UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Variação fenotípica e estrutura da comunidade de califorídeos (Insecta: Diptera) necrófagos: perspectivas ecológicas e considerações para prática forense

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Tese apresentada ao programa de Pós-Graduação em Ecologia da Universidade de Brasília, sob a orientação da Profª. Drª Rosana Tidon como critério para obtenção do título de Doutor em Ecologia

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Sumário

RESUMO

A entomologia forense consiste na interpretação de vestígios entomológicos no curso de investigações criminais. Profissionais dessa área tem por objetivo inferir o tempo transcorrido entre a prática de um crime e a coleta da evidência com base em características dos indivíduos ou da comunidade de insetos coletados. Para a predição acurada, é importante que os profissionais conheçam o grau de variação dos fenótipos avaliados, bem como os fatores que levam a variação fenotípica e em que magnitude esses fatores operam. Sabe-se que fatores bióticos e abióticos interferem nos indivíduos levando à variabilidade de tamanhos e formas em insetos e que essas influências estão desigualmente distribuídas entre as espécies e os sexos. O presente trabalho investigou (1) como a variação de tamanho e a resposta do tamanho à variação de densidade estão estruturadas entre os sexos em três espécies de califorídeos necrófagos; (2) como a morfologia alar de diferentes espécies de califorídeos necrófagos reage a situações de competição intra- e interespecífica; (3) como a comunidade de califorídeos necrófagos e suas variações fenotípicas estão temporalmente e espacialmente estruturadas em uma área ambientalmente protegida no Distrito Federal; e (4) como a morfologia alar de duas espécies de califorídeos necrófagos (uma nativa e outra exótica) respondem à variação temporal (estação seca x chuvosa) e vegetacional (mata de galeria x campo sujo) nesta área protegida. Os resultados apontam que o tamanho e a forma das asas de califorídeos machos e fêmeas são diferentes e reagem de forma diversa ao aumento de densidade, e que as três espécies analisadas reagem de forma distinta à competição interespecífica. Mostramos ainda que a composição da comunidade de califorídeos necrófagos varia em função do tempo e das coberturas vegetais, com diferenças nas abundâncias e dominância de espécies em função do tempo e da cobertura vegetal, e que algumas espécies têm potencial promissor como indicadores de ambientes. Ainda, as duas espécies examinadas no Cerrado se comportaram de forma independente entre os meses de seca e de chuva, porém não apresentaram níveis diferentes de assimetria flutuante em suas estruturas alares. Esses resultados apontam para a necessidade de abordagens específicas e individuais para cada indicador forense e em função das suas respectivas trajetórias ecológicas e evolutivas. Apenas por meio de uma análise sistemática e da delimitação clara das populações de interesse será possível reduzir os graus de incerteza associados à prática forense.

Palavras-chave: Entomologia forense, morfometria geométrica, insetos necrófagos

ABSTRACT

Forensic entomology consists in the interpretation of entomological evidence in criminal investigations. Forensic entomologists' aim is to infer the period between the committing a crime and the collection of the evidence based on individual or communitarian features of the specimens. For accurate prediction, it is important that entomologists know the degree of phenotypic variation and what factors influence in that trait. Biotic and abiotic factor interfere in individuals, leading to variation in shape and size in insects, and this influence yield different results in males and females. The present work investigated (1) Sexual size dimorphism in three species of necrophagous calliphorids in response to density; (2) how those three species respond in wing morphology to intra and interspecific competition; (3) the temporal and spatial structure of necrophagous calliphorids community in an environmentally protected area in the Federal District and the morphological variation of two species (a native and an exotic) in different seasons and vegetation covers, and; (4) how these two species alter their wing morphology in response to temporal (dry and rainy season) and spatial (grassland and forest) heterogeneity. The results show that wing morphology in males and females of three species of necrophagous calliphorids display different responses to density, as well as interspecific competition. We also showed that community composition changes in response to time e vegetation cover, with abundance and dominance differences regarding both seasons and vegetation types, and some species have potential as indicators of environments. Furthermore, the two species examined in the Cerrado behave differently between the months of the dry and the rainy season, but display no differences in fluctuating asymmetry levels in their wing structures These results point to the necessity of individual approaches, specific for each forensic indicator due to its ecological and evolutionary trajectories. Only through a systematic analysis and clear delimitation of population it is going to be possible to reduce uncertainty in forensic practice.

Keywords: Forensic entomology, geometric morphometrics, necrophagous insect, ensemble,

INTRODUÇÃO

Entomologia Forense

Entomologia forense é a área de estudo que analisa evidências entomológicas em casos judiciais. De forma geral, essa atividade se baseia na estimativa da idade larval por meio do peso ou tamanho dos indivíduos coletados em uma cena de crime. O principal uso de dados entomológicos refere-se ao cálculo do intervalo pós morte mínimo (IPM_{min}), que diz respeito ao tempo mínimo em que uma pessoa está morta, que normalmente coincide com a idade do inseto mais velho coletado (Amendt et al. 2011). Esse cálculo se baseia na premissa de que insetos necrófagos depositam ovos em uma carcaça assim que o recurso se torna disponível e os imaturos se desenvolvem em taxas conhecidas para um determinado conjunto de variáveis ambientais (e.g., temperatura, umidade, fotoperíodo) (Harvey, *et al.*, 2016). O cálculo do IPM pode fornecer estimativas precisas acerca do momento em que uma pessoa foi morta, auxiliando os investigadores criminais a incluir ou excluir pessoas no rol de suspeitos.

Diversos métodos de cálculo do IPM já foram reportados, cada um com suas limitações e vantagens (Sharma, *et al.*, 2015). Contudo, a literatura tem se mostrado incapaz de identificar as fontes de incertezas associadas com esse cálculo, e por isso esse tipo de evidência ainda é questionado por muitos profissionais. Por exemplo, a taxa de desenvolvimento dos insetos sofre influência da temperatura, variando significativamente em função do ambiente e regulando o ajuste dos organismos às condições vigentes (Kingsolver & Huey 2008). Portanto, estimativas de IPM devem considerar não apenas características do organismo indicador, mas também do ambiente onde ele está inserido.

Ou seja, a taxa de desenvolvimento é um fenótipo variável que reflete complexas interações entre genótipo e ambiente.

Se por um lado a diversidade fenotípica de uma população é vista com otimismo para fins conservacionistas, para os profissionais da entomologia forense ela alerta para uma possível fonte de erro que aumenta a incerteza nos modelos preditivos (Tomberlin, Mohr, et al. 2011). Desta forma, em espécies de interesse forense é necessário avaliar como os fenótipos de uma população se distribuem no tempo e no espaço, e como expressão fenotípica das populações pode ser influenciada pelos diversos fatores ambientais.

As diferentes taxas de desenvolvimento que um mesmo genótipo pode produzir sob diferentes condições ambientais podem ser ilustradas por uma norma de reação (Fig. 1b). Contudo, populações apresentam genótipos variados, cada um deles com sua própria norma de reação, e por isso as representações de uma espécie são, na verdade, valores médios populacionais. Por conseguinte, o padrão de variação fenotípica pode divergir entre diferentes genótipos em função das condições ambientais (Fig. 1a). Nesse contexto, áreas de alta sobreposição fenotípica (i.e., diversos genótipos produzindo o mesmo fenótipo) indicam baixo grau de incerteza associado ao cálculo do IPM, enquanto a baixa sobreposição representa altos índices de incerteza.

Profissionais da entomologia forense aos poucos vêm internalizando a diversidade intraespecífica, e encaram essa variedade como adaptações locais. Apenas recentemente a literatura tem reconhecido à importância de se compreender os aspectos ecológicos e evolutivos das espécies de interesse forense (Tomberlin, *et al.* 2011).

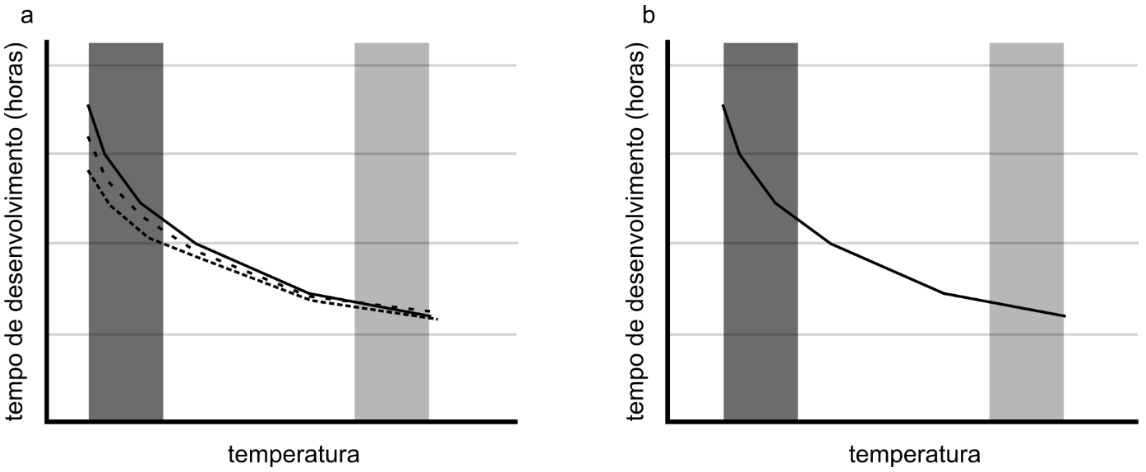

Figura 1. Representação de Normas de Reação para (a) um conjunto de genótipos de uma população e (b) um único genótipo. À esquerda (a), temos em cinza escuro uma região onde diferentes genótipos resultam fenótipos muito diversos, enquanto a área mais clara representa um intervalo de condições ambientais onde os genótipos da população produzem fenótipos muito próximos entre si. À direita (b), em cinza escuro ilustra-se uma área de alto índice de incerteza devido à alta variação fenotípica em relação à variação ambiental, enquanto a área em cinza claro representa o oposto.

Invasões Biológicas

As espécies invasoras são responsáveis por substanciais impactos econômicos e ecológicos à biodiversidade. Elas alteram o equilíbrio ecológico das comunidades deslocando funções das espécies nativas, interferindo em seus processos ecológicos e evolutivos, e causando extinções (Mack *et al.*, 2005). Nesse contexto, uma das diretrizes da Convenção sobre a Diversidade Biológica é a promoção da conservação *in situ* por meio do estudo e combate às introduções de espécies exóticas invasoras. A Convenção reconhece as espécies invasoras como uma das principais causas da redução global da diversidade biológica.

O processo de invasão de um habitat por uma espécie invasora pode ser estruturado em quatro etapas, intervaladas por filtros a serem transpostos pelo invasor. Esse processo compreende o transporte da espécie invasora, o estabelecimento de ao menos um propágulo no novo ambiente, a expansão da população advinda desse propágulo e, por fim, o produto do impacto da população invasora (Lockwood, 2007). Colautti e MacIsaac (2004) propuseram um quadro prático estruturado em seis estágios, interconectados por filtros, para representar esse processo (Fig. 2). Os autores ressaltam que o processo de invasão é influenciado por três categorias de variáveis: a) pressão de propágulo; b) demandas físico-químicas da espécie invasora (fatores abióticos), e; c) interações comunitárias (fatores bióticos) (Colautti & MacIsaac 2004).

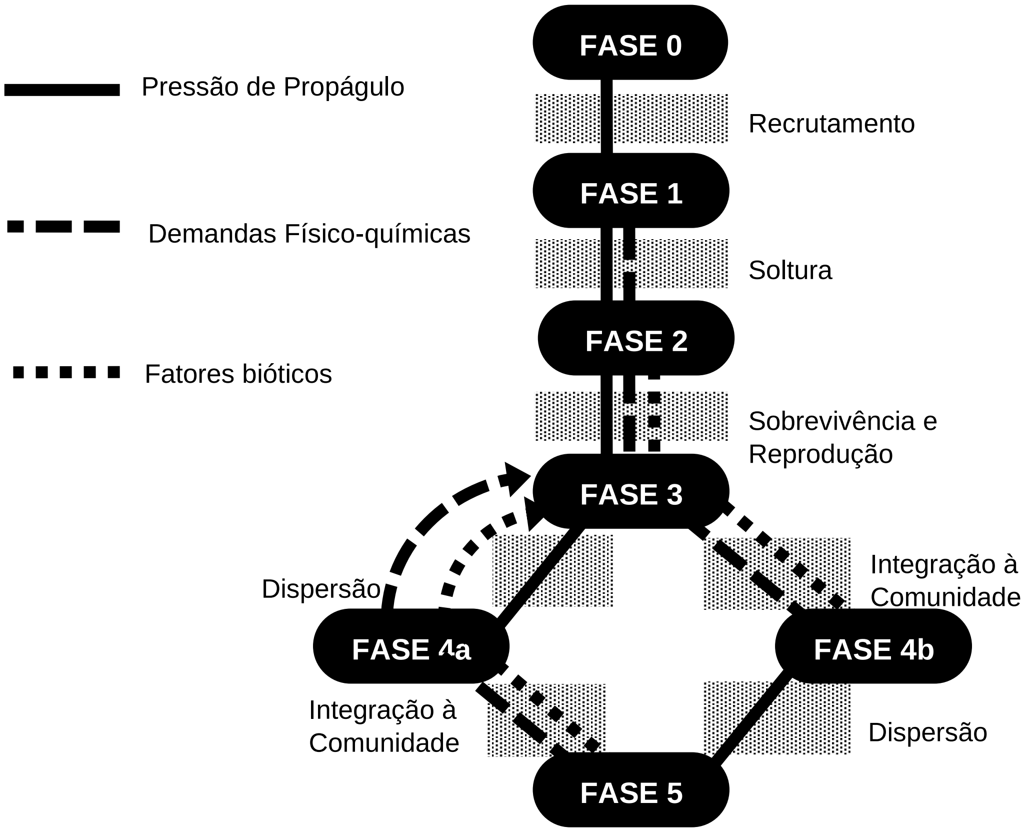

Figura 2. Adaptado de Colautti & MacIsaac (2004) - Fases do processo de invasão biológica. Áreas hachuradas representam filtros à espécie invasora, linhas sólidas apontam influência preponderante da pressão de propágulo, linhas sólidas indicam pressão de propágulo, linhas tracejadas indicam preponderância de demandas físico-químicas da espécie exótica e linhas pontilhadas indicam preponderância de fatores bióticos.

Além das abordagens do processo de invasão focadas nas espécies invasoras e nos processos que elas desempenham para atingir esse status, é importante compreender como a comunidade residente reage a esse processo (Peacor *et al.*, 2006). Neste sentido, é relevante investigar os ajustes ecológicos e evolutivos envolvidos na integração das espécies recém-chegadas às espécies nativas da comunidade na qual se inserem. Ante a chegada de uma espécie exótica, as espécies nativas se sujeitam a mudanças comportamentais, morfológicas, fisiológicas e em caracteres de história de vida. Tais mudanças ocorrem por rápida adaptação evolutiva (i.e. seleção de genótipos de maior valor adaptativo) ou por plasticidade fenotípica, situação na qual a variação das frequências dos fenótipos é dissociada da mudança na frequência alélica correspondente (Berthon, 2015). Nesse contexto, o conceito de invasão também tem sido interpretado como resultado de uma competição interespecífica, a qual resulta da superioridade local de uma espécie sobre a outra (Valéry *et al.*, 2008). A superação de um ou de vários obstáculos pela espécie invasora (e.g. a retirada ou adição de um predador de topo de cadeia, ocorrência de queimadas extensas, aporte ou retirada severa de nutrientes) pode resultar na exclusão ou redução populacional de espécies residentes.

A literatura acerca da biologia da invasão é extensa e diversificada. Contudo, é oportuno interpretar um evento específico de invasão sob uma perspectiva mais ampla, indissociável do histórico da comunidade. A chegada de uma espécie exótica é um evento pontual na montagem de uma comunidade, o qual reorganiza as pressões bióticas e reconfigura a rede de interações entre as espécies. Essas alterações no sistema biológico, por sua vez, podem alterar o balanço energético das espécies residentes pela reorganização de alocação energética (Prior *et al.*, 2015). As espécies envolvidas deparam-se com novas situações, o que pode conduzir à uma reestruturação da comunidade. Ao longo do processo de redução das pressões totais sobre as espécies no ambiente invadido, podem ocorrer alterações do nicho percebido bem como situações de competição. Por vezes, havendo disponibilidade de recursos ou sendo as espécies equivalentes competitivos, a chegada de uma espécie nova exerce influência desprezível na comunidade local, e a espécie apenas se conforma aos limites abióticos e passa a integrar a comunidade (Bugnot *et al.* 2016). Nesse cenário, o estabelecimento de espécies exóticas diz respeito à formação das comunidades locais, e os mecanismos acima descritos representam duas possibilidades teóricas, uma delas associada à Teoria do Nicho, de ênfase determinística, e a outra de natureza preponderantemente estocástica, característica da Teoria Neutra.

Formação de Comunidades

As regras de montagem vinculadas ao processo de formação de comunidades tem sido investigadas e debatidas pelos ecólogos há décadas. Apesar de não haver consenso a respeito da magnitude das influências bióticas, abióticas, e de eventos estocásticos, admite-se que se trata de um processo complexo e iterativo: a cada vez que se repete gera um resultado parcial, que será o ponto de partida para a etapa seguinte.

Duas classes de teorias buscam explicar os mecanismos que regulam a montagem das comunidades. As teorias do nicho enfatizam os aspectos bióticos que regulam interações entre os indivíduos da comunidade em análise. Nesse caso, entende-se que as espécies têm diferenças fundamentais entre si e que, havendo competição por recursos, a superior levará a inferior à extinção local. A teoria neutra, por outro lado, pressupõe que as espécies de um mesmo nível trófico são equivalentes competitivos, e que a persistência de cada uma na comunidade depende da capacidade de suporte do ambiente, dos limites ambientais impostos à espécie, e de eventos estocásticos.

