts was described by Srygley (1994) as one possible solution for finding evidence of convergence on wing shape between comimetics, separating one from the other effects. Moreover, it is important to choose appropriate tools, since the non-convergence in the shape of the wing (Srygley, 1994), may be the result of inadequate tools. For butterflies, this is an emerging avenue of research, and many recently published papers addressing the evolution of wing shapes and its relation to environmental transitions and mimicry (e.g. Cespedes et al., 2015; Chazot et al., 2016; DeVries et al., 2010; Jones et al., 2013; Merot et al., 2016; Rossato et al., 2018). In this review, we present the recent advances in the study of wing shapes and discuss the different aspects that act on wing morphology in butterflies, taking into account: (i) ontogenetic drivers (Fig. 1, step A); (ii) impact of the biotic and abiotic filters (Fig. 1, steps A–C); and (iii) the macroevolutionary process (Fig. 1, step D). Finally, we present an eco-evolutionary framework identifying what is potentially driving the evolution of butterfly wing shape diversity.

Wing shape and function

The primary function of the wing is locomotion. In this sense, much of the wing shape studies are focused on flight physics, material properties and aerodynamics (revised in Wootton, 1992, 2002). Affecting drag or lift, and thus flight behaviour, the wing size and shape are strongly related to physical wing properties (Berwaerts et al., 2002; Chazot et al., 2016; Dudley, 2000; Wootton, 1992). Moreover, wing shape is related to many other ecological functions, as sexual dimorphism and adaptive convergence to mimetic ring (Rossato et al., 2018), being a sound predictor of functional adaptation.

Thus, wing shape can be a good proxy of different processes and can be used to answer an array of questions in winged organisms (Johansson et al., 2009).

Evaluating the Wing Shape

Usually wing shape information is widely evaluated by means of the aspect ratio index—proportion between length and wing area (Betts and Wootton, 1988; Bots et al., 2012; Hassall, 2015; Johansson et al., 2009), or through the use of the elliptical Fourier (Ferson et al., 1985; Holwell and Herberstein, 2010; Jones et al., 2013). However, the best way to obtain the shape is by removing the size, rotation and translation information (Bookstein, 1991), though of generalized procrustes analysis (GPA) (Rohlf and Slice, 1990) in geometric morphometrics (GM) (Adams et al., 2004). GM uses a Cartesian landmark coordinates to obtain the shape of the wing set, solving the problems of linear measurement when the same set of linear measurements can be obtained from different shapes (Van der Molen et al., 2007). This technique is widely used in many research areas in biology, including ecology, evolution, development, zoology, botany, taxonomy and even palaeontology.

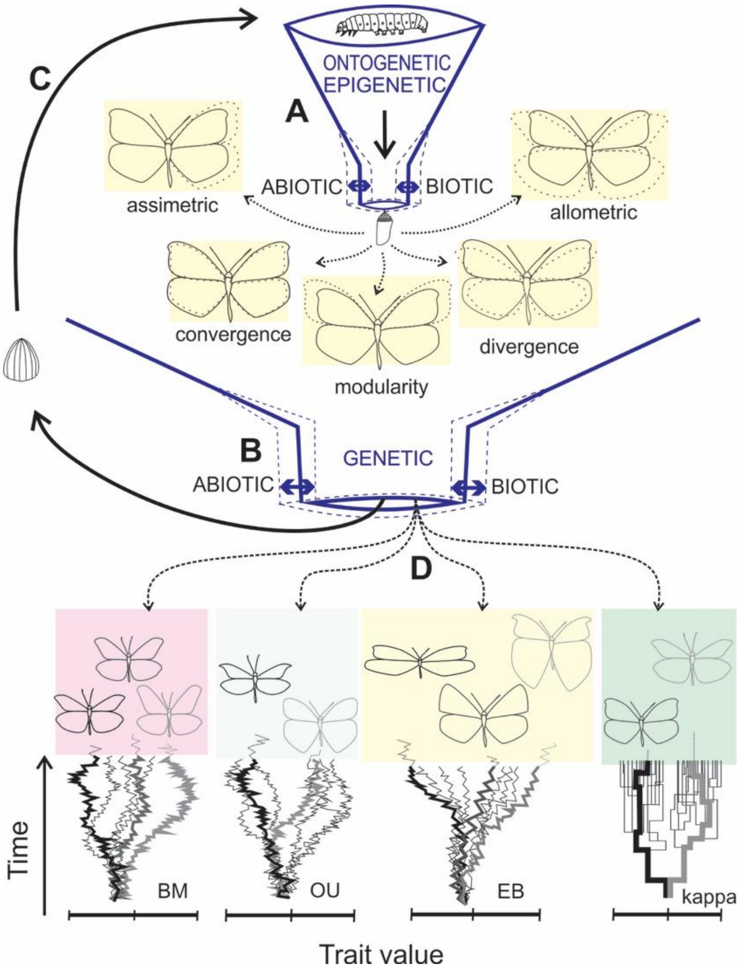


Fig. 1 The eco-evolutionary framework depicted micro- and macroevolutionary processes acting on wing shape diversity in butterflies. At the top, the selective pressures that could act on a small scale of individuals level are represented (steps A, B and C), which with much repeated time could result in macroevolutionary scenarios described by distinct models of evolution (step D). Step 1A starts in the immature stages, which are affected by biotic and abiotic conditions through ontogeny and is the main stage for the epigenetic controls. Arrow inside the filter represents the ontogenetic framework being molded by biotic (e.g. predation) and abiotic (e.g. food quality, environmental conditions) filters. The interaction of these external factors with individuals features may influence the gene expression, also by the epigenetic control. Dotted arrow, after to pupal stage, shows that similar genotypes could result in distinct wing shapes (asymmetry, convergence, modularity, divergence, allometry) depending of the power of the selective forces. Step B starts with adult genetic information to be selected for biotic (e.g. predation) and abiotic (e.g. habitat type) factors, this step ends at the egg stage as result of mating with genetic information selected and survival to predation. Step C is the restart of this cycle of the selections acting on individuals. Step D starts the macroevolutionary process, a consequence of several recurrent cycles of microevolutionary steps (A, B and C) through evolutionary time of lineages. Dotted arrow shows the possibility of distinct evolution models occur, each one reflects different evolutionary hypotheses on the time (BM, Brownian model; OU, Ornstein–Uhlenbeck; EB, early-burst; kappa).

GM analysis revolutionized the way that we study the shape morphology in many organisms, particularly in butterflies. This emergence increases the knowledge to a better understanding of the different selective pressures acting on morphological traits that can be a result of evolutionary history and ecological processes. Thus, for butterflies this method is crucial, since the evaluation of phenotypic plasticity of the wing shape of *Pararge aegeria* (Breuker et al., 2010), to study biogeographic scenario including cryptic species combined with genetic markers to explain the relationship between historical dispersal and morphological variation (Dincă et al., 2011). In mimetic *Heliconius* butterflies, Jorge et al. (2011) evaluated the wing variation of *Heliconius erato* rearing on distinct host plant species, and Jones et al. (2013) evaluated the wing shape variation and convergence in a complex of mimicry butterflies. Also, Merot et al. (2016) evaluated the phenotypic resemblance between comimics in different localities with varying species composition. Finally, Rossato et al. (2018) evaluated the resemblance of the wing and red band between the spatial cooccurrence of comimetic species.

Allometry

The morphological variation in organism bodies is integrated (Olson and Miller, 1958). The study of the different growth rates between parts of the individual and the corresponding impact of these changes under the perspective of ecology and evolution is known as allometry (Huxley and Teissier, 1936). Allometric variation can be evaluated at different levels, between individuals of the same population and the same stage of development (static), between different stages of development (ontogenetic) and throughout the evolutionary history (phylogenetic) (see Cock, 1966;

Klingenberg and Zimmermann, 1992; Moreira et al., 2017). Rossato et al. (2018) used the allometric index to understand differences between the red band and wing size in males and females in some butterfly species in a mimetic ring. Furthermore, we can also call allometry the relationship between size and shape since these parts must vary in a coordinated manner, change in size will result change in shape, so that each organism forms a completely functioning whole (Cheverud, 1996; Olson and Miller, 1958). The GPA of GM removes the isometric effect, but not the allometric relationships. Thus, this approach does not consider the changes in shape when the size has changed. In a critical review, Outomuro and Johansson (2017) compared different studies on GM analysis and the relationship between shape and size, and found that size explains 88% of shape variation, suggesting the importance in incorporating the size in order to understand shape variation. In addition, Outomuro et al. (2013) studying wing shape allometry and aerodynamics in calopterygid damselflies suggest that the relationship between wing shape and size is taxa-specific and differs among closely related lineages.

Still, to analyse wing shape and the factors that impact it, the allometry is probably the best way to assess the potential contribution of size on the shape variation. This inclusion will not only be more informative and will enable a better understanding of the relationship between shape and size (Outomuro and Johansson, 2017), but also the variation on the growth rate between different parts of butterflies related to different selective pressures.

Modularity

Another important aspect of the wing shape is the modularity: the idea that some parts or regions (modules) of a structure are internally integrated by interactions among traits but are relatively independent from other modules (Klingenberg, 2008). The role of developmental and genetic integration in evolution is contentious. One hypothesis states that integration acts as a constraint on evolution, whereas an alternative is that developmental and genetic systems evolve to match the functional modularity of organisms (Klingenberg et al., 2010). This question was studied by Klingenberg et al. (2010) who examined morphological structures using GM in the cricket wing where developmental and functional modules are discordant, making it possible to distinguish between the two alternatives. The patterns of genetic, phenotypic and developmental integration were clearly similar, but not identical. In this sense, the hypothesis that genetic and developmental integration evolve to match functional modularity can be rejected (Klingenberg et al., 2010), on this case. Fore and hind wings originate from distinct imaginal discs, so it is expected that wings are developmentally autonomous. These two pairs of wings constitute two developmental modules in which groups of traits evolve relatively independently of each other (Breuker et al., 2006), that is, different wings can play different functions in flight (Grodnitsky et al., 1994). To understand the integration of wings and the extent to which it relates to ecology is a fundamental question for understanding the evolution of butterfly wings. We can assume different functions during flight may have led to a different pattern of wing size and drive diversification (Klingenberg et al., 2001). It is known that forewings drive flight in butterflies (Dudley, 2000) and when the hind wings are removed, the butterfly is still able to fly, so hind wings do not contribute to normal flight (Jantzen and Eisner, 2008), suggesting that hind wings can be related to increase flight speed and manoeuvrability. Therefore, Chazot et al. (2016), while evaluating shapes using morphometric tools, found that both fore and hind wings were similarly affected by microhabitat, both were more elongated in canopy and more narrow rounded in understory, even though they have different involvements in flight. Moreover, they found that wings are more integrated rather than modular, showing similar functionality. The combination of fore and hind wings can change in different microhabitats, and this disappears when the phylogeny effects are controlled. The covariation of fore and hind wings is the result of functional constraints driven by microhabitat; it cannot be distinguished from a neutral phylogenetic signal (Chazot et al., 2016). Focusing on the degree of modularity of different regions of the wings and across fore and hind wings at various levels (intra- vs interspecific, Klingenberg, 2009), should provide future insight into the developmental and functional integration of butterfly wings (Allen et al., 2008; Frankino et al., 2007). Additional to this, could be interest to see the modularity into the same wing, for understand how the veins and parts of the wing answer for different environmental conditions, and for different selective pressures, to mimicry butterflies, could getting the aspect of belonging to ring mimic, like the red band shape in *Heliconius* postman, compared with whole wing shape.

Phylogenetic Relationships

The differences in wing shapes analysed in a phylogenetic context can clarify many functional aspects, since the species are not independent entities. Consequently, the observed traits can be biased due to shared ancestry rather than evolutionary differences (Harvey and Pagel, 1991). In other words, wing shape can be a phylogenetic constraint and not moulded by interaction with the environment. Thus, when we intend to understand whether the change in wing shape is a result of the interaction with ecological and evolution processes, it is important to consider the relationship between the phylogeny and traits, since the trait values can have a high phylogenetic signal and not be the result of the selective pressure on the trait, but of shared evolutionary history. Thus, incorporating phylogenetic correction can help us to understand the evolutionary history and ecological processes on trait evolution (Blankers et al., 2012; Magurran, 2004). Looking at the phylogenetic analysis acting upon the community context, the taxa that are more phylogenetically distant in their composition will have greater phylogenetic diversity in relation to the other community that has closely related taxa (Sobral and Cianciaruso, 2012). This diversity of evolutionary scenarios can reflect trait diversity when these traits evolve for drift, without selective pressure. However, to evaluate if some selective pressure acting on wing shape is important to remove the effect of shared evolutionary history from the trait.

Ontogenetic drivers

Understanding the origin of morphological variation is the main goal of evolutionary developmental biology (evo-devo). Derived from a precursor group of 30 cells that invaginates from the embryonic ectoderm, the butterfly wing is a morphological structure with quantitative multivariate change both at intraspecific and interspecific levels (Houle et al., 2003). Specifically, the wing shape can show some parts with strong patterns of covariation (Klingenberg and Zaklan, 2000), whereas others are independent (Weber, 1992). The shape of the wing discs changes throughout their growth phases in a species-specific pattern. These changes, in the aforementioned instance, must be related to changes in cell processes such as proliferation, shape change, movement and death. Additionally, the wing shape could be affected by genes with prominent developmental roles outside the wing, such as the transcription factors invected, engrailed and scalloped, as well wingless, decapentaplegic and vestigial (Baena-Lopez and Garcia-Bellido, 2006; Carreira et al., 2011; Huang and Huang, 2005). In this view, most of the growth and differentiation of the wing occurs during the late-larval, prepupa and pupa stages; and the orientation of division plays a key role in determining organ shape (Gillies and Cabernard, 2011). Therefore, the evolution in the shape of the wing begins with a change in the progressive spatial pattern of the cell division. But, although the differentiation of the vein morphology occurs during the stage of development of the pupa, it is still in the larval stage that the group of cells expresses the vein-specific proteins during the larval phase and its patterns are generally used to characterize the wing shape through GM tools. These stages begin at the molecular level with highly polygenic genetic inheritance making it difficult to attribute a general understanding of variation in wing size and shape to just one gene. Jones et al. (2012, 2013) suggest that the *P* supergene can be involved in the control of the wing shape among morphs in *Heliconius numata*. This genetic information affects the growth and several cellular processes controlled by the patterned distribution of morphogenesis. However, it is difficult to establish a real causal relationship between specific morphogenesis distribution and specific tissue shapes. In this view, about 50% of the transcriptome exhibits changes in expression during the time course of wing development (O'Keefe et al., 2012).

To understand how the wing evolves in butterfly ontogenetic growth, Nijhout et al. (2014) evaluated the growth pattern on the wing, using landmarks, in two distantly related species with a different shape. The authors found the anterior portion of the wing disc was under stress, supporting the idea that cell elongation in this region is due to stretching imposed by growth and elongation of the more posterior portion of the wing. Growth and cell division in lepidopteran wing discs require both ecdysone and insulin signalling (Nijhout and Grunert, 2002; Nijhout et al., 2007). Likewise, the evolution of wing shapes must come about through evolutionary changes in the spatial and temporal pattern of cell division. These changes, in turn, are likely due to the evolution of the spatial arrangement of the hormone response elements that control the forms of cell division and orientation and the mechanical forces (Marinari et al., 2012; Vincent et al., 2013).

The developmental constraints in the caterpillar stage are relevant factors that can affect the adult butterfly wing shape. Caterpillars, generally, have low mobility and generally feed on a single plant during development. The specialized diet of caterpillars is reported to be a limiting factor shaping the distributions patterns in butterflies (Graça et al., 2015) even though adults may be mobile and forage across extensive areas (Marchant et al., 2015). In this view, Jorge et al. (2011) found a variation of size and wing shape among individuals that fed on different plant species, reinforcing the role of plasticity on host–plant use. In fact, the host plant is a paramount environmental factor in the ecology of herbivorous insects, indicating that a large proportion of total morphological variation has an environmental origin (Jorge et al., 2011). Besides that, the biotic factor as the presence of predators can induce the phenotypic plasticity in larvae, causing changes on their morphology without differentiation at the genetic level, inducing one adaptive response to development program (epigenetic control).

Phenotypic plasticity occurs when the same genotype produces different phenotypes in response to varying environmental conditions (Pfennig et al., 2010; Whitman and Agrawal, 2009). Some evolutionary events are assigned to phenotypic plasticity, such as the origin of novel phenotypes, the divergence among population and species, the formation of new species and adaptive radiation (Pfennig et al., 2010). Thus, the environment could act together with genotypes and induce a change in morphology, but it could also be accompanied by a change in behaviour and physiology (Bourdeau, 2009; Cornwallis and Birkhead, 2008; Pfennig et al., 2010). Phenotypic plasticity could promote genetic accommodation and genetic assimilation. Genetic accommodation occurs when mutation or environmental changes result in novel phenotypes which result in an adaptive phenotype through quantitative genetic changes (Suzuki and Nijhout, 2006). On the other hand, when new phenotypes do not change under different environments, in this case, it is genetic assimilation and the trait will be expressed constitutively over evolutionary time (Waddington, 1953). One common kind of phenotypic plasticity in butterflies is polyphenism, when individuals with identical genomes respond to different environmental cues by expressing alternatively developmental pathways (Aubin-Horth and Renn, 2009; Snell-Rood et al., 2010), which results in distinctively different adaptive phenotypes (Pfennig et al., 2010). The study of polyphenism in butterflies has been analysed from the point of view of variation in the seasonal wing colour pattern (e.g. Canfield and Pierce, 2010; Nijhout, 2003; Ruszczyk et al., 2004; Shapiro, 1976), but how wing shapes respond to polyphenism variation is still a poor-studied issue.

Another informative way to understand the genetic and environmental factors acting on wing shapes is analysing fluctuating asymmetry (FA) (Møller and Swaddle, 1997; Palmer and Strobeck, 1986). Knowing that both sides of the body of an individual share the same genome and nearly the same environment, FA results from small random perturbations in developmental processes that take place on the left and right sides of the body (Klingenberg and Nijhout, 1999). If the development of two traits is linked, the same perturbations can have an effect on both of them simultaneously and, hence, can generate a correlation between their asymmetries.

Biotic filters

Direct and indirect interactions between individuals are an important component of biological systems that connect different species in the eco systems (Jordano, 2016). Biotic filters are considered the results of ecological interactions between organisms and are divided into interactions between individuals of the same species (intraspecific) or between individual from different species (interspecific) (Fig. 1).

Intraspecific Interaction

Sexual selection has a relevant influence on the evolution of wing shapes, mainly when wings interact with reproductive behaviour and sexual communication. Due to the reproductive roles of each sex, the divergence in flight and wing shape can be found between females and males. While males spend more time obtaining mates (e.g. defending territories), females typically focus on the search for suitable foraging and oviposition sites. Additionally, females show a typical flight, being slower than males which are more mobile and has a stronger flights for escape from predators (Joron, 2005; Jones et al., 2013). Consequently, the difference in behaviours can reflect on morphological differences because males and females interact differently with biotic and abiotic factors.

Therefore, Chazot et al. (2016) suggest that the existence of strong sexual selection could contribute to wing size and shape variation among sexes. An interesting discovery is that changes in forewing shape were associated with dispersal ability only in females of the Nearctic butterfly *Melitaea cinxia* (Breuker et al., 2007). On the other hand, the aspect ratio and wing centroid measurement showed to be sex-specific in tropical forest Morpho butterflies (DeVries et al., 2010). For this group of butterflies, the wing shape was shown to depend on body size only in females (DeVries et al., 2010), and the aspect ratio proxy is lower in females than males of Nearctic *P. aegeria* (Berwaerts et al., 2002). In contrast, at the community level, Graça et al. (2017b) evaluating the correlation between wing-thorax ratio and forest stratum between sex in a tropical rainforest founded that was stronger in females than in males, suggesting that females a stronger relationship between phenotypic and habitat conditions than males. Moreover, the sexual dimorphism is more accentuated in understory species (Graça et al., 2017b). Taking into account the research on how the colour pattern covaries with both wing size and shape, Chazot et al. (2016) found that forewing shape dimorphism between sexes is higher in species that show strong colour dimorphism, and flight behaviour may be selected jointly with cryptic colouration, thereby influencing both wing shape and wing colour evolution. These studies could will shed light on the extent to which different dimensions of butterfly wings respond independently to different selective pressures or are instead constrained by functional or developmental components. In addition, when females visit different microhabitats, those males and these places have Müllerian mimetic rings; there may be a differential selective pressure on females that converge for Batesian mimicry, generating sex-limited mimicry (Fig. 2). In this sense, sex-limited mimicry is more related to natural selection than sexual selection and depends on the spatial variation of the frequency of aposematic models (Kunte, 2008). This phenomenon seems to be favoured in species that already have sexual dimorphism (Timmermans et al., 2017).

Interspecific Interaction

One interesting system that can be used to evaluate wing shape is the mimicry ring. The convergence between different species for one wing pattern can be the result of a few factors, including toxicity, superficial similarity and cooccurrence with other aposematic species. In addition, to the great diversity in colour and size, convergence of the wing shape at the community level is one very interesting opportunity to evaluate mimicry (Johansson et al., 2009), which can make it possible to understand these factors separately through the resemblance of mimicry rings. Recent studies have unravelled the mechanisms of structural mimetic ring communities, revealing important roles in niche differentiation and speciation processes (see Aubier et al., 2017; Joshi et al., 2017). In Müllerian rings, comimic butterfly species share chemical defence traits which are avoided by predators, imposing strong selective pressure and thus leading to convergence onto a shared warning colour signal (Müller, 1878, 1879). It is important to note that while Müllerian mimicry is a mutualistic interaction, since all members of the ring may have benefits for survival, Batesian mimicry by palatable species is an antagonistic interaction (but see Rowland et al., 2010). Generally, the older or most abundant species in a mimicry ring drives the evolution of phenotypic resemblance in other species (Franks and Sherratt, 2007; Mallet, 1999; Ruxton et al., 2008; Turner, 1977). Thus, the warning signal in diverse communities is strongly frequency-dependent on comimetics (Chouteau et al., 2016; Joshi et al., 2017). Additionally, mimicry benefits are associated with a given warning signal, depending on the relative numbers of prey sampled compared to the available ones, and are usually driven by the most toxic or the most abundant prey species (Mallet and Joron, 1999). The ability of predators to recognize the signals of toxic prey is an important determinant of selection on resemblance in a mimicry system (Ihalainen et al., 2012; Rowe et al., 2004). Ihalainen et al. (2008) found that phenotypic similarity is influenced not only by natural selection favouring accurate mimicry but also by the genetic architecture underlying variation in phenotypes, increasing predation against imperfect mimics.

One trade-off of selective pressure on imperfect mimicry is the ability of the butterflies' escape from the predation (Fig. 1B). Butterflies with longer and larger area wings flew more slowly and were captured more easily by predators when chased (Svensson and Friberg, 2007). On the other hand, the butterflies with shorter and smaller wings tended to fly faster and more erratically and frequently escaped from predators. Graça et al. (2017a) found that tropical butterflies and birds are similar when the body size (functional component) variation is analysed, but this relationship is not detected when looking at taxonomic butterfly species composition. Bates (1862) suggested that mimicry in insects should extend to their physical motions, as should the convergence of the locomotory behaviour as well as colour patterning. Studies that compared the type of movement between two comimetic species and two other phylogenetically closely related species found that the comimetics *Heliconius cydno* and *Heliconius sapho* beat their wings more slowly and with more symmetrical motion than their respective sister species, the comimetics *Heliconius melpomene* and *H. erato* (Srygley and Ellington, 1999). Thus, wing motion appears to be one important component of the overall mimetic signal and may serve to identify mimetics before the predator can see details of the wing pattern (Srygley, 2007). This fact made wing shape variation an important factor for understanding the mimetic ring in addition to the other factors that act on this structure. Additionally, spatial cooccurrence can reinforce the phenotypic resemblance in mimicry rings (Joron and Iwasa, 2005).

Some structures that give extraordinary shapes to lepidopteran wings are related to escape strategies, such as the long tails present in different lineages of butterfly and moths (see Barber et al., 2015; López-Palafox and Cordero, 2017; Robbins, 1981; Zhong et al., 2016). The escape may also be related to mimicry, although little studied, escape or invasive mimicry has been increasingly considered as a real possibility in butterflies (see Pinheiro and Freitas, 2014; Pinheiro et al., 2016). In these mimetic systems, the fastest, best escapes are “models” for species with similar (Müllerian) or less (Batesian) effective escaping tactics (Pinheiro and Freitas, 2014). Like aposematic based mimicry, convergence in escape mimicry occurs in the wing colour patterns, but considering the key aspect of flight in this kind of mimicry, a strong convergence in wing shape or others flight-related traits would be expected. Moreover, Pinheiro et al. (2016) suggest that some mimicry rings can contain species combining the two kinds of defence and signs for predators, unpalatability and escape ability. A comparative analysis of wing shape can be a promising approach to disentangle the selective pressures in these complex mimetic systems.