Teoria do nicho

Segundo a Teoria do Nicho, a estrutura de uma comunidade depende da relação entre os traços das espécies que a compõem e características do ambiente. Nesse cenário, a competição entre espécies regula a diversidade biológica em função da composição da comunidade (Brannstrom *et al.*, 2012; Chesson, 2000). Tal arcabouço estrutura-se sobre à visão de Hutchinson (1959), de que a sobreposição de nichos leva à exclusão dos competidores menos adaptados. A perda de espécies por exclusão competitiva é compensada pelo aumento da diversidade proporcionado pela diferenciação e especialização das espécies que sobrevivem em nichos distintos (Hutchinson, 1959; Macarthur & Levins, 1967; Macarthur, 1970).

Para a ecologia da invasão a Teoria do Nicho tem implicações diretas, em especial na predição do resultado da chegada de um propágulo em um novo habitat, que se traduz em sucesso ou insucesso da invasão. Trata-se de uma visão com ênfase determinística, que valoriza as diferenças entre as espécies, as quais podem se manifestar de duas formas distintas. Na primeira dessas, as diferenças entre as espécies são responsáveis por permitir que uma delas explore um nicho até então não explorado – hipótese do nicho vago. Alternativamente, a espécie recém chegada pode ser distinta de eventuais residentes pela sua superior capacidade de exploração de um recurso compartilhado e escasso (Ricciardi *et al.* 2013). Esse processo levará à exclusão competitiva dos residentes que sejam incapazes de resistir com a fração de nicho restante, ou de alterar seu nicho percebido

(Gidoin *et al.*, 2015). Nesse contexto, o processo de invasão biológica pode ser encarado como um conjunto de etapas sequenciais, determinístico ou, ao menos ponderável, a depender das diferenças entre espécies invasoras e residentes.

Teoria neutra

Os defensores da teoria neutra consideram não haver diferença competitiva significativa entre indivíduos e espécies de um mesmo nível trófico, não havendo, portanto, vantagem ou desvantagem de uma espécie em relação às demais. Dessa forma, indivíduos seriam equivalentes e intercambiáveis, e eventos aleatórios estocásticos, bem como a confluência das forças ambientais, atuariam na formação e manutenção das comunidades (Hubbel 2005; Gotzenberger *et al.*, 2012). Neste cenário, o estabelecimento de uma espécie exótica em um novo local só dependeria de condições ambientais favoráveis para sua sobrevivência, e não da composição da comunidade local.

As predições baseadas na teoria neutra, em contexto de invasões biológicas, foram examinadas por Daleo *et al.* (2009), os quais assumiram que: (1) o sucesso de um invasor recém chegado independe da diversidade de espécies em que chega o propágulo; (2) ambientes com maior disponibilidade total de recursos e/ou maior frequência de distúrbio são mais suscetíveis a invasões; (3) todas as espécies são igualmente invasoras; (4) a similaridade entre invasores e nativos não inibe o estabelecimento de um invasor; e (5) espécies invasoras não afetam a abundância relativa de espécies (Daleo *et al.*, 2009). É importante notar que a equivalência funcional (3) ocorre dentro de um nível trófico (Hubell, 2005), mas distúrbios em um nível trófico (2) podem exercer influência sobre os demais. Esses outros níveis tróficos afetados podem sofrer alterações que exercerão efeitos nos próprios níveis onde se originaram os distúrbios em primeiro lugar (Davidson et al. 1984), levando a situações que refutam a teoria neutra da biodiversidade de Hubell. Assim, Daleo e colaboradores (2009) concluíram que a teoria da neutralidade não é suficiente para explicar completamente a ocorrência de invasões biológicas. Contudo, os autores ressaltam que algumas predições da teoria neutra são consistentes com observações empíricas.

Uma visão unificada

A Teoria do Nicho e a Teoria Neutra contrastam em seus aspectos mais fundamentais, e ambas são capazes de explicar, ao menos em parte, as leis e processos que regem a montagem de comunidades, com especial ênfase na consubstanciação de uma invasão biológica. Observados os aspectos determinísticos da Teoria do Nicho e os eventos estocásticos da Teoria Neutra, ambas são verdadeiras em parte, configurando os extremos em um contínuo. A montagem de uma comunidade, portanto, é regida por aspectos objetivos e sujeitos à mensuração, e também por flutuações randômicas, sendo as contribuições desses fatores essencialmente imprevisíveis em cada caso concreto.

Para Valladares *et al.* (2015), espécies coexistem em um local se a diferença de nicho entre elas supera a diferença entre seus valores adaptativos. Nesse sentido, espécies com nichos muito próximos coexistem caso suas habilidades competitivas sejam também próximas (Valladares *et al.* 2015). Contudo, à medida em que diferenças entre esses últimos valores se acentuam, espécies com nichos próximos se excluem competitivamente. Ambos os fatores são gradientes que, se representados em um espaço bidimensional, denotam regiões de coexistência e regiões de exclusão (Fig. 3) (MacDougall *et al.* 2009). Este arcabouço conceitual acomoda as teorias de nicho e neutra, transformando-as em configurações espacial e temporalmente discretas, o que é de grande utilidade em estudos de ecologia da invasão porque permite a avaliação e predição de eventos.

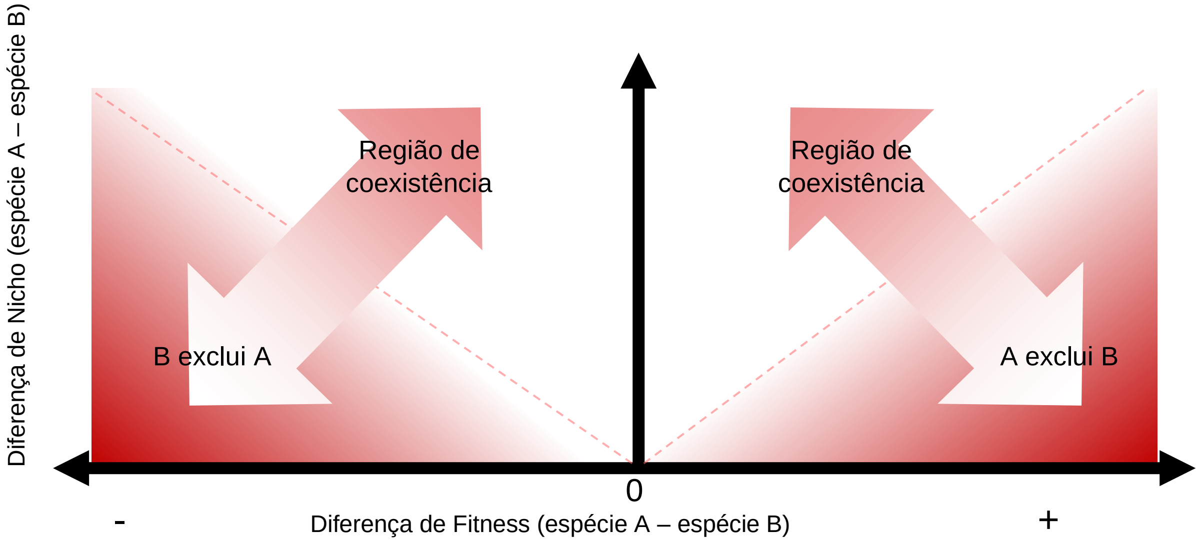

Figura 3. Adaptado de MacDougall (2009). As diferenças entre os nichos ocupados por duas espécies (eixo vertical) e entre os valores adaptativos (eixo horizontal) dispostas em um plano bidimensional possuem regiões de coexistência e regiões de exclusão como resultado da interação entre as espécies.

Diversidade Biológica e a Distribuição de Fenótipos nas Populações

Biodiversidade, ou diversidade biológica, é a propriedade multidimensional que se refere à variação nos sistemas biológicos desde os níveis mais internos (molecular) e individuais (genéticos) de cada organismo até as complexas interações ecossistêmicas (Morris et al. 2014). A forma pela qual esses níveis se interconectam, bem como a influência dessas conexões na estabilidade ou produtividade dos ecossistemas, ainda são alvos de discussão (Tilman et al. 2001, Craven et al. 2018). Contudo, sabe-se que a redução da diversidade tem profundos efeitos negativos nas comunidades (Tilman and Downing 1994, McKinney and Lockwood 1999, Olden 2008).

A variação intraespecífica é a base para a seleção natural e o processo adaptativo. A variedade de fenótipos em uma população é o que permite que os indivíduos reajam de formas diversas a um estímulo ou conjunto deles e através desse processo, entre outros, as gerações seguintes são diferentes das anteriores. Apesar do reconhecimento da existência e importância da variação populacional, entretanto, ainda é difundido o pressuposto de que indivíduos da mesma espécie podem ser tratados como equivalentes na formulação de modelos ecológicos (e.g. competição, predação). Sabe-se que tais pressupostos são falsos, mas os admitimos quando exploramos questões gerais sobre a interação entre as espécies e dessas com o ambiente.

O avanço em nossa compreensão sobre essas questões permite que busquemos respostas mais específicas sobre como os modelos que baseiam nosso entendimento se comportam com a quebra das premissas da homogeneidade (Salguero-Gómez et al. 2018). Novos modelos têm sido propostos para compreender a variação intraespecífica de caracteres e seus efeitos ecológicos (Bu et al. 2011, Xu 2017). Neste aspecto, reconhece-se que populações distintas de uma mesma espécie respondem de forma tão diferente a fatores ambientais quanto populações de diferentes espécies. Isso demonstra que a capacidade de alteração de caracteres fenotípicos excede as barreiras das espécies, sugerindo que a espécie pode não ser um bom preditor das características fenotípicas de um indivíduo (Mclean et al. 2018).

Forsman (2014) observou que a variabilidade genotípica e fenotípica são fatores determinantes no sucesso de processos de estabelecimento e perpetuação em ambientes novos. O tamanho corporal, um importante traço fenotípico ligado à história de vida, regula processos ecológicos de interações intra- e interespecíficas, dinâmicas populacionais e estrutura de teias alimentares (Cohen et al. 1993, Roos et al. 2003). Portanto, a estruturação do tamanho corporal em uma população influencia a cadeia trófica em uma comunidade (Bolnick et al. 2003). Todos os níveis tróficos respondem a diferenças na variação de tamanho do consumidor (Carlson and Langkilde 2017), demonstrando que variabilidade fenotípica intrapopulacional pode alterar significativamente interações ecológicas interespecíficas e consequentemente interferir na estruturação de comunidades ecológicas (Gibert and Delong 2017).

A diferença mais óbvia de tamanho corporal, bem como de outros caracteres fenotípicos, é o dimorfismo sexual. Esse é um padrão bem documentado por toda a classe dos insetos (Esperk et al. 2007). Ainda assim, uma pequena parte dos estudos em ecologia e em entomologia forense leva em consideração essa variabilidade de tamanhos e formas.

Assimetria Direcional, Antissimetria e Assimetria Flutuante

Os organismos com simetria bilateral, regulam o desenvolvimento de caracteres morfológicos em ambos os lados do corpo através de um único complexo gênico. Dessa forma, espera-se que na ausência de forças disruptivas tais caracteres (e.g. asas, pernas, olhos) apresentem simetria especular, ou seja, que um lado seja a imagem especular do outro (Clarke, 1995). Contudo, não é isso que se observa na realidade. Raramente organismos são capazes de manter tal simetria, apresentando-se normalmente assimétricos.

Há basicamente três tipos de assimetria, que variam de acordo com sua origem e significado biológico em populações naturais. A simetria direcional ocorre quando uma característica bilateral é sempre maior em um dos lados do que no outro, e o lado maior é geralmente o mesmo em todos os indivíduos da população. Um exemplo clássico é o coração dos mamíferos, onde o lado esquerdo é maior que o direito (Van Valen, 1962). De forma geral, esse tipo de assimetria tem significado biológico expressivo e é objeto de seleção, apresentando vantagem para a população em questão. A antissimetria, por sua vez, pode ser representada por uma distribuição bimodal. Ela ocorre em casos de dimorfismo sexual, ou quando fenótipos extremos são favorecidos em relação aos fenótipos intermediários (i. e. Seleção Disruptiva). Da mesma forma que a assimetria direcional, a antissimetria pode ser resultado de pressão seletiva. Por último, existem os casos em que desvios randômicos a partir da simetria total resultam da incapacidade do organismo de tamponar pressões externas aleatórias. Essa situação pode ser representada como uma distribuição normal de desvios, cuja média é igual a zero. Nesses casos, trata-se de assimetria flutuante (*fluctuating asymmetry*), que não é resultado de pressão seletiva, mas apenas de instabilidade de desenvolvimento (Clarke, 1995; Klingenberg 2015).

A quantificação dos níveis de assimetria flutuante (FA) tem sido usada como um indicativo de stress, ambiental ou genético, ao qual uma determinada população esteja submetida. Presume-se que, sob condições estressantes, parte da energia que seria usada no desenvolvimento de um indivíduo perfeitamente simétrico é desviada para lidar com o estresse. Contudo, há críticas ao uso da FA devido à forma instrumental de se acessar os níveis de assimetria flutuante em estudos de ecologia e biologia evolutiva (Abeli *et al.* 2015; Kozlov & Zvereva 2015). Os primeiros estudos nessa área baseavam-se em uma ou algumas poucas medidas lineares ou angulares de caracteres anatômicos dos organismos investigados. A dificuldade, então, era dissociar o desvio da simetria derivada de erro de medida do observador (pesquisador que efetua as medidas) do desvio realmente originário da incapacidade do organismo de manter a simetria em seu desenvolvimento embrionário, pois esses índices são geralmente muito pequenos e acabam se perdendo no erro instrumental. A capacidade de contornar essa dificuldade recai na robustez de métodos de morfometria geométrica, cuja popularidade aumentou sensivelmente nas últimas décadas (Adams *et al.*, 2004). Substitui-se o uso de algumas poucas medidas lineares pela análise de configurações planares ou espaciais, oriundas de diversos marcos anatômicos ("*landmarks*"), submetidos a uma vasta gama de ferramentas de análises multivariadas a fim de se caracterizar os reais desvios de simetria, excluídos os desvios direcionais e os de antissimetria (Klingenberg, 2015).

É importante ressaltar que a constatação de assimetria flutuante em um estudo pode ter quatro origens distintas: heterogeneidade nos produtos do ruído de desenvolvimento; variância por conta de amostragem em ambos os lados; variância nos erros de medição dos caracteres em foco, e; variabilidade na capacidade individual de suprimir consequências de perturbações ambientais randômicas (Lens & van Dogen 2001). A probabilidade de erro oriundo de medições em conjunto com a dificuldade de se separar entre os diferentes tipos de assimetria são fatores sensíveis ao se abordar a relação entre FA, instabilidade de desenvolvimento e estresse ambiental. Ainda assim, a análise de FA, se criteriosa e baseada em traços significantes, pode prover informações valiosas sobre valores adaptativos em populações e em indivíduos (Leung *et al.*, 2000; de Coster *et al.*, 2013).

A maior parte dos estudos populacionais envolvendo assimetria flutuante se refere aos efeitos de fatores abióticos (e.g. estresse hídrico, nutricional, osmótico ou térmico). Poucos se referem a fatores bióticos, como a competição (Hochwender & Fritz 1999; Handy *et al.*, 2004), e não estamos cientes de nenhum relato do emprego desse índice em estudos sobre invasões biológicas. O nível de assimetria flutuante de uma população em processo de invasão biológica pode refletir sua tolerância a estresses abióticos (e. g. variação térmica e estresse hídrico), mas também ao estresse biótico que a presença de uma espécie invasora exerce sobre as espécies nativas, e vice-versa (assimetria flutuante correlacionada com a composição da comunidade). Tais parâmetros, se comparados a características associadas ao valor adaptativo (e. g. número de ovos e carga alar), podem revelar os impactos da interação entre as espécies em um processo de invasão biológica. Por isso, é relevante acessar a assimetria flutuante em comunidades invadidas.

Asas e voo em insetos

A capacidade de voo está entre as novidades evolutivas mais importantes que conhecemos até hoje. Ao desenvolver asas e alçar voo, diversos grupos animais adquiriram imensa capacidade dispersiva e sofisticaram comportamentos de corte e mecanismos de termorregulação, entre outros. Na história evolutiva, a capacidade de voar apareceu ao menos em quatro oportunidades: nos insetos, nos pterossauros, nas aves e nos mamíferos (Engel et al. 2013). Os insetos foram os primeiros animais a apresentar estruturas para o voo e, ao contrário do que ocorreu nos vertebrados, essa capacidade surgiu uma única vez, por volta de 400 milhões de anos atrás (Engel and Grimaldi 2004).

Voar é uma capacidade complexa que envolve não apenas as asas, mas uma série de adaptações morfológicas e fisiológicas voltadas à otimização da performance de voo. Os insetos, por apresentarem tamanho reduzido em comparação aos demais animais com capacidade de voo, enfrentam condições específicas para vencer a gravidade. Enquanto mamíferos e aves evoluíram táticas para vencer a inércia de seus próprios corpos, os insetos tem na viscosidade do ar o maior obstáculo ao voo livre (Fry et al. 2003). A fim de transpor esse obstáculo, os insetos desenvolveram voo baseado em um acelerado e constante batimento de asas com movimentos translacionais e rotacionais capazes de gerar força suficiente para manter os inseto no ar (Dickinson et al. 1999).