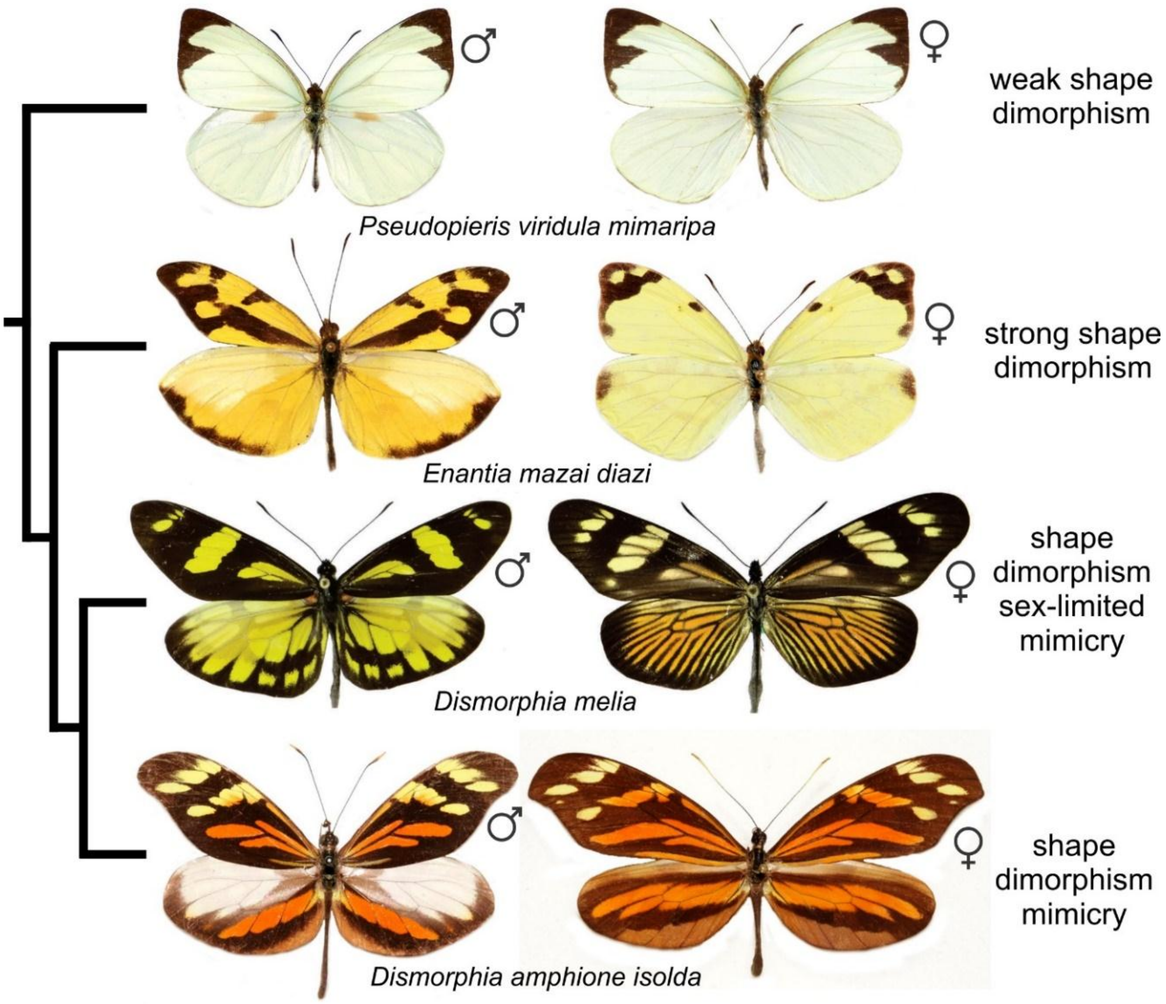


Fig. 2 Phylogenetic relationships between some species of Dismorphiinae (Lepidoptera: Pieridae), showing a small sample of the wing shape and colour variation associated with occurrence in different microhabitats and Batesian mimicry. Note that sexual dimorphism can occur in both the wing shape and colour, including sex-limited mimicry in *Dismorphia melia* (female is a mimic of the Actinote species in Southeast Brazil). Phylogenetic relationships based on Wahlberg et al. (2014).

Abiotic filters

The emergence of wing shape diversity throughout evolution is a result of a different selective processes, such as biotic interactions and stochastic processes, but also by environmental pressure (Fig. 1). The species are locally grouped taking into consideration their traits to the environmental conditions (Cavender-Bares et al., 2009; Ricklefs, 2004), and the functional traits from species must be compatible with the habitat (Arnan et al., 2013; Graça et al., 2015). Therefore, the wing shape is partly moulded by the affinity of an organism, population or community in respect to the habitat conditions.

Wing functionality associated with wing shape that can be strongly affected by environment conditions is the capacity of the flight. Changes in wing shape allow for different aerodynamics of flight and therefore, an occurrence of diverse flight types. Considering species of bats, birds and butterflies that forage near flat and smooth surfaces, they all have modified body aerodynamics to glide in ground effect and enhance flight performance at lower costs (Cespedes et al., 2015; Kim et al., 2014). Usually, the elongate wing shape (high values of aspect ratio), mainly in the forewing, is more related with gliding flight. While the rounded overall wing shape (lower values for aspect ratio) predicted to be more manoeuvrable (Betts and Wootton, 1988) and employ flapping flight to a greater degree (Dudley, 2000).

Additionally, the wings of migratory individuals frequently have a more elongate forewing apex, which allows for a more energetic and aerodynamic flight (DeVries et al., 2010; Johansson et al., 2009; Johnson, 1969). Individual migrants, usually across very diverse places, have some special wing characteristics for flight. In *Danaus plexippus*, a population that shows migratory behaviour has larger and more elongated forewings and a higher aspect ratio than resident individuals (Satterfield and Davis, 2014). Like birds (Egbert and Belthoff, 2003), the wing shape of migrations differs considerably among dragonflies species (Johansson et al., 2009). Bird studies demonstrated that migrating species tend to have longer and more pointed wings (Kaboli et al., 2007; Monkkonen, 1995; Voelker, 2001). Another interesting observation is that the effects of migration and mate guarding on wing shape appear to evolve (partly) independently (Johansson et al., 2009).

Long-term studies in tropical rainforest sites show that butterfly communities present vertical stratification, including the iconic Morpho butterflies (DeVries and Walla, 2001; DeVries et al., 1997). DeVries et al. (2010) found that spatial use of forest microhabitats differs among Morpho species and that natural selection has acted to influence flight behaviours and wing morphology relative to the costs of flights. The difference in wing shape on stratification has only been observed in males (DeVries et al., 2010), suggesting that sexual dimorphism may modulate such stratification patterns (Graça et al., 2017b). The difference in sunlight level and host plant vertical distribution (Beccaloni, 1997) can be explained by understory/canopy segregation (DeVries, 1988). The evolution of wing shape has been driven by microhabitat use (Chazot et al., 2016), and flight morphology explains in part the vertical stratification of tropical fruit-feeding Nymphalidae (Graça et al., 2017b). According to Papageorgis (1975), wing colour patterns play a key role in defence, but also have to be in accordance with habitat use and the ability to regulate body temperature to prevent collapse. The relation between colour and wing shape from the perspective of thermoregulation is a fundamental subject but still little studied.

Although size variation in herbivorous species related with host plants can be attributed directly to nutritional quality (Nylin and Gotthard, 1998), this relationship does not work with shape variation. However, this shape variation is involved in static allometry (Gould, 1966), and the shape differences could be attributed to size differences resulting from starvation (Jorge et al., 2011). An extreme case of abiotic filter affecting wing shape is the evolution of brachyptery and flightlessness. Although it is a recurring phenomenon in moths, it is recorded for only one genus of Andean butterflies (Pyrcz et al., 2017; Viloria et al., 2003). This brachypterous butterfly evolved in Páramo vegetation, a high mountain habitat which presents extreme weather conditions, with strong winds and low daily temperatures, making flight dangerous and expensive. Interestingly, the reduction of the wings occurs only in females, indicating that there are constraints that keep the males with functional wings (Viloria et al., 2003).

Macroevolutionary processes

All these previous selective pressures (ontogenetic, biotic and abiotic filters) acting repeatedly over a large amount of time must result in different evolutionary patterns in both species and traits. Distinct selective pressures acting on microevolutionary processes repeatedly result in different evolutionary scenarios, which take into account the occurrence of selective or nonselective pressures. The habitat can act on the trait, from sudden (natural catastrophic event), to regular (seasonal variation), or progressive events (global warming or urbanization), changing the history of wing evolution (Hill et al., 2002; Vitousek et al., 1997). In butterflies, this effect can be easily seen through the close relationship of these organisms with the environment.

Identifying exactly which factors or selective pressure acted on wing is paramount to define primary wing features, but it is not easy, due to the wide functionality attributed to this structure, since the species traits and occurrence patterns result from a series of ecological and evolutionary processes (Ackerly, 2003; Thomas et al., 2016). Nevertheless, one way to clarify how these selective pressure act on distinct aspects of the wing is observing the different facet of the wings which are related to some dimension of the fitness (growth, reproduction and survival) and access the allometric and assimetric relationships and the modularity between these functional components of wings. To obtain just the effect of these selective factors is important to remove the shared evolutionary history between the species, allowing to get the trait value without the effect of the phylogenetic relationship (Webb, 2000). One way to access ecologically functional traits is using morphological characteristics, which are usually good predictors of functional diversity in winged animals. Understanding to what extent wings evolved under different selective pressures is important to understand which factor could affect wing evolution. The capacity to fly is one of the most important functions of wings, and by using phylogenetic comparative analysis, it is possible to infer mechanisms underlying trait evolution. Therefore, we can infer the evolutionary history from the traits using some models with different evolutionary scenarios in order to understand how time, the mode and the phylogeny molded trait evolution. To test for this, these models must take into account phylogeny relationships among species and/or selective pressure on the traits.

An interesting model that can be used to simulate the evolution of the traits that consider phylogeny, but not selective pressure, was the Brownian model (BM) (Felsenstein, 1985) and the lambda model (Pagel, 1999). In the BM, change in the trait values occurred at a constant rate, without directional pressure, like genetic drift. In this view, the lambda model was frequently used for the inference of phylogenetic signals, so it is used to evaluate whether closely related species showed similar trait values. On the other hand, some models take into account selective pressures scenarios. If the attributes evolved as a result of the emergence of some barrier, for example, then the occurrence of stabilizing pressure and the attributes of each side of the barrier evolved to optimal values was considered. In this case, the evolutionary model of the attribute was the Ornstein-Uhlenbeck (OU) (Butler and King, 2004). However, when the change in the traits is exponential in terminal or ancestor branches the best fit will be the early burst (EB) (Harmon et al., 2010). Moreover, the change in the trait can be more strongly related to the speciation process, which can be tested using a kappa model (Pagel, 1999), and, finally, the null model (white noise) to compare with other models. To understand how the traits evolved on the phylogenetic context allows us to know which evolutionary processes can act on the evolution of ancient lineages and then consider the recent processes that can act.

The estimation of the phylogenetic constraint in morphological traits and in trait–habitat relationships helps us to understand the mechanisms underlying evolutionary selection and its impact on current ecological patterns (Ackerly, 2009). Some processes, like adaptive radiation, suggest that diversification is due to changes in species traits in response to ecological opportunities promoted by available niche space, while niche conservatism describes the tendency of closely related species to retain similar characteristics, minimizing evolutionary change (Ackerly, 2003, 2009; Harvey and Pagel, 1991; Losos, 2010). In part, niche conservatism reflects phylogenetic signal; however, phylogenetic signal alone does not directly denote niche conservation (Blomberg and Garland, 2002; Blomberg et al., 2003).

Studies using the comparative phylogenetic methods have increased in winged animals, for example, in birds (Claramunt et al., 2012), in bats (Santana and Cheung, 2016) and in butterflies. Chazot et al. (2016) evaluated the process that drove the diversification of wing size and shape in Morpho using the macroevolutionary perspective, but not using these evolutionary models. Furthermore, wing shape is one of the most interesting forms for understanding the differences in flight ability occurrence, and the aspect ratio is one way to obtain the wing shape for butterflies (Betts and Wootton, 1988; Breuker et al., 2007). More research, however, is needed to be performed to clarify our understanding of the evolution of the traits in butterflies.

Concluding remarks

In this brief review, we have shown how an intricate eco-evolutionary history can explain the amazing morphological patterns displayed in butterfly wings (Fig. 1) by discussing some recent advances in this subject. As we have seen, the diversity of wing shape and colour patterns could evolve from different selective pressures. These pressures begin in the early stages, when biotic and abiotic filters constrain the ontogenetic process which will reflect on structures of adult butterflies, including wing morphology. After becoming adults, butterflies have no opportunity to change their exoskeleton; however, other selective forces can act at this stage, such as sexual selection, when adaptive shapes could to be selected for sexual partners, or through the natural selection exerted by predators where some genes associated with wing shape could to be removed or remain in the population over time. Apart from these pressures, environmental conditions impose a strong abiotic filter on aerodynamic traits, including wing shape. In summary, the interaction between genotype, phenotype and environment could answer many questions about the evolutionary history of wing shapes. Obviously, these features are not restricted to butterflies, but we propose that they can be more easily understood by using these organisms as models.

Nevertheless, disentangling which factors are operating in these different contexts is a major challenge. The use of the phylogenetic approach is fundamental to tease apart ecological factors operating in wing shapes from shared evolutionary history. Moreover, some morphological tools allow for understanding the asymmetric, allometric and modularity effects that act on the wing shapes, while separating each effect. Allometry allows for the comparison of growth differentials between different parts of the body in males and females, in different stages in ontogenies, or different structures (modularity). In the mimicry context, for example, the allometric analysis shows that conspicuous aposematic red spots and wing shapes grow in a similar way, but sex-related differences can occur between species in the *Heliconius* postman ring Müllerian mimicry (Rossato et al., 2018). This kind of approach can be one important way of understanding the selective pressures acting in both intra- and interspecific communications.

The convergence in mimicry rings has a clear spatial structure, consequently, the spatial distribution of mimetic rings and predators can help us to understand the role of space in wing convergence. The use of GM as a morphoecological tool to study wing shape is quite recent, especially in the context of mimicry evolution (Jones et al., 2013; Merot et al., 2016; Rossato et al., 2018). A good way to begin a wing shape study is to choose an informative aspect of wing functionality (e.g. reproduction, dispersion, thermoregulation, communication) to be investigated in a mimicry ring and look for others selective pressures that can act on it and include it in the analysis, and trying to separate the factors and see how they affect the trait. Thus, if an investigator chooses to evaluate wing shape regarding spatial phenotypic convergence, it is not only important to compare similarities between species, but the flight capacity to escape the predator must be taken into account, since this selective pressure acts together with convergence pressure. Therefore, it cannot be easily understood how these factors work: however, the wing shape will be selected like a trade-off between the importance of a certain feature to perform the communication function or to the flight. In the end, the wing will be the result of these two, or more, pressures acting on the shape of the wing.

Although butterflies are models in many areas of evolutionary biology, most studies are restricted to a few well-studied lineages, such as the large swallowtail butterflies (Papilionidae), or the well-known *Heliconius*. In fact, these model groups are only a small fraction of the great radiation of butterflies that have occurred since the Cretaceous (Heikkila et al., 2012). When we speak of understanding the wing shape diversity in butterflies, we would like to include all of this variation. In this sense, we need to know more about some neglected lineages, to unravel the ecological and evolutionary patterns that operate in the less studied families. In recent years, robust phylogenetic hypotheses are being obtained for almost all butterfly families, which can serve as the basis for these evolutionary studies (e.g. Sahoo et al., 2016; Seraphim et al., 2018; Wahlberg et al., 2014). Identifying new model systems is also crucial, for example, the Dismorphiinae that illuminated early ideas about mimicry (Bates, 1862) are a candidate group to study the evolution of wing shapes (Fig. 2). This group includes species with slight sexual dimorphism in wing shape, such as the Pseudopieris species, which present a typical pierid bauplan and occur in more naturally disturbed open edge environments. Also, it includes species with strong sexual dimorphism, such as Enantia species, whose males present unusually short fore wings. And finally, it includes Dismorphia and other genera which are Batesian mimics (Bates, 1862; LeCrom et al., 2004), including species with both sex-limited dimorphism (males flying in distinct microhabitats from females) or without dimorphism in the colour pattern (males and females flying in same microhabitats), but with marked dimorphism in the wing shape due to male investment in androconial scales.

In addition to these evolutionary studies with closely related lineages, there are major questions about the wings of butterflies. For example, what are the great convergences and divergences imposed by different biotic (e.g. host plants, interaction with ants, predation rates) and abiotic filters (e.g. insularity, humidity, altitude, etc.) that explain this diversity of wing shape in butterfly families? How is this shape diversity restricted by the evolutionary history of the groups, including phylogenetic constraints and biogeography? The answers to these considerable issues are unlikely to be obtained from specific studies on models, but can be revealed by comparative studies on a large scale.

The emergence of next-generation sequencing can allow us to find specific parts of the genome that can be related to wing shape, though, for example, the quantitative trait locus (Lynch and Walsh, 1998). Another promising approach is evaluating the epigenetic factors that can act on the genome, resulting in wing shape changes in different environments (Jablonka, 2017). The possibility of integrative studies joining genomics, bioinformatics, field experiments and mathematical modelling seems almost infinite and points to a promising future in all areas of evolutionary biology, including ecomorphology and ecophysiology. Almost two centuries after Bates's insights about the biological relevance of studying the butterfly wings, we can verify that he was right, and that finally scientists are close to solving many mysteries that are still waiting to be unveiled in the flapping wings of butterflies.

Acknowledgements

We would like to thank Richard ffrench-Constant for the invitation to write about some interesting issue. D.O.R. and L.A.K. are indebted to Gilson R.P. Moreira for his introduction to study insect morphology. We are also grateful to Derrick Mulder for editing the text. D.O.R. was supported by a CAPES PhD Program Fellowship. L.A.K. was supported by PNPD/CAPES Program Fellowship. L.D. research activities have been supported by CNPq Productivity Fellowship (Grant 307886/2015-8). L.D. research has been developed in the context of National Institutes for Science and Technology (INCT) in Ecology, Evolution and Biodiversity Conservation, supported by MCTIC/CNPq (proc. 465610/2014-5) and FAPEG.

References

Ackerly, D.D., 2003. Community assembly, niche conservatism and adaptive evolution in changing environments. Int. J. Plant Sci. 164, S165–S184.

Ackerly, D.D., 2009. Conservatism and diversification of plant functional traits: evolutionary rates versus phylogenetic signal. PNAS 106, 19699–19706.

Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: ten years of progress following the "revolution". Ital. J. Zool. 71, 5–16.

Alexander, D.E., 2015. On the Wing: Insects, Pterosaurs, Birds, Bats and the Evolution of Animal Flight. Oxford University Press, Oxford.

Allen, C.E., Beldade, P., Zwaan, B.J., Brakefield, P.M., 2008. Differences in the selection response of serially repeated color pattern characters: standing variation, development and evolution. BMC Evol. Biol. 8, 94.

Arnan, X., Cerda´, X., Rodrigo, A., Retana, J., 2013. Response of ant functional composition to fire. Ecography 36, 1182–1192.

Aubier, T.G., Elias, M., Llaurens, V., Chazot, N., 2017. Mutualistic mimicry enhances species diversification through spatial segregation and extension of the ecological niche space. Evolution 4, 826–844.

Aubin-Horth, N., Renn, S.C.P., 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol. Ecol. 18, 3763–3780.

Baena-Lopez, L.A., Garcia-Bellido, A., 2006. Control of growth and positional information by the graded vestigial expression pattern in the wing of *Drosophila melanogaster*. PNAS 103, 13734–13739.

Bai, M., Beutel, R.G., Song, K.Q., Liu, W.G., Malqin, H., Li, S., Hu, X.Y., Yang, X.K., 2012. Evolutionary patterns of hind wing morphology in dung beetles (Coleoptera: Scarabaeinae). Arthropod Struct. Dev. 41, 505–513.

Baker, R.R., 1972. Territorial behaviour of the nymphalid butterflies, *Aglais urticae* (L.) and. *Inachis io* (L.). J. Anim. Ecol. 41, 453–469.

Barber, J.R., Leavell, B.C., Keener, A.L., Breinholt, J.W., Chadwell, B.A., McClure, C.J.W., Hill, G.M., Kawahara, A.Y., 2015. Moth tails divert bat attack: evolution of acoustic deflection. PNAS 112, 2812–2816.

Bates, H.W., 1862. Contributions to an insect fauna of the Amazon valley (Lepidoptera: Heliconidae). Trans. Linn. Soc. Lond. 23, 495–566.

Bates, H.W., 1864. The Naturalist on the River Amazons. A Record of Adventures, Habits of Animals, Sketches of Brazilian and Indian Life, and Aspects of Nature Under the Equator, During Eleven Years of Travel. J. Murray, London.

Beccaloni, G.W., 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.

Beldade, P., Brakefield, P.M., 2002. The genetics and evo-devo of butterfly wing patterns. Nat. Rev. Genet. 3, 442–452.

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

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

Blankers, T., Adams, D.C., Wiens, J.J., 2012. Ecological radiation with limited morphological diversification in salamanders. J. Evol. Biol. 25, 634–646.

Blomberg, S.P., Garland, T., 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J. Evol. Biol. 15, 899-910.

Blomberg, S.P., Garland, T., Ives, A.R., 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evol. 57, 717-745.

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

Bookstein, F.L., 1991. Morphometric Tools for Landmark Data Geometry and Biology. Cambridge University Press, Cambridge.

Bots, J., Breuker, C.J., Kaunisto, K.M., Koskimaki, J., Gossum, H.V., Suhonen, J., 2012. Wing shape and its influence on the outcome of territorial contests in the damselfly *Calopteryx virgo*. J. Insect Sci. 12, 96.

Bourdeau, P.E., 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology 90, 1659–1669.

Breuker, C.J., Debat, V., Klingenberg, C.P., 2006. Functional evo-devo. TREE 21, 488–492.

Breuker, C., Brakefield, P.M., Gibbs, M., 2007. The associations between wing morphology and dispersal is sex-limited in the *Glanville fritillary* butterfly. Eur. J. Entomol. 104, 445–452.

Breuker, C.J., Gibbs, M., van Dongen, S., Merckx, T., Van Dyck, H., 2010. The use of geometric morphometrics in studying butterfly wings in an evolutionary ecological context. In: Elewa, A.M.T. (Ed.), Morphometrics for Nonmorphometricians. Springer Verlag, Berlin, pp. 271–287.

Brown Jr., K.S., 1997. Diversity, disturbance, and sustainable use of Neotropical forests: insects as indicators for conservation monitoring. J. Insect. Cons. 1, 25–42.

Butler, M.A., King, A.A., 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. Am. Nat. 164, 683–695.

Cadotte, M.W., Davies, J., 2016. Phylogenies in Ecology: A Guide to Concepts and Methods. Princeton University Press, Princeton and Oxford.

Canfield, M.R., Pierce, N.E., 2010. Facultative mimicry? The evolutionary significance of seasonal forms in several Indo-Australian butterflies in the family Pieridae. Trop. Lepid. 20, 1–7.

Carreira, V.P., Soto, I.M., Mensch, J., Fanara, J.J., 2011. Genetic basis of wing morphogenesis in *Drosophila*: sexual dimorphism and non-allometric effects of shape variation. BMC Dev. Biol. 11, 32.

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. Ecol. Let. 12, 693–715.

Cespedes, A., Penz, C.M., DeVries, P.J., 2015. Cruising the rain forest floor: butterfly wing shape evolution and gliding in ground effect. J. Anim. Ecol. 84, 808–816.

Chazot, N., Panara, S., Zilbermann, N., Blandin, P., Le Poul, Y., Cornette, R., Elias, M., Debat, V., 2016. Morpho morphometrics: shared ancestry and selection drive the evolution of wing size and shape in *Morpho* butterflies. Evolution 70, 181–194.

Cheverud, J.M., 1996. Developmental integration and the evolution of pleiotropy. Am. Zool. 36, 44–50.

Chouteau, M., Arias, M., Joron, M., 2016. Warning signals are under positive frequency-dependent selection in nature. PNAS 113, 2164–2169.

Claramunt, S., Derryberry, E.P., Brumfield, R.T., Remsen, J.V., 2012. Ecological opportunity and diversification in a continental radiation of birds: climbing adaptations and cladogenesis in the Furnariidae. Am. Nat. 179, 649–666.

Cock, A.G., 1966. Genetical aspects of metrical growth and form in animals. Q. Rev. Biol. 41, 131–190.

Cornwallis, C.K., Birkhead, T.R., 2008. Plasticity in reproductive phenotypes reveals status-specific correlations between behavioral, morphological, and physiological sexual traits. Evolution 62, 1149–1161.

Davies, N.B., 1978. Territorial defense in the speckled wood butterfly (*Pararge aegeria*): the resident always wins. Ani. Behav. 26, 138–147.

De Keyser, R., Breuker, C.J., Hails, R.S., Dennis, R.L.H., Shreeve, T.G., 2015. Why small is beautiful: wing colour is free from thermoregulatory constraint in the small lycaenid butterfly, *Polyommatus icarus*. PLoS One 10, e0122623.

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

DeVries, P.J., Walla, T.R., 2001. Species diversity and community structure in neotropical fruit-feeding butterflies. Biol. J. Linn. Soc. 74, 1–15.

DeVries, P.J., Murray, D., Lande, R., 1997. Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. Biol. J. Linn. Soc. 62, 343–364.

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

Dincaˇ, V., Dapporto, L., Vila, R., 2011. A combined genetic-morphometric analysis unravels the complex biogeographical history of *Polyommatus icarus* and *Polyommatus celina* common blue butterflies. Mol. Ecol. 20, 3921–3935.

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

Egbert, J.R., Belthoff, J.R., 2003. Wing shape in house finches differs relative to migratory habitat in eastern and western North America. Condor 105, 825–829.

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15.

Ferson, S., Rohlf, F.J., Koehn, R.K., 1985. Measuring shape variation of two-dimensional outlines. Syst. Zool. 34, 59–68.

Frankino, W.A., Zwaan, B.J., Stern, D.L., Brakefield, P.M., 2007. Internal and external constraints in the evolution of morphological allometries in a butterfly. Evolution 61, 2958–2970.

Franks, D.W., Sherratt, T.N., 2007. The evolution of multicomponent mimicry. J. Theor. Biol. 244, 631–639.

Freitas, A.V.L., Marini-Filho, O.J., 2011. Plano de Ação Nacional para a Conservação dos Lepidópteros Ameaçados de Extinção. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brasília.

Gillies, T.E., Cabernard, C., 2011. Cell division orientation in animals. Curr. Biol. 21, R599–R609.

Gould, S.J., 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. 41, 587–638.

Graça, M.B., Morais, J.W., Franklin, E., Pequeno, P.A.C.L., Souza, J.L.P., Bueno, A.S., 2015. Combining taxonomic and functional approaches to unravel the spatial distribution of an Amazonian butterfly community. Environ. Entomol. 45, 301–309.

Graça, M.B., Pequeno, P.A.C.L., Franklin, E., Souza, J.L.P., Morais, J.W., 2017a. Taxonomic, functional, and phylogenetic perspectives on butterfly spatial assembly in northern Amazonia. Ecol. Entomol. 42, 816–826. https://doi.org/10.1111/een.12454.

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

Greenslade, P., Farrow, R.A., Smith, J.M.B., 1999. Long distance migration of insects to a subantarctic island. J. Biogeogr. 26, 1161–1167.

Grimaldi, D., Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press, Cambridge, New York, Melbourne.

Grodnitsky, D.L., Dudley, R., Gilbert, L., 1994. Wing decoupling in hovering flight of swallowtail butterflies (Lepidoptera: Papilionidae). Trop. Lepid. 5, 85–86.

Harmon, L.J., Losos, J.B., Davies, T.J., Gillespie, R.G., Gittleman, J.L., Jennings, W.B., Kozak, K.H., McPeek, M.A., Moreno-Roark, F., Near, T.J., Purvis, A., Ricklefs, R.E., Schluter, D., Schulte, J.A., Seehausen, O., Sidlauskas, B.L., Torres-Carvajal, O., Weir, J.T., Mooers, A., 2010. Early bursts of body size and shape evolution are rare in comparative data. Evolution 64, 2385–2396.

Harvey, P.H., Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, New York.

Hassall, C., 2015. Strong geographical variation in wing aspect ratio of a damselfly, *Calopteryx maculata* (Odonata: Zygoptera). PeerJ 3, e1219. https://doi.org/10.7717/peerj.1219.