Desta forma, não surpreende que as asas tenham sido estruturas essenciais na capacidade de diversificação dos insetos, assumindo diversas funções e se tornando uma característica chave na sistemática de dípteros (Stark et al. 1999, Nicholson et al. 2014). Por outro lado, as asas estão intrinsicamente associadas com outras estruturas para o voo (Dickinson and Tu 1997), e isso atua no sentido de constranger mudanças bruscas naquelas estruturas. Esse fato coaduna com a observação de que mesmo mudanças sutis nas estruturas alares podem ter significado biológico e estar ligadas a diferenças genéticas entre populações (Haas and Tolley 1998, Laparie et al. 2016).

Por fim, asas são estruturas consideravelmente bidimensionais e isso as torna um ótimo modelo para estudos morfométricos modernos. O advento de ferramentas analíticas baseadas em morfometria geométrica e análises estatísticas multivariadas possibilitou que estudos sobre a estrutura alar focassem separadamente tamanho de forma e examinassem mudanças muito súbitas nesses parâmetros (Klingenberg 2010, Adams et al. 2013a).

Diptera: Calliphoridae

Calliphoridae (Schizophora, Calyptratae, Oestroidea) é a família de dípteros conhecidos como moscas varejeiras. É um grupo cosmopolita, que compreende 150 gêneros e mais de 1000 espécies. Diagnose para a família e chaves de identificação para os principais gêneros para as regiões Neotropical e Neártica foram apresentadas por Shewell (1987), Vargas e Wood (2010) e Kosmann *et al.* (2013). Um grupo específico de califorídeos tem grande afinidade por áreas urbanas e rurais em que as condições de higiene são precárias, sendo encontrados em baixos números quando a situação é a inversa (Greenberg, 1973). Espécies desse grupo são conhecidas por carrearem grande diversidade de patógenos, bem como de serem responsáveis por numerosos casos de miíase. Trata-se, portanto, de uma família com grande importância médica e sanitária (Greenberg, 1971; Kosmann, 2013). Em adição, Calliphoridae é a principal família de dípteros encontrada em carcaças animais e cadáveres humanos, sendo, em geral, o principal tipo de evidência entomológica coletada em locais de crimes contra a vida (Biavatti *et al.*, 2010). Via de regra, são os primeiros indivíduos a acessarem o cadáver após sua morte, atraídos pelo odor produzido nos estágios mais cediços do processo de decomposição (Wall & Warnes, 1994), o que permite o estimar o intervalo pós-morte.

Um dos gêneros mais representativos de Calliphoridae é *Chrysomya*, representado no Brasil por três espécies: *Chrysomya albiceps* (Weidemann 1819), *C. megacephala* (Fabricius, 1974) e *C. putoria* (Weidemann, 1818). Originalmente ausente das regiões Neotropical e Neártica (Guimarães & Papavero 1999), se estabeleceu nelas com notável sucesso. Indivíduos desse gênero são coletados em quantidades maiores quando comparados aos de espécies nativas no território nacional, incluindo o Cerrado.

O primeiro relato de ocorrência do gênero *Chrysomya* no Brasil ocorreu em 1975, quando Imbiriba *et al.* (1977) registraram *C. chloropyga* no Estado do Paraná (posteriormente reconhecida como *C. putoria*). A entrada dessa espécie no país se deve à pronunciada chegada de refugiados africanos na década de 1970, os quais trouxeram consigo animais domésticos e, com eles, espécimes de *Chrysomya* spp. (Guimarães & Papavero 1999). Populações desse gênero se dispersaram rapidamente (Guimarães *et al.* 1979, Prado & Guimarães 1982) e, atualmente, espécies de *Chrysomya* ocorrem em todos os estados brasileiros e em vários países do Novo Mundo, da Argentina aos Estados Unidos da América (Kosmann *et al.* 2013).

Grella *et al.* (2015) reportaram que espécimes de *C. albiceps* coletados em território nacional apresentam polimorfismo fenotípico em situações em que nenhum polimorfismo genético pode ser observado para mtDNA. Esses autores salientam ainda que Ribeiro *et al.* (2013) registraram a presença de *Chrysomya rufifacies* (Macquart, 1842) erroneamente para o território brasileiro, ao negligenciar o polimorfismo fenotípico de *C. albiceps*. Esses fatos apontam a necessidade de abordagens multifatoriais para caracterização das espécies desse gênero no Brasil.

Este é o primeiro estudo abordando as espécies de *Chrysomya* no Cerrado, sob a perspectiva da ecologia da invasão. Os resultados obtidos devem fornecer subsídios para o entendimento da relação entre a diversidade e variação fenotípica com a estrutura da comunidade, contribuindo, portanto, para se compreender os fatores preponderantes na formação da comunidade de califorídeos necrófagos no Cerrado.

Considerações adicionais

Invasões biológicas são eventos pontuais na formação de assembleias cujo sucesso depende, dentre outros fatores, de como as espécies nativas se relacionam entre si e com os fatores abióticos, e de quanto essas relações comportam alterações. Nesse contexto, o estabelecimento de espécies exóticas nos locais invadidos depende da composição da comunidade local, das interações entre as espécies, e de condições abióticas tais como limites de temperatura, regime de chuvas e disponibilidade de recursos, dentre outras.

Invasões são frequentemente seguidas por uma rápida mudança fenotípica das espécies invasoras em resposta ao novo ambiente. Os invasores que possuem genótipos com alta capacidade de plasticidade fenotípica são capazes de contrapor os efeitos da sua baixa diversidade genética. As espécies nativas reagem às invasões pelo mesmo mecanismo de aceleradas mudanças em sua forma de se relacionar com o meio físico e com as demais espécies da comunidade.

Os mecanismos ecológicos e evolutivos mais atuais, em especial no que diz respeito à Síntese Evolutiva Estendida (Pigliucci and Muller 2010), não foram ainda internalizados pelos profissionais da entomologia forense. O estudo na variação da taxa de desenvolvimento em função da temperatura se relaciona intimamente com a ideia de plasticidade fenotípica e normas de reação, um dos pilares da Síntese Estendida. O resultado dessa abordagem incompleta é a incapacidade de identificar e estimar as fontes de incerteza no cálculo do IPM, o que leva a apresentação de informações incorretas ou incompletas nos tribunais de justiça. Compreender esses mecanismos é imprescindível para o aprimoramento das técnicas de estimativa de IPM a fim de fornecer dados acurados e de confiabilidade estabelecida.

ESTRUTURA DA TESE

A presente tese divide-se em quatro capítulos e tem como objetivos gerais compreender a variação fenotípica em populações de califorídeos necrófagos como resposta às interações bióticas entre essas populações e, ainda, em função da heterogeneidade temporal e espacial. Ainda tivemos por objetivo avaliar a composição das comunidades de califorídeos necrófagos em uma área protegia de Cerrado em função da heterogeneidade temporal e espacial.

Os objetivos específicos foram abordados em cada um dos capítulos separadamente. No primeiro tratamos do dimorfismo sexual de espécies de califorídeos de interesse forense e da resposta dessas espécies à competição intra- e interespecífica. Em 2018 esse capítulo foi publicado como artigo no periódico *Forensic Science International*, um dos mais lidos na área de Ciências Forenses. O título do artigo é “Sexual size dimorphism in three species of forensically important blowflies (Diptera: Calliphoridae) and its implications for postmortem interval estimation”.

O segundo capítulo aborda as respostas distintas que espécies de califorídeos de interesse forense apresentam em sua morfologia alar, quando submetidas a situações de competição intra e interespecífica. Ambos os artigos dos capítulos 1 e 2 foram fruto da reanálise de material biológico coletado pelo perito criminal da Polícia Civil do Distrito Federal, Luciano Chaves Arantes, em sua dissertação de mestrado desenvolvida no King’s College, Londres, Reino Unido, intitulada *Asymmetric competition among three different species of carrion breeding blowflies*, o qual se encontra armazenado nos laboratórios do Instituto de Criminalística da Policia Civil do Distrito Federal. O manuscrito referente ao segundo capítulo está em fase final de revisão no periódico

Journal of Medical Entomology, e o título do artigo é “Contrasting responses of wing morphology of three blowfly (Diptera: Calliphoridae) species to competition”

O terceiro capítulo avalia a estrutura da comunidade de califorídeos necrófagos da Reserva Ecológica do IBGE, pela perspectiva ecológica funcional de *ensemble* (Fauth et al. 1996). São focalizadas as dinâmicas temporal (estação seca x chuvosa) e espacial (floresta x vegetação aberta) do ambiente, e as relações entre diversidade de comunidade e variação fenotípica (espalhamento dos fenótipos em um espaço teórico).

Por fim, o quarto capítulo avalia as respostas da morfologia alar de duas espécies de califorídeos necrófagos do Cerrado, uma nativa – *Chloroprocta idioidea* Wulp, 1896 – e uma invasora – *Chrysomya albiceps* –, à heterogeneidade temporal e espacial característica do Cerrado. São analisados também os níveis de assimetria flutuante (FA) a que estão submetidas as referidas espécies, e como isso as afeta. É sabido que espécies de drosofilídeos nativos e invasores apresentam reações distintas a esses estímulos e supõe-se que califorídeos possam apresentar o mesmo padrão.

Detalhes dos métodos empregados em cada capítulo e do material analisado estão individualizados nos próprios capítulos. As numerações de figuras e tabelas, bem como as referências bibliográficas seguem divididas por manuscritos (capítulos).

Sexual size dimorphism in three species of forensically important blowflies (Diptera: Calliphoridae) and its implications for postmortem interval estimation

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Abstract

Forensic entomologists rely on insects present in human remains to establish a minimum postmortem interval (PMI_{min}). Blowflies have been widely used in these estimates because they lay eggs on the victim`s body shortly after death and, within hours, larvae hatch and grow at predictable rates. Hence, isomegalen diagrams based on larval size and local temperature are considered good models to estimate PMI_{min}. Still, most professional do not account for size sexual dimorphism in blowflies, which add uncertainty to predictive models by two mechanisms: 1) males and females might grow up to different sizes, and; 2) males and females might grow at different rates. In this study, we investigate sexual dimorphism and biases on the prediction of adult size in three species of blowflies (*Lucilia sericata*, *Calliphora vicina* and *C. vomitoria*) reared under different larval densities. Estimated size range, stablished with and without sex discrimination, showed that females were larger than males in the three species. The ultimate size of adult stage, however, was more difficult to predict. *Calliphora vicina* and *C. vomitoria* decrease as density raises but at different rates, and even males and females of the same species react differently to density increase. Adult size of *Lucilia sericata*, in contrast, shows a slight increase with density. Except for *C. vomitoria* females, estimated size ranges are lower when species are divided by sex. Our results show that sex is an important factor to consider in PMI_{min} estimates. Scenarios for all three species shorten their estimated size ranges when compared to databases with no sex identification. Therefore, computing data by sex raises accuracy in size based predictive models.

Keywords: *Lucilia sericata, Calliphora vicina, Calliphora vomitoria,* size range

1. INTRODUCTION

Forensic entomologists rely on insects present in human remains to establish a minimum postmortem interval (PMI_{min}). Colonization is assumed to initiate shortly after death and blowflies are usually the first insects to arrive and lay eggs on the victim's body. Within hours, larvae hatch and start post-embryonic development, feeding on the human remains. These larvae are supposed to grow at predictable rates and forensic entomologists estimate their age based on their sizes or lengths.

Isomegalen diagrams are sophisticate models to estimate larval age (*i.e.* PMI_{min}) based on larval size and local temperature (which is a function of environmental temperature and larval density) (Amendt et al. 2011). These diagrams assume that a group of larvae will take a specific amount of time to reach a given size at a specific temperature. Through careful examination of some specimens, an entomologist might be able to fit real case data into an isomegalen based model to yield a PMI_{min} estimate. In that context, intraspecific phenotypic variation is equivalent to "uncertainty" in predictive models (Tomberlin, Benbow, et al. 2011), therefore predictions based on likelihood ratios must embody this diversity to quantify uncertainty.

Sexual size dimorphism (SSD) is well spread through the animal kingdom and has caught the attention of evolutionary biologists since 19th century (Smith 1999). Insects display remarkable SSD, with females being larger than males in many groups. Sukhodolskata *et al.* (2016) reported dimorphism in size in 12 carabid beetles species (Coleoptera: Carabidae) (Sukhodolskaya et al. 2016). In the Diptera order, Nunez & Liria (2017) and Hightower et al. (1972) reported the same pattern of SSD in four species of blowflies (Calliphoridae) of forensic interest (B.G. Hightower, G, E. Spates 1972, Nuñez-Rodríguez and Liria 2017), and Hu *et al.* (2011) reported both SSD in the oriental latrine blowfly, *Chrysomya megacephala*, and its decrease in higher temperatures (Hu et al. 2011).

Despite of the pivotal role of size in PMI_{min} estimation, most professional do not account for sexual dimorphism in blowflies' size. We believe that this gap may increase error and uncertainty to predictive models by two mechanisms: 1) males and females might grow up to different sizes, and 2) males and females might grow at different rates even when developed at the same temperature. In both scenarios, researchers might be dealing with subpopulations that display distinct size parameters.

In this study, we used de Wit replacement series in three blowfly species to test if: (a) males and females grow up to different sizes (SSD), and (b) males and females react similarly to larval density (i.e., if SSD respond to an environmental factor).

2. MATERIAL AND METHODS

2.1. Fly population stocks

Lucilia sericata adults were collected on Bermondsey (SE) and Acton (NW), London, using liver-baited traps. *Calliphora vicina* adults were obtained from caged populations maintained in the laboratory and from Bermondsey. *Calliphora vomitoria* third-instar larvae were purchased from a fishing bait shop and reared to adult in the laboratory. Adult fly populations were maintained as single-species cultures in cages. Water and granulated sugar were provided *ad libitum*. Fresh pig liver was offered as a protein source to stimulate ovarian development and oviposition.

2.2. Experimental design

The three blowfly species were reared in pairs at initial densities of 1, 3 and 9 larvae per gram of fresh pig liver until the emergence of adults. For each density, species were combined in five different proportions – 100% of larvae from one species (1:0) and 100% of larvae from the other species (0:1) – and three mixed-species cultures at different proportions – 2/3:1/3, 1/2:1/2, 1/3:2/3 (de Wit 1960).

2.3. Wings preparation

Left wings from all emerged flies were cut and glued with transparent nail vanish on a white paper covered with transparent Scotch tape. Papers with glued wings were oven dried at 40-50ºC for more than 24 h. From the specimens that had no left wing or had it damaged, we proceeded to remove and fix the right wing and the digitalized image was horizontally inverted for later use in morphometric analyses, as no directional asymmetry is reported for the species used in the present study.

The sheets of paper with the wings were set over a flat transilluminator and flattened with a glass barrier on top. Then, the wings were individually photographed using a Canon T4i DSLR camera coupled with a Canon MP-E 65mm f/2.8 1-5x macro photo lens. The camera and transilluminator levels were set in two orthogonal axes with a hydraulic leveler.

2.4. Morphometric and statistical analysis

A total of 3167 wings were analyzed, 1223 from *C. vicina* (584 females and 639 males), 1044 from *C. vomitoria* (525 females and 519 males) and 900 *L. sericata* (469 females and 431 males). Each wing was classified according to its species, sex, and larval density. A library with all wings was built using TPSUtil and 11 landmarks were positioned in each wing using TPSDig (Rohlf 2015).

Morphometric analysis of each group consisted in Procrustes superimposition, where the landmark coordinates were superimposed so that the centroids overlap, scaling the unit to centroid size. Then we determined the centroid size of each individual, the square root of the sum of squared distances of the centroid to the 11 landmarks (Bookstein 1997). Centroid size was taken as a proxy to ultimate adult body size, and body size range was estimated as the interval encompassing from the 2.5% to the 97.5% quantile of each category of species, sex and density. As we did not assume any populational distribution of our data and populational structure was not fixed, the effect of species, sex and density on adult size was then tested with Scheirer-Ray-Hare extension of the Kruskal Wallis test (Scheirer et al. 1976). The influence of sex (males, females and both together) on estimated range values, in turn, was tested with a nested ANOVA considering that sex is a factor nested into density levels, which in turn is nested into species. The effect of density on size was accessed with a simple linear regression analysis.

Morphometric analyses were executed in MorphoJ software (Klingenberg 2011), and linear model calculations were performed in R, stats package (R Core Team, 2016). Graphic material was produced in R, ggplot2 Package (Wickham 2016), environment and refined in Inkscape (v 0.91) graphic software.

3. RESULTS

A total of 3167 size measures were taken into 9 categories (three species in three densities each), and in all scenarios males were smaller than females ($H = 263.9$, $p < 0.001$) at a 1:1.1 ratio. Size range was also affected by sex ($F_{2,27} = 15.38$, $p < 0.001$), with larger ranges in groups without discrimination between males and females. The only exception was *Calliphora vomitoria* bred at 9 flies/g, where the size range was slightly higher in females than in individuals not divided by sex (Figure 1, Table1).