Heikkila, M., Kaila, L., Mutanen, M., Peña, C., Wahlberg, N., 2012. Cretaceous origin and repeated tertiary diversification of the redefined butterflies. Proc. R. Soc. B 279, 1093–1099.

Hill, J.K., Thomas, C.D., Fox, R., Telfer, M.G., Willis, S.G., Asher, J., Huntley, B., 2002. Responses of butterflies to twentieth century climate warming: implications for future ranges. Proc. R. Soc. B 269, 2163–2171.

Holwell, G.I., Herberstein, M.E., 2010. Chirally dimorphic male genitalia in praying mantids (Ciulfina: Liturgusidae). J. Morphol. 271, 1176–1184.

Houle, D., Mezey, J., Galpern, P., Carter, A., 2003. Automated measurement of *Drosophila* wings. BMC Evol. Biol. 3, 25.

Huang, S.S., Huang, J.S., 2005. TGF-beta control of cell proliferation. J. Cell. Biochem. 96, 447–462.

Huxley, J., Teissier, G., 1936. Terminology of relative growth rates. Nature 137, 780–781.

Ihalainen, E., Lindstroom, L., Mappes, J., Puolakkainen, S., 2008. Butterfly effects in mimicry? Combining signal and taste can twist the relationship of Müllerian co-mimics. Behav. Ecol. Sociobiol. 62, 1267–1276.

Ihalainen, E., Rowland, H.M., Speed, M.P., Ruxton, G.D., Mappes, J., 2012. Prey community structure affects how predators select for Müllerian mimicry. Proc. R. Soc. B 279, 2099–2105.

Jablonka, E., 2017. The evolutionary implications of epigenetic inheritance. Interface Focus 7, 20160135.

Jantzen, B., Eisner, T., 2008. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. PNAS 105, 16636–16640.

Johansson, F., Soderquist, M., Bokma, F., 2009. Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. Biol. J. Linn. Soc. 97, 362–372.

Johnson, C.G., 1969. Migration and Dispersal of Insects by Flight. Meuthen, London.

Jones, R.T., Salazar, P.A., ffrench-Constant, R.H., Jiggins, C.D., Joron, M., 2012. Evolution of a mimicry supergene from a multilocus architecture. Proc. R. Soc. B 279, 316–325.

Jones, R.T., Le Poul, Y., Whibley, A.C., Merot, C., ffrench-Constant, R.H., Joron, M., 2013. Wing shape variation associated with mimicry in butterflies. Evolution 67, 2323–2334.

Jordano, P., 2016. Chasing ecological interactions. PLoS Biol. 14, e1002559.

Jorge, L.R., Cordeiro-Estrela, P., Klaczko, L.B., Moreira, G.R.P., Freitas, A.V.L., 2011. Host-plant dependent wing phenotypic variation in *Heliconius erato*. Biol. J. Linn. Soc. 102, 765–774.

Joron, M., 2005. Polymorphic mimicry, microhabitat use, and sex-specific behaviour. J. Evol. Biol. 18, 547–556.

Joron, M., Iwasa, Y., 2005. The evolution of a Müllerian mimic in a spatially distributed community. J. Theor. Biol. 237, 87–103.

Joshi, J., Prakash, A., Kunte, K., 2017. Evolutionary assembly of communities in butterfly mimicry rings. Am. Nat. 189, E58–E76.

Kaboli, M., Aliabadian, M., Guillaumet, A., Roselaar, C.S., Prodon, R., 2007. Ecomorphology of the wheatears (genus *Oenanthe*). Ibis 149, 792–805.

Kim, E.J., Wolf, M., Ortega-Jimenez, V.M., Dudley, R., 2014. Hovering performance of Anna's hummingbirds (*Calypte anna*) in ground effect. J. R. Soc. Interface 11, 20140505.

Kingsolver, J.G., 1985. Butterfly engineering. Sci. Am. 253, 106–113.

Klingenberg, C.P., 2008. Morphological integration and developmental modularity. Annu. Rev. Ecol. Evol. Syst. 39, 115–132.

Klingenberg, C.P., 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evol. Dev. 11, 405–421.

Klingenberg, C.P., Nijhout, H.F., 1999. Genetics of fluctuating asymmetry: a developmental model of developmental instability. Evolution 53, 358–375.

Klingenberg, C.P., Zaklan, S.D., 2000. Morphological integration between development compartments in the *Drosophila* wing. Evolution 54, 1273–1285.

Klingenberg, C.P., Zimmermann, M., 1992. Static, ontogenetic and evolutionary allometry: a multivariate comparison in nine species of water striders. Am. Nat. 140, 601–620.

Klingenberg, C.P., Badyaev, A.V., Sowry, S.M., Beckwith, N.J., 2001. Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. Am. Nat. 157, 11–23.

Klingenberg, C.P., Debat, V., Roff, D.A., 2010. Quantitative genetics of shape in cricket wings: developmental integration in a functional structure. Evolution 64, 2935–2951.

Kronforst, M.R., Papa, R., 2015. The functional basis of wing patterning in *Heliconius* butterflies: the molecules behind mimicry. Genetics 200, 1–19.

Kunte, K., 2008. Mimetic butterflies support Wallace's model of sexual dimorphism. Proc. R. Soc. B 275, 1617–1624.

LeCrom, J.F., Llorente-Bousquets, J., Constantino, L.M., Salazar, J., 2004. Mariposas de Colombia, Tomo 2: Pieridae. Carlec Ltd.; Bogotá

López-Palafox, T.G., Cordero, C.R., 2017. Two-headed butterfly vs. mantis: do false antennae matter? Peer J 5, e3493.

Losos, J.B., 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. Am. Nat. 175, 623–639.

Lynch, M., Walsh, J.B., 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland.

Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell, Maldan. Mallet, J., 1999. Causes and consequences of a lack of coevolution in Müllerian mimicry. Evol. Ecol. 13, 777–806.

Mallet, J., Joron, M., 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance and speciation. Annu. Rev. Ecol. Syst. 30, 201–233.

Marchant, S., Moran, A.L., Marko, P.B., 2015. Out-of-the tropics or trans-tropical dispersal? The origins of the disjunct distribution of the gooseneck barnacle *Pollicipes elegans*. Front. Zool. 12, 39.

Marinari, E., Mehonic, A., Curran, S., Gale, J., Duke, T., Baum, B., 2012. Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. Nature 484, 542–545.

Mérot, C., Poul, Y.L., Therv, M., Joron, M., 2016. Refining mimicry: phenotypic variation tracks the local optimum. J. Anim. Ecol. 85, 1056–1069.

Møller, A.P., Swaddle, J.P., 1997. Asymmetry, Developmental Stability and Evolution. Oxford University Press, Oxford.

Monkkonen, M., 1995. Do migrant birds have more pointed wings? A comparative study. Evol. Ecol. 9, 520–528.

Moreira, G.R.P., Silva, D.S., Gonçalves, G.L., 2017. Comparative morphology of the prothoracic leg in heliconian butterflies: tracing size allometry, podite fusions and losses in ontogeny and phylogeny. Arthropod Struct. Dev. 46, 462–471.

Müller, F., 1878. Uber die vortheile der mimicry bei schmetterlingen. Zool. Anz. 1, 54 –55.

Müller, F., 1879. Ituna and Thyridia: a remarkable case of mimicry in butterflies. Trans. Entomol. Soc. Lond. 1879, 20–29.

Nijhout, H.F., 2003. Development and evolution of adaptive polyphenisms. Evol. Dev. 5, 9–18.

Nijhout, H.F., Grunert, L.W., 2002. Bombyxin is a growth factor for wing imaginal disks in Lepidoptera. PNAS 99, 15446–15450.

Nijhout, H.F., Smith, W.A., Schachar, I., Subramanian, S., Tobler, A., Grunert, L.W., 2007. The control of growth and differentiation of the wing imaginal disks of *Manduca sexta*. Dev. Biol. 302, 569–576.

Nijhout, H.F., Cinderella, M., Grunert, L.W., 2014. The development of wing shape in Lepidoptera: mitotic density, not orientation, is the primary determinant of shape. Evol. Dev. 16, 68–77.

Norberg, U.M., 1990. Vertebrate Flight: Mechanics, Physiology, Morphology, Ecology and Evolution. Springer, Berlin.

Nylin, S., Gotthard, K., 1998. Plasticity in life-history traits. Ann. Rev. Entomol. 43, 63–83.
O’Keefe, D., Thomas, S., Bolin, K., Griggs, E., Edgar, B., Buttitta, L., 2012. Combinatorial control of temporal gene expression in the *Drosophila* wing by enhancers and core promoters. BMC Genomics 13, 498.

Olson, E., Miller, R., 1958. Morphological Integration. University of Chicago Press, Chicago.

Outomuro, D., Johansson, F.A., 2017. Potential pitfall in studies of biological shape: does size matter? J. Anim. Ecol. 86, 1447–1457. https://doi.org/10.1111/1365-2656.12732.

Outomuro, D., Adams, D.C., Johansson, F., 2013. Wing shape allometry and aerodynamics in calopterygid damselflies: a comparative approach. BMC Evol. Biol. 13, 1–11.

Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877–884.

Palmer, A.R., Strobeck, C., 1986. Fluctuating asymmetry: measurement, analysis, patterns. Ann. Rev. Ecol. Syst. 17, 391–421.

Papageorgis, C., 1975. Mimicry in Neotropical butterflies: why are there so many different wing-colocation complexes in one place? American Sci. 63, 522–532.

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., et al., 2010. Phenotypic plasticity’s impacts on diversification and speciation. TREE 25, 459–467.

Pinheiro, C.E.G., Freitas, A.V.L., 2014. Some possible cases of escape mimicry in Neotropical butterflies. Neotrop. Entomol. 43, 393–398.

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.

Pyrcz, T.W., Lorenc-Brudecka, J., Zubek, A., Boyer, P., Gabaldon, M.C., Mavarez, J., 2017. Taxonomy, phylogeny and distribution of the genus *Steromapedaliodes* sensu novo in the Cordillera de Merida, Venezuela (Lepidoptera: Nymphalidae: Satyrinae: Satyrini). Art. Syst. Phylo. 75, 195–243.

Regal, P.J., 1977. Ecology and evolution of flowering plant dominance. Science 196, 622–629.

Ricklefs, R.E., 2004. A comprehensive framework for global patterns in biodiversity. Ecol. Lett. 7, 1–15.

Robbins, R.K., 1981. The “false head” hypothesis: predation and wing pattern variation of Lycaenid butterflies. Am. Nat. 118, 770–775.

Rohlf, F.J., Slice, D., 1990. Extension of the Procrustes method for the optimal superimposition of landmarks. Syst. Zoo. 39, 40–59.

Rossato, D.O., Boligon, D., Fornel, R., Kronforst, M.R., Gonçalves, G.L., Moreira, G.R.P., 2018. Subtle variation in size and shape of the whole forewing and the red band among comimics revealed by geometric morphometric analysis in *Heliconius* butterflies, Ecol. Evol. 8, 3280–3295.

Rowe, C., Lindstrom, L., Lyytinen, A., 2004. The importance of pattern similarity between Müllerian mimics in predator avoidance learning. Proc. R. Soc B271, 407–413.

Rowland, H.M., Mappes, J., Ruxton, G.D., Speed, M.P., 2010. Mimicry between unequally defended prey can be parasitic: evidence for quasi-batesian mimicry. Ecol. Lett. 13, 1494–1502.

Ruszczyk, A., Motta, P.C., Barros, R.L., Araú jo, A.M., 2004. Ecological correlates of polyphenism and gregarious roosting in the grass yellow butterfly *Eurema elathea* (Pieridae). Braz. J. Biol. 64, 1–14.

Ruxton, G.D., Franks, D.W., Balogh, A.C.V., Leimar, O., 2008. Evolutionary implications of the form of predator generalization for aposematic signals and mimicry in prey. Evolution 62, 2913–2921.

Sahoo, R.K., Warren, A.D., Wahlberg, N., Brower, A.V.Z., Lukhtanov, V.A., Kodandaramaiah, U., 2016. Ten genes and two topologies: an exploration of higher relationships in skipper butterflies (Hesperiidae). PeerJ 4, e2653.

Santana, S.E., Cheung, E., 2016. Go big or go fish: morphological specializations in carnivorous bats. Proc. R. Soc. B 283, 20160615.

Satterfield, D.A., Davis, A.K., 2014. Variation in wing characteristics of monarch butterflies during migration: earlier migrants have redder and more elongated wings. Anim. Migr. 2, 1–7.

Seraphim, N., Kaminski, L.A., DeVries, P.J., Penz, C.M., Callaghan, C., Wahlberg, N., Silva-Brandão, K.L., Freitas, A.V.L., 2018. Molecular phylogeny and higher systematics of the metalmark butterflies (Lepidoptera: Riodinidae). Syst. Entomol. doi:10.1111/syen.12282.

Shapiro, A.M., 1976. Seasonal polyphenism. Evol. Biol. 9, 259–333.

Snell-Rood, E.C., Cash, A., Han, M.V., Kijimoto, T., Andrews, J., Moczek, A.P., 2010. Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn-polyphenic beetles. Evolution 65, 231–245.

Sobral, F.L., Cianciaruso, M.V., 2012. Estrutura filogenética e funcional de assembleias: (re) montando a ecologia de comunidades em diferentes escalas espaciais. Biosci. J. 28, 617–631.

Srygley, R.B., 1994. Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. Philos. Trans. R. Soc. Lond. Ser. B. 343, 145–155.

Srygley, R.B., 2007. Evolution of the wave: aerodynamic and aposematic functions of butterfly wing motion. Proc. R. Soc. B 274, 913–917.

Srygley, R.B., Ellington, C.P., 1999. Discrimination of flying mimetic, passion-vine butterflies *Heliconius*. Proc. R. Soc. B 266, 2137–2140.

Suzuki, Y., Nijhout, H.F., 2006. Evolution of a polyphenism by genetic accommodation. Science 311, 650–652.

Svensson, E.I., Friberg, M., 2007. Selective predation on wing morphology in sympatric damselflies. Am. Nat. 170, 101–112.

Thomas, M.K., Kremer, C.T., Litchman, E., 2016. Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. Glob. Ecol. Biogeogr. 25, 75–86.

Timmermans, M.J., Thompson, M.J., Collins, S., Vogler, A.P., 2017. Independent evolution of sexual dimorphism and female-limited mimicry in swallowtail butterflies (*Papilio dardanus* and *Papilio phorcas*). Mol. Ecol. 26, 1273–1284.

Turner, J.R.G., 1977. Butterfly mimicry—the genetical evolution of an adaptation. Evol. Biol. 10, 163–206.

Van der Molen, S., Martínez, N.A., González-Jose, R., 2007. Introducción a la Morfometría Geometrica. Curso teórico práctico. Centro Nacional Patagónico and University of Barcelona, Barcelona.

Viloria, A.L., Pyrcz, T.W., Wojtusiak, J., Ferrer-Paris, J.R., Beccaloni, G.W., Sattler, K., Lees, D.C., 2003. A brachypterous butterfly? Proc. R. Soc. B 270, 21–24.

Vincent, J.P., Fletcher, A.G., Baena-Lopez, L.A., 2013. Mechanisms and mechanics of cell competition in epithelia. Nat. Rev. Mol. Cell Biol. 14, 581–591.

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

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of Earth's ecosystems. Science 277, 494–499.

Voelker, G., 2001. Morphological correlates of migratory distance and flight display in the avian genus *Anthus*. Biol. J. Linn. Soc. 73, 425–435.

Waddington, C.H., 1953. Genetic assimilation of an acquired character. Evolution 7, 118–126.

Wahlberg, N., Rota, J., Braby, M.F., Pierce, N.P., Wheat, C.W., 2014. Revised systematics and higher classification of pierid butterflies (Lepidoptera: Pieridae) based on molecular data. Zool. Scr 43, 641–650.

Webb, C.O., 2000. Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. Am. Nat. 156, 145–155.

Weber, K.E., 1992. How small are the smallest selectable domains of form? Genetics 130, 345–353.

Whitman, D.W., Agrawal, A.A., 2009. What is phenotypic plasticity and why is it important? In: Whitman, D.W., Ananthakrishnan, T.N. (Eds.), Phenotypic Plasticity of Insects. Science Publishers, CRC Press, Enfield, pp. 1–63.

Wootton, R.J., 1992. Functional morphology of insect wings. Annu. Rev. Entomol. 37, 113–140.

Wootton, R.J., 2002. Design, function and evolution in the wings of holometabolous insects. Zool. Scr. 31, 31–40.

Zhong, M., Hill, G.M., Gomez, J.P., Plotkin, D., Barber, J.R., Kawahara, A.Y., 2016. Quantifying wing shape and size of saturniid moths with geometric morphometrics. J. Lep. Soc. 70, 99–107

CAPÍTULO 2

The impact of agroforestry and conventional banana plantations on genomic and species diversity of butterfly

Dirleane O. Rossato[1]*, Nicola Nadeau[2], Cristiano A. Iserhard[3], Leandro Duarte[1]

*dirleane.ottonelli@gmail.com

[1] Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil

[2] University of Sheffield, United Kingdom

[3] Universidade Federal de Pelotas (UFPEL), Pelotas, Rio Grande do Sul, Brazil

----

Artigo preparado para submissão na revista Journal of Applied Ecology

Abstract

1. Environmental changes, particularly, those related to land use are directly linked to biodiversity loss. The lack of connectivity between the habitats patches and decrease of genetic and species diversity affect different biological aspects, which are essential for to shape and to structure the populations and the communities' dynamics. Molecular markers in individual and population levels, associate to the community information, can help us to elucidate how distinct ecoevolutionary level responds to different banana plantation.

2. We evaluated the effects generated by conventional and agroforestry banana plantations acting in butterflies. Different ecoevolutionary aspects were used as response variable. In the individual and the population levels, we access the genomic information from *Heliconius ethilla narcae* through the ddRAD sequencing method. This specie was the most equal distribution between the sites. Then, we evaluate the effect of the treatment type, the morphological dispersal trait and geographic spatial occurrence in genomic (individual and population) level. On the other hand, in a community level, we evaluate the effect from the treatment type in species diversity by Nymphalidae group. Finally, we evaluate the correlation between genomic and species diversity in relationship to the treatment effect.

3. In the individual level, the treatment and block influenced the genomic information. However, no effect was found by morphological dispersal traits or geographic spatial occurrence in this level. Take into account the treatment type in another levels, no effect was found in the genomic population in each site neither the species diversity. Besides that, the genomic information in conventional treatment showed a

simplification genomic, and, the most distinct genomic cluster was shared only between the native and agroforestry sites, being absent in the conventional plantation.

Synthesis and applications. We found some evidence of land use effect in individual genomic level. The most distinct and rare genotypes was shared between individuals from native and agroforestry sites. The conventional plantation, showed a genomic simplification. Nevertheless, the effect by distinct treatment did not affect the population or community level, showing that the genomic analysis are important for evaluate the anthropogenic factors. Different approach and integration for distinct ecoevolutionary level must to be used associate for evaluate and distend the impacts on different level.

Introduction

Anthropogenic changes related to the agriculture and infrastructure development are among the main drivers of biodiversity loss, due to reducing natural habitat and causing biotic homogenization (1). The loss of connectivity between different places depend how much different are the native in relationship to altered area (2). The anthropogenic changes could work as barrier for dispersal in distinct ecological levels, since species until community. In a community level, the set of local community connected by multiple species by dispersal is knower as metacommunity (3) . Besides the spatial patchiness caused by human activities modifies species and communities, affecting mate and food finding, predator avoidance and patch colonization (4,5), the insecticide use associate to food production further reduces the biodiversity (6).

Taking into account that the global population is increasing and consequently demand for food (7) and the pesticide use. Insecticide resistance or population responses to the herbicides use may have an effect on the genetic diversity in this system (8). Microevolution process, acting into the species, could be extrapolated for explain the macroevolution differences between species and another taxa (9). Saunders, Ries, Oberhauser, Thogmartin, & Zipkin (2017) found the first empirical evidence of a negative association between glyphosate level application and local abundance of adult monarchs (*Danaus sp.*). Taking into account, that the local community formation was the result of the local interaction and regional process (11) is important the inclusion of different perspectives to understand the local community formation. Therefore, environmental changes, such as habitat fragmentation and pesticide use, could affect different aspects of biodiversity, as the dispersal capacity and the diversity, in distinct ecology level, for example, in population and community.

The movement of individuals and propagules, with potential consequences for gene flow (12), is one central factor that modulates the ecological and evolutionary dynamic of population and communities, shaping demography as a consequence of gene flow. Dispersal capacity is a complex and multidimensional phenotype, involving morphological, physiological and behavioural traits (13) and it is correlated with many others aspects of life history. The dispersal capacity in flying insects can be predicted by morphological trait, as wing loading (proportion between thorax and wing area) and thorax volume (14). Frequently, evaluated by morphological traits the dispersal has a genetic basis (see Saastamoinen et al., 2018). Evaluate the genetic variability, from individual in population (16), could indicate how the environment affect the biodiversity taking into account how much is the genetic variability in distinct population.

The emergence of next generation sequencing (NGS) methods makes possible to evaluate the dispersal rate and the genomic diversity associate to land use. Little is currently know about the long-term effects of insecticides on non-pest insects, and hence the effect on biodiversity. Genomic population techniques, such as RADseq, offer a great opportunity to measure and understand the genomic diversity and dispersal between distinct populations. In addition, could be combined with species and community information allow to access the effect of environment variables acting at multiple ecoevolutionary level. NGS technologies also open new possibilities to look for adaptive drivers that predict the performance of individuals in different environmental conditions (17). The population and the species shared the same process of evolution, like birth, death and immigration. Because that, access the impact of different farms on distinct ecoevolutionary level allow to access how distinct level answered to distinct land use (18).

Several organisms are particularly sensitive to the environmental change, like the butterflies (19). Their close relationship with resources and biotic and abiotic factors, making them good indicators of the environmental quality, in distinct scales, from micro to macro scale (20,21). Therefore, this system can help us to understand the impact of agricultural and chemical practices on genomic and species levels.

In Brazil, the pesticide use increased exponentially, being among the five highest insecticide consumers worldwide in the last 15 years (22). Besides that, in Brazil the Atlantic Forest is a biodiversity hotspot (23), being the one of the most threatened and impacted biomes, due to changes in land use, with only 14% of its natural areas preserved (24). Agriculture is one of the principal factors responsible for the loss of Atlantic Forest with banana among the main crops replacing natural Atlantic Forest. Large-scale banana production was introduced in south Brazil during the 60s, and involved widespread use of pesticides. Since 1991, several banana producers have adopted organic farming methods without the use of pesticides or other synthetic chemicals.

Our general goal is to evaluate if different farming techniques, as agroforestry and conventional banana plantations (treated with the pesticide, fungicides, and herbicides), acted as barriers to dispersal or affect the genomic diversity in *Heliconius ethilla narcaea* populations and in species diversity level from Nymphalidade. Specifically, we tested if: (i) The genomic information in individuals from *H. ethilla narcaea* respond to different treatment, to distinct morphological dispersal traits and if was related to geographic occurrence; (ii) The genomic information from individuals are structured in genomic population; and, (ii) The genomic and species diversities was affected and in a similar way by distinct treatment.

Material and Methods

Sample collection

Firstly, the blocks and sites terms will be used representing distinct aspects. The blocks represent the data set formed by the set of three treatments, totally nine blocks of community sampled. On the other hand, the site representing each place were the butterflies were collected, totally 27 sites. Take into account the blocks and sites where occur individuals from *Heliconiu ethilla narcaea* we used eight blocks and 21 sites for the next analysis in this paper.

Sampling from the butterflies occurred from January to April 2016, in the northeast portion of the southernmost state of Brazil, Rio Grande do Sul (Figure 1). We collected butterflies in a total of nine different areas, each one designated as a block. Each block was composed of three treatments: native forest, agroforestry system and conventional banana farm (Figure 1). Therefore, each treatment type was sampled nine times. The conventional banana crops are treated with the pesticide, Furadan; fungicides, Manzate and Tilt; and herbicides, Tordon and Roundup-Glifosato. On the others hand, the agroforestry is a type of farm where no synthetic chemically treated was used and the banana are cultivated amongst other plants with the intend to maintain the forest structure.

We sampled the butterfly communities in each of the 27 sites. All treatment from the same block was sampled in the same day. The sample order was randomly without repositioning. Each site was sampled three times and by three collectors, a total of 13 hours and 30 minutes. The collectors, through a transection into each site using hand nets, performed sampling. All specimens were maintained in envelope, for next conduce the morphological measurement. Most of the butterflies collected belong to Nymphalidae family, totalizing 1543 individuals. Therefore, we limited our investigation in this group. The corresponding distribution from individuals' level is in Table S1. The species that show the most equal distribution, 21 of 27 sites, was the *Heliconius ethilla narcaea* (Table S2) because that, it is the selected species for conduct the genomic analysis.

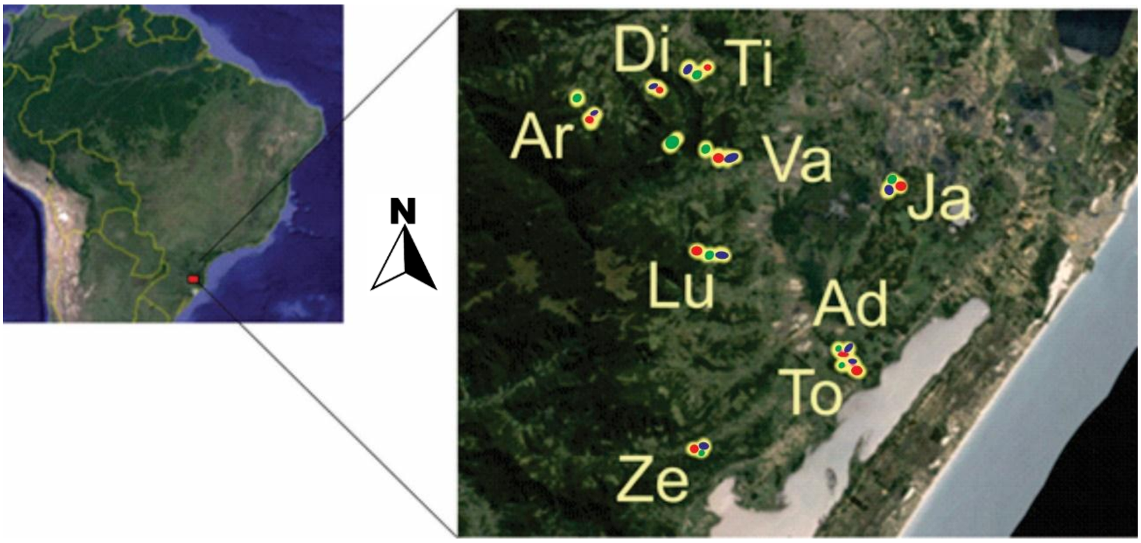


Figure 1. Location of the sampling sites in the northeast of the Rio Grande do Sul state (Brazil). The yellow circles enclose the treatment plots: native forest (green), agroforestry (blue) and conventional (red) banana crop. Each two-letter code represents a farmer name and corresponds to a block containing these three treatments.