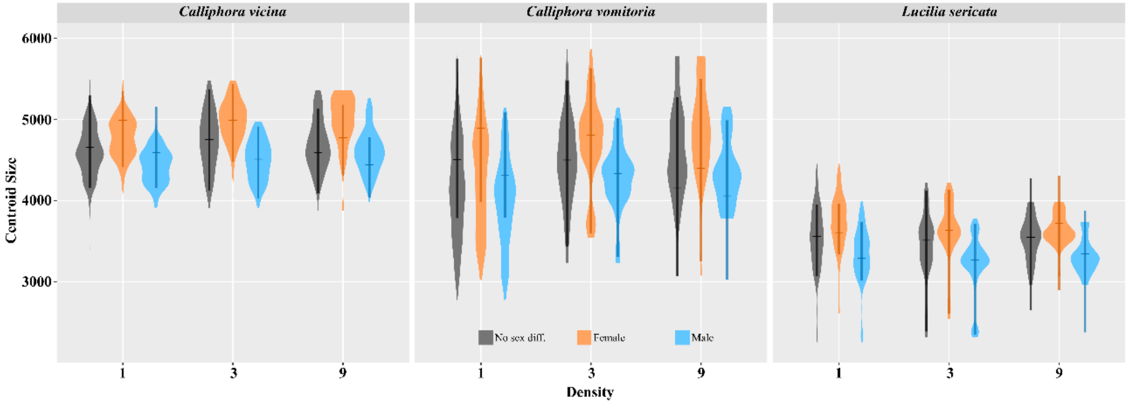

Figure 1. Effect of larval density on size (Centroid Size) and size range of *Calliphora vicina*, *Calliphora vomitoria e Lucilia sericata* with (orange for females and blue for males) and without (grey) sex differentiation. Vertical lines inside violin plot show centroid size range from the 2.5% to 97.5% quantiles and horizontal dash crossing the vertical line shows median values.

Table 1. Size range considered as the values from the 2.5% (lower limit) to 97.5% upper limit) percentiles of centroid size for the three species studied. Data in classified according to species and density (larvae/gram) and presented without (on the left) and with (on the right) sex differentiation.

Species	Density	Lower limit		Upper limit		Range	
Calliphora vicina	9	4087.9	♀4309.5	5130.2	♀5182.0	1042.3	♀872.5
			♂4037.7		♂4782.5		♂744.8
	3	4123.3	♀4476.5	5373.4	♀5437.6	1250.1	♀961.0
			♂4031.1		♂4913.7		♂882.6
	1	4159.4	♀4417.9	5299.8	♀5350.0	1140.5	♀932.1
			♂4159.3		♂5158.1		♂998.9
Calliphora vomitoria	9	3071.3	♀3253.0	5275.3	♀5496.6	2204.1	♀2243.6
			♂3030.9		♂4989.2		♂1958.3
	3	3431.6	♀3596.0	5479.3	♀5629.8	2047.7	♀2033.8
			♂3305.5		♂5015.3		♂1709.7
	1	3785.4	♀3982.0	5749.9	♀5761.2	1964.5	♀1779.2
			♂3786.5		♂5085.7		♂1299.2
Lucilia sericata	9	2651.5	♀2894.4	4272.5	♀4307.9	1620.9	♀1413.4
			♂2377.8		♂3875.0		♂1497.3
	3	2388.7	♀2606.4	4119.7	♀4137.1	1731.0	♀1530.8
			♂2347.9		♂3717.7		♂1369.8
	1	3073.5	♀3336.8	3954.2	♀3964.0	880.8	♀ 627.2
			♂3015.1		♂3733.4		♂ 718.4

Density had a negative effect on size for *C. vicina* (t = -6.03, p < 0.001) and *C. vomitoria* (t = -9.25, p < 0.001), but a slightly positive effect for *L. sericata* (t = 2.49, p = 0.012) (Figure 2, Table 2). Linear coefficients of these models show *C. vicina* with 0.3% size reduction for unitary increase in larval density, *C. vomitoria* displays 1.1% size decrease and *L. sericata* 0.3% size increase for the same density variation (Table 2). Density had no significant effect on size ratio ($F_{2,27}$ = 1.121, p = 0.411) neither on size range ($F_{2,27}$ = 1.532, p = 0.246).

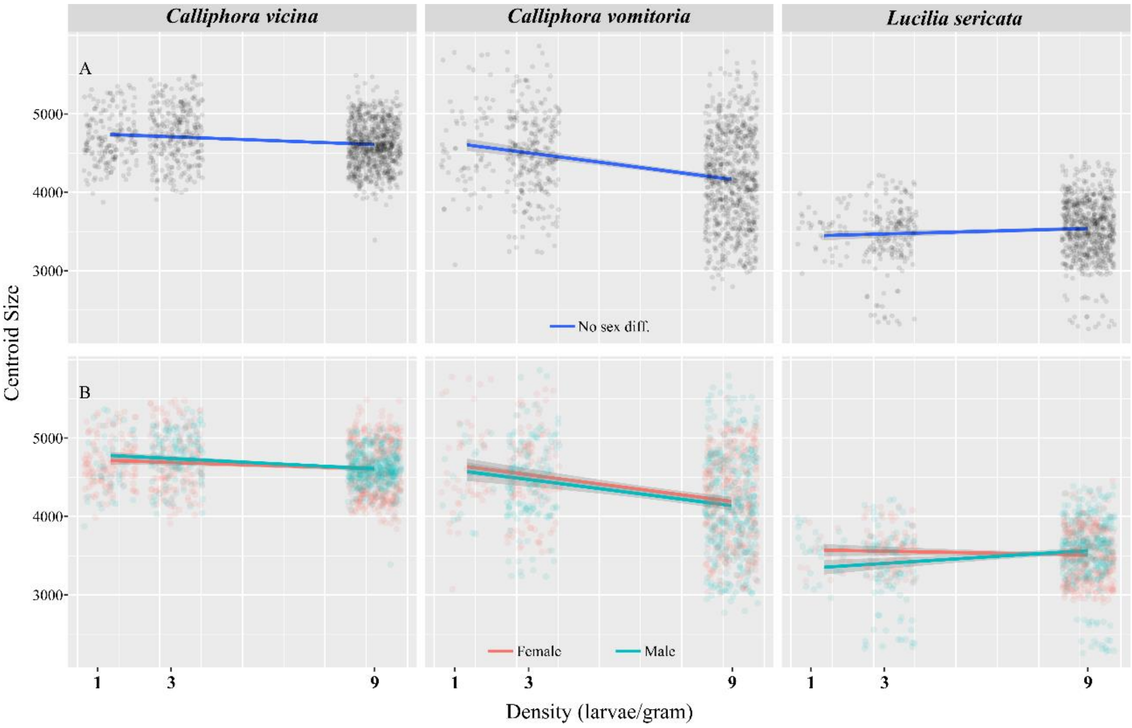

Figure 2. Linear models for the effect of larval density on the three studied species. Data is presented without sex differentiation (A) and with sex classification (B). Males are shown in light blue and females in light red.

Table 2. Linear Regression coefficients (intercept and angular) for the effect of density on the three studied species.

Species	Intercept		Angular coef.	
C. vicina	4753.1	♀ 5058.7 ♂ 4589.5	-15.9	♀ -31.8 ♂ -18.7
C. vomitoria	4658.8	♀ 4930.9 ♂ 4426.5	-54.8	♀ -68.9 ♂ -46.3
L. sericata	3437.6	♀ 3604.7 ♂ 3203.2	11.2	♀ 14.0 ♂ 15.3

4. DISCUSSION

In this study we investigated sexual size dimorphism in three blowfly species of forensic interest, focusing on the influence of sex and larval density in their individual body size and body size ranges. Our results showed that SSD is present in the three species. Females are 10% larger than males and this relation is not affected by density or mean size. Sexual size dimorphism is well documented through insect orders and bears both ecological and evolutionary features (Picaud and Petit 2009, Sukhodolskaya et al. 2016). In this aspect these results are not surprising, they are aligned with published literature.

Responses of sexual size dimorphism to density, however, were more complex. Density influenced size in the three species, but not in the same manner and neither at the same rate. *Caliphora vomitoria* was the most influenced by density, decreasing adult size as larval density raises (Figure 2). This result supports previous observations that density, but not food type, affects the size of *C. vomitoria* adults (Ireland and Turner 2006). *Calliphora vicina* presented the same trend, but with a milder reduction in size.

On the other hand, linear models show that density had a positive effect on size in *Lucilia sericata*, but with the mildest tendency of the three species. In fact, the slope of that model (Figure 2, Table 2) is so mild that we are not convinced that it has a considerable biological meaning, despite p-values of the regression models ($p = 0.01$). Our observation partially contrasts from what we expected due to previous data showing reduction on pupal length and weight of *L. sericata* in response to temperature (Tarone et al. 2011) and food moisture (Tarone and Foran 2006); nevertheless, we analyzed size response to density, and different mechanisms might influence the outcome of the experiments.

There are previous reports of size reduction in blowflies as a consequence of competition with other blowflies species (So and Dudgeon 1989, Von Zuben et al. 2000), families of Diptera, and insect orders (Teder and Tammaru 2005). The effect of density on adult size might be explained by at least two mechanisms: (a) larval aggregation induces heat emission, raising local temperature, in what is known as larval-mass effect (Charabidze et al. 2011), and; (b) reduced nutrient availability to individuals results in smaller adults, due to resource scarcity. Both factors lead to size reduction and probably are present in our experimental design. Therefore, we feel comfortable to compare our results with published data regarding effects of temperature on related species only with the due caveats

Size range is smaller for datasets divided by sex than for datasets with no sex classification. However, our findings show that size range is not influenced by density, in contrast to previous finding that report not only SSD for the forensically important blowfly *Chrysomya megacephala* but also different size ranges for higher temperatures (Hu et al. 2011). Despite many studies that investigate the bionomy of insect species regarding density (Peters and Barbosa 1977), specially of those of forensic interest (Sullivan and Sokal 1963, Al-Misned 2002, Barros-Souza et al. 2012, da Silva Xavier et al. 2015), we are not aware of other studies investigating the effect of density on body size range.

As pointed above, sexual size dimorphism is well documented in insects. Nevertheless, most forensic entomology studies do not account for sexual dimorphism in calliphorid flies. In fact, sexual dimorphism is a common topic in ecological and evolutionary studies for decades, and this draws attention to Tomberlin and his coworkers call for basic research in ecology and evolution of these flies to improve accuracy and reliability in forensic entomology (Tomberlin, Benbow, et al. 2011).

Predictive models in forensic entomology relates larval size to its age. Thus, differences in larval size from a specimen to parameters of a dataset that subsidizes a model lead to errors on age prediction. Furthermore, the present study shows that to ignore sex when constructing models provide the researcher with a broader dataset of which mean (or any other central tendency measure) does not correspond to the true mean and neither dispersion measures corresponds to the true dispersion of the actual population the specimen should be assigned to, resulting in higher uncertainty levels. Therefore, predictive models based on size can reduce uncertainty if provided with data computed by sex instead of general data with no sex discrimination.

In short, size sexual dimorphism is present in the three species analyzed, and size range is narrower when females and males are computed as subsets of a specific population. To take heed of this is crucial to reduce error and uncertainty when formulating predictions based on size.

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Contrasting responses of wing morphology of three blowfly (Diptera: Calliphoridae) species to competition

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Abstract

Competition influences the expression of morphological, physiological and behavioral traits and regulates ecological and evolutionary dynamics. This study aims to identify and characterize changes in wing morphology in response to intra- and interspecific competition in three necrophagous blowfly species. Using geometric morphometry, we analyzed 3238 wings from *Lucilia sericata* (Meigen, 1826), *Calliphora vicina* Robineau-Desvoidy, 1830 and *C. vomitoria* (Linnaeus, 1758) raised under cloistered and pairwise conditions. The three species reacted similarly to intraspecific competition — reducing wing size with increased competition — but displayed contrasting patterns of response to interspecific competition. *Lucilia sericata* displayed a directional change in wing shape in response to an interspecific competitor, while *C. vicina* increased the scattering of individuals across the morphospace, and *C. vomitoria* displayed no significant change in response to the same stimulus. Our results show that the same stimulus yields distinctive responses; thus, different competition-related strategies are expected to occur in the three species.

Key words: Wing shape, necrophagous insects, geometric morphometrics

Introduction

Competition is one of the most important biotic factors regulating ecological and evolutionary dynamics (Rabosky 2013). Population response to competition lies on a spectrum from the pure scramble scenario, where individuals share available resources equally and die when the resources run out, to a pure contest scenario, in which resources are unequally distributed among individuals (Nicholson 1954). Stochasticity can influence the outcome of competitive scenarios, especially in cases of ephemeral and fragmented resources, such as the colonization of carcasses by blowflies. Along the scramble–contest spectrum, fly populations tend toward the former: changes in longevity, mortality, and body size tend to be similarly distributed among individuals of a population under competition (So and Dudgeon 1989, Prinkkila and Hanski 1995).

Competition can influence several traits, including morphology. For example, the planthoppers *Prokelisia marginata* (Van Duzee, 1897) and *P. dolus* Wilson, 1982 respond to intraspecific competition by shifting the wing shape from short-winged morphs (flightless) to long-winged morphs (migratory) as density increases (Denno and Roderick 1992). Similarly, the polyembryonic wasp *Copidosoma floridanum* Ashmead, 1900 produces distinct morphological castes, altering caste ratios in response to intraspecific competition. Other biological interactions, such as mutualism, predation risk, and parasitism, can also induce rapid morphological responses in several species (Agrawal 2001). For example, in the presence of a predator, the water flea *Daphnia lumholtzi* Sars, 1885 develops a sharp helmet and extended tail spine (Green 1967). Not all morphological responses are so abrupt; blowflies, for example, respond more subtly to environmental factors (Laparie et al. 2016).

The flies and mosquitoes of the order Diptera have been widely used as models to study eco-evolutionary phenomena. Several species display environmentally induced variation in a wide range of traits (Reeves et al. 2000; Hoffmann and Shirriffs 2002; Aytekin et al. 2007; Kjærsgaard et al. 2007; Soto et al. 2008; Marsteller et al. 2009; Devicari et al. 2011; Demirci et al. 2012; Hidalgo et al. 2015), demonstrating their usefulness as bioindicators. Specifically, chironomids (Chironomidae) have been proposed as indicators of eutrophication of water streams (Machado et al. 2015) and drosophilids as indicators of atmospheric pollution (Parsons 1991), urbanization level (Ferreira and Tidon 2005), and habitat quality (Mata et al. 2008). Blowflies (Calliphoridae) are widely used as indicators of time of death in criminal investigations (Amendt et al. 2011).

Wing morphology is a key feature in Diptera systematics (Stark et al. 1999), and changes in wing structure have been important in the evolution of maneuverability and precision in insect flight (Ennos 1987). In *Drosophila lumei*, even subtle changes in wing structure can be linked to genetic differences among populations (Haas and Tolley 1998). With the development of powerful analytical tools based on multivariate statistics and geometric morphometrics, the components of shape and size can be separated (Adams et al. 2013a), making it feasible to detect even subtle differences in form (Strauss and Bookstein 1982, Klingenberg 2010). Despite several reports of morphological changes induced by biotic interactions, we are not aware of any studies describing changes in wing size and shape in response to intra- and interspecific competition in the Calliphoridae family.

In this study, we used geometric morphometrics to characterize the wing morphology of *Lucilia sericata* (Meigen, 1826), *Calliphora vicina* Robineau-Desvoidy, 1830, and *Calliphora vomitoria* (Linnaeus, 1758) in response to pairwise interspecific competition and compared it with wing morphology in single-species experiments under the same conditions. We then analyzed the response patterns displayed by those species.

Materials and Methods

The basis of our experimental design has already been described in a previous paper (Macedo et al. 2018), where we investigated the sexual dimorphism of these three blowfly species. Here, we provide additional details about the samples and procedures.

Fly stocks

Lucilia sericata and *Calliphora vicina* adults were collected in Bermondsey (SE) and Acton (NW), London, using liver-baited traps between May and June 2004. *Calliphora vomitoria* third instar larvae were obtained from a fishing bait shop and reared to adult in the laboratory. Adult fly specimens were placed in single-species population cages at a 1:1 female:male ratio, with water and granulated sugar provided *ad libitum*. Adult cages and rearing boxes were maintained at room temperature (19ºC ±1.5ºC). Fresh pig liver was offered as a protein source to stimulate ovarian development and oviposition. All experiments were conducted with eggs laid by the collected specimens, that is, the F_1 generation.

Experimental design

De Wit replacement series were used to evaluate intra- and interspecific competition effects (de Wit, 1960). Immatures of the three species were reared in pairwise arrangements at initial densities of 1, 3, and 9 larvae per gram of fresh pig liver. At each density, two species were combined at the following ratios: two single-species cultures (100% of larvae from one species [1:0] and 100% of larvae from the other species [0:1]) and three mixed-species cultures (2/3:1/3, 1/2:1/2, and 1/3:2/3). For statistical analysis, data from the mixed-species cultures were combined to allow the comparison between two groups: with and without the presence of an interspecific competitor.

Wing preparation

The left wings from all flies were removed and attached with transparent nail varnish to white paper, which was then oven dried at 40–50°C for more than 24 h. For flies with no left wing or a damaged left wing, we used the right wing and horizontally inverted the digital image for morphometric analyses because no directional asymmetries have been reported for these species.

The sheets of paper with the attached wings were set on a flat transilluminator and flattened with a glass barrier. The wings were individually photographed using a Canon T4i DSLR camera coupled with a Canon MP-E 65 mm f/2.8 1–5× macro photo lens. The camera and transilluminator levels were set according to two orthogonal axes with a hydraulic leveler.

Morphometric and statistical analysis

A total of 3167 wings were analyzed, 900 from *L. sericata* (469 females and 431 males), 1223 from *C. vicina* (584 females and 639 males), and 1044 from *C. vomitoria* (525 females and 519 males). Each wing was classified according to species, sex, presence/absence of an interspecific competitor and larval density, and a library with all wings was created using tspUtil software (Rohlf, 2015). Eleven landmarks were digitized on each wing (Figure 1) using tspDig (Rohlf, 2013).