Library preparation

The DNA extraction was performed from butterfly thorax tissue using the Qiagen DNeasy Blood & Tissue Kit. Samples were quantified using a Qubit fluorimeter. ddRAD libraries were prepared as described by (25) with some modifications. Each library was prepared from 100ng of starting genomic DNA. Not all collected individuals yielded this much DNA (likely because butterflies were not stored under ideal conditions following collection), therefore from 99 collected individuals libraries were prepared from 88 individuals. ddRAD uses the sequence specificity of restriction endonucleases to fragment the genomic DNA at specific locations and therefore create reduced representation sequencing libraries. We used the restriction enzymes *Sbf*I-HF and *EcoR*I-HF, P1 adaptors with a unique 8bp molecular identifier barcode for each sample (Table S3) were ligated to S*bf1* cut site, and generic P2 adaptors were ligated to the *EcoR1* cut site. Fragments of 300bp were selected by running each sample on an agarose gel, and were then amplified with 24 cycles of PCR. Then, the products were purified with AMPure XP beads and quantified with qPCR, before being pooled in equimolar amounts. The pooled libraries were sequenced on a single lane of Illumina HiSeq v4 to generate 460M 125 base paired-end sequence reads.

Bioinformatics analysis

Individuals were de-multiplexed and the adaptor barcodes removed using the process_radtags program within the STACKS package (26), which also removes reads lacking a barcode or restriction site and with low quality or missing data (with default values and allowing correction of errors in the barcode and restriction site). Sequencing quality was checked with FastQC, which revealed some remaining adaptor sequence. We therefore trimmed remaining adaptor sequence with the program cutadapt (v1.2.1) (27). *H. ethilla narcaea* sequences were aligned to the *H. melpomene* (v2) reference genome (28) (29) downloaded from Lepbase (30) using the program Stampy (v1.0.22) (31), with substitution rate set to 0.05. This aligner was used because it has been shown to work well when aligning data to non-conspecific genomes (28). Samtools was used to sort, index and convert SAM file to BAM files. We then called SNPs using UnifiedGenotyper in GATK (v2.5.2) with the heterozygosity prior set to 0.01, based on previous genomic studies in *Heliconius* (28). VCF tools (v0.1.12b) (32) were then used to process and filter the SNPs. We firstly removed 4 individual for which very few sites had been called. We then removed the sequences with depth of coverage greater than 200 across all individuals, as these likely represent repetitive regions. Finally, we performed stricter filtering removing individuals and positions with more than 50% missing data, and positions with a minor allele count below 2 and any indels, to leave a high quality set of 6213 SNPs in 65 individuals. Figure S1 shows the representative read depth across all sites and individuals in the final filtered data set (33).

Effects on genomic individuals information

To evaluate how the treatment, the dispersal capacity and geographic localization, affect the genomic information in *Heliconius ethilla narcaea* individuals, we assess the genomic distance between the individuals in TASSEL software (Trait Analysis by aSSociation, Evolution and Linkage v. 5.2.43) (34). The genomic distance in TASSEL is calculates as 1 - IBS (identity by state) similarity, with IBS is defined as the probability that alleles drawn at random from two individuals at the same locus are the same. The geographic distance was measured take into account the geographic distance between each site to another's. Finally, the morphological dispersal trait was measured by thorax volume index, which was positively related to dispersal capacity (14). The thorax volume value was inferred by thorax length (TL) and width (TW), using this formula:

$$\text{Thorax Volume} = 4\frac{\pi}{3} * \frac{TW^2}{2} * \frac{\text{TL}}{2}$$

The mantel test was used for evaluate the effect of geographic and morphological dispersal trait on genomic information, which as distance matrix between individuals. Additional, we used the *adonis* function and Bray-Curtis distance for conduct the PERMANOVA analysis (35) with 999 permutation, for evaluate the treatment and block effects in genome level.

Population structure analysis

For evaluate if the genomic individual level from *H. ethilla narcaea* are structured in genome population we used the individual admixture coefficients from the genotype matrix (36,37) in Landscape and Ecological Association Studies (LEA) package (38), which is similar to Bayesian clustering algorithm STRUCTURE (36). To assess the ancestral population structure we used the entropy criterion, which evaluates the quality of fit of a statistical model to the data using a cross-validation technique. In this way, the entropy criterion can be used to choose the number of ancestral populations that best explains the genotypic data (39,40), being the number of principal components that best explain variation in the genomic data. We then evaluated whether we could discern the different genomic clusters through a discriminate analysis (DAPC) (41). We tested all 21 possible populations from *Heliconius ethilla narcaea* in DAPC analysis. The best number of the cluster was determined as that giving the lowest BIC value. We then used these to predict cluster and membership probability for each individual.

The genomic and species diversity

The genomic information by each individual was used to predicted the membership probability of the sample belong to the same genomic group (Table S4) by DAPC (42). We evaluate that for individual and population levels. Then, we predict the genomic diversity into *Heliconius ethilla narcaea* and species diversity from Nymphalidae, which represent different types of diversity: (i) the equitability of samples, Shannon; (ii) the dominance of one sample, Simpson; and: (iii) the richness with uniform evenness, not being affected by sampling effort, Inverse of the Simpson index. Both diversity indexes, genomic and species, was used for evaluate the effect of the treatment and block factors by PERMANOVA, using the *adonis* function and Bray-Curtis distance from *vegan* package (35) with 999 permutation.

Finally, for accesses the relationship between genomic and species diversity we run the linear regression by *lm* function, from *stats* package (version 3.4.4, Core Team, 2018), for that we also evaluate the block as random effects and the best model was selected by Akaike Information Criteria (AIC).

Results

Individual and population genomic level

The treatment type affects the genomic distance when comparing between individual genomic information. However, the result from the treatment depend of the blocks ($F_{11, 44}$ = 1.10, R^2 = 0.18, *p* = 0.001). On the others hand, the morphological dispersal trait did not affect the individual genomic level (r^2= 0.01, *p* = 0.41) and no autocorrelation spatial was found in individual level. However, we found a significant association between genomic and geographic information (r^2 = 0.1181, *p* = 0.001), it relationship is not clear (see the Figure S2). Maybe, the significance could be result of multiple comparisons between the individuals.

These individuals are not structured into genomic population. The LEA package (38) results suggest only one ancestral population within the genomic data (Figure S3A). Therefore, high gene flow was found between the individuals. However, the Discriminant Analysis of Principal Component (DAPC, (42) suggest six possible genomic clusters (Figure S3B) take into account a minimum BIC value (Figure 2A). On this way, the most common genomic information shared was the clusters called one, four and six, being the two, three and five the less frequent genetic group in our set sample (Figure S4, Figure 2B). Besides that, the most distinct genomic group was the two and three. These genomic clusters less frequent occur only in native and agroforestry sites from distinct blocks (Figure 2B).

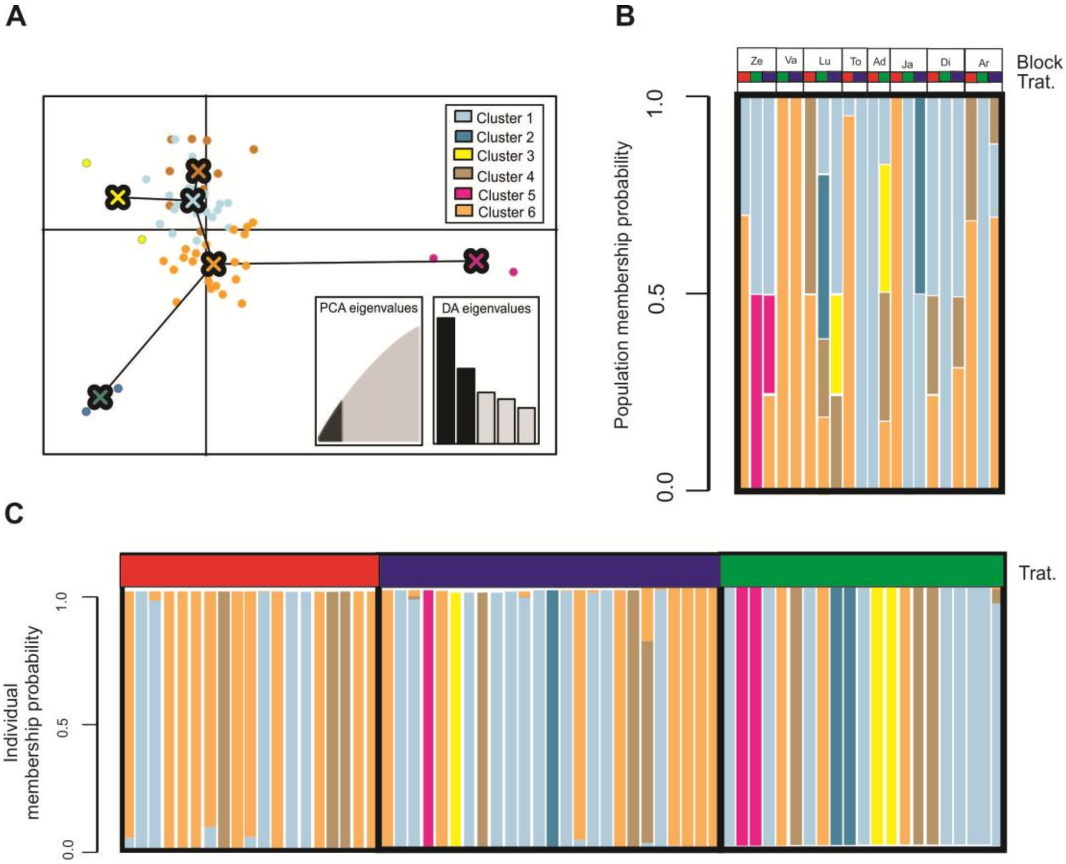


Figure 2. Genetic clusters from *Heliconius ethilla narcaea*. (A) The Discriminant Analysis of Principal Components (DAPC) result and respective PCA eigenvalues used and Discriminant Analysis eigenvalue used in black to infer the DAPC clusters. Totality, 15 PCs (black filled) which representing 37% of cumulative variation. (B) The population membership probability from six genetic group from DAPC analysis. Each column represent a genetic information in a subpopulation, individuals collected in each 21 sites. The two letter codes correspond to the block names from Figure 1. (C) The individual membership probability to six clusters identified from the DAPC. The respective treatment from each individual and population are represented by: native forest (green), agroforestry (blue) and conventional (red) treatments.

Effects at genomic site information and diversity

Using the membership probability from each individual to belong to the six genetic clusters (Figure 2B) we measured the membership probability from each subpopulation (21 sites) to belong to the six genetic clusters and evaluate the treatment effect in genomic subpopulation. Considering the membership probability rate from each subpopulation, we did not found effect from geographic localization ($r^2 = -0.06$, $p = 0.84$) and the treatment type ($F_{2, 18} = 1.33$, $R^2 = 0.13$, $p = 0.26$) (Table 1).

On the other hand, the most abundant in a species level was *Tegosa sp.*, but was largely limited to the banana farms and less abundant in the forest patches. On the other hand, the species with more equals distribution between the treatments in *Heliconius erato phyllis*, *H. ethilla narceae*, *Mechanitis lysimnia* and *Morpho epistrophus.*

There are no relation between genomic and species diversity (Table S5) when comparing the treatments and/or blocks (Figure S5; Table S6). However, both genomic and species diversity values, appear to be higher in agroforestry than in conventional and native areas (Figure 3).

Table 1. The effect of the treatment on genetic and species diversity by linear analysis.

	Linear Model		
Diversity	$F_{2,18}$	R^2	*p*
Genomic			
Shannon	0.28	-0.08	0.75
Simpson	0.28	-0.08	0.76
Inverse of Simpson	1.63	-0.07	0.72
Species			
Shannon	1.79	0.07	0.19
Simpson	0.19	-0.09	0.82
Inverse of Simpson	0.20	-0.08	0.81

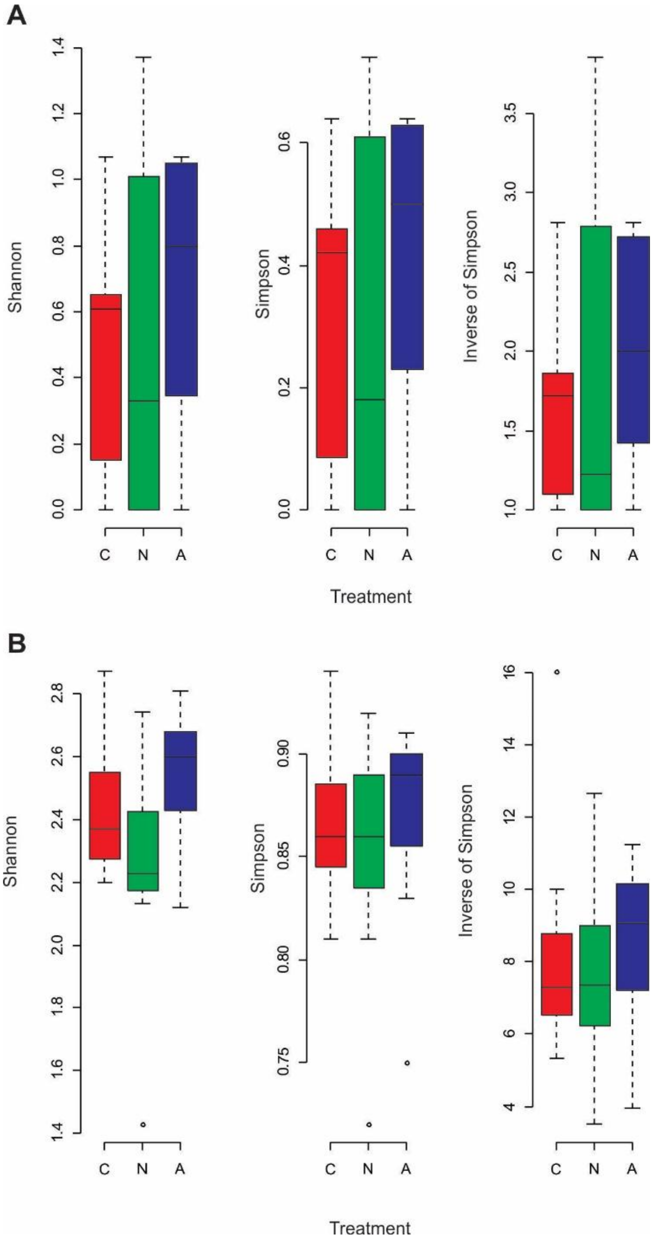


Figure 3. The genetic (A) and species (B) diversity result take into account the treatments. The conventional is in red (C), native in green (N) and agroforestry in blue (A) treatment.

Discussion

Taking into account, that the banana plantation type affected the individual genomic information and the most different genome sequence is shared only between individuals from agroforestry and native place, we could suggest that the decrease of complexity environment in the conventional plantation simplify also the genomic information, which could simplify the answer to adverse environment. Besides that, probably, the most frequent genotypes from *Heliconius ethilla* found in the conventional farm could be less affected by insecticides use (8). On this way, the treatment and block acted together to modulate the genome information in individual level. On the other hand, the dispersal ability, evaluated using the thorax volume (15) did not related to genomic information. This could be due the high connected populations from distinct treatment place suggesting that the treatment type did not worked as barrier to dispersal capacity. The intense gene flow between individuals in a genome level could be the reason that no association between genome and morphological dispersal trait in *Heliconius ethilla* was found. Besides that, recently, (15) reviewed dispersal in a genetic context and found that heritability of dispersal in insects is on average 0.35. (43) Studying *D. melanogaster*, found that 192 genes were associated with locomotion phenotype and from these only 12 SNPs explained about 60% of the variation observed in both sexes. They suggest that the litter genetic changes could strongly affect dispersal rate. In butterflies, (44) evaluating 222 SNPs, found fifteen loci related to flight. On the other hand, study in Glanville fritillary butterfly (*Melitaea cinxia*) found allelic variation in a single gene for the enzyme phosphoglucose isomerase (Pgi) was associate with dispersal rate (reviewed by Niitepõld & Saastamoinen, 2017). Our sampling of a relative small proportion of the genome makes it unlikely that we could detect specific associations between a few genes and dispersal capacity. Besides that, the phenotypic variation may have evolved in response to multiple, conflicting, biotic and abiotic factors (46). Therefore, the dispersal inferred by thorax volume, have others functions associate such as physiological and behavioural traits (47) as reproductive traits (48).

Therefore, for the first time, we evaluate the effect in different levels of biological diversity within butterflies were used for evaluate the impact of banana plantations. We combined the genomic in individual and population level to community information for clarifying the impact from the agroforestry systems and conventional plantations at these distinct biological levels. However, there are no relation between genomic and species diversity suggesting that different indices in different biological levels capture distinct information. However, previous works found that similar species was shared between agroforestry and native forestry (49–51), but, in the present work we did not find. Maybe, the diversity indices resume a lot of information as indices that could cache important aspects from the community (Cianciaruso et al. 2009) in the present case. Maybe exploring in a phylogenetic and functional aspect could show the pattern more clearly. Besides that, this result could be related of relatively small sample size to detecting some affects if is there.

Acknowledgements

We would like to thank you the Professor Vera Valente from UFRGS for *Drosophila* laboratory dependence for DNA extraction. We would like say thanks to field works helpers: Lucilene, Naka, Andressa, Taína, Ane, Nicolas, Júnia, Marina, Luan, Lucas, Wilian e Nádia. Thanks for Giovanna and Caroline from UFRGS and Érico from INPA for the important discussion about next generation analysis. DOR was supported by CAPES fellowship. This works was supported by NERC Science of the environment NBAF Grant 1058/2017. We thank the staff at NBAF Sheffield for their assistance with the ddRAD library preparation and Edinburgh Genomics for performing the sequencing. L.D. research activities have been supported by CNPq Productivity Fellowship (Grant 307886/2015-8). L.D. research has been developed in the context of National Institutes for Science and Technology (INCT) in Ecology, Evolution and Biodiversity Conservation, supported by MCTIC/CNPq (proc. 465610/2014-5) and FAPEG.

Reference

1. Curado N, Hartel T, Arntzen JW. Amphibian pond loss as a function of landscape change - A case study over three decades in an agricultural area of northern France. Biol Conserv [Internet]. 2011;144(5):1610–8. Available from: http://dx.doi.org/10.1016/j.biocon.2011.02.011
2. Marín L, Léon-Cortés JL, Stefanescu C. The effect of an agro- pasture landscape on diversity and migration patterns of frugivorous butterflies in Chiapas, Mexico. Biodivers Conserv. 2009;18:919–34.
3. Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, et al. The metacommunity concept: A framework for multi-scale community ecology. Ecol Lett. 2004;7(7):601–13.
4. Bowler DE, Benton TG. Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. Biol Rev Camb Philos Soc. 2005;80(2):205–25.
5. Schtickzelle N, Joiris A, Van Dyck H, Baguette M. Quantitative analysis of changes in movement behaviour within and outside habitat in a specialist butterfly. BMC Evol Biol. 2007;7:1–15.
6. Isenring R. Pesticides and the loss of biodiversity. Pesticide Action Network Europe (PAN); 2010.
7. Tilman D, Balzer C, Hill J, Befort BL. Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci. 2011;108:20260–4.
8. Fouet C, Atkinson P, Kamdem C. Human Interventions: Driving Forces of Mosquito Evolution. Trends Parasitol [Internet]. 2018;xx:1–13. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1471492217302684

9. Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, et al. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematic. Annu Rev Ecol Evol Syst. 1987;18:489–522.

10. Saunders SP, Ries L, Oberhauser KS, Thogmartin WE, Zipkin EF. Local and cross-seasonal associations of climate and land use with abundance of monarch butterflies Danaus plexippus. Ecography (Cop). 2017;278–90.

11. Wilson D. Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. Ecology. 1992;73:1984–2000.

12. Ronce O. How Does It Feel to Be Like a Rolling Stone? Ten Questions About Dispersal Evolution. Annu Rev Ecol Evol Syst [Internet]. 2007;38(1):231–53. Available from: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.38.091206.095611

13. Clobert J, Le Galliard JF, Cote J, Meylan S, Massot M. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. Ecol Lett. 2009;12(3):197–209.

14. Turlure C, Schtickzelle N, Baguette M. Resource grain scales mobility and adult morphology in butterflies. Landsc Ecol. 2010;25(1):95–108.

15. Saastamoinen M, Bocedi G, Cote J, Legrand D, Guillaume F, Wheat CW, et al. Genetics of dispersal. Biol Rev. 2018;93(1):574–99.

16. Solbrig OT. From genes to ecosystems: a research agenda for biodiversity. Solbrig OT, editor. Cambridge: Paris: IUBS- SCOPE-UNESCO; 1991. 128 p.

17. Capblancq T, Luu K, Blum M, Bazin E. How to make use of ordination methods to identify local adaptation: a comparison of genome scans based on PCA and RDA. bioRxiv [Internet]. 2018;33(0):258988. Available from: https://www.biorxiv.org/content/early/2018/02/03/258988

18. Marquet PA. Integrating macroecology through a statistical mechanics of adaptive matter. Proc Natl Acad Sci [Internet]. 2017;114(40):10523–5. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1713971114

19. Diekötter T, Haynes KJ, Mazeffa D, Crist TO. Direct and indirect effects of habitat area and matrix composition on species interactions among flower-visiting insects. Oikos. 2007;116(9):1588–98.

20. Freitas AVL, Marini-Filho OJ. Plano de ação nacional para a conservação dos Lepidópteros. 2011. 124 p.

21. Machado ABM, Drummond GM, Paglia AP. Livro vermelho da fauna brasileira ameaçada de extinção. Vol. 2, Livro vermelho da fauna brasileira ameaçada de extinção. 2008. 9-19 p.

22. Dos Santos CF, Otesbelgue A, Blochtein B. The dilemma of agricultural pollination in Brazil: Beekeeping growth and insecticide use. PLoS One. 2018;13(7):1–13.

23. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature [Internet]. 2000;403(6772):853–8. Available from: http://www.nature.com/articles/35002501

24. Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol Conserv [Internet]. 2009;142(6):1141–53. Available from:

http://dx.doi.org/10.1016/j.biocon.2009.02.021

25. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One. 2012;7(5).

26. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. *Stacks* : Building and Genotyping Loci *De Novo* From Short-Read Sequences. G3&#58; Genes|Genomes|Genetics [Internet]. 2011;1(3):171–82. Available from: http://g3journal.org/lookup/doi/10.1534/g3.111.000240

27. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal [Internet]. 2011;17(1):10. Available from: http://journal.embnet.org/index.php/embnetjournal/article/view/200

28. The Heliconius Genome Consortium. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature [Internet]. 2012;487(7405):94–8. Available from: http://dx.doi.org/10.1038/nature11041

29. Davey JW, Chouteau M, Barker SL, Maroja L, Baxter SW. Major Improvements to the Heliconius melpomene Genome Assembly Used to Con fi rm 10 Chromosome Fusion Events in 6 Million Years of Butter fl y Evolution. G3 Genes, Genomes, Genet. 2016;6(March):695–708.

30. Challis RJ, Kumar S, Dasmahapatra KK, Jiggins CD, Blaxter M. Lepbase: The Lepidopteran genome database. bioRxiv [Internet]. 2016;056994. Available from: http://biorxiv.org/lookup/doi/10.1101/056994

31. Lunter G, Goodson M. Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res. 2011;21(6):936–9.

32. Auton A, Marcketta A. VCF tools. 2009.

33. Knaus BJ, Grünwald NJ. vcfr: a package to manipulate and visualize variant call format data in R. Mol Ecol Resour. 2017;17(1):44–53.

34. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23(19):2633–5.

35. Oksanen A, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, et al. R package ‘ vegan ’ [software]. 2017; Available from: https://cran.r-project.org/web/packages/vegan/index.html

36. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155(2):945–59.

37. François O, Durand E. Spatially explicit Bayesian clustering models in population genetics. Mol Ecol Resour. 2010;10(5):773–84.

38. Frichot E, Francois O. LEA: an R package for Landscape and Ecological Association Studies. 2018;

39. Alexander DH, Lange K. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC Bioinformatics. 2011;12.

40. Frichot E, Mathieu F, Trouillon T, Bouchard G, François O. Fast and efficient estimation of individual ancestry coefficients. Genetics. 2014;196(4):973–83.

41. Jombart T. A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 1 . 3-4. Rvignette. 2012;1–37.

42. Jombart T, Devillard S, Balloux F, Falush D, Stephens M, Pritchard J, et al. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet [Internet]. 2010;11(1):94. Available from: http://bmcgenet.biomedcentral.com/articles/10.1186/1471-2156-11-94

43. Jordan KW, Carbone MA, Yamamoto A, Morgan TJ, Mackay TFC. Quantitative genomics of locomotor behavior in Drosophila melanogaster. Genome Biol. 2007;8(8).

44. Fountain T, Nieminen M, Sirén J, Chong S, Lehtonen R, Hanski I. Correction for Fountain et al., Predictable allele frequency changes due to habitat fragmentation in the Glanville fritillary butterfly. Proc Natl Acad Sci [Internet]. 2016;113(36):E5363–E5363. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1613041113

45. Niitepõld K, Saastamoinen M. A candidate gene in an ecological model species: Phosphoglucose isomerase (Pgi) in the Glanville fritillary butterfly (Melitaea cinxia). Ann Zool Fennici [Internet]. 2017;54(1–4):259–73. Available from: http://www.bioone.org/doi/10.5735/086.054.0122

46. Rossato DO, Kaminski LA, Iserhard CA, Duarte L. More Than Colours: An Eco-Evolutionary Framework for Wing Shape Diversity in Butterflies. Adv In Insect Phys [Internet]. 2018;1–31. Available from: http://linkinghub.elsevier.com/retrieve/pii/S006528061730036X

47. Clobert J, Baguette M, Benton TG, Bullock JM. Dispersal Ecology and Evolution. 2012;2012.

48. Stevens VM, Trochet A, Van Dyck H, Clobert J, Baguette M. How is dispersal integrated in life histories: A quantitative analysis using butterflies. Ecol Lett. 2012;15(1):74–86.

49. Schroth G, Harvey CA. Biodiversity conservation in cocoa production landscapes: An overview. Biodivers Conserv. 2007;16(8):2237–44.

50. Williams-Guillén K, McCann C, Martínez Sánchez JC, Koontz F. Resource availability and habitat use by mantled howling monkeys in a Nicaraguan coffee plantation: Can agroforests serve as core habitat for a forest mammal? Anim Conserv. 2006;9(3):331–8.

51. Harvey CA, González Villalobos JA. Agroforestry systems conserve species-rich but modified assemblages of tropical birds and bats. Biodivers Conserv. 2007;16(8):2257–92.

Supplementary material

Table S1. Species composition in each site, taking into account subfamilies of Nymphalidae. The blocks here are represented by set of two letter, which the localization correspond to the Figure 1, in different treatment type: conventional (C), native (N) and agroforestry (A). In the right side has a resume from the species concentration by the treatments.

		Ze			Val			Lu			To			Ad			Ja			Di			Ar			Ti			Treatment		
Subfamily	Species	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A	C	N	A
Heliconiinae																															
	Actinote sp.	0	3	3	2	0	0	2	4	13	0	0	0	2	3	0	1	3	2	0	0	4	2	0	4	2	1	1	11	14	27
	Agraulis vanillae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2	1	0
	Heliconius besckei	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Heliconius erato	0	5	3	2	0	1	2	5	0	3	1	9	2	15	10	2	11	5	1	9	5	1	3	6	1	4	7	14	53	46
	Heliconius ethilla	10	6	5	0	1	1	2	5	5	1	0	3	4	9	0	2	3	2	4	4	8	4	3	10	1	4	2	28	35	36
	Dione juno	0	0	0	0	0	1	0	0	1	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2	2
	Dione moneta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
	Eueides aliphera	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	10	0	0	0	4	5	11
	Eueides isabella	0	0	0	0	0	2	0	0	10	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	3	0	12
	Philaethria wernickei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0
Limenitidinae																															
	Adelpha cocala	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Adelpha lycorias	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
	Adelpha mythra	0	0	0	0	0	0	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
	Adelpha plesaure	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Adelpha serpa	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	0
	Adelpha syma	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Adelpha thessalia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Adelpha zea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Smyrna blomfildia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Libythea carineta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Danaidae																														
Aeria olena	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	19	1	0	8	0	0	0	0	0	0	0	0	31	1
Danaus eresimus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	3	0	1
Danaus erippus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	1	1	0	1	1	0	5
Danaus gillipus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2
Danaus plexippus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Lycorea halia	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	3	0	3
Episcada hymenaea	0	0	1	1	0	2	0	0	2	0	0	2	0	0	0	0	2	1	0	0	1	0	0	1	0	0	0	1	2	10
Epityches eupompe	1	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	6	1	2	1	1	4	10	3
Ithomia agnosia	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5	1
Ithomia drymo	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	5	1
Mechanitis lysimnia	2	2	8	7	3	3	1	5	15	2	6	1	0	6	4	1	7	1	2	0	2	1	8	2	6	4	1	22	41	37
Methona themisto	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	5	0
Placidina euryanassa	1	0	11	1	0	0	0	9	5	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	3	2	0	6	11	17
Pseudoscada erruca	0	3	0	0	3	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	2	9	2
Pteronymia sp	0	1	0	0	1	0	0	0	4	0	0	0	0	1	1	0	0	0	0	1	0	1	2	0	0	1	1	1	7	6
Dircenna dero	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	1
Nymphalinae																														
Colobura dirce	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	3	0	0	7	1	0
Chlosyne lacinia	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	1	0	5
Ortilia ithra	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Tegosa sp	8	0	2	28	0	3	6	0	3	9	0	14	1	2	10	3	0	1	4	0	53	23	2	50	22	0	18	104	4	154
Hypanartia bella	1	0	0	2	0	3	0	0	0	1	0	0	3	0	4	0	0	0	0	0	0	0	1	0	3	0	1	10	1	8

Hypanartia lethe	1	0	0	0	0	2	0	0	0	2	0	0	1	0	4	1	0	0	3	0	0	2	0	3	4	0	0	14	0	9
Anartia amathea	4	0	0	18	1	4	0	0	7	3	0	0	0	0	1	0	0	2	1	0	1	0	0	54	1	0	1	27	1	70
Anartia jatrophae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Siproeta epaphus	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	1	4
Siproeta stelenes	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Eresia lansdorfi	0	0	0	4	0	1	1	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	4
Junonia sp.	0	0	0	2	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	1	1	0	1	1	0	0	12	0	2
Vanessa braziliensis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Vanessa myrinna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Satyrinae																														
Blepolenis sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Caligo eurilochus	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Caligo martia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Catoblepia amphirhoe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1
Dasyopthalma creusa	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0
Opsiphanes sp	0	0	0	0	0	1	0	0	0	1	0	1	1	0	2	0	0	0	0	0	0	1	0	0	1	0	0	4	0	4
Morpho anaxibia	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Morpho epistrophus	0	2	5	6	4	3	0	0	0	1	0	1	1	0	0	0	1	0	5	0	4	3	3	6	1	0	3	17	10	22
Capronnieria galesus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	1	0	0	4
Godartiana muscosa	0	0	0	2	0	1	1	0	0	0	0	3	0	1	4	0	0	2	0	0	0	0	0	1	1	2	2	4	3	13
Moneuptychia paeon	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0	0	0	1	1	6
Moneuptychia soter	1	0	0	1	0	2	4	0	0	0	0	1	0	0	2	0	0	1	0	0	3	0	0	4	0	1	11	6	1	24
Paryphthimoides phronius	2	0	0	0	0	0	0	0	0	4	0	4	1	0	4	1	0	1	1	0	1	3	0	8	2	2	5	14	2	23
Paryphthimoides poltys	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	4
Pedaliodes phanias	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pedaliodes phronius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0

Taygetis ypthima	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0
Yphthimoides sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Hermeuptychia sp.	19	0	3	7	0	14	8	1	21	13	2	8	9	4	39	1	0	11	12	0	9	4	2	8	3	1	10	76	10	123
Apaturinae																														
Doxocopa laurentia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Doxocopa zunilda	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
Dryas iulia	2	0	0	1	0	1	0	0	0	0	0	0	1	2	2	0	1	0	0	0	0	4	0	0	1	1	4	9	4	7
Biblidinae																														
Hamadryas amphinome	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1
Hamadryas epinome	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	4	0	0	0	0	0	0	0	0	2	1	2	7
Hamadryas februa	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2
Hamadryas fornax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	1
Callicore phygas	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Catonephele sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Dynamine myrrhina	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	3	1
Dynamine postverta	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	0	0	1	1	0	0	1	1	9
Diaethria clymena	0	0	1	2	0	0	2	0	0	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	8	0	3
Biblis hyperia	0	0	4	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	6
Ectima thecla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Haematera pyrame	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	5	0	0	0	1	2	6	2
Temenis laothoe	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2	0	3
Charaxinae																														
Consul fabius	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2
Memphis moruus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	3	0
Archaeoprepona amphimachus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Archaeoprepona demophoon	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1

Cyrestinae																															
	Marpesia chiron	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Marpesia petreus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
	Marpesia zerynthia	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1
TOTAL		57	38	55	94	23	50	30	36	100	47	11	59	42	53	93	25	55	50	40	24	102	55	41	182	71	33	77	461	314	768

Table S2. Number of *Heliconius ethilla narcaea* individuals in each site and treatment. The site listed as two letter corresponds to the same site from Figure 1. Each block contains three treatment plots, conventional (C), agroforestry system (A) and native (N). 99 individuals were sampled during field collecting (Col_99), 88 individuals had DNA of sufficient quantity and quality to be included in the sequencing (Lib_88), and 65 individuals were included in the final analysis following quality filtering of the sequence data (Filt_65). Figure S1 shows the data quality for the final filtered set.

Sites	**Col_99**				**Lib_88**				**Filt_65**			
	C	A	N	Total	C	A	N	Total	C	A	N	Total
Ze	10	5	6	21	10	5	5	20	7	4	4	15
Ti	1	2	4	7	1	1	3	5	0	0	0	0
Ar	4	10	3	17	4	8	3	15	3	6	1	10
Di	4	8	4	16	4	7	2	13	4	6	1	11
Já	2	2	3	7	1	2	3	6	1	2	3	6
Ad	4	0	9	13	4	0	8	12	1	0	6	7
To	1	3	0	4	1	3	0	4	1	2	0	3
Lu	2	5	5	12	2	4	5	11	2	4	5	11
Va	0	1	1	2	0	1	1	2	0	1	1	2
TOTAL	28	34	34	99	27	31	30	88	19	25	21	65

Table S3. Sequence barcode from 88 sample of individuals sent to be sequence.

Individuals	Barcode
1NILuci01	CGTTGGTT
1OIGa10	TTCTGGTT
3CILuan04	GCATGGTT
3CMDi07	GTTCGGTT
3NIDi03	TGGCGGTT
3NIDi06	AACCGGTT
3NILuci05	TCTAGGTT
3NMLuci02	GATGCGTT
3NMMa04	CGCTCCTT
3OFDi14	AGGAGATT
3OMDi03	ATCTCATT
3OMDi26	CCTATGGT
3OMWi22	TTGATGGT
4CIMa08	TTCCTCGT
4OFJu13	TCTCGCGT
4OFJu24	AGCCTAGT
4OMDi29	TGGTCAGT
5CIDi03	TTAGAAGT
5CIDi09	CCGGTTCT
5CIDi17	GTCGTTCT
5CINi04	CATAACCT
5NFDi07	GCATAACT
5NFDi09	CGTAATAT
5NFDi14	GACTGCAT
5NFDi15	GATGGTTG
5NFLuca03	AGAGGTTG
5NMDi09	TATTATTG
5NMGa04	GTCCGCTG
5NMJu10	CTGGTTGG
6CMLuca02	ACTCTTGG
6NIDi11	CCAGCTGG
6NINi05	CTATATGG
6NMGa05	AGGATCGG
6OFLuca04	TCGCTAGG
6OMAnd02	CGTCGAGG
7CFDi15	AGTTCAGG
7CFDi17	GTACCAGG
7CMDi01	CAGCTTCG
7CMMa06	CCGACTCG
7NILuci03	ATGATGCG
7NIMa02	TAGCGGCG
7OFDi02	GTCGAGCG
7OFDi05	AGCGAACG
7OFDi06	TGCCTACG
7OFLuci13	ATAACGAG

7OIDi39	GAACTCAG
7OMAne03	TTATCCAG
7OMDi27	GACTCAAG
8CIDi08	AACGGTTC
8CIMa06	CAGCGTTC
8CMLuci10	ATACGTTC
8CMLuci11	CTTAGTTC
8NFLuci05	CGAGCTTC
8NFMa01	TACTCCTC
8NMDi01	TAAGAATC
8OFAne07	GCTAACGC
8OFAne26	TACGTAGC
8OFAne34	TGACCAGC
8OFDi22	CCAATTCC
8OFLuci17	ATTAATCC
8OIGa03	GCAACGCC
8OILuci05	AGAGAGCC
8OMDi25	CGCCGACC
9CIDi02	GACTAACC
9NIDi08	GCTGAACC
9NIGa14	CCGGCTAC
9NILuci10	GAGAATAC
9OILuci09	AAGGCGAC
10CFDi03	GCCAAGAC
10CFDi07	AACTTAAC
10CFLuan05	TATTGAAC
10CFDi09	GTTGCAAC
10CFWi01	CGACGTTA
10CIDi13	AGTAGGTA
10CIDi14	CAAGTTGA
10CIGa09	CAGTAGGA
10CIJu05	ATGACCGA
10CMTai09	TCGAACGA
10NFDi03	CGAATAGA
10NFDi07	ATATAAGA
10NFGa01	TTCTTGCA
10NFJu03	ACCTTCCA
10NMDi01	AATTACCA
10OIDi10	TCATACCA
10OIWi03	CAGGACCA
10OMDi09	GTCAACCA
10OMDi10	ACGCTTAA
10OMGa12	TCATCGAA
1NILuci01_2	CCGACGAA
5CIDI03_2	GGTCTCAA
10NFDi03_2	TGGATCAA

Table S4. Genomic membership in each site take into account distinct the clusters from DAPC. The genomic information by site was measured, taking into account the membership values of each individual from each site (Figure S4). The blocks are represented by the two letter codes used in Figure 1 and treatment type: conventional (C), native (N) and agroforestry (A).

Block	Treat.	Genomic Clusters					
Ze		1	2	3	4	5	6
	C	0.3	0	0	0	0	0.7
	N	0.5	0	0	0	0.5	0
	A	0.5	0	0	0	0.3	0.3
Val							
	C	0	0	0	0	0	0
	N	0	0	0	0	0	1
	A	1	0	0	0	0	0
Lu							
	C	0	0	0	0.5	0	0.5
	N	0.2	0.4	0	0.2	0	0.2
	A	0.5	0	0.3	0.3	0	0
To							
	C	0.1	0	0	0	0	1
	N	0	0	0	0	0	0
	A	1	0	0	0	0	0
Ad							
	C	1	0	0	0	0	0
	N	0.2	0	0.3	0.3	0	0.2
	A	0	0	0	0	0	0
Ja							
	C	0	0	0	0	0	1
	N	1	0	0	0	0	0
	A	0.5	0.5	0	0	0	0
Di							
	C	0.5	0	0	0.3	0	0.3
	N	1	0	0	0	0	0
	A	0.5	0	0	0.2	0	0.3
Ar							
	C	0	0	0	0.3	0	0.7
	N	0.9	0	0	0.1	0	0
	A	0.2	0	0	0.1	0	0.7
Ti							
	C	0	0	0	0	0	0
	N	0	0	0	0	0	0
	A	0	0	0	0	0	0

Table S5. Genomic and species diversity from each site. The diversity indices used was Shannon (Shan), Simpson (Simp) and Inverse of Simpson (InvSimp). The diversity was measured in each place take into account distinct treatament (Trat.): conventional (C), native (N) and agroforestry (A).

		Diversity					
		Genomic			Species		
Block	Trat.	Shan	Simp	InverSimp	Shan	Simp	InverSimp
Ze							
	C	0.61	0.42	1.72	2.20	0.83	5.77
	N	0.69	0.50	2.00	2.74	0.92	12.70
	A	1.07	0.64	2.81	2.60	0.90	10.30
Val							
	N	0.00	0.00	1.00	2.22	0.88	8.14
	A	0.00	0.00	1.00	2.69	0.89	9.06
Lu							
	C	0.69	0.50	2.00	2.25	0.87	7.50
	N	1.33	0.72	3.57	2.23	0.86	7.36
	A	1.07	0.64	2.81	2.67	0.90	10.10
To							
	C	0.30	0.17	1.20	2.40	0.86	7.29
	A	0.00	0.00	1.00	2.55	0.88	8.68
Ad							
	C	0.00	0.00	1.00	2.70	0.90	10.00
	N	1.37	0.74	3.85	2.34	0.86	7.11
Ja							
	C	0.00	0.00	1.00	2.87	0.94	16.00
	N	0.00	0.00	1.00	2.13	0.81	5.39
	A	0.69	0.50	2.00	2.81	0.91	11.30
Di							
	C	1.07	0.64	2.81	2.37	0.86	7.27
	N	0.00	0.00	1.00	1.43	0.72	3.51
	A	1.03	0.62	2.63	2.12	0.75	3.94
Ar							
	C	0.61	0.42	1.72	2.30	0.81	5.32
	N	0.33	0.18	1.22	2.51	0.90	9.83
	A	0.80	0.46	1.85	2.31	0.83	5.72

Table S6. The linear regression between species and genomic diversity.

Species Diversity		Genomic Diversity		
		Shannon	Simpson	Inverse of Simpson
Shannon				
	β_0	0.50	0.24	1.83
	β_1	0.02	0.04	0.01
	$F_{1,19}$	0.00	0.03	0.00
	R^2	-0.05	-0.05	-0.05
	p	0.94	0.85	0.98
Simpson				
	β_0	0.78	0.40	1.95
	β_1	-0.26	-0.07	-0.10
	$F_{1,19}$	0.01	0.00	0.00
	R^2	-0.05	-0.05	-0.05
	p	0.89	0.95	0.97
Inverse of Simpson				
	β_0	0.74	0.43	2.12
	β_1	-0.02	-0.01	-0.03
	$F_{1,19}$	0.37	0.25	0.21
	R^2	-0.03	-0.03	-0.04
	p	0.54	0.62	0.64

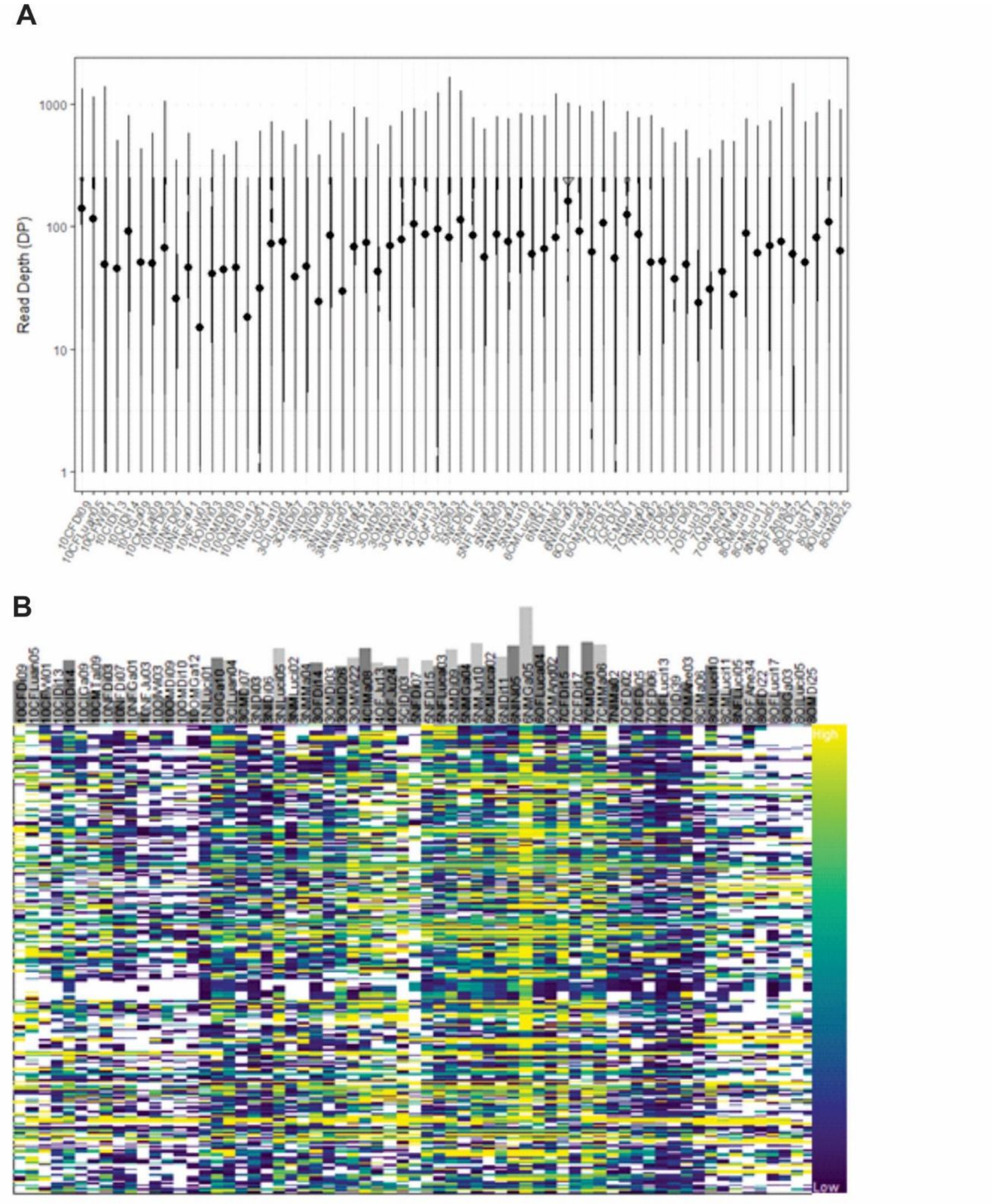


Figure S1. Quality of the data set following filtering with VCF tools. This set only includes individuals with less than 50% missing data per individual (65), and positions with less than 50% missing data across all individuals, a minor allele count of at least 2 and no indels (10245 SNPs). (A) The read depth from each of the 65 individuals. (B) The read depth in each individual at each position across the genome, high and low depth are represented by yellow and blue color, respectively and missing data in the position in white

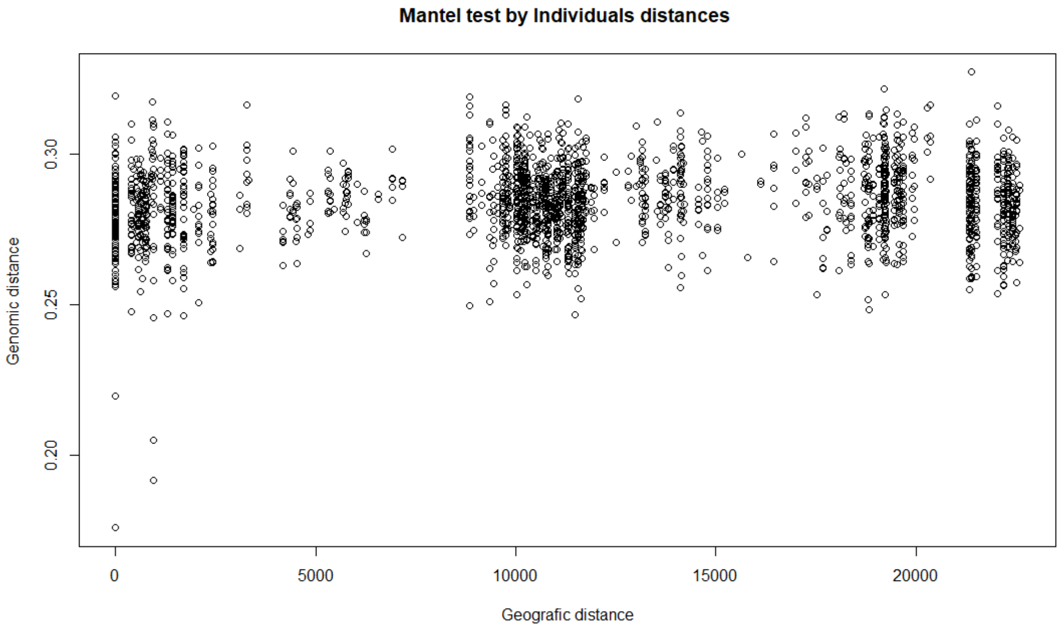


Figure S2. Mantel test result from pairwise between individual genomic and geographic distance in metres.

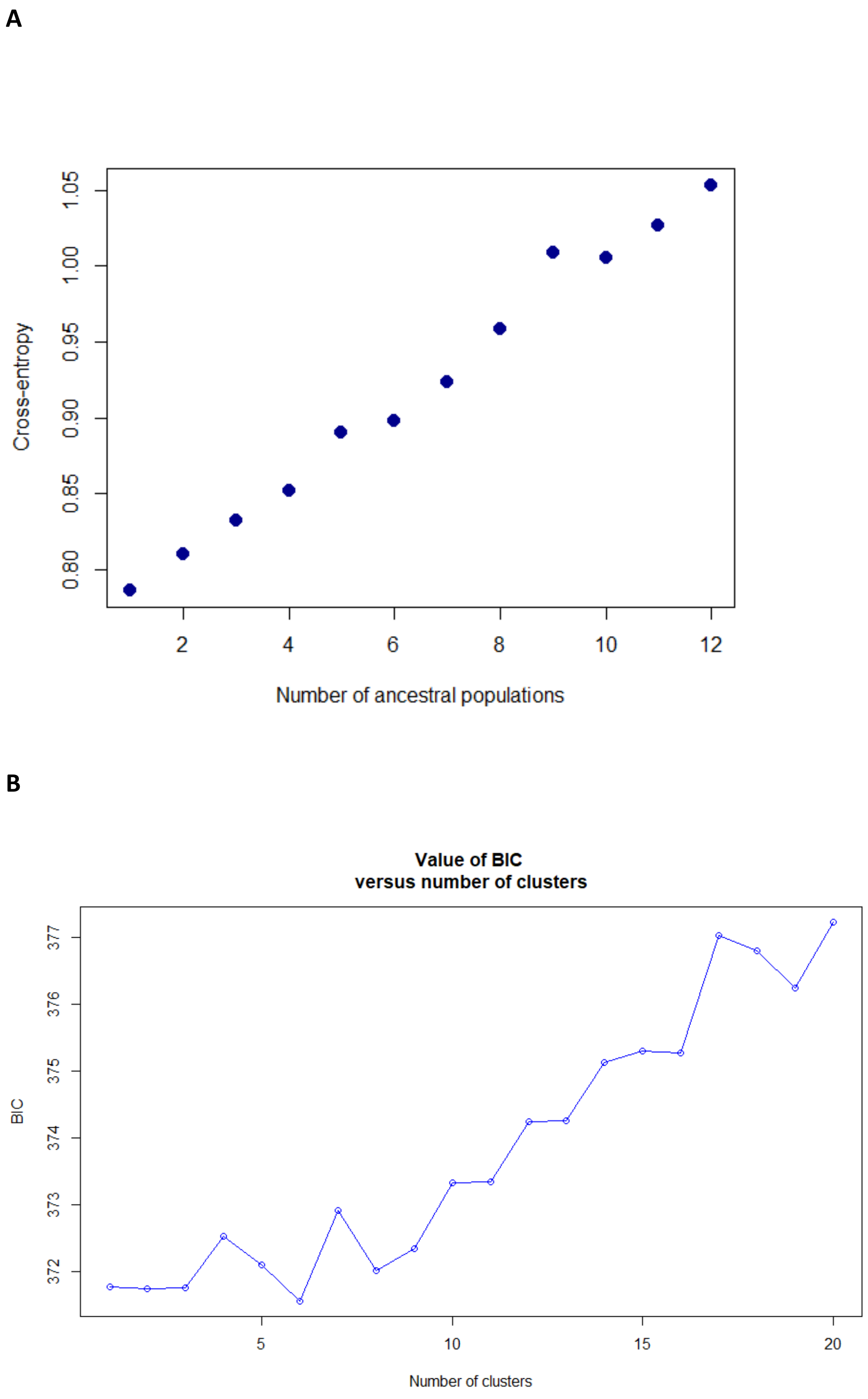


Figure S3. The population number predict by LEA package and the number of genomic clusters predict by discriminant analysis (DAPC). (A) Cross-entropy found by the ddRAD data set from with genomic by individuals suggesting only one population ancestral. (B) DAPC results where the lower BIC value suggest six genomic clusters in our set data.