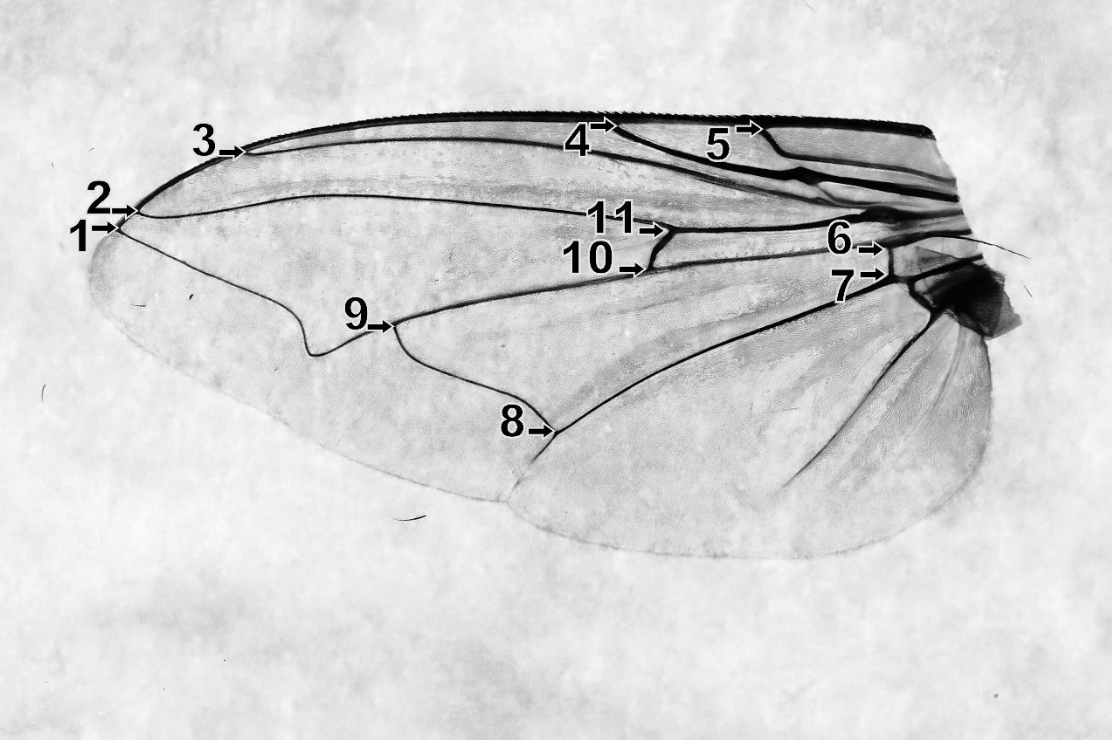

Figure 1. Dorsal view of a calliphorid wing with the 11 landmarks used in this study.

Morphometric analysis consisted of Procrustes superimposition, which is a method that aims to retain only shape-related information in a dataset through the removal of information concerning position, orientation and size (Rohlf and Slice 1990, Zelditch et al. 2004). This procedure is based on operations of translation, scaling, and rotation of the configuration matrices and generates two primary outcomes in the MorphoJ environment: centroid size, which is a measure of wing size mathematically independent of wing shape, and wing shape coordinates (Klingenberg 2011).

To estimate measurement error due to image acquisition and landmark digitization, we selected a subsample of 30 wings and photographed and digitized the landmarks twice on different occasions on distinct days by the same operator. We then used Procrustes analysis of variance (ANOVA) with the landmark positioning date as a factor to test for deviations from randomness.

To test for allometry, i.e., whether size influences shape, we used multivariate regression to test whether the Procrustes coordinates behave as dependent variables associated with centroid size with permutation tests (10,000 randomization rounds). The tests were conducted for each of the six groups separately (males and females of three species) because sexual size dimorphism has been reported for these species (Macedo et al. 2018), and size and shape differences among species are expected.

From the Procrustes coordinates, we built a covariance matrix and then used a principal component analysis (PCA) to explore the distribution of individuals in the morphospace based on landmark configuration.

The effect of competition on wing morphology was tested by Procrustes ANOVA and visualized by canonical variate analysis (CVA). To evaluate the effects of intraspecific competition, we tested the effect of larval density on wing size and shape in single-species experiments. To evaluate the effects of interspecific competition, we evaluated subsets of individuals reared in mixed-species experiments versus individuals raised in cloistered conditions. In the evaluation of interspecific competition, the effect of larval density was excluded from the analysis, credited as a source of error. Morphometric analyses were performed in MorphoJ software (Klingenberg 2011), and factorial ANOVA calculations were performed in the R Stats Package (R Development Core Team 2015). Graphics were produced in MorphoJ and the R ggplot2 package (Wickham 2016) and refined with Inkscape graphic software.

Results

Repeatability tests showed that image acquisition and redigitization of landmarks on the same wing on separate days did not change the Procrustes coordinates and were not a source of error ($F = 0.18$ d.f. $= 18$, $p > 0.99$). Shape prediction by allometric analysis was 0.27% ($p = 0.24$) for females and 0.59% ($p = 0.03$) for males of

L. sericata, 1.08% for females and 3.41% for males of *C. vicina* ($p < 0.001$), and 2.40% for females and 1.90% for males of *C. vomitoria* ($p < 0.001$). These results demonstrate that differences among individuals are not due to error in image acquisition or landmark digitation and that the influence of size on wing shape is mild but significant for *C. vicina* and *C. vomitoria*.

Variation in wing size and shape for the whole dataset was influenced by species (size: $F = 2019.5$, d.f. 2; shape: $F = 1628.8$, d.f. = 36) and sex (size: $F = 612.5$, d.f. = 1; shape: $F = 2178.0$, d.f. = 18) ($p < 0.001$ for all factors). Therefore, the analyses were performed separately for each group (Table 1). The effect of intraspecific competition on wing size and shape was significant for all three species (both males and females); however, this effect was more pronounced for wing size (i.e., smaller wings under higher competition) than for wing shape (Table 1). Our results indicate that interspecific competition influenced wing shape in females and males of *L. sericata* ($p < 0.001$), females and males of *C. vicina* ($p < 0.001$), and males of *C. vomitoria* ($p < 0.05$) (Table 1).

Table 1. Univariate analysis of variance on wing size (centroid size) and multivariate analysis of variance on wing shape variables (shape) of female and male *Lucilia sericata*, *Calliphora vicina*, and *Calliphora vomitoria* under intraspecific and interspecific competition.

			Intraspecific competition		Interspecific competition	
			d.f.	F	d.f.	F
Lucilia sericata	♀ (n = 469)	centroid size	2	4.23 (p=0.01)	1	5.99 (p=0.01)
		shape	36	2.26***	18	13.85***
	♂ (n = 431)	centroid size	2	4.86 (p=0.01)	1	3.37 (p=0.07)
		shape	36	3.07***	18	9.18***
Calliphora vicina	♀ (n = 584)	centroid size	2	39.75***	1	2.11 (p=0.15)
		shape	36	6.87***	18	5.86***
	♂ (n = 639)	centroid size	2	14.82***	1	24,76***
		shape	36	10.28***	18	14.80***
Calliphora vomitoria	♀ (n = 525)	centroid size	2	32.08***	1	7.97 (p=0.01)
		shape	36	9.75***	18	1.12 (p=0.32)
	♂ (n = 519)	centroid size	2	21.55***	1	1.03 (p=0.31)
		shape	36	7.02***	18	1.75 (p=0.03)

p-values are indicated in parentheses;
*** = p < 0.001;
d.f. = degrees of freedom

The PCA results for the whole dataset demonstrated that 79.7% of the morphological variation was represented in the first three principal components, with 47.16% in the first PC, 20.79% in the second, and 11.74% in the third. The main differences were due to species differences and sexual dimorphism, with clear visualization of six groups (Figure 2).

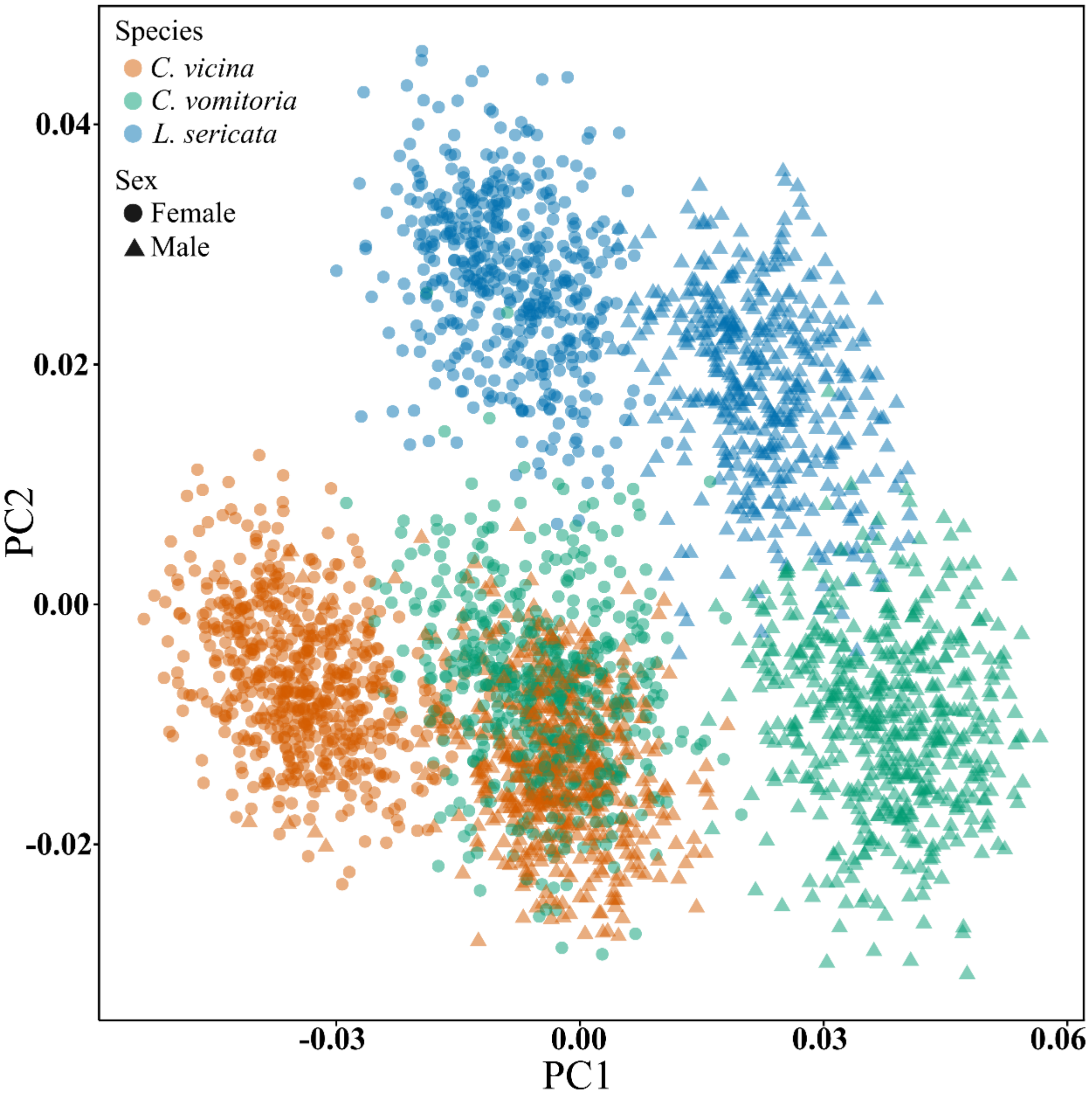

Figure 2. Two-dimensional plot showing the distribution in the morphospace of *Calliphora vicina* (orange), *Calliphora vomitoria* (green), and *Lucilia sericata* (blue) specimens based on a principal component analysis.

Because Procrustes ANOVA results showed only subtle effects of competition on wing morphology, these effects were further explored by CVA. The CVA showed that, in all three species, more than 96% of the variation among groups was explained by the first canonical variate (97.6% for *L. sericata*, 96.1% for *C. vicina* and 99.6% for *C. vomitoria*), which was related to sexual dimorphism. The second canonical variate accounts for considerably less variation among groups (2.2% for *L. sericata*, 3.2% for

C. vicina and 0.3% for *C. vomitoria*) (Figure 3). However, the nature of the competition (second canonical variate) also influenced wing morphology, especially in *C. vicina* and *L. sericata* (Figure 3).

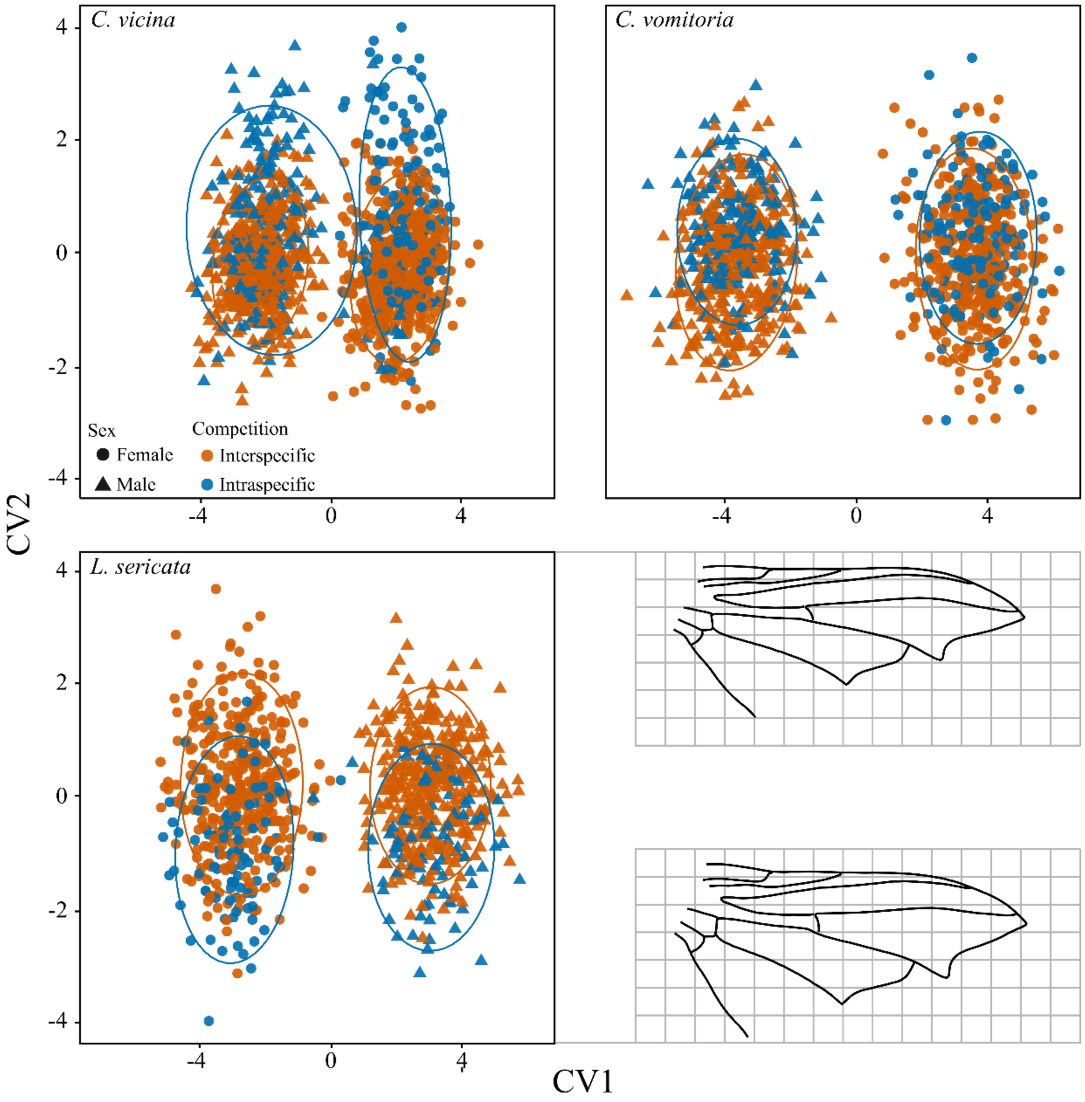

Figure 3. Scatterplot of the first canonical variates (CV1 and CV2) for *Calliphora vicina*, *Calliphora vomitoria*, and *Lucilia sericata* specimens under interspecific (orange) and intraspecific (blue) competition, with the visual representation of shape change detected on CV2 for *L. sericata* specimens. Shape changes are shown at the proximal-distal axis extremities with no magnification.

Discussion

Density and allometric effects

In this study of three blowfly species, we observed similar changes in wing size in response to intraspecific competition. Specifically, higher intraspecific competition resulted in smaller wings (except for male *L. sericata*, as discussed below). This might be explained by the increased temperature caused by metabolic activity at higher larval densities, known as the larval mass effect (Charabidze et al. 2011), as well as a decrease in nutritional resource availability. Both factors are known to lead to smaller flies in several Calliphoridae species and other Diptera families (Ireland and Turner 2006, Ribeiro and Von Zuben 2010, Amendt et al. 2011, Yeap et al. 2013, Sharma et al. 2015, Gruner et al. 2017). In a previous study (Macedo et al. 2018), we found that density had a minor positive impact on *L. sericata* size, but the slope of that regression model was so small that any biological meaning is unclear. Nevertheless, size differences in response to density may derive from different mechanisms than differences originating from temperature; thus, future studies should clarify this effect.

Although shape displays significant correlations with size for *C. vicina* and *C. vomitoria*, we have not excluded the allometric component in our analysis because the values are very low (less than 3.41%), and we dealt with static allometry. The exclusion procedure, performing morphometric analysis based on the residuals of multivariate regression, is widespread, especially in taxonomic studies and in cases where ontogenic allometry is considered (Klingenberg 2016). We analyzed individuals of the same age but different sizes (static allometry) on an ecological time scale; thus, we considered a more conservative approach to report shape changes regardless of correlation with size. Nevertheless, we presented the statistical values of allometry-related aspects of our data in case readers do not agree with our premises.