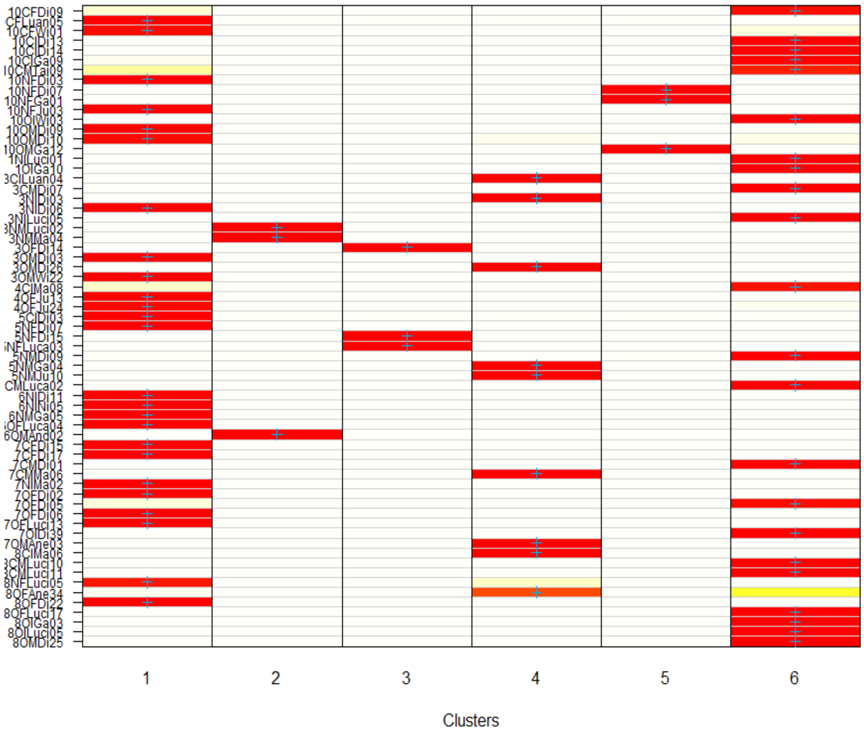


Figure S4. The membership of each individual to each genomic cluster. The membership probability to distinct clusters are strong (red) or weak (yellow).

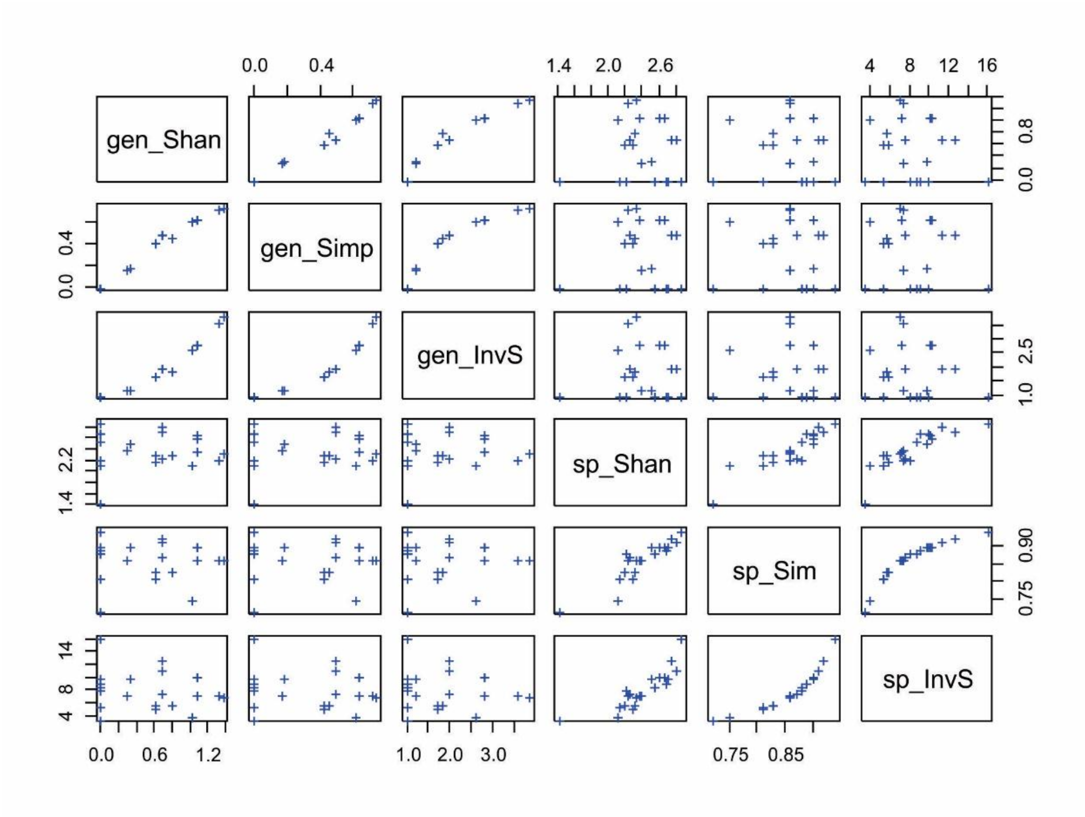


Figure S5. Relation between genomic and species diversity index. To diversity index used was Shanonn, Simpson and Invers of Simpson which was respectively evaluate in genomic and species diversity as gen_Shan, gen_Simp, gen_InvrS, sp_Shan, sp_Sim and sp_InvS.

CAPÍTULO 3

Macroevolutionary patterns of eco morphological traits related to dispersal in Nymphalidae butterflies

Dirleane Ottonelli Rossato[1*], Vanderlei Júlio Debastiani[1], André Victor Lucci Freitas[2],

Cristiano Agra Iserhard[3], Leandro Da Silva Duarte[1]

[*] dirleane.ottonelli@gmail.com

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

[2] Programa de Pós-graduação em Ecologia, Departamento de Biologia Animal, Universidade Estadual de Campinas, São Paulo, Brazil

[3] Programa de Pós-graduação em Biologia Animal, Departamento de Ecologia, Zoologia e Genética, Universidade Federal de Pelotas, Pelotas, Brazil

----

Artigo preparado para submissão na revista Proceedings of the Royal Society B: Biological Sciences.

Abstract

We evaluated the causes and consequences of butterflies dispersal in a macroevolutionary perspective. The dispersal capacity was inferred using different morphological traits as thorax volume, wing loading, aspect ratio and wing shape. To access the forces that shaped the dispersal traits along of Nymphalidade evolution, we compared different evolutionary models. Moreover, we evaluated the dispersal effects on speciation and extinction rates using the QuaSSE and ES-*sim* methods. We found no evidence that directional selective pressures shaped the evolution of dispersal traits: the changes in dispersal traits were equivalent with the Brownian Model expectation. On the other hand, the impact of dispersal capacity on speciation and extinction was unclear, considering the effect from dispersal traits to speciation and extinction rate and comparing distinct traits acting on these processes. The difference between dispersal trait affecting the speciation and extinction rate could be related to specifics aspects of dispersal, as speed and flight performance, which are, respectively related to, wing loading and aspect ratio indexes. While, the flight speed decreased the speciation rate, the flight performances increase the speciation in the extreme values representing distinct wing shape. On the others hand, the ES-*sim* result suggest the dispersal did no effect the speciation rates. However, the BM model could explain the evolution from these dispersal trait. The wing loading and aspect ratio affect differently the speciation and extinction rate. Finally, our work adds an important contribution to understand the wingers dispersal in a macroevolutionary perspective.

Keywords: Quasse, ES-*sim*, phylogenetic comparative methods, morphometric geometric, aspect ratio, wing loading

Introduction

The possibility of flying is one of the most important aspects of wings, since they enable exploring new habitats across space, time and seasons (1,2). On this way, the dispersal capacity evolved as cause, but also, consequence of ecology and evolutionary dynamics. Using the phylogenetic comparative methods is possible to investigate how dispersal traits change over time considering the occurrence or not of some selective pressure during evolution. Using this methods we can discriminate concurrent evolutionary processes underlying evolutionary dynamics (3) and infer some ecological aspects related to adaptation. The impacts generate by different selective pressures acting repeatedly in microevolution along of different developmental stages in butterflies considering the depth time, could result in some different evolutionary models selected. Some models could explained by the drift, the vicariance, the adaptive radiation and the speciation process (4).

Besides that, the flying capacity influenced the emergence and evolution of new ecological interactions, such as the pollination. For example, the coevolution between insects and plants influenced the angiosperms radiation (5). Besides that, the increase of dispersal capacity is likely to strongly influence the wingers evolution, which brings potential consequences for gene flow across space (6), and ultimately for population genetics (7) which impact the diversification rate. On this way, the effect of dispersal on speciation rate was already evaluated for different organisms (8–12). In a scenario of intense dispersal followed by reduced gene flow, strong natural selection (13), population isolation (14), an increased speciation rates are expected. However, one interesting dispersal scenario is the "intermediate dispersal" model, where the intermediate dispersal capacity is related to high speciation rate. (15) found that intermediate dispersal was more strongly related to speciation rates while high and low dispersal capacity reduces the speciation rates.

In the butterflies group, the diversification rates was already documented as related to climate change, host plant use and geological events (16–18). Although all these factors are affected by dispersal capacity, until de moment, little is known about the direct effects from the dispersal on butterfly diversification. Therefore, Nymphalidae is an interesting winged group for evaluate the causes and consequences of dispersal in butterflies. Widely distributed across the globe, this family show a high species diversity levels and an amazing variety of morphological features and habitat uses. The big associations between the environments factors allowed many cases of coevolved between butterflies and host plant (19). Understand the butterflies' dispersal trait evolution and the effects from these processes to Nymphalidae evolution allow us to clarify the drivers that impact the butterflies' evolution and then to another's related groups.

Our goals in this study were: (i) to evaluate the most plausible evolutionary model underlying the variety of dispersal-related traits in Nymphalidae; (ii) access the effect of the dispersal on speciation and extinction rates in Nymphalidade. We hypothesize that (i) all dispersal traits evolved under some selective pressures to one optimal value and the same evolutionary model could explain the evolution from the different dispersal traits; and, (ii) the intermediate dispersal capacity will be related to high speciation rate, while the dispersal capacity will be negatively association to extinction rate.

Material and Methods

Species sample

Morphological traits were obtained from 830 individuals from 280 butterfly species, around 50% of the species included in the Nymphalidae phylogeny (20). Dry mounted specimens were photographed using a Canon camera (EOS 550D, Rebel T2i). Most samples (806 specimens) were obtained in the Zoology Museum UNICAMP (Campinas, São Paulo, Brazil) and additional 24 samples were obtained from internet repositories (Table S1). Only images containing a reference scale were used for trait measurements based on either dorsal and/or ventral photo side. The number of individuals analysed for each species varied from one to six, and took into account the proportion of males and females (Table 1). The taxonomic representativeness (based on genera) in relation to the Nymphalidae phylogeny proposed by (20) is presented in Figure S1.

Dispersal traits measurements

The dispersal is a complex process (21). For better understand distinct dimensionalities of that, four different morphological dispersal traits were selected as proxies, the aspect ratio (AR), the wing loading (WL), the thorax volume (TV) and the wing shape (WS). However, all traits were related to dispersal, each one capture distinct functional aspects of dispersal in butterflies (sensu (22)). The AR combines information on forewing length (FWL) and forewing area (FWA) (see Figure 1) and is the most traditional trait used for estimate dispersal capacity of wingers (11,23–25). The AR is associated with the WS (Betts & Wootton, 1988) and both are related to flight performance in butterflies (27–29). However, while the AR is negatively related to speed (30) the indexes that take into account the muscular allocation for flight, accessed by thorax volume (TV), alone or associate to wings area value by WL index, was positively related to speed fight. The TV values was predict by the thorax length [TL] and width [TW]) (31), which was used associate to wings area (forewing - [FWA] and hindwing - [BWA]) for infer the WL value (32). The AR, WL and TX measurement were performed by AxionVision software (release 4.9.1; Carl Zeiss) and were infer by the respectively formula, as follows:

$$AR = 4\,\frac{\mathrm{FWL}^2}{\mathrm{FWA}}$$

$$WL = \frac{TV}{(\mathrm{FWA} + \mathrm{BWA})}$$

Where, $TV = \frac{4\pi}{3} \times \left(\frac{TW}{2}\right)^2 \times \frac{\mathrm{TL}}{2}$

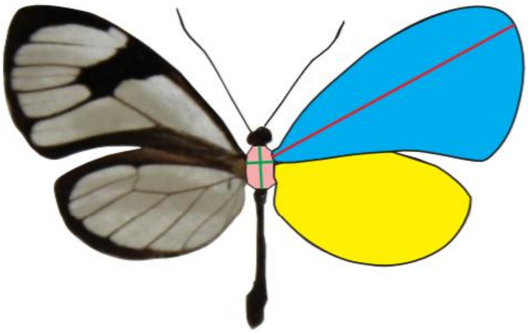


Figure 1. Morphological dimensions of butterflies used to calculate aspect ratio (AR) and wing loading (WL): forewing area (FWA) in blue; hindwing area (BWA) in yellow. The red line indicate the length of forewing and the two green lines are thorax length (TL, the vertical line) and width (TW, the horizontal line), both measures used to infer the thorax volume (TV, in pink).

Considering that the flight in butterflies is mostly driven by forewings (33,34) we measured the forewing wing shape (WS) by geometric morphometric (GM) tools. Therefore, the image from each individual was incorporated in TPSDig (version 2.17; (35)). In order to identify the landmarks in distant related species from Nymphalidae we used nine landmarks, the same used by (28) (Figure 2). The landmarks are homologues among the species, having the biological correspondence in anatomic position between the forms (36). The landmarks are located on the forewing margin and were marked by the same person (DOR). We overlap the landmark coordinates using Generalised Procrustes Analysis (GPA) (37) in TPSRewl 1.49 (38). The GPA removes differences not related to the shape, like the scale, the position and the orientation (39,40). The wing shape mean was obtained firstly into each sexes and after for each species. After that, we calculated, in R software (41), the mean wing shape for all species and the difference between the wing shape mean to each species as used as residual matrix (42), which was used in a Principal Components Analysis (PCA) as explore analyses. This procedure allows us to reduce the dimensionality of data set and work the variable independently (43).

For evaluate the phylogenetic and morphological traits autocorrelation, we evaluate the pairwise relationship between the linear traits using the Phylogenetic Generalized Least Squares models (PGLS; (44)) which were log transformed and Partial Least Squares Regression (PLS) from geomorph package (45) in R, from evaluate the correlation between linear to multivariate trait, as WS.

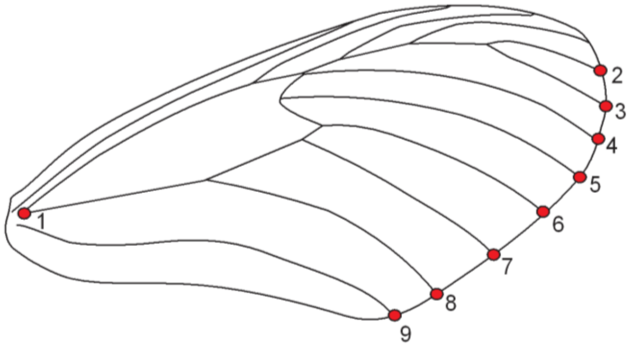


Figure 2. Landmarks used for inter the wing shape in Nymphalidade group. The determined marks are in the meeting between the veins.

Table 1. The distribution of the samples into different taxa levels.

Subfamily	Tribe	Genus	Species	Individual
Apaturinae	1	1	3	5
Biblidinae	6	17	46	153
Charaxinae	3	12	29	86
Cyrestinae	1	1	2	4
Danainae	2	25	39	162
Heliconinae	4	10	24	74
Limentidinae	1	1	17	31
Nymphalinae	5	16	25	74
Satyrinae	6	53	91	237
Limenitidinae	2	2	2	2
Libytheinae	1	1	1	1
Calinaginae	1	1	1	1
TOTAL	33	140	280	830

Nymphalidae phylogeny

We used the original dated Nymphalidae phylogeny proposed by (20), which was built considering the 10 genes and 235 morphological characters. For to obtain a most robust phylogeny, eleven new genera (*Taygetomorpha*, *Blepolenis*, *Capronnieria*, *Cybdelis*, *Eryphanis*, *Selenophanes*, *Splendeuptychia, Archeuptychia, Cissia, Carminda, Pseudodebis and Paryphthimoides*), did not include in the original phylogeny, were added. The inclusion kept the correct phylogenetic relationship between the related species. Information on the phylogenetic position of these genera in the phylogeny was obtained from (18,46–48), Freitas et al. (in prep.), and Barbosa et al. (in prep.). The genus information from the original phylogeny was refining to species levels. In cases where two or more species from the same genus were including, we kept the phylogenetic relationships according the literature and resolving the polytomies (Figure S2). Finally, we removed all species which we had no information about the traits. The final phylogeny used is on Figure S2.

Estimating evolutionary model for dispersal-related traits

To estimate the best model which explain the morphological dispersal traits evolution in Nymphalidae we used the continuous models of evolution based on maximum likelihood (49,50) available in the ‘fitContinuous’ function implemented in the R package *geiger* (51). The analysis was conducted taking into account distinct evolutionary scenarios. The simplest evolutionary model analysed was the Brownian Motion (BM, (52)), according the trait value change as a result of time and taxa. In this case, as many as no selective pressure could act along of the trait evolution, but no to one optimal value. The selective pressure acting on the trait evolution that conduct to one optimal value as function of a stabilizing selection strength was evaluate by the (1) the Ornstein-Uhlenbeck (OU, (53)) model. On the other hand, on the Early-Burst model (EB,(50)), the trait value increases or decreases the evolution rates exponentially along time. Finally, to evaluate possible influences of punctuated equilibrium on dispersal evolution we tested the kappa model (49), where changes in trait values are related to the speciation time. Moreover, we compared all models to a white noise process where the trait is assumed to evolve independently of phylogenetic relationships among species, following a normal distribution with mean = 0 and variance = 1. Considering that the measurement errors, intraspecific and sex variation, can inflate the variation measurement and the estimated models, we calculate the standard error to morphological dispersal trait for each sex, firstly, and then for each species. Finally, we selected the best models by Akaike Information Criterion (AIC)(54).

Diversification in Nymphalidae by dispersal traits

For evaluate the relationship between morphological dispersal traits affecting the Nymphalidae diversification (speciation minus extinction) we used the QuaSSE method (Quantitative State Speciation and Extinction (55)) from Diversitree (56) package in R software. However, take into account it high Type I error [58], [59] we also run also the ES-*sim* [60] test, which was evaluate considering only the speciation rate. The relationship between each dispersal trait and Nymphalidae diversification was investigated independently for each trait.

The QuaSSE method considers that the speciation and extinction are like birth-death process, which are functions of the continuous variable that evolves according to a diffusion process (55). Therefore, for each morphological dispersal trait four different functions to the speciation rates and two for extinction rates were built. The models built to understand how dispersal traits are associated with speciation rates were: (i) Constant, the speciation rate remains constant along of the trait values, therefore the dispersal did not affect the speciation rate; (ii) Linear, the speciation rate changes linearly according the trait values; (iii) Sigmoidal, trait values changes according to the speciation rate in a sigmoidal relation; and, (iv) Unimodal, dispersal affect the speciation rate as the Gaussian model (55). On the other hand, the models built to understand the dispersal affect the extinction rates were: (i) Continuous, where the dispersal did not affect the extinction rate; and: (ii) Linear, where the traits values are a linear relation to extinction rate. All functions were created in QuaSSE models and estimated using maximum likelihood were part of Diversitree (56) package in R. We also used the AIC information-theoretic approach to compare the models (54). In addition, the relative supports for particular parameters model averaging approach across the models were assessed by summing the probabilities of the models in which a particular parameter was present.

Results

Morphological dispersal trait relations and evolution

Considering all morphological dispersal traits, the PGLS results show a strong correlation between WL and TV and between AR and WS (Table S2; Figure S3). Our results also suggest a negative relationship between WL and TV to AR. The Partial Least Squares regression (PLS) result suggest that WS are more strongly related to AR to others dispersal trait (r = 0.87) (Figure S3). The scatter plot to all correlation according each species in Nymphalidade is showed in Figure 3.

The morphological dispersal traits evolved mainly by Brownian model (BM), take into account the selected model and/or parameter value from the best support models (Table 2). Although the BM is the most plausible, the different morphological dispersal traits evolved under another's evolutionary models, probability related to distinct dimensionality include in these indexes. The EB (Early-Burst) model was the unique selected model to explain the thorax volume (TV) evolution, therefore, the parameter value decreased slowly though the phylogeny. Besides the TV, the wing loading (WL) also evolved by EB model, however, the parameter value are more related to EB model in TV than in WL (Table 2). On the other hand, the traits related only to the wings aspects captured by aspect ratio (AR) and wing shape (WS) evolved also by kappa and OU (Ornstein-Uhlenbeck) models (Table 2), respectively, related to stochasticity and some selective pressure occurrence. However, the OU was most strongly related to the WS considering the weight and parameter value (Table 2). However, these another evolutionary trait affected the morphological dispersal trait evolution, the BM is the best and congruent model for explain the dispersal evolution.

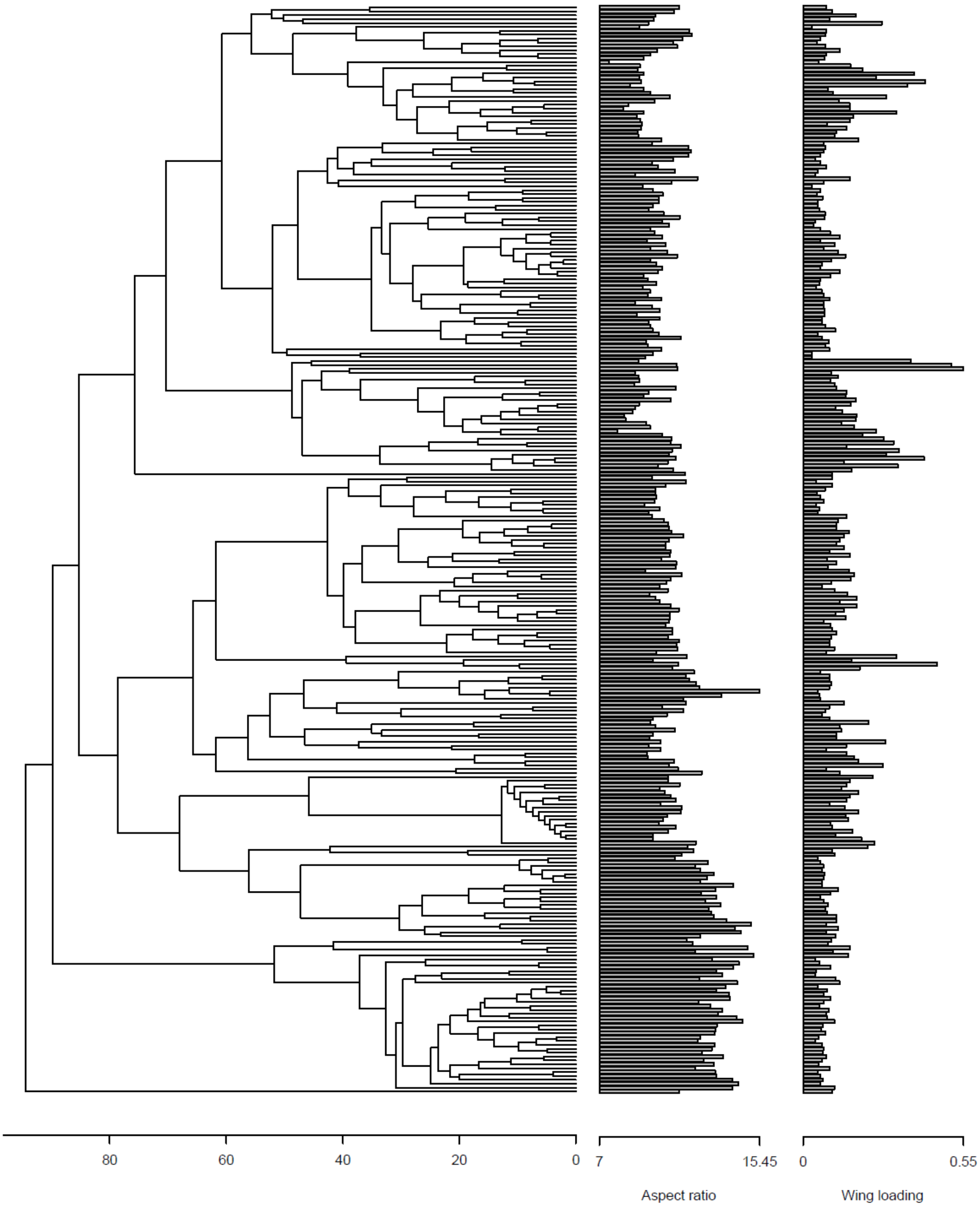


Figure 3. Trait values from aspect ratio and wing loading along the Nymphalidae phylogeny.

Table 2. Evolutionary model selected and respectively parameters from the morphological dispersal trait: the aspect ratio (AR), the wing loading (WL), the thorax volume (TV) and the wing shape (WS). The model tested were Brownian Motion (BM), kappa, the Ornstein–Uhlenbeck (OU), Early Burst (EB) and *white noise* (white). The corresponding the diffusion rate (σ^2), the number of the parameter (k), the standard error (SE), the Akaike information criterion (AICc) and delta AIC (ΔAIC) and it respectively weight (ω) from each model were included.

Model	σ^2	parameter	k	SE	AICc	ΔAIC	ω
Aspect ratio							
kappa	**0.0317**	**k = 0.802**	**4**	**0.592**	**668.865**	**0.000**	**0.356**
BM	**0.0202**		**3**	**0.596**	**668.904**	**0.039**	**0.349**
OU	**0.0218**	**α = 0.003**	**4**	**0.589**	**670.353**	**1.488**	**0.169**
EB	0.0202	r = 0.000	4	0.596	670.963	2.098	0.125
white	2.2635		3	0.000	1037.123	368.258	0.000
Wing loading							
BM	**0.0001**		**3**	**0.065**	**-858.697**	**0.000**	**0.435**
EB	**0.0002**	**r = -0.011**	**4**	**0.060**	**-857.614**	**1.083**	**0.253**
OU	0.0001	α = 0.000	4	0.065	-856.638	2.058	0.156
kappa	0.0001	k = 1.000	4	0.065	-856.638	2.058	0.156
white	0.0027		3	0.100	-692.103	166.594	0.000
Thorax volume							
EB	**0.1241**	**r = -0.035**	**4**	**0.291**	**1476.168**	**0.000**	**0.824**
BM	0.0149		3	0.160	1480.395	4.227	0.100
kappa	0.0212	k = 0.867	4	0.109	1482.191	6.023	0.041
OU	0.0149	α = 0.000	4	0.160	1482.454	6.285	0.036
white	0.3969		3	1.168	1553.462	77.293	0.000
Wing shape							
OU	**0.0001**	**α = 0.006**	**3**	**NA**	**-1102.670**	**0**	**0.4**
kappa	**0.0001**	**k = 0.814**	**3**	**NA**	**-1102.100**	**0.562**	**0.3**
BM	**0.0001**		**2**	**NA**	**-1101.430**	**1.235**	**0.22**
EB	0.0001	**r =** 0.000	3	NA	-1099.390	3.279	0.08
white	0.0037		2	NA	-766.310	336.3	0

Speciation and extinction rate

Take into account that the WL was related to TV and AR to WS, we run the speciation and extinction analysis only for WL and AR. The respective values from this trait are represented in Figure 3. However, while by QuaSSE method, the WL and AR affect the speciation, by *ES*-sim method no.

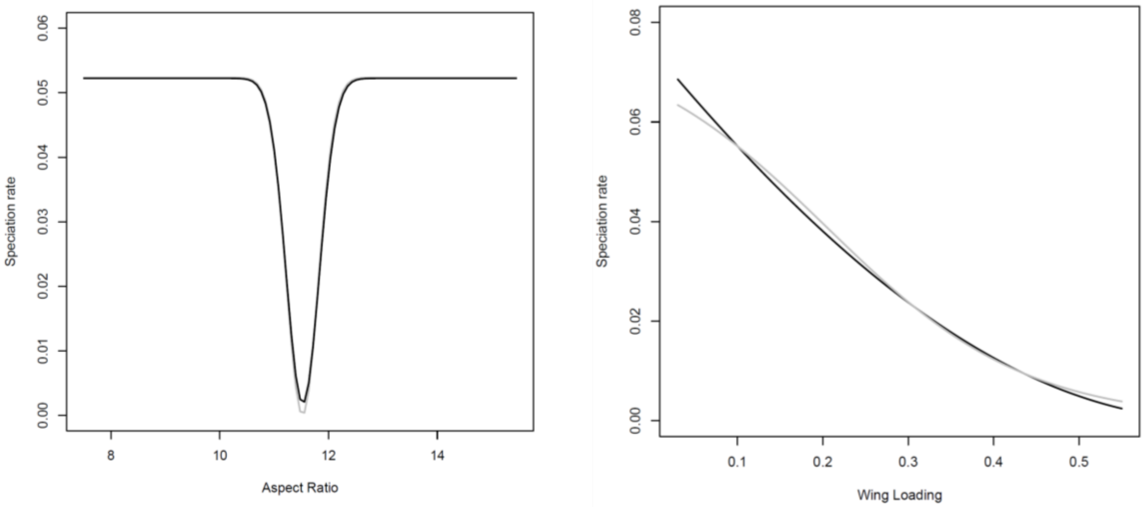
The results found by QuaSSE method suggest that the AR and WL affected differently relationship to Nymphalidae speciation rate. There is a negative and unimodal relationship between AR to speciation rate. Therefore, both low and high AR values were related to high speciation rate comparing to intermediate dispersal (Table 3; Figure 4). On the other hand, the relationship between AR to extinction rate appears to be less important, which could be constant or linear, however, in a linear the slope is weak (Table S3; Figure S5). On the other hand, the WL impacts the speciation rate like a negative exponential relationship (Figure 4). Although, the selected models were the unimodal or sigmoidal (Table 3), both are congruent to a negative exponential relationship (Figure 4), once the selected unimodal model was restricted to the values of the WL. We could do not run a model where the speciation and extinction are linear, probably due to poor fit as predict by (56). Besides that, contrarily to AR, the WL impact the extinction rate as linear relationship, however, the slope is weak (Table S5).

On the other hand, the ES-*sim* results suggested that the WL and AR did not affect the speciation and extinction process, showing no significance in *p* neither in rho value. However, the relation was more strongly in WL (-0.108, *p* = 0.37) than in AR (0.013, *p* = 0.91).

Table 3. Diversification models for the morphological dispersal trait, the wing loading (WL) and the aspect ratio (AR, and respective results. The combination between different speciation and extinction rate to dispersal trait to each model: the number of parameters (K); the log likelihood value; the Akaike Information Criteria (AIC); the delta AIC and respective weight (ω). The NA represents the model with poor fit.

Dispersal Trait	Speciation	Extinction	K	Log likelihood	AIC	ΔAIC	ω
Wing loading							
	constant	constant	3	-732.23	1470.47	37.83	0.000
	linear	constant	4	-729.79	1467.58	34.94	0.000
	sigmoid	constant	6	-728.54	1469.09	36.45	0.000
	unimodal	constant	6	-730.12	1472.24	39.60	0.000
	constant	linear	4	-732.23	1472.46	39.83	0.000
	linear	linear	NA	NA	NA	NA	NA
	sigmoid	**linear**	**7**	**-709.52**	**1433.04**	**0.41**	**0.449**
	unimodal	**linear**	**7**	**-709.32**	**1432.64**	**0.00**	**0.551**
Aspect ratio							
	constant	constant	3	-1494.71	2995.42	9.47	0.006
	linear	constant	4	-1494.66	2997.32	11.37	0.002
	sigmoid	constant	6	-1494.63	3001.26	15.31	0.000
	unimodal	**constant**	**6**	**-1486.97**	**2985.95**	**0.00**	**0.716**
	constant	linear	4	-1494.68	2997.36	11.41	0.002
	linear	linear	5	-1495.12	3000.24	14.29	0.001
	sigmoid	linear	7	-1494.68	3003.36	17.41	0.000
	unimodal	**linear**	**7**	**-1486.94**	**2987.88**	**1.93**	**0.272**

Figure 4. The results between morphological trait, the aspect ratio (AR) and the wing loading (WL), to speciation rate. The bold line is the model with the weight is high and the grey line represent the second best model.



Discussion

We evaluate for the first time the morphological dispersal trait evolution and it effects to speciation and extinction rate in Nymphalidae butterflies. The dispersal capacity was accessed by four different morphological traits, which capture distinct aspects. The dispersal traits evolved mainly by Brownian motion, but few changes in each trait evolution was found. The relationship between dispersal capacities to diversification process are unclear, through the QuaSSE method the dispersal affect the speciation, but by ES-*sim* method no. However, differently from ES-*sim*, the QuaSSE method considers the extinction rate which could make some effect mainly in wing loading which shows a correlation to extinction. Our result agree with Furnariid birds, both in relation to ES-*sim* (57) as to Quasse methods (11). By QuaSSE the wing loading (WL) and aspect rate (AR) showed a different relationship to speciation and extinction rate. Probability, distinct aspects from these morphological traits affect the speciation rate in distinct ways, the WL more related to speed and AR to flight performance.

However, we selected four different morphological traits for infer the dispersal capacity, we found a significate association between WL to TV and AR to WS. These correlation suggest these traits capture different dimensionalities from de dispersal, the WL and TV associated to dispersal speed (32) and the AR and WS associated to flight performance (27–29). Besides that, as (32), our data set suggest a negative relationship between AR to WL and TV. This was also confirmed by the evolutionary model. In fact, we believe, that the dispersal, as all, evolved by Brownian motion model and these traits evolved with no directional selective pressure. Until we know, the dispersal evolved randomly in wingers, once (11) found a similar result in birds. Besides that, taking into account that these traits captured different dimensionalities from the dispersal, others evolutionary model were selected besides the BM. The unique evolutionary model selected by TV, the Early Burst, affected also the WL evolution model. On this way, the variation in TV value decreased through Nymphalidae history, contrarily the increase of changes from adaptive radiation, typically related in some butterfly groups (58–60). The association to more functionalities from wing (4,26,32,59,61,62) could explain the selection from many evolutionary model while, the TV, for example are related as territorialism behaviours (32). Therefore, the OU model selected by wing shape could predict the selective pressure acting along wing evolution, where the microhabitat, as the host plant used in the caterpillar stage (63), could be related to distinct wing aspects, both in taking into account the vertical stratification (2), (27) and the geographic occurrence (61).

On the other hand, the relationship between dispersal capacity and speciation rate in Nymphalidae is unclear. The same result found by Quasse (55) and ES-*sim* (57) method, was found in birds (11,57). However, the result found using the WL in butterflies is compared to AR in birds [14]. Therefore, if dispersal process affected the winger speciation rate in the same way, a similar pattern was found between these morphological traits. In butterflies the WL are related to speed dispersal (32). Probability the association between QuaSSE and ES-*sim* methods could help us to clarify the dispersal effect considering distinct dimensionalities, while the QuaSSE show a high Type I Error the ES-*sim* consider only the speciation rate. In this ways, looking to QuaSSE results the distinct effect was found between WL and AR to speciation rate, which could result of the distinct dispersal aspects captured in each trait. The negative relationship between WL to speciation rate suggest that the less dispersers have weak mobility, however, when they could move to a new places, no others could, therefore start the genetic differentiation in distinct populations, which result in high speciation rate. On the other hand, the high dispersal affected negatively the speciation rate, suggesting the high dispersers may result in genetic homogenization (11). The high dispersal capacity will connect distinct populations. On the other hand, the disruptive relation between AR to speciation rate in Nymphalidae, reinforce the idea that the distinct wing shape was selected by different habitats (2,27,61), affecting the speciation rate. Thus, butterflies with intermediate AR values could fly between distinct habitats and no selective pressures to one form could be found. This will keep the genetic connection and without generating of new species. However, the extreme AR value, are related to different WS and probably could affect the speciation rate take into account distinct habitats. However, the weak slope between dispersal trait and extinction rate suggested the low impact of this dispersal to escape from predators and to avoid unfavourable conditions (64–66). However, the WL show a linear effect to extinction rate, with high values comparing to AR reinforce the importance to measure the extinction rate, however, it is difficult to have a clearly comprehension about the real extinction process.

Conclusions

For the first time we evaluated the dispersal trait evolution and its influence on butterfly diversification from a macro evolutionary perspective. Dispersal speed and performance flight evolved randomly, like observed before in birds. Others functions, such as the territoriality and environment could affected its evolution. Despite the opposite results from ES-*sim* and QuaSSE method, both are very useful to understand distinct aspects of the eco evolutionary dynamics and could clarify the trait effect in speciation rates. The dispersal trait affected differentially the speciation rate, probably as result of distinct aspects of the dispersal as performance and flight speed. In fact, as (4) proposed different filters work in the trait evolution, probably the WL affected mainly by biotic and AR by abiotic filter. Finally, the dispersal in a macroevolution perspective add the importance of traits along the evolution of insects lineage (e.g., (16,67,68) and wingers. The macroevolution allows us to find a good conservation strategy for maintaining biodiversity and predict how much the individual could support distinct impacts.

Competing interests

No competing interests

Authors' contributions

LSD and DOR design the research. DOR measured, analysed and interpreted the linear and geometric dispersal trait. DOR and VJD run the macro evolutionary analysis. AVLF as curate from specimens' museum. DOR oversaw manuscript preparation. All authors contributed in preparing the manuscript.

Acknowledgements

We would like to thank Milton Mendonça, Lucas Kaminski and Jorge Bernardo-Silva for the good contribution and suggestion to this article. We also grateful to Jessie Pereira, Tamara Aguiar and Eduardo Barbosa for the important help to access the specimens used in the present work from Laboratory of Lepidoptera in UNICAMP.

Funding

D.O.R. was supported by a CAPES PhD Program Fellowship. V.J.D. was supported by PNPD/CAPES Program Fellowship. L.D. research activities have been supported by CNPq Productivity Fellowship (Grant 307886/2015-8). L.D. research has been developed in the context of National Institutes for Science and Technology (INCT) in Ecology, Evolution and Biodiversity Conservation, supported by MCTIC/CNPq (proc. 465610/2014-5) and FAPEG.

Reference

1. Johnson CG. Mechanisms of Insect Dispersal. Science. 1969;166:592.
2. DeVries PJ, Penz CM, Hill RI. Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies. J Anim Ecol. 2010;79(5):1077–85.
3. Pyron RA, Burbrink FT. Phylogenetic estimates of speciation and extinction rates for testing ecological and evolutionary hypotheses. Trends Ecol Evol [Internet]. 2013;28(12):729–36. Available from: http://dx.doi.org/10.1016/j.tree.2013.09.007
4. Rossato DO, Kaminski LA, Iserhard CA, Duarte L. More Than Colours: An Eco-Evolutionary Framework for Wing Shape Diversity in Butterflies. Adv In Insect Phys [Internet]. 2018;1–31. Available from: http://linkinghub.elsevier.com/retrieve/pii/S006528061730036X
5. Armbruster WS. Floral specialization and angiosperm diversity: Phenotypic divergence, fitness trade-offs and realized pollination accuracy. AoB Plants. 2014;6:1–24.
6. Ronce O. How Does It Feel to Be Like a Rolling Stone? Ten Questions About Dispersal Evolution. Annu Rev Ecol Evol Syst [Internet]. 2007;38(1):231–53. Available from: http://www.annualreviews.org/doi/10.1146/annurev.ecolsys.38.091206.095611
7. Vellend M. Conceptual synthesis in community ecology. Q Rev Biol. 2010;85:183–206.
8. Mayr E. Animal species and their evolution [Internet]. Trends in Ecology & Evolution. 1963. p. 797 pages. Available from: http://linkinghub.elsevier.com/retrieve/pii/0169534794901872
9. Gavrilets S. Perspective: Models of Speciation: What Have We Learned in 40 Years? Evolution (N Y) [Internet]. 2003;57(10):2197–215. Available from: http://doi.wiley.com/10.1111/j.0014-3820.2003.tb00233.x

10. Kisel Y, Barraclough TG. Speciation Has a Spatial Scale That Depends on Levels of Gene Flow. Am Nat [Internet]. 2010;175(3):316–34. Available from: http://www.journals.uchicago.edu/doi/10.1086/650369

11. Claramunt S, Derryberry EP, Remsen J V., Brumfield RT. High dispersal ability inhibits speciation in a continental radiation of passerine birds. Proc R Soc B Biol Sci [Internet]. 2012;279(1733):1567–74. Available from: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2011.1922

12. Igea J, Miller EF, Papadopulos AST, Tanentzap AJ. Seed size and its rate of evolution correlate with species diversification across angiosperms. PLoS Biol. 2017;15(7):1–16.

13. Garcia-R. JC, Trewick SA. Dispersal and speciation in purple swamphens (Rallidae: *Porphyrio*). Auk [Internet]. 2015;132(1):140–55. Available from: http://www.bioone.org/doi/abs/10.1642/AUK-14-114.1

14. Butlin RK, Galindo J, Grahame JW. Sympatric, parapatric or allopatric: the most important way to classify speciation? Philos Trans R Soc B Biol Sci [Internet]. 2008;363(1506):2997–3007. Available from: http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2008.0076

15. Paulay G, Meyer C. Dispersal and divergence across the greatest ocean region: do larvae matter? Integr Comp Biol. 2006;46:269–81.

16. Condamine FL, Rolland J, Höhna S, Sperling FAH, Sanmartín I. Testing the Role of the Red Queen and Court Jester as Drivers of the Macroevolution of Apollo Butterflies. Syst Biol [Internet]. 2018; Available from: https://academic.oup.com/sysbio/advance-article/doi/10.1093/sysbio/syy009/4847851

17. Chazot N, De-Silva DL, Willmott KR, Freitas AVL, Lamas G, Mallet J, et al. Contrasting patterns of Andean diversification among three diverse clades of Neotropical clearwing butterflies. Ecol Evol. 2018;(October 2017):3965–82.

18. Peña C, Wahlberg N. Prehistorical climate change increased diversification of a group of butterflies. Biol Lett [Internet]. 2008;4(3):274–8. Available from: http://rsbl.royalsocietypublishing.org/cgi/doi/10.1098/rsbl.2008.0062

19. Ehrlich PR, Raven PH. Butterflies and Plants : A Study in Coevolution. Evolution (N Y). 1964;18(4):586–608.

20. Wahlberg N, Leneveu J, Kodandaramaiah U, Pena C, Nylin S, Freitas AVL, et al. Nymphalid butterflies diversify following near demise at the Cretaceous/Tertiary boundary. Proc R Soc B Biol Sci [Internet]. 2009;276(1677):4295–302. Available from: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2009.1303

21. Clobert J, Le Galliard JF, Cote J, Meylan S, Massot M. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. Ecol Lett. 2009;12(3):197–209.

22. Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, et al. Let the concept of trait be functional! Oikos. 2007;116:882–92.

23. Hassall C. Strong geographical variation in wing aspect ratio of a damselfly, *Calopteryx maculata* (Odonata: Zygoptera). PeerJ [Internet]. 2015;3:e1219. Available from: https://peerj.com/articles/1219

24. Hodgkison R, Balding ST, Zubaid A, Kunz TH. Habitat structure, wing morphology, and the vertical stratification of Malaysian fruit bats (Megachiroptera: Pteropodidae). J Trop Ecol. 2004;20(6):667–73.

25. Findley JS, Studier EH, Wilson DE. Morphologic properties of bat wings. J Mammal. 1972;53:429–44.

26. Betts CR, Wootton RJ. Wing Shape and Flight Behaviour in Butterflies (Lepidoptera: Papilionoidea and Hesperioidea): A Preliminary Analysis. J Exp Biol [Internet]. 1988;138(1):271–88. Available from: http://jeb.biologists.org/content/138/1/271.abstract

27. Chazot N, Panara S, Zilbermann N, Blandin P, Le Poul Y, Cornette R, et al. Morpho morphometrics: Shared ancestry and selection drive the evolution of wing size and shape in Morpho butterflies. Evolution (N Y). 2016;70(1):181–94.

28. Cespedes A, Penz CM, Devries PJ. Cruising the rain forest floor: Butterfly wing shape evolution and gliding in ground effect. J Anim Ecol. 2015;84(3):808–16.

29. Berwaerts K, Aerts P, Dyck HVAN. On the sex-specific mechanisms of butterfy fight: fight performance relative to fight morphology, wing kinematics, and sex in Pararge aegeria. Biol J Linn Soc. 2006;89:675–87.

30. Dudley R, Srygley R. Flight Physiology of Neotropical Butterflies: Allometry of Airspeeds During Natural Free Flight. J Exp Biol [Internet]. 1994;191(1):125–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9317473

31. Dempster JP, King ML, Lakhani KH. The status of the swallowtail butterfly in Britain. Ecol Entomol. 1976;1(2):71–84.

32. Turlure C, Schtickzelle N, Baguette M. Resource grain scales mobility and adult morphology in butterflies. Landsc Ecol. 2010;25(1):95–108.

33. Dudley R. The Biomechanics of Insect Flight: Form, Function and Evolution. 1st ed. Dudley R, editor. Princeton: Princeton University Press; 2000.

34. Jantzen B, Eisner T. Hindwings are unnecessary for flight but essential for execution of normal evasive flight in Lepidoptera. Proc Natl Acad Sci U S A [Internet]. 2008;105(43):16636–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2575472&tool=pmcentrez&rendertype=abstract

35. Rohlf JF. TPSDig. New York: Department of Ecology and Evolution, State University of New York, Stony Brook; 2013.

36. Sneath PHA, Sokal RR. Numerical Taxonomy. San Francisco: Freeman; 1973.

37. Dryden IL, Mardia K V. Statistical shape analysis. New York, NY: John Wiley & Sons Inc;

1998. 380 p.

38. Rohlf JF. TPSRewl. New York: Department of Ecology and Evolution, State University of New York at Stony Brook; 2010.

39. Rohlf FJ, Slice D. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst Zool. 1990;39:40–59.

40. Adams CD, Rohlf FJ, Slice ED. Geometric morphometrics: Ten years of progress following the “revolution.” 2004;71:5–16.

41. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2018.

42. Jolliffe IT. Principal component analysis. Geneva: New York Springer; 1986.

43. Cordeiro-Estrela P, Baylac M, Denys C, Marinho-Filho J. Interspecific Patterns of Skull Variation Between Sympatric Brazilian Vesper Mice: Geometric Morphometrics Assessment. J Mammal. 2006;87(6):1270–9.

44. Grafen A. The phylogenetic regression. Philos Trans R Soc London Ser B. 1989;326:119–57.

45. Adams DC, Collyer M, Sherratt E. geomorph 3.0.3: Software for geometric morphometric analyses. 2016.

46. Matos-Maraví PF, Peña C, Willmott KR, Freitas AVL, Wahlberg N. Systematics and evolutionary history of butterflies in the “Taygetis clade” (Nymphalidae: Satyrinae: Euptychiina): Towards a better understanding of Neotropical biogeography. Mol Phylogenet Evol. 2013;66(1):54–68.

47. Peña C, Nylin S, Freitas AVL, Wahlberg N. Biogeographic history of the butterfly subtribe euptychiina (lepidoptera, nymphalidae, Satyrinae). Zool Scr. 2010;39(3):243–58.

48. Penz CM, Mohammadi N. Wing pattern diversity in Brassolini butterflies (Nymphalidae, Satyrinae). Biota Neotrop. 2013;13(3):154–80.

49. Pagel M. Inferring the historical patterns of biological evolution. Nature. 1999;401(6756):877–84.

50. Harmon LJ, Losos JB, Jonathan Davies T, Gillespie RG, Gittleman JL, Bryan Jennings W, et al. Early bursts of body size and shape evolution are rare in comparative data. Evolution (N Y). 2010;64(8):2385–96.

51. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. GEIGER: Investigating evolutionary radiations. Bioinformatics. 2008;24(1):129–31.

52. Felsenstein J. Maximum-likelihood estimation of evolutionary trees from continuous characters. Am J Hum Genet. 1973;25(5):471–92.

53. Butler MA, King AA. Phylogenetic Comparative Analysis: A Modeling Approach for

Adaptive Evolution. Am Nat [Internet]. 2004;164(6):683–95. Available from: http://www.journals.uchicago.edu/doi/10.1086/426002

54. Burnham K, Anderson D. Model selection and multimodel inference: a practical information-theoretic approach. 2002.

55. Fitzjohn RG. Quantitative traits and diversification. Syst Biol. 2010;59(6):619–33.

56. Fitzjohn RG. Diversitree: Comparative phylogenetic analyses of diversification in R. Methods Ecol Evol. 2012;3(6):1084–92.

57. Harvey MG, Rabosky DL. Continuous traits and speciation rates: Alternatives to state-dependent diversification models. Methods Ecol Evol. 2017;2018(November 2017):984–93.

58. Peña C, Witthauer H, Klečková I, Fric Z, Wahlberg N. Adaptive radiations in butterflies: evolutionary history of the genus *Erebia* (Nymphalidae: Satyrinae). Biol J Linn Soc [Internet]. 2015;116(2):449–67. Available from: http://doi.wiley.com/10.1111/bij.12597

59. Kozak KM, Wahlberg N, Neild AFE, Dasmahapatra KK, Mallet J, Jiggins CD. Multilocus species trees show the recent adaptive radiation of the mimetic heliconius butterflies. Syst Biol. 2015;64(3):505–24.

60. Jiggins CD, McMillan WO. The genetic basis of an adaptive radiation: warning colour in two Heliconius species. Proc R Soc B Biol Sci [Internet]. 1997;264(1385):1167–75. Available from: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.1997.0161

61. Rossato DO, Boligon D, Fornel R, Kronforst MR, Gonçalves GL, Moreira GRP. Subtle variation in size and shape of the whole forewing and the red band among co-mimics revealed by geometric morphometric analysis in *Heliconius* butterflies. Ecol Evol [Internet]. 2018;(August 2017):1–16. Available from: http://doi.wiley.com/10.1002/ece3.3916

62. De Keyser R, Breuker CJ, Hails RS, Dennis RLH, Shreeve TG. Why small is beautiful: Wing colour is free from thermoregulatory constraint in the small lycaenid butterfly, Polyommatus icarus. PLoS One. 2015;10(4):1–13.

63. Jorge LR, Cordeiro-Estrela P, Klaczko LB, Moreira GRP, Freitas AVL. Host-plant dependent wing phenotypic variation in the neotropical butterfly Heliconius erato. Biol J Linn Soc. 2011;102(4):765–74.

64. Kingsolver JG, Watt WB. Thermoregulatory Strategies in Colias Butterflies: Thermal Stress and the Limits to Adaptation in Temporally Varying Environments. Am Nat [Internet]. 1983;121(1):32. Available from: http://www.jstor.org/stable/2461044

65. Srygley RB. The aerodynamic costs of warning signals in palatable mimetic butterflies and their distasteful models. Proc R Soc B Biol Sci [Internet]. 2004;271(1539):589–94. Available from: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2003.2627

66. Niitepõld K, Mattila ALK, Harrison PJ, Hanski I. Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. Oecologia. 2011;165(4):847–54.

67. Grimaldi D, Engel MS. Evolution of the Insects. 1st ed. Grimaldi D, Engel MS, editors. Cambridge: Cambridge University; 2005.

68. Bai M, Beutel RG, Song KQ, Liu WG, Malqin H, Li S, et al. Evolutionary patterns of hind wing morphology in dung beetles (Coleoptera: Scarabaeinae). Arthropod Struct Dev [Internet]. 2012;41(5):505–13. Available from: http://dx.doi.org/10.1016/j.asd.2012.05.004

Supplementary material

Table S1. Online data sources on butterfly traits accessed in this study.

Subfamily	Tribe	Species	Web Site
Apaturinae	Apaturini	*Asterocampa celtis*	1
Calinaginae	-	*Calinaga buddha*	2
Charaxinae	Charaxini	*Agatasa calydonia*	3
Charaxinae	Charaxini	*Polyura cognatus*	3
Heliconiinae	Argynnini	*Boloria alaskensis*	1
Libytheinae	-	*Libytheana carinenta*	1
Limenitidinae	Adoliadini	*Dophla evelina*	3
Nymphalinae	Nymphalini	*Aglais milberti*	1
Charaxinae	Preponini	*Anaeomorpha splendida*	1
Satyrinae	Satyrini	*Auca coctei*	1
Satyrinae	Satyrini	*Cercyonis pegala*	1
Satyrinae	Satyrini	*Cosmosatyrus leptoneuroides*	1
Satyrinae	Satyrini	*Eretris maria*	1
Satyrinae	Haeterini	*Haetera sp*	1
Satyrinae	Satyrini	*Manataria hercyna*	1
Satyrinae	Satyrini	*Manarebia monopis*	1
Satyrinae	Satyrini	*Mygona sp*	1
Satyrinae	Satyrini	*Neominois ridingsii*	1
Satyrinae	Satyrini	*Oeneis jutta*	1
Satyrinae	Satyrini	*Lethe anthedon*	1
Satyrinae	Satyrini	*Oressinoma typhla*	1
Satyrinae	Satyrini	*Neorina sp*	2
Satyrinae	Amathusiini	*Thauria aliris*	2
Satyrinae	Satyrini	*Coenonympha pamphilus*	4

Web site: (1) Butterflies of America; (2) Ifoundbutterflies; (3) Wikipidia; (4) Boldsystems.

Table S2. The relation between distinct morphological dispersal traits. The linear traits, the wing loading (WL), the aspect ratio (AR) and the thorax volume (TV) were log transformed and evaluate by PGLS. The results of these relations are represented as *t* value, being the OU model the best adjustment. The relationship between the linear to multivariate traits, the wing shape (WS), was evaluate by PLS and the table value represented the *rho* value. All relationship was significate; the bold values represent the values with more strong correlation between the morphological dispersal traits.

Morphological Traits	WL	AR	TV
WL			
AR	-3.44		
TV	**23.83**	-5.47	
WS	0.51	**0.87**	0.60

.