Intraspecific to interspecific competition effects

The effect of interspecific competition on wing shape differed among the fly species, with no general pattern observed. *Calliphora vicina* showed an increased scattering of individuals across the morphospace under intraspecific competition in comparison to the pattern under interspecific competition (Figure 3). This finding suggests that a high density of individuals of the same species could reduce the individual's ability to repress environmental interference in the production of a phenotype (buffer effect). This idea is supported by reports of increased fluctuating asymmetry (Beasley et al. 2013) and increased phenotypic variance in quantitative traits (Forbes et al. 1995) in response to environmental stress. The increased homogeneity in wing shape under interspecific competition may be a result of selection pressure on that trait.

Calliphora vomitoria displayed no significant change in wing shape from intraspecific to interspecific competition. Despite F and p-values of 1.75 and 0.03, d.f. = 18, for male *C. vomitoria* (Table 1), the sample size in the present study and the multivariate statistics framework of morphometric analysis indicate extremely subtle differences; thus, we interpreted these F and p-values conservatively and comparatively between size and shape within each species. Visual inspection of the CV plots for *C. vomitoria* (Figure 2) revealed no directional shift or increased scattering under interspecific competition. It is important to note that CV analysis maximizes between-group variation, supporting our interpretation of no actual change despite the Procrustes ANOVA values.

Lucilia sericata was the only species that displayed directional change in wing shape in response to an interspecific competitor. That change consisted of an elongation of the proximal-distal axis (Figure 3), although the wing area remained fairly unchanged

(Table 1). Although the biological mechanism underlying these changes is unclear, two hypotheses may explain this observation: (a) selection pressures favor phenotypes that do not overlap between two species (character displacement) or (b) immature stages detect the presence of another species and alter the ontogenic process in response to this stimulus (phenotypic plasticity).

Is the morphological change in Lucilia sericata adaptive?

In insects, wing shape influences flight performance. Slender, elongated wings increase the wingspan, increasing the glide ratio and favoring migratory flight. On the other hand, a more rounded wing, close to a half ellipse shape, lowers induced drag and the lift-to-drag ratio in the wing structure, reducing energy costs for flight. These alternative phenotypes may have a significant impact on an organism's lifestyle (Hoffmann et al. 2005). Environmental changes such as the colonization of novel territories or seasonality have been shown to rapidly modify wing morphology (Laparie et al. 2016); thus, *L. sericata* may develop wings favoring migratory flight in the presence of an interspecific competitor to facilitate dispersal and competition avoidance strategies. This hypothesis should be tested with further observational studies.

The mechanisms underlying changes in wing phenotype in *L. sericata* in the presence of another species are not well understood. Divergent selection acting on genetic variability is a well-known evolutionary force in competitive scenarios involving shared resources (Pfennig and Pfennig 2010, Abrams and Cortez 2015). However, phenotypic plasticity has been acknowledged as an important force driving community dynamics (Berg & Ellers 2010) because it allows rapid phenotypic change in the absence of genetic diversity (Pigliucci 2005, West-Eberhard 2005, Beldade et al. 2011). Although we believe that the phenotypic plasticity hypothesis is a more straightforward explanation than selection for the observed changes in *L. sericata* wing morphology, further studies are needed.

Final remarks

Unraveling the ecological and evolutionary aspects of organisms is important because it allows us to rely on predictions yielded by real-life scenarios. Forensic estimates based on Calliphoridae flies depend on the eco-evolutionary features of those species; thus, an understanding of these patterns is critical to the standardization of forensic practice (Tomberlin, Benbow, et al. 2011). Our results should raise a flag to these professionals, who rely on phenotype expression in changing environments, to estimate the postmortem interval. Their analyses must take into account the presence of a competitor, which could alter the fly phenotype or the variance in a quantitative trait (e.g., developmental rate).

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Temporal and spatial community structure and morphological variation of necrophagous blowflies (Diptera: Calliphoridae) in a protected area in the Brazilian Cerrado

Abstract

Blowflies (Diptera: Calliphoridae) are an important group due to its habits of feeding and breeding in decomposing organic matter. To understand the ecological dynamics of this group is vital to approach their validity as biological indicators and to understand their role as pathogens vectors. This study aim is to comprehend the influence of temporal and spatial heterogeneity in the community structure of necrophagous calliphorids in an environmentally protected area in the Brazilian Cerrado, and the amount of morphological variation in wing shape among seasons and vegetation cover. We analyzed 5506 specimens of calliphorids collected from June 2017 to February 2018 in the Ecological Reserve of IBGE, in the Federal District – Central Brazil. A total of nine species was registered in the community of necrophagous calliphorids, and *Chloroprocta idioidea* was the most representative species (60.2%), especially in the rainy season. *Chrysomya albiceps* was the second most representative species in the total sample (31.5%), and it dominated the community on the dry season. The species *Chrysomya putoria,* and *Mesembrinella bicolor* were collected in low numbers (5 and one specimens respectively), raising questions regarding its status as necrophagous in the Cerrado biome. We did not find correlations between Shannon-Weaver (H') and Simpsons (C) indexes and the values of morphological variation. Thus, we conclude that community diversity is not linked to intraspecific morphological diversity.

Keywords: calliphorids; forensic entomology, community diversity

Introduction

Forensic entomology is based on the interpretation of insect evidence to elucidate criminal cases (Amendt et al. 2011). Generally, forensic entomologists estimate larval age through the analysis of its size or weight because blowflies grow at predictable rates in a given set of environmental conditions (e.g., temperature, humidity). Assuming that insects start to colonize a cadaver shortly after its death, the oldest larvae collected will allow the experts to estimate the minimum postmortem interval.

Intraspecific variation occurs in all living organisms, and it is the base of the natural selection and a cornerstone of the evolutionary process. Nevertheless, this trait variation among individuals of the same species is synonymous with uncertainty in predictive models. Thus, comprehend trait variation associated with ecological and evolutionary aspects of forensically important species is pivotal to unravel the amount of uncertainty in real cases, increasing the reliability of forensic entomology (Tomberlin, Benbow, et al. 2011, Tomberlin, Mohr, et al. 2011)

Blowflies (Diptera: Calliphoridae) constitute a widely spread group with more than 1,000 species encompassed in nearly 150 genera in all biogeographic regions. Calliphorids are medium size flies with diverse habits and high synanthropy, they encompass myiasis causing, pathogen vectors and decomposers species, attributing economic and ecological importance to that family (Baumgartner and Greenberg 1984, Kosmann et al. 2013). Hence, a high number of studies regarding the family are available, especially concerning its taxonomy and applied features about some species as biological indicators or disease causers, and yet there is a lack of studies regarding its species diversity in tropical biomes and ecological aspects of the family (Kosmann et al. 2013, 2017).

The Brazilian Savanna, also known as Cerrado biome, occupies approximately a quarter of its country territory. It is a patchy landscape encompassing forests, woodlands, savannas, and grassland habitats in a mosaic of well-drained interfluvial zones interleaved with moist formations, generally gallery forests, that follows the watercourses (Oliveira and Marquis 2002). The Cerrado climate is markedly divided into two seasons: the rainy season (from November to April), and the dry season (from May to October). That edaphic and topographic diversity is associated with variable fire regimes (Furley 1999) and seasonality, giving origin to high levels of biological diversity, of endemism, and to complex ecological relationships.

On top of this intrinsic richness, the Cerrado (in fact, the Neotropical region) has been a stage of a biological invasion of the carrion feeding blowflies from the *Chrysomya* genus, especially for *Chrysomya megacephala* (Fabricius, 1794), *Chrysomya albiceps* (Wiedemann, 1819), and *Chrysomya putoria* (Wiedemann, 1818). The first species was reported as having high biotic potential, and all the three species display high dispersion capabilities, causing the displacement of native calliphorids (Gagné 1981, Prado and Guimarães 1982, Barbosa et al. 2016). Nevertheless, the mechanisms and ecological consequences of the arrival of *Chrysomya* species in the Brazilian Cerrado is still poorly known, especially on the spatio-temporal dynamics of the ensemble constituted by the carrion feeding blowflies and on the phenotypic variation levels of populations.

Here we characterized the community structure and diversity of necrophagous calliphorids in an environmentally protected area in the Brazilian

Cerrado, regarding spatial (vegetation cover) and temporal (contrasting seasons) heterogeneity. We also investigated the morphological variation levels in populations of two species from the Chrysomyinae subfamily: the invasive *C. albiceps*, and the native *Chloroprocta idioidea* (Robineau_Devoidy, 1830).

Material and Methods

Study site and fly collection

Adult calliphorids were collected in the Ecological Reserve of IBGE (Instituto Brasileiro de Geografia e Estatística) (RECOR), located 35km south of Brasilia (15° 56' S; 47° 53' W) from June 2017 to February 2018, encompassing the dry and the rainy seasons. This ecological reserve is part of a complex of environmentally protected areas that partially surround the city, covering nearly 10,000 ha and representing the mosaic of vegetations of the Cerrado biome.

We selected four collection sites in that area, two in gallery forests (gf1 and gf2) and the other two (cs1 and cs2) in a formation locally known as *campo sujo*, a grassland field with few shrubs and small trees scattered across the landscape (Oliveira and Marquis 2002); from now on, it will be referred to as grassland. In each of the four collection sites, a Shannon trap (a cubic tent with 1.8m side) was assembled and, as bait, a pig carcass (*Sus scrofa domesticus*) of 2 kg was exposed inside a metal cage to prevent access of necrophagous mammals to the carcass. Each Shannon trap was placed in the same site three times (triplicates) in different months of the season (dry: June, July, August; rainy: December, January, February), encompassing a total of 24 traps. In each replicate, we actively collected adult flies with an entomological net three times (pseudoreplicates) in different days (day 1, day 3, and day 5, after the carcass exposition). The specimens were placed in plastic containers labeled with the collection site and date. All calliphorid specimens were identified to the species level, according to taxonomical keys (Carvalho and Mello-Patiu 2008, Kosmann et al. 2013), and then stored in 70% ethanol at -20ºC in the Laboratory of Evolutionary Biology at the University of Brasília.

Community characterization

Species diversity was individually accessed in each of the four collection sites (gf1, gf2, cs1, and cs2) two times: once in the rainy and once in the dry season. For each sampling unit, the three months in each season were considered replicates. The calculations were based on the raw species occurrence data, with no transformation, for both Shannon-Weaver(H’) and Simpson (C) indexes.

Ensemble composition was compared between communities with Nonmetric Multidimensional Scaling (NMDS) in a two-dimensional representation of the ecological distances among ensembles. The NMDS was performed on a dissimilarity matrix and used the Bray-Curtis dissimilarity index with a maximum of 100 restarts. NMDS consist of an ordination method used in ecological studies. It does not assume any data distribution and is an iterative process, to determine the best position of a given number entities on as many dimensions the operator chooses, with the lowest stress of the n-dimensional configuration. A visual representation of the relative position of each species and each collection site was assembled, and the representations regarding the effects of seasonality and vegetation cover were estimated as the vector from the origin to the centroid of the group of collection sites under the influence of these factors (grassland/forest or dry/rainy).

Wing preparation and image acquisition,

For the morphometric characterization, we selected 539 specimens from two species: the invasive *Chrysomya albiceps* (n = 291) and the native *Chloroprocta idioidea* (n = 248). We removed both wings from each individual and placed them in dorsal view on a glass microscope slide topped with a glass coverslip. Mineral oil was used to fix the wings and nail polisher to fix the edges of the coverslip. Glass slide and slip were pressed together with a plastic press for 24 hours for the drying of the nail polisher.

The wings were placed in the center of the visual field of a Stereomicroscope (Leica, model MZ16) and photographed with a Canon T4i DSLR coupled to the microscope with an adaptor on the right ocular. The images of the left wings were horizontally flipped for the digitation of landmarks.

Morphometric characterization, morphological variation, and statistical analysis

We built a library consisting of 1078 wings using tpsUtil (Rohlf 2015), and digitized twelve type I landmarks in each wing using tpsDig software (Figure 1) (Rohlf 2015). All wings were classified according to its species, side, season and vegetation cover. As measurement error in image acquisition and landmark digitation are the primary concern in the preparation of morphometric data (Arnqvist and Martensson 1997), we conducted a repeatability test consisting in the random selection of ten wings and the repetition of the photographing and landmarks digitation on four different days, by the same operator. Then we used the date of image acquisition as a factor to test deviation from randomness in wing shape.

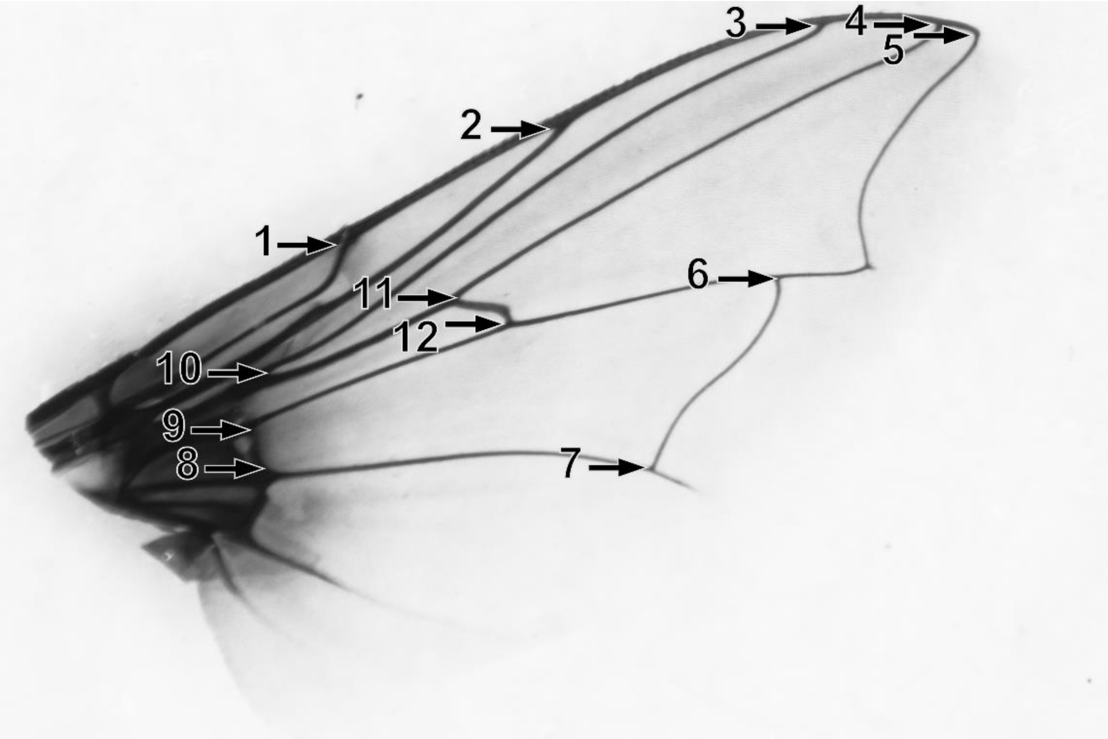

Figure 1. Dorsal view of a right wing of *Chrysomya albiceps* and the 12 digitized landmarks.

After image acquisition, we considered each specimen as the average of its right and left wings and then performed a Procrustes superimposition. This process relies on translation, scaling, and rotation of the configuration matrices to remove all non-shape information from the dataset and has two main outputs for each individual in MorphoJ environment (Klingenberg 2011): the Procrustes coordinates, which characterize wing shape, and centroid size, which is a proxy for wing size.

The Procrustes coordinates were used to project all individuals in the tangent morphospace – a theoretical representation of all forms an organism can have. Each individual is represented as a point in this space, and the centroid of a population (set of points) is the average shape that can be used as the representation of the population. Then, we calculated the Value of morphological variation (V_{mv}) for populations of *C. albiceps* and *C. idioidea* in each of the four collection sites individually, considering the seasonal effect (i.e., for each collection site we calculated a V_{mv} for the dry and another for the rainy season), in a total of eight units and 16 populations.

The V_{mv} was calculated as the mean value of the root of the squared distances from each individual point to its respective centroid, and represents the expected deviance from the average shape displayed by an individual shape. The higher the V_{mv} is, the more dispersed the individuals are in the morphospace representation, and, thus, higher is the morphological variation.

Spearman correlations tests between morphological variation values and Shannon-Weaver and Simpson diversity indexes for communities were performed to evaluate the association between morphological variation and community diversity. Chi-squared tests were used to test species preference between different vegetation cover.

The Procrustes superimposition was performed in MorphoJ software (Klingenberg 2011), Procrustes coordinates were exported from MorphoJ environment, and morphological variation calculations were performed in R (Core Team 2015). Correlation tests were performed in Hmisc stats package, and Chi-squared test was performed in R stats package (Core Team 2015).

Results

A total of 5506 calliphorids from nine species were collected, three from the invasive genus *Chrysomya* (*C. albiceps, C. megacephala,* and *C. putoria*). *Chloroprocta idioidea* dominated the assemblage (60.2%), followed by *C. albiceps* (31.5%). Those two species combined account for 91.7% of the specimens collected, while *Mesembrinella bicolor* Fabricius, 1805 was the rarest species, represented by only one specimen (Table 1).

Table 1. Species absolute abundances (and relative in parenthesis) of the nine necrophagous calliphorid species collected from June 2017 to February 2018. White white rows indicate the dry season and gray rows indicate the rainy season.