Table S3. The respective parameters estimate for trait-dependent speciation and extinction from QuaSSE models related to wing loading (WL) and aspect ratio (AR). The specific parameters to different speciation (λ) and extinction (μ) process take into account each dispersal trait. About the λ the distinct model were: (l.c) constant speciation rate; (l.y0) linear speciation rate at the morphological trait is 0, mean the intercept of linear models, or the rate at lowest values of the index for sigmoid and unimodal models; (l.y1) the speciation rate at highest values of the index; (xmid) inflection point of the sigmoid or the place of the maximum for modal models; (l.r) steepness of the sigmoid function; (l.s2) width (variance) of the Gaussian function; (l.m) slope of linear models; (σ^2) Brownian diffusion rate of trait evolution. On the other hand the parameters from the μ: (m.c) constant extinction rate and (m.m) slope of linear model. The results from speciation and extinction linear did not work which was representing by NA. The bold models are the most explicative models defined by AIC criteria.

		λ					μ	
speciation	extinction	l.c l.y0	l.y1	l.xmid	l.r l.s2 l.m	σ^2	m.c	m.m
Wing Loading								
constant	constant	0.046				0.0001	0	
linear	constant	0.059			-0.098	0.0001	0	
sigmoid	constant	0.05	0	0.315	17.712	0.0001	0	
unimodal	constant	0.169	0	0.984	1.160	0.0001	0	
constant	linear	0.046				0.0001	-0.01	-0.0657
linear	linear	NA			NA	NA	NA	NA
sigmoid	**linear**	**0.117**	**0**	**0.081**	**6.187**	**0.0001**	**8.32**	**-2.831**
unimodal	**linear**	**0.438**	**0**	**0.658**	**1.161**	**0.0001**	**9.029**	**-3.170**
Aspect Ratio								
constant	constant	0.046				0.0257	0	
linear	constant	0.038			0.001	0.0257	0	
sigmoid	constant	0.044	0.05	11.402	0.52	0.0257	0	
unimodal	**constant**	**0.052**	**0.002**	**11.526**	**0.092**	**0.0258**	**0**	
constant	linear	0.046				0.0259	0.029	-0.0081
linear	linear	0.014			0.003	0.0259	-0.009	-0.0153
sigmoid	linear	0.046	0.012	30.649	4.197	0.0259	-0.026	-0.0591
unimodal	**linear**	**0.052**	**0**	**11.521**	**0.086**	**0.0258**	**0.023**	**-0.0615**

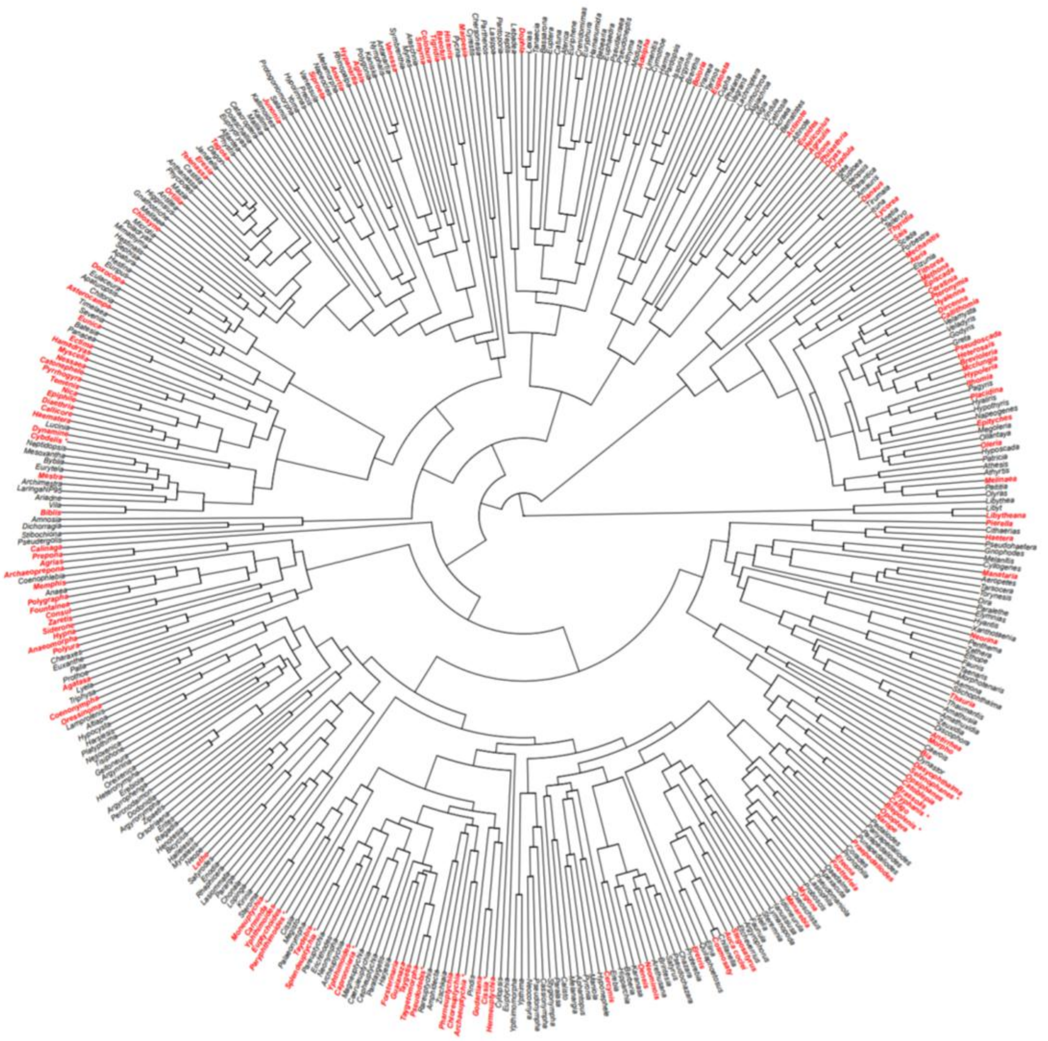


Figure S1. Phylogenetic representativeness of the species sample analysed in this study related to Nymphalidae phylogenetic hypothesis follows Wahlberg et al. (2009) is in red colour name.

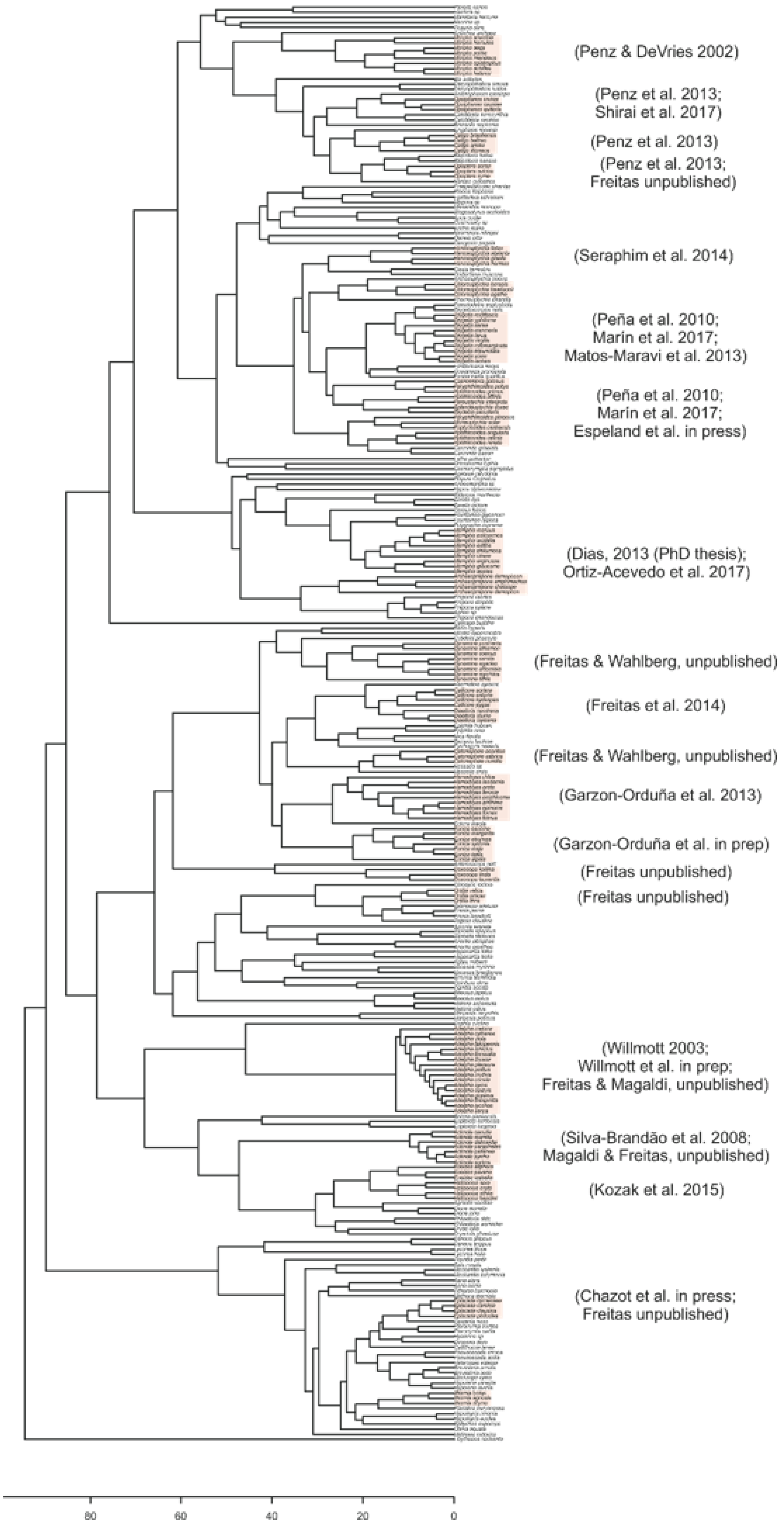


Figure S2. Phylogenetic relationships between Nymphalidae species analysed in this study and relative references used for the insertion of the species in the phylogeny.

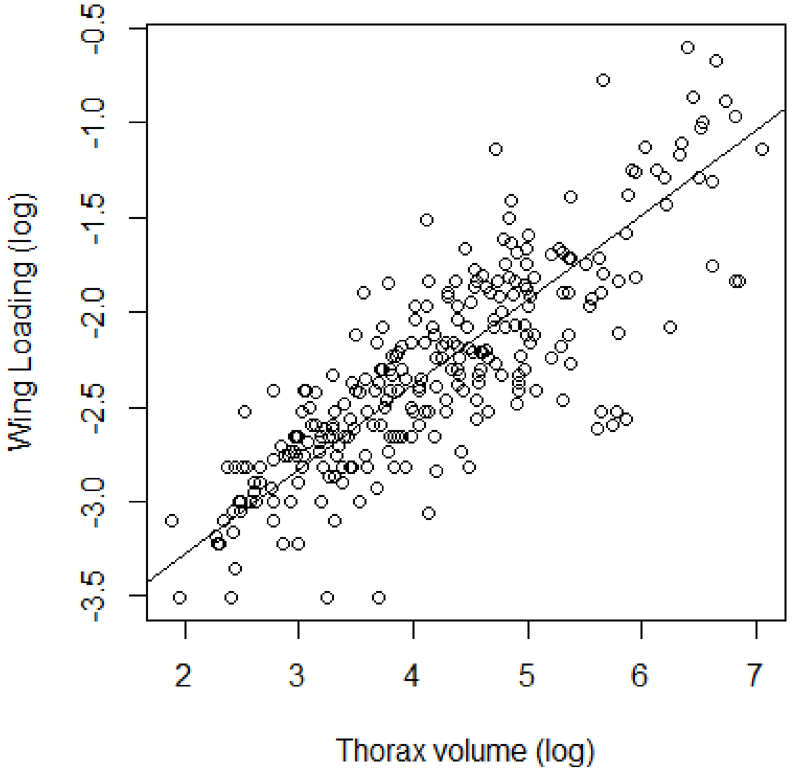


Figure S3. The Phylogenetic Generalized Least-Squares (PLGS) result from the correlation between Wing Loading and thorax volume.

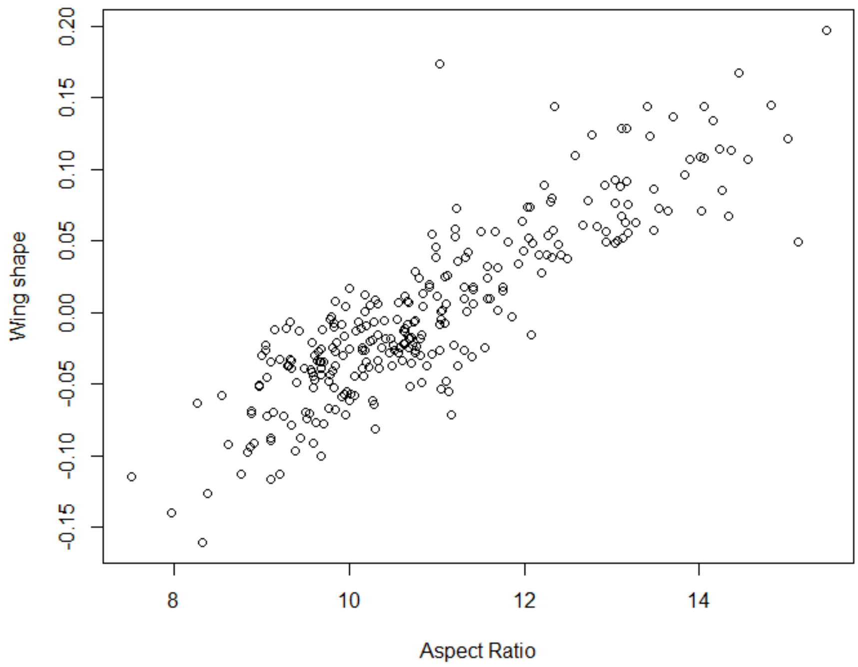


Figure S4. The Partial Least Squares regression (PLS) results, from the wing shape obtained by geometric morphometric and aspect ratio.

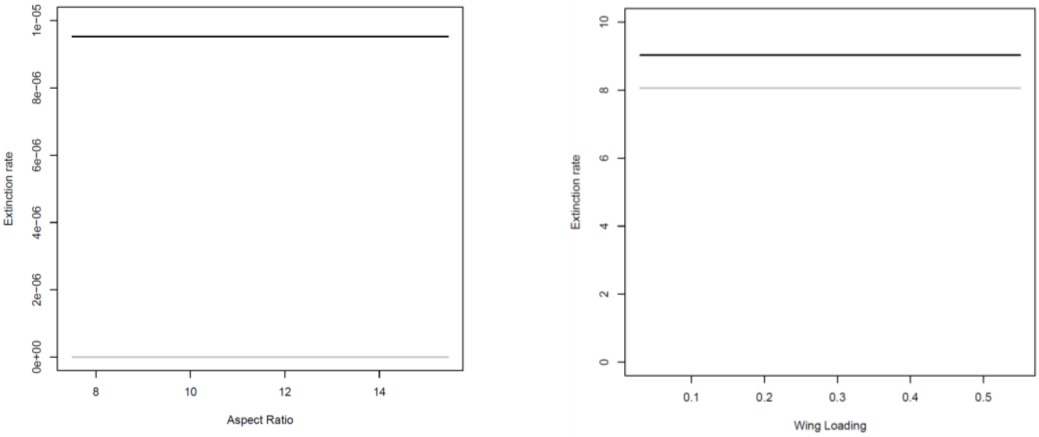


Figure S5. The relationship between aspect ratio (AR) and wing loading (WL) to extinction rate. The bold line is the model with more weight and the grey line are the model with second best weight.

CONSIDERAÇÕES FINAIS

A dispersão afeta e é afetada por distintas dinâmicas ecológicas e evolutivas tanto em escala micro quanto macroevolutiva. A presente tese acessou os processos dispersivos nestas escalas, o que possibilitou a proposição de um arcabouço teórico para entender a variação na forma da asa nestes distintos níveis ecoevolutivos considerando as distintas etapas de desenvolvimento das borboletas. Além disso, testarmos empiricamente como alguns filtros, bióticos e abióticos, preditos no arcabouço teórico, afetaram a capacidade de dispersão, inferida através de informação genética e morfológica nas distintas escalas evolutivas. Numa escala microevolutiva avaliamos como diferentes ambientes, tipos de cultivo de banana, com e sem uso de agrotóxicos, afetaram: (i) a dispersão de indivíduos de uma população, (ii) a formação de populações genéticas e (iii) a diversidade genética e de espécies em cada local. Numa escala de tempo maior, macroevolução, acessamos: (i) os modelos evolutivos que estariam explicando a evolução dos atributos preditores da capacidade da dispersão, e: (ii) os impactos da dispersão sobre processos de especiação e extinção em Nymphalidade. Por fim, encontramos evidência da atuação de filtros bióticos e abióticos sobre a informação genética e morfológica em Nymphalidade, respectivamente, avaliado a nível micro e macroevolutivo. Ambientes mais similares à área nativa (Mata Atlântica), como o sistema agroflorestal, apresentaram indivíduos com maior similaridade genética. A informação genética correspondente a grupos genéticos mais distintos ocorreram apenas em indivíduos que ocorreram na área de cultivo agroflorestal e área nativa, estando ausentes em indivíduos que ocorreram nas áreas onde o cultivo de banana era convencional, o que sugere homogeneização genética em indivíduos que ocorreram em áreas com utilização de agrotóxico. Por outro lado, considerando o nível macroevolutivo, encontramos que atributos morfológicos relacionados à dispersão evoluíram por deriva, principalmente segundo o modelo Browniano e os diferentes aspectos morfológicos preditores da capacidade de dispersão afetaram de maneira distinta os processos de especiação e extinção de Nymphalidae, provavelmente devido à diferença funcional desses atributos morfológicos para a eficiência do processo dispersivo. A carga alar, proporção entre volume do tórax e área da asa, diferencia-se do *aspect ratio*, pois esta mais relacionada à velocidade de voo enquanto o *aspect ratio* da asa captura a forma da asa e está mais relacionada ao tipo de voo. Neste sentido, várias questões inovadoras e pertinentes foram exploradas pela primeira vez. Os quais possibilitaram uma avenida de novas direções a ser explorada, a fim de ampliar o entendimento da dispersão e processos associados, tanto considerando uma perspectiva micro como macroevolutiva.

Neste sentido, o arcabouço teórico proposto na presente tese com o objetivo de entender as principais etapas que moldam a forma da asa, pode ser estendido para outros aspectos dispersivos, morfológicos ou não, e para diferentes escalas ecológicas e evolutivas. Neste sentido, um novo arcabouço teórico poderia ser proposto para o entendimento da dispersão em borboletas. Para isso, uma profunda revisão poderia enfocar no entendimento dos processos que moldam os atributos dispersivos em distintos níveis ecológicos e evolutivos como: (i) a dispersão a nível genômico, dentro do genoma dos indivíduos, de população e de metapopulações, (ii) a dispersão num viés de comunidades e metacomunidades, (iii) a inclusão de estudos a nível de família e diversificação e, por fim, (iv) os efeitos dos processos macro sobre os microevolutivos. Um enfoque para esses fatores através de uma revisão sobre a dispersão pode ampliar o entendimento deste importante processo considerando as distintas escalas ecoevolutivas. Aspectos recorrentes nos distintos níveis podem ser processos chaves para o entendimento da dispersão desde uma escala micro como macroevolutiva.

O entendimento da dispersão em sua menor unidade evolutiva poderia ser explorado levando em consideração o movimento de fragmentos de DNA/RNA dentro do genoma e entre genomas. Por exemplo, os elementos de transposição (TEs) caracterizados por serem fragmentos de DNA ou RNA que se movem no genoma (dentro e entre), sejam por replicar uma cópia de si mesmo ou ser excisado e inserido em outro local, são apontados como uma importante força evolutiva e a intensa movimentação dos TEs é afetada pelos eventos estressantes do ambiente. O estudo destes fragmentos móveis já foi reportado em borboletas. O clássico caso do melanismo industrial encontrado em *Biston betularia* na Inglaterra está relacionado ao aumento de *transposons* e maior ativação da expressão do gene *cortex*, o qual está relacionado à pigmentação das borboletas (van't Hof et al 2016). Além disso, considerando o movimento destes, os *transposons* já foram associados com o aumento de hiperatividade em borboletas (Talla et al 2017). Associados a importantes aspectos ecológicos e evolutivos os TEs apresentam várias semelhanças com sistemas ecológicos (Brookfield, 2005) embora permaneçam pouco explorados num contexto ecológico. Portanto, estudos ecológicos, incluindo o entendimento da dispersão dentro do genoma, podem ajudar a desvendar importantes fronteiras do impacto dos TEs sobre a dispersão em níveis taxonômicos maiores. Além disso, os TEs estão associados a controles epigenéticos como a metilação. Neste sentido, tanto considerando a revisão dos principais fatores que afetam a forma da asa (capítulo 1) como na revisão da genética da dispersão feita por Saastamoinen et al. (2018), estudos epigenéticos são apontados como fatores com grande potencial para entender o efeito do ambiente sobre os processos dispersivos tanto a nível molecular como morfológico.

Ainda dentro de uma perspectiva microevolutiva, a inclusão de informação genômica a nível individual e dentro de populações é extremamente relevante para entender os fatores ecoevolutivos que são moldados por variáveis abióticas, como os diferentes tipos de cultivos agrícolas utilizados na presente tese. Isso viabilizaria entender como diferentes partes do genoma respondem e se adaptam a diferentes fatores como o uso ou não de agrotóxico em diferentes cultivos agrícolas. Essa questão é bastante relevante para entender as dimensões ecoevolutivas que são afetadas por distintos fatores abióticos. Somado a isso, os processos microevolutivos de indivíduos de *Heliconius ethilla narcaea,* pode ser mais bem explorado através da localização de variações nucleotídicas relacionadas à capacidade de dispersão em borboletas (Saastamoinen et al. 2018), as quais já foram descritas para outras borboletas como relacionadas ao processo dispersivo como o gene *Pgi*. Sendo essa outra questão que pode ser mais bem explorada para entender os impactos dos tratamentos agrícolas, com e sem uso de agrotóxico, sobre a genômica da dispersão, tanto no nível de indivíduos como de populações.

Numa escala local, mas passando para o nível de comunidade e de metacomunidade, novas métricas para mensurar a biodiversidade podem ser utilizadas para avaliar efeito dos filtros bióticos e abióticos, como a funcional e a filogenética, as quais podem nos ajudar a entender melhor as diferentes dimensões da biodiversidade. O entendimento dos fatores que moldam a formação das comunidades locais adiciona importante informação para ressaltar a importância das distintas forças, como fatores ambientais, a equivalência ecológica e a dispersão, podem explicar melhor a formação das comunidades locais. Finalmente, experimentos avaliando os impactos gerados a partir do uso de agrotóxico utilizados em distintas intensidades podem ser realizados considerando diferentes estágios de desenvolvimento de borboletas e em diferentes espécies de borboletas. Somado a isso, incluir nas análises informações de ecologia de paisagem considerando os aspectos abióticos, tanto coletados no momento da coleta como das áreas do entorno, os quais possibilitarão entender como as características da paisagem local e regional poderiam estar atuando e moldando mais fortemente a informação genômica, filogenética, funcional e taxonômica, assim como diferentes níveis ecológicos desde população, espécies até de comunidades, por exemplo.

Finalmente, considerando os aspectos macroevolutivos, uma avenida enorme de possibilidades pode ser explorada para o entendimento da dispersão, seja considerando aspectos associados como características comportamentais e do ambiente onde as espécies ocorrem. As diferenças no processo dispersivo podem ser moldadas considerando características ambientais. Tanto a forma da asa (Capítulo 1) como as características moleculares da dispersão (Saastamoinen et al. 2018) já foram reportadas como possibilidade de ser moldada pelo ambiente. A necessidade de entender como o ambiente molda os atributos morfológicos preditores da capacidade de dispersão torna-se bastante importante. Além disso, estudos que incorporem a ocorrência de características comportamentais, sejam comparando machos e fêmeas ou tipos distintos de voos e diferentes estratégias permitirão separar e avaliar pressões seletivas conflitantes ao longo da filogenia e entender como elas podem ter atuado para moldar as características morfológicas. Diferentes aspectos morfológicos podem atuar de maneira conjunta para desempenhar uma determinada função, neste caso são conhecidos como módulos. O entendimento de como as distintas partes morfológicas atua possibilitam separar e relacionar os distintos aspectos a suas funções seja formando módulos distintos (características morfológicas que apresentam funções distintas) ou diferentes aspectos que atuam conjuntamente formando um módulo (atributos apresentam funções que evoluíram para desempenhar um papel similar).

Portanto, uma nova avenida de possibilidades pode ser explorada para aumentar o entendimento da dispersão tanto ao nível micro como macroevolutivo, como, por exemplo, acrescentando outras informações intrínsecas ao processo dispersivo, como: (i) aspectos comportamentais, (ii) inclusão de outras pressões seletivas, antagônicas ou não ao processo dispersivo, (iii) a modularidade dos atributos morfológicos para realização deste movimento e (iv) como os diferentes módulos podem responder as pressões seletivas distintas. Já numa perspectiva morfológica, o entendimento da forma da asa como preditor do tipo de voo pode ser mais bem explorado através do estudo mais aprofundado da enervação da asa, que poderia estar relacionado ao melhor direcionamento de voo e especialização quanto ao tipo de recurso utilizado. A capacidade de ser especialista, por exemplo, poderia estar associada a um maior controle no voo através da disposição das nervuras, pois esta possibilita acessar o alvo desejado de maneira mais direcional. Isso poderia afetar outras dimensões da dispersão como a escolha de planta hospedeira e a escolha de parceiro para copular. Além disso, a inclusão de estudos considerando a forma da asa posterior, o qual poderia estar associado à dinâmica de voo, possibilitaria um entendimento mais completo das diferentes dimensões da asa.

Referências bibliográficas

Talla V, Suh A, Kalsoom F, Dincă V, Vila R, Friberg M, Wiklund C, Backström N (2017) Rapid Increase in Genome Size as a Consequence of Transposable Element Hyperactivity in Wood-White (*Leptidea*) Butterflies, *Genome Biology and Evolution*, **9**: 2491 – 2505.

Saastamoinen M, Bocedi G, Cote J, Legrand D, Guillaume F, Wheat CW, Fronhofer EA, Garcia C, Henry R, Husby A, Baguette M, Bonte D, Coulon A, Kokko H, Matthysen E, Niitepõld K, Nonaka E, Stevens VM, Travis JMJ, Donohue K, Bullock JM, del Mar Delgado M (2018) Genetics of dispersal. *Biological Review*, **93**: 574–599.

Brookfield JFY (2005) The ecology of the genome mobile DNA elements and their hosts. *Nature*, **6:** 128-136.