Site	*Chrysomya albiceps*		*Chrysomya megacephala*		*Chrysomya putoria*		*Hemilucilia semidiaphana*		*Hemilucilia segmentaria*		*Lucilia eximia*		*Chloroprocta idioidea*		*Cochliomyia macellaria*		*Mesembrinella bicolor*	
cs 1 dry	369	(82.6)	4	(0.9)	2	(0.4)	0	(0.0)	0	(0.0)	10	(2.2)	27	(6.0)	35	(7.8)	0	(0.0)
gf 1 dry	159	(51.6)	7	(2.3)	0	(0.0)	5	(1.6)	4	(1.3)	32	(10.4)	101	(32.8)	0	(0.0)	0	(0.0)
cs 2 dry	521	(91.1)	4	(0.7)	2	(0.3)	0	(0.0)	0	(0.0)	2	(0.3)	10	(1.7)	33	(5.8)	0	(0.0)
gf 2 dry	79	(56.0)	0	(0.0)	0	(0.0)	0	(0.0)	14	(9.9)	12	(8.5)	31	(22.0)	5	(3.5)	0	(0.0)
cs 1 rny	206	(45.3)	28	(6.2)	0	(0.0)	0	(0.0)	0	(0.0)	9	(2.0)	208	(45.7)	4	(0.9)	0	(0.0)
gf 1 rny	185	(11.2)	22	(1.3)	0	(0.0)	26	(1.6)	24	(1.5)	41	(2.5)	1342	(81.5)	7	(0.4)	0	(0.0)
cs 2 rny	124	(23.5)	15	(2.8)	0	(0.0)	0	(0.0)	6	(1.1)	23	(4.4)	356	(67.4)	4	(0.8)	0	(0.0)
gf 2 rny	94	(6.7)	18	(1.3)	1	(0.1)	15	(1.1)	21	(1.5)	13	(0.9)	1241	(88.1)	4	(0.3)	1	(0.1)

The temporal distribution of blowflies differed between seasons. The abundance was higher in the rainy season ($X^2 = 1198.6$, d.f. = 1, $p < .001$), with 4038 specimens (73.3%) collected from December to February. This result, however, is due to flies collected in forests (gf 1 – $\chi^2 = 915.73$, d.f. = 1, $p < .001$; and gf 2 – $\chi^2 = 1034.7$, d.f. = 1, $p < .001$) and not in grasslands (cs 1 – $\chi^2 = 0.05$, d.f. = 1, $p = 0.81$; and cs 2 – $\chi^2 = 1.68$, d.f. = 1, $p = 0.19$). From the nine species, only three are more abundant in the dry season: the invasives *C. albiceps* and *C. putoria*, and the native *Cochliomyia macellaria* (Fabricius, 1775). All native species, except for *C. macellaria* (higher abundance in the dry season) and *M. bicolor* (low sampling number), are more abundant during the rainy season (Figure 2).

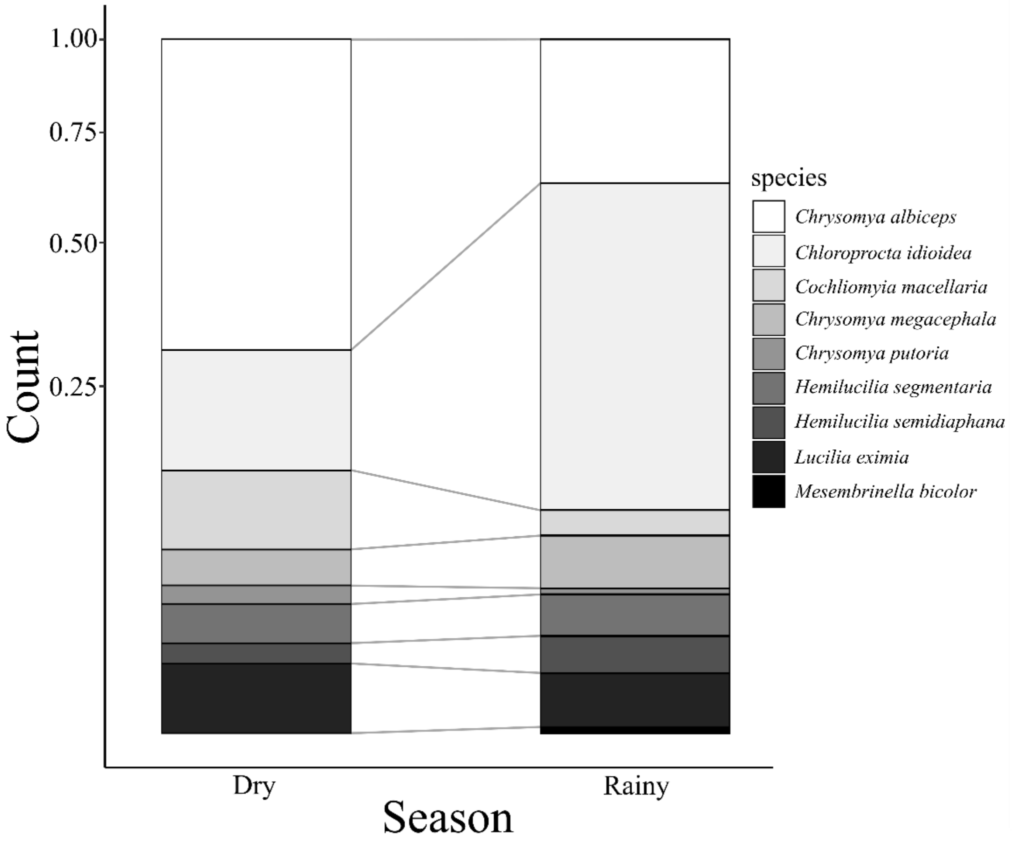

Figure 2. Seasonal variation of the ensemble composition of necrophagous calliphorids in the RECOR environmental protectetion area.

Abundance in forest was higher than in grassland (*campo sujo*) (χ^2 = 409.19, d.f. = 1, p-value < .001) but that difference is significantly influenced by the populational increase of *C. idioidea* populations from grasslands to gallery forests and from dry to rainy season (Table 1Community diversity indexes, both Shannon-Weaver (H’) and Simpson (C), showed higher diversity in forest sites, but results were not significant. Seasonality acted in a contrasting pattern: in grasslands, we noted a rise in diversity indexes from the dry to the rainy season, while in the forests, we noted a fall in these indexes in the same period (Table 2). There was no correlation at all between morphological variation and community diversity for both species, considering both Shannon-Weaver (H`) and Simpson (C) indexes (Table 3).

Table 2. Shannon-Weaver (H’) and Simpson (C) diversity index and morphological variation values for *Chrysomya albiceps* ($V_{mv\ CALB}$) and *Chloroprocta idioidea* ($V_{mv\ CIDI}$).

Site	**H’**	**C**	$\mathbf{V_{mv\ CALB}}$ ($x10^{-2}$)	$\mathbf{V_{mv\ CIDI}}$ ($x10^{-2}$)
cs 1 dry	0,679	0,308	1,965	1,915
cs 1 rny	1,007	0,582	1,901	2,066
gf 1 dry	1,152	0,614	2,257	1,976
gf 1 rny	0,712	0,322	2,013	2,113
cs 2 dry	0,395	0,167	2,069	1,889
cs 2 rny	0,932	0,487	1,890	2,093
gf 2 dry	1,215	0,619	2,068	2,005
gf 2 rny	0,529	0,218	1,908	2,101

Table 3. Pairwise Spearman correlation index from Shannon-Weaver (H') and Simpson (C) diversity index and morphological diversity values for *Chrysomya albiceps* ($V_{mv\ CALB}$) and *Chloroprocta idioidea* ($V_{mv\ CIDI}$). Correlation indexes are below diagonal, to the left, and p-values are above the diagonal, to the right.

	H'	C	$V_{md\ CALB}$	$V_{md\ CIDI}$
H'	-	0	0,82	0,82
C	1	-	0,82	0,82
$V_{md\ CALB}$	0,10	0,10	-	0,18
$V_{md\ CIDI}$	0,10	0,10	-0,52	-

There was no consistent pattern in species preference among vegetation cover. Nevertheless, the native species *Hemilucilia semidiaphana* (Rondani, 1850), *Hemilucilia segmentaria* (Fabricius, 1805) and *Lucilia eximia* (Weideman, 1819) tend to prefer forest formation. On the other hand, the native *C. macellaria* and the exotic, *C. albiceps* showed preference for grasslands. *C.megacephala*, also an exotic species, was present in equal proportion among grassland and forest. The exotic *C. putoria* and the native *M. bicolor* were collected in numbers that did not yield significative results (Table 4).

Table 4. Habitat preference of the collected blowfly species, their respective Chi-squared and p-values (in all cases degrees of freedom = 1).

	n	**Grass.**	**Glry. Fst.**	X^2	**p**
Chrysomya albiceps	(1737)	X		283.7	< .001
Chrysomya megacephala	(98)	Ø	Ø		
Chrysomya putoria	(5)	Ø	Ø		
Hemilucilia semidiaphana	(46)		X	44.02	< .001
Hemilucilia segmentaria	(69)		X	45.55	< .001
Lucilia eximia	(142)		X	37.84	< .001
Chloroprocta idioidea	(3316)		X	1346.4	< .001
Cochliomyia macellaria	(92)	X		37.83	< .001
Mesembrinella bicolor	(1)	Ø	Ø		

X – preferred habitat
Ø – no habitat preference

NMDS ordination across the eight units performed for two dimensions returned a stress value of 0.038. Differences in species composition due to seasonality and vegetation cover was noticed (Figure 3). The influence of rainy season and the influence of forest are similar on the first dimension, but opposite in the second. The grouping formed by the invasive species, the *Chysomya* genus, was centered nearly ate the same point that represented the influence of grassland, while native species behaves oppositely, except for *C. macellaria* (Figure 3).

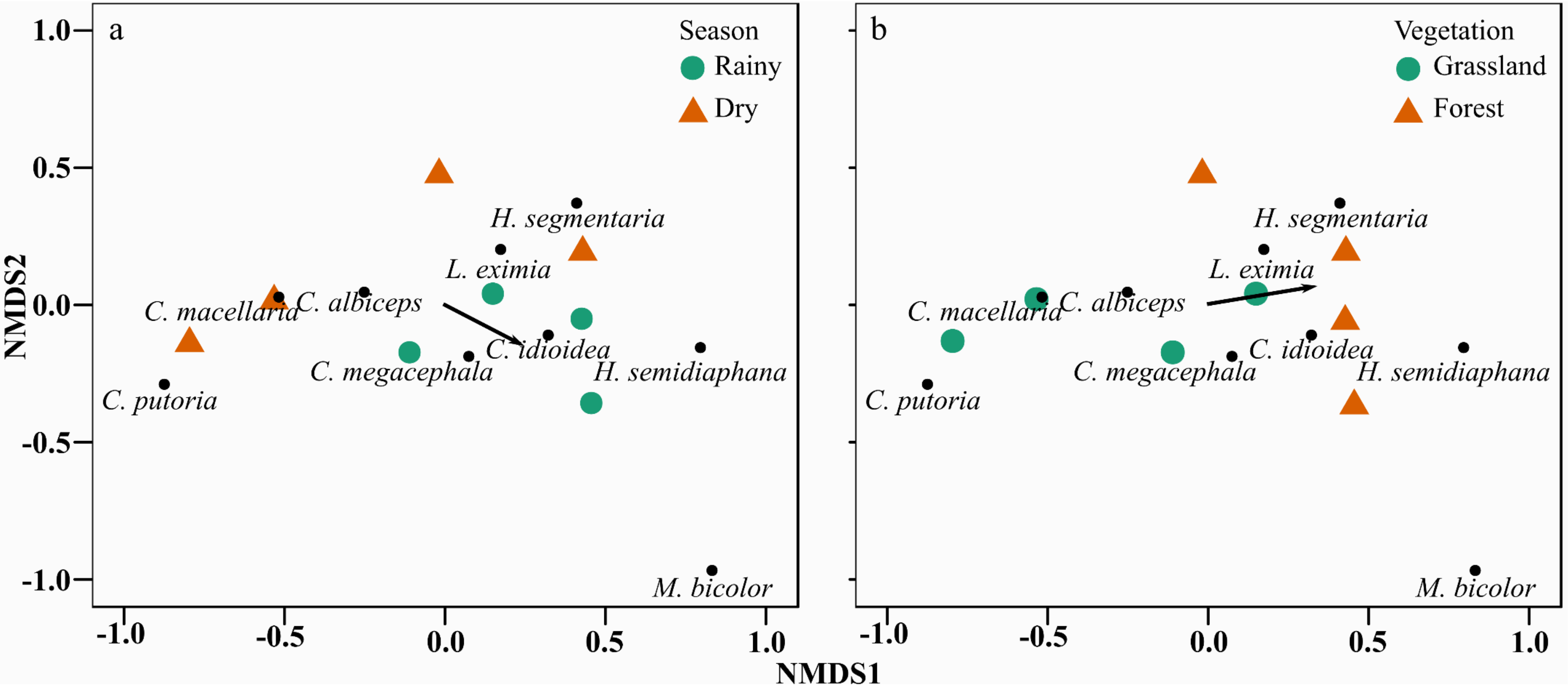

Figure 3. NMDS plots of the study sites and species distribution on the ordinated dimensions. Effects of seasonality (a) and vegetation cover (b) are indicated by a vector from the origin to the centroid of the selected group.

Discussion

Our study recorded nine species of necrophagous calliphorids in the Ecological Reserve of IBGE. The native *Chloroprocta idioidea* and the invasive *Chrysomya albiceps* dominated the ensemble, and summed they represent more than 90% of species abundance. On the other hand, the abundance of other two species was lower than 0.1%, the exotic *Chrysomya putoria* (0.09%) and the native *Mesembrinela bicolor* (0.02%). Yet, these have been associated with animal carcasses in previous studies; for that reason, we are convinced that they fit the resource use criteria for an ensemble (Fauth et al. 1996).

Abundance is influenced by season (rainy season accounts for nearly three-quarters of the specimens) and by vegetation cover (rainy season accounts for nearly two-thirds of the collected specimens). Ensemble dominance also shifts from dry to rainy season and from grassland to forestall vegetation. The invasive *C. albiceps* dominates the ensemble in the dry season and in grasslands sites while *C. idioidea* dominates in the rainy season and gallery forests. Seasonality is almost ubiquitous in insects to the point that a species might be considered rare in one season and abundant in another (Wolda 1988), and the calliphorids seem to be no exception to that. Consistently, similar results were observed in Rio de Janeiro, where *C. megacephala* dominate the necrophagous Calliphoridae ensemble in the summer but is almost absent in the winter (Barbosa et al. 2010). Many studies point to communities of calliphorid flies dominated by species from the *Chrysomya* genus, although it was introduced in the Neotropics only a few decades ago. The contrasting results (*C. idioidea* dominance) might be explained by low urbanization of the study area, as *C. albiceps* and *C. megacephala* display high synanthropism.

Community diversity indexes did not diverge between seasons nor vegetation cover, neither for Shannon-Weaver and for Simpson indexes (Table 3), implying that ensemble diversity did not vary according to environmental factors. In fact, we noted that community diversity indexes vary according to the interaction of seasonality and vegetation cover effects. In grasslands, the communities got more diverse from the dry to the rainy season, while in forests communities got less diverse in the same period. This observation might be explained by *C. idioidea* the dramatically increased its abundancy in forests during the rainy season (table 1).

No correlation was found between ensemble diversity and morphological variation within *C. albiceps* and *C. idioidea* populations. Therefore, we conclude that, for the necrophagous Calliphoridae, those levels of biological diversity are not connected. Nevertheless, the relationship between levels of biodiversity, as well as between them and ecological features such as stability or productivity, are multifactorial (Tilman 1996, Craven et al. 2018). Our results showed that specific experimental designs should be considered to access interconnections among diversity in multiple levels of biological organizations in calliphorids.

The NMDS plots revealed segregation between grasslands and gallery forests as well as between dry and rainy seasons. As discussed above, native species dominate both forest sites and the rainy season. A key component to both factors is an elevation in humidity. In that sense, our observations coincide with previous findings for Calliphoridae in south Brazil (Azevedo & Krüger, 2013) but contradict other studies in Rio de Janeiro (Marinho et al., 2006) and Thailand (Sontigun et al., 2018). Nevertheless, humidity related aspects seem to play an essential role in community structures for calliphorids, but its mechanisms remain unclear.

Cochliomyia macellaria is an exception to the prevalence of native species in forests. It was collected prevalently in grasslands and during the dry season, overlapping with the invasives *C. albiceps*, *C. megacephala* and *C. putoria*. This finding is in accordance with previous reports of ecological displacement of the native species by the invasive *C. megachephala*. Albeit we have found no reports on *C. albiceps* influence on *C. macellaria* populations, we assume that its high reproductive potential and voracious feeding habits of immature forms constitutes a threat to the populations of that native species (Faria et al. 1999).

Hemilucilia semidiaphana was collected exclusively in forests, whereas most of *H. segmentaria* specimens were collected in this environment (note that nearly 10% of the samples were collected in grassland habitat). Both species are considered forensic indicators of rural areas (Oliveira-Costa 2011), and *H. semidiaphana* is an indicator of forest environment. Even though we note a widespread belief that *Hemilucilia* species are indicators of forest habitats among forensic entomologists, we have previously described *H. segmentaria* larvae in human remains in an urbanized grassland formation in the Cerrado (Kosmann et al. 2011). That observation, combined with the present results, advise for careful consideration when drawing conclusions in forensic practice for *H. segmentaria*, as well as the need of more strong validation to establish the usefulness of *H. semidiaphana* as a species characteristic from forest areas.

It is worth to notice that we did not collect any specimen from *Lucillia cuprina* (Weidemann, 1830), even though we noticed adults in the vicinities of the experiment. In the literature, *L. cuprina* is sometimes present in surveys of necrophagous insects communities (Centeno et al. 2004, Vianna et al. 2004, Ferraz et al. 2009, Gonçalves et al. 2011, Alvarez Garcia et al. 2019). That inconstancy may be due to the lack of standardization of practice, especially regarding the use of baits, which hinder conclusions. In our study, even though we did use entire piglets, not stillborn (i.e., with intestinal microbiota), they did not attract this species to the traps. A previous study on the Cerrado did collect *L. cuprina* in pig carcass (Biavati et al. 2010), and it considers it as an important forensic species, based on previous literature. Nevertheless, they collected only four specimens out of 14,910 calliphorids. Thus, we recommend caution when considering this species as a necrophagous indicator in the Cerrado (e.g., forensic indicator).

Also, we collected just one *M. bicolor* specimen out of 5506 calliphorids raising questions whether to recommend treating this species as a forensic indicator or not. Furthermore, we are aware of sufficient evidence to acknowledge family status to the Mesembrinellidae group, apart from Calliphoridae (Wolff and Kosmann 2016, Marinho et al. 2017). Nevertheless, we opted to present our results in that way because of the significant niche overlap between *M. bicolor* and the necrophagous calliphorids as well as the traditional approach of forensic entomologists to treat M. bicolor as a forensic indicator (Soares and Vasconcelos 2016).

In conclusion, necrophagous calliphorids in the Cerrado display seasonal fluctuations in abundance and ensemble composition. The invasive *C. albiceps* dominates the ensemble in the dry season, and *C. idioidea* in the rainy season. These species also display habitat preferences; while *C. idioidea* occupy forest formations preferentially, *C. albiceps* prefers grasslands. Morphological variation is not linked to ensemble diversity. Although most studies on Calliphoridae species focus on practical aspects of forensic or medical and veterinary aspects and bionomy of these species (for obvious reasons), we consider that ecological and evolutionary aspects of this family need to be taken into account in further studies.

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Morphological shift in wing structures between seasons in an invasive versus a native calliphorid (Insecta, Diptera) species

Abstract

Seasonality plays an important role in community structure in the Brazilian Cerrado, but few studies have been published regarding its effects on blowflies (Diptera: Calliphoridae) communities. These flies are frequently used as indicators in forensic practice, and their size is a trait commonly used by forensic entomologists. Nevertheless, the effects of seasonality on morphological traits of calliphorids remain unexplored in neotropical savannas, as the Cerrado. In this study, we investigated if wing morphology and levels of fluctuating asymmetry of two calliphorid species, the exotic *Chrysomya albiceps* (Weideman, 1819) and the neotropical *Chloroprocta idioidea* Wulp, 1896, respond to temporal and spatial variation in vegetation type. We found that wing size was affected by temporal heterogeneity in both species but in contrasting patterns. While *C. idioidea* increased wing size from rainy season to dry season, *C. albiceps* displayed size reduction. This pattern leads to equal wing size in the dry season and pronounced differences in the rainy season. Wing shape was affected only in *C. albiceps* populations. No difference in fluctuating asymmetry was detected neither for temporal nor for spatial heterogeneity. The results show that natural populations of these species do not respond in the same way to temporal heterogeneity and specific studies for both of them are needed to access variation patterns of morphological change.

Introduction

Seasonality is a fundamental aspect in a wide range of ecological interactions. It regulates how species reproduce, migrate, and diapause; thus, influencing community assemblage through the ways that species deal with the repetitive temporal heterogeneity. Insects respond to seasonality in many ways: lepidopterans display polyphenism throughout summer and winter forms, some hemipterans shift reproductive behavior form vivipara in summer to oviparae in autumn, and calliphorids may display larval diapause due to short day length (Saunders 2002).

In neotropical savannas, drosophilid community responds to seasonality with exotic species dominance in one season and neotropical species dominance in the other season (Roque et al. 2017). Drosophilid larval assemblages also respond to seasonality, with abundance peaks in the rainy season and strong bottlenecks in the dry season, in response to fluctuation in resource (fruits) availability (Mata et al., 2015). Calliphorids are also known for responding to seasonality in the neotropics through a variety of mechanisms. The secondary screwworm *Cochliomyia macellaria* (Fabricius, 1775) populations display a bimodal abundance variation in the Brazilian Pantanal (Koller et al. 2012). In the Rio de Janeiro State, populational parameters in calliphorid communities respond to seasonality in well-preserved areas (Marinho et al. 2006), and urban areas (Pires et al. 2008, Barbosa et al. 2010). Also, the abundance and richness of calliphorid communities in the southern Brazilian grasslands fluctuate according to the seasons (Azevedo and Krüger 2013) but not in the Amazon rainforest (Ururahy-Rodrigues et al. 2013).

Tropical savannas cover a large portion of South America, and the Cerrado is the largest savanna region in the Neotropics. This biome covers nearly a quarter of

Brazilian territory and extends marginally into Paraguay and Bolivia. It is a patchy landscape composed by a mosaic of forests, woodlands and grassland habitats. Interfluvial zones are well-drained areas interleaved with moist vegetal formation, generally gallery forests, that follows the watercourses (Oliveira and Marquis 2002). Biodiversity of the Cerrado is amplified by vast topographic and edaphic diversity and by a range of fire frequencies (Furley 1999). Its climate is markedly seasonal with a distinct dry season in the winter (from May to October) and a rainy season in the summer (from November to April). This temporal and spatial heterogeneity leads to idiosyncratic and complex ecological relations (Oliveira and Marquis 2002). As a consequence of that environmental diversity, the Cerrado displays high endemism and, thus is considered a hotspot of biodiversity but only nearly 20% of its original area remaining (Myers et al. 2000). This scenery brings forth ecological features that are unique to that biome, begetting the necessity of specific studies to understand ecological processes in the Cerrado.

Blowflies (Diptera: Calliphoridae) are a well-spread group, present in all biogeographic regions. Currently including more than 1,000 species, calliphorids are well known for its synanthropy and feeding and breeding behavior associated with decomposing organic matter. Pathogen vectors and myiasis causers, the blowflies raises medical-veterinary concern (Baumgartner and Greenberg 1984, Kosmann et al. 2013).

The genus *Chrysomya* was first introduced in the Neotropical region in the mid-1970s, in southern Brazil, coming from the southwestern coast of Africa along waves of refugees from Angola. *Chrysomya* species are flies of medical-veterinary importance (causing myiasis and acting as pathogens vectors) and also have displaced the endemic calliphorids *Lucillia eximia* (Wiedemann, 1819) and *Cochliomyia* *macellaria* (Prado and Guimarães 1982). By the early 1980s, those flies had spread throughout the neotropics reaching the Neartic region, in the south of the USA, and strengthening the hypothesis of multiple introductions (propagule pressure) (Gagné 1981).

Chloroprocta Wulp, 1896, is a monotypic genus of the Chrysomyinae subfamily. Its unique species is *Chloroprocta idioidea* (Robineau-Desvoidy, 1830), it is a Neotropical species present from the Mexican territory to Argentina.

Studies on the traits that made *Chrysomya* invasion successful in the New World are still scarce, and while a combination of high biotic potential (Barbosa et al. 2016) and dispersal capability (Baumgartner and Greenberg 1984) seems to be pivotal for that process, none of these studies focused the invasion of the Cerrado nor other Neotropical savannas. Likewise, studies on the population dynamics of *Chrysomya* in face of seasonality are rare and usually focused on physiological aspects (Reigada and Godoy 2005). These studies investigated the relation of *Chrysomya* species just with *C. macellaria*, and they were performed in the laboratory (Aguiar-Coelho and Milward-de-Azevedo 1995, Faria et al. 1999, Reis et al. 1999) or based on experimental manipulation of the necrophagous fly community.

Flight capability is an important evolutionary novelty. It has appeared around 400 million years ago and allowed insects to sophisticate their performance, leading to great diversification of that taxon (Engel et al. 2013). A complex set of structures mediates flight directly and indirectly (Dickinson and Tu 1997), imposing constraints to drastic alterations in isolate structures, such as wings. Thus, even the very subtle changes in wing morphology have significant biological meaning and can be linked to genetic differences among populations (Haas and Tolley 1998, Laparie et al. 2016).

This feature makes wing morphology a key aspect in evolutionary and ecological studies, and the recent development of geometric morphometrics and multivariate statistics paved the way for modern studies in ecology and evolution based on morphology(Adams et al. 2013b, Klingenberg 2016)

Organisms with bilateral symmetry rely on the same genotype to symmetrically express both sides of their structural features (e.g., the left and right wings of the same individual). Thus, it is expected that those structures will be specular images of one another. Nevertheless, total symmetry is extremely rare in real life, and the majority of animal populations display some level of asymmetry. Directional asymmetry is characterized when some trait is wider in one side than in the other (e.g., heart structure in mammals, and antisymmetry, in case of the trait is bimodally distributed in the population (e.g., sexual dimorphism). These deviations from symmetry have been widely discussed in the literature, and result from directional or disruptive selection modes (Valen 1962, Klingenberg et al. 2002).

On the other hand, deviations from symmetry may occur randomly due to the organism incapability to buffer environmental stress. That fluctuating asymmetry is the result of developmental instability and not of selective pressure (Klingenberg 2003, 2015b). Several studies associate fluctuating asymmetry with stressful situations, thus, it has been used as a proxy to environmental stress (Parsons 1992, Hoffmann et al. 2005, Beasley et al. 2013, Lajus et al. 2015, Michaelsen et al. 2015, Pertoldi and Kristensen 2015, Tabugo et al. 2016). Nevertheless, there is an intense debate regarding the usefulness of that type of asymmetry and stressful situations during organism development, because many studies did not find a link between stress and fluctuating asymmetry (Arambourou et al. 2012, 2014, 2015).

This study investigated if wing morphology (size and shape) and levels of fluctuating asymmetry of two calliphorid species, the exotic *Chrysomya albiceps* (Weideman, 1819) and the native *Chloroprocta idioidea*, respond to temporal (dry vs. rainy season) and spatial variation vegetation type (savanna vs. forest) in a protected area located in the Brazilian Cerrado.

Material and Methods

Study sites and fly populations

The flies were collected in the Ecological Reserve of IBGE (Instituto Brasileiro de Geografia e Estatística) (RECOR), from June 2017 to February 2018. This Reserve, located 35km south of Brasilia (15º 56' S; 47º 53' W), is part of an environmental protection area covering 10,000 ha and situated in the middle of the Brazilian Cerrado. Two areas of collection were selected, distant two kilometers of each other (area 1 and area 2). In each area, two collection sites (200 meters apart) were established: one in a gallery forest (gf 1 and gf 2) and the other in the *campo sujo*, a savanna vegetation with few shrubs and small trees scattered across a grassland field (cs 1 and cs 2) that, from now on, we will designate it just as grassland.

In each of the four collection sites, a Shannon trap (a cubic tent with 1.8m side) was assembled and, as bait, a pig carcass (*Sus scrofa domesticus*) of 2 kg was exposed inside a metal cage to prevent access of necrophagous mammals to the carcass. Each Shannon trap was placed in the same site three times (triplicates) in different months of the season (dry: June, July, August; rainy: December, January, February), encompassing a total of 24 traps. In each replicate, we actively collected adult flies with an entomological net three times (pseudoreplicates) in different days (day 1, day 3, and day

5, after the carcass exposition). All *Chloprocta idioidea* and *Chrysomya albiceps* specimens were identified with the use of taxonomical keys (Mello 2003, Carvalho and Mello-Patiu 2008, Kosmann et al. 2013) and stored in 70% ethanol at -20º C in the Laboratory of Evolutionary Biology at the University of Brasília.

Wings preparation

Both wings from all the female flies were cut and placed over a glass microscope slide topped with a glass coverslip. The wings were placed on dorsal view fixed on mineral oil and the edges of the slip were sealed with nail polisher. Glass slide and slip were pressed together with a plastic press for 24 hours for the drying of the nail polisher.

The slides were placed in a Leica stereomicroscope, and the images were obtained with a Canon T4i DSLR camera coupled with a DSLR camera adapter for microscopes placed on the right ocular. All wings were centered at the same spot at the visual field to avoid lens distortion, and images were captured remotely with Cannon remote shooting software. Images of the left wings were horizontally flipped at the landmark positioning process, with tpsDig software (Rohlf 2015).

Morphometric and statistical analysis

A total of 1078 wings were analyzed, 582 from *C. albiceps* and 496 from *C. idioidea*. Each wing was classified according to its species, side, vegetation cover, and season of collection (table 1). A library with all wings was built using tpsUtil (Rohlf 2015). We digitized 12 type I landmarks in each of the wing using tpsDig software (Rohlf 2015) (Figure 1).

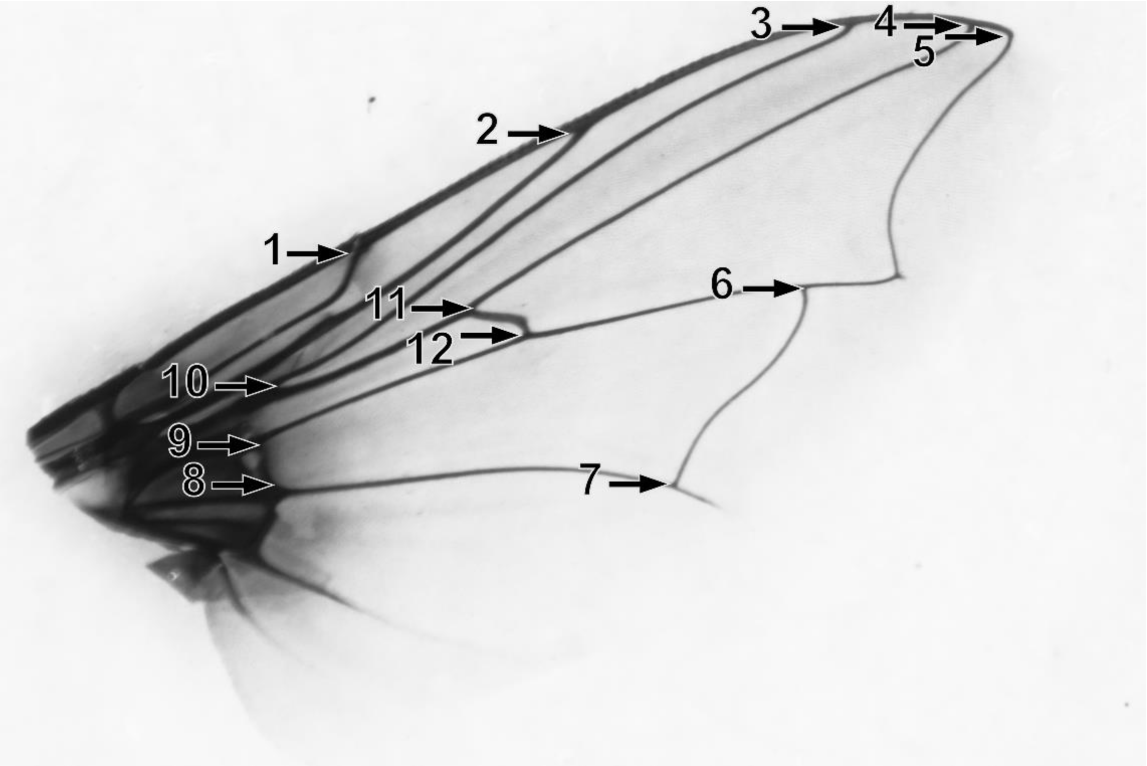

Figure 1. Dorsal view of a *Chrysomya albiceps* wing with the 12 landmarks digitized as used in this study.

To estimate measurement error caused by image acquisition or by the digitation of landmarks, we conducted a repeatability test. It consists in randomly selecting ten wings to go through the complete photograph process and the digitation of landmarks, four different times at separate days, by the same operator. We then applied a Procrustes ANOVA using the landmark positioning data as a factor to test for deviations from randomness.

Morphometric analysis consisted in Procrustes superimposition, a method relying on operations of translation, scaling, and rotation of the configuration matrixes to remove non-shape information from the dataset. This process removes characteristics of orientation, size, and position from the digitized images (Rohlf and Slice 1990, Zelditch et al. 2004). Each individual was computed as a mean configuration matrix of their left and right wing, except for fluctuating asymmetry tests, as mentioned further.

We performed allometry tests to evaluate the influence of size on shape. Allometry tests consisted in multivariate regression with the configuration matrix (i.e., the Procrustes coordinates) as dependent variables to centroid size (as a proxy to wing size) with 10,000 randomization rounds, in permutation tests against the null hypothesis of independence. Tests were conducted in two individual datasets grouped by species because size and shape differences among species are expected.

Fluctuating asymmetry was obtained as the variation within sides of the same individual (individual-by-side interaction). It is taken as a scalar-valued trait associated with those side differences in a Procrustes ANOVA (Klingenberg 2015a).

To estimate the relative amount of variation in wing shape due to seasonality and to vegetation cover, we did rely on Procrustes ANOVA tests. To access those effects, we tested the influence of vegetation cover and seasonality on the Procrustes coordinates and the centroid size of the specimens. Then, to visualize the variation in cases where significative effect was detected, we opted for a canonical variate analysis (CVA) to enhance discriminatory powers of the tested effect (i.e., vegetation cover or seasonality).

All morphometric analyses were performed in MorphoJ Software (Klingenberg 2011) with statistical corrections for non-parametric data (Pillai 1961) and factorial ANOVA calculations were performed in the R stats package. Graphic material was produced in MorphoJ and R, ggplot2 package (Wickham 2016), environment and refined in Inkscape graphic software.

Results

Repeatability tests showed that photographing and digitation of landmarks on the same subject on separate days did not significantly change the Procrustes coordinates ($F = 0.02$, $p = 1.00$). Shape prediction by allometric analysis was 2.61% ($p < 0.001$) for *C. albiceps* and 6.46% for *C. idioidea* ($p < 0.0001$). These results demonstrate that differences among individuals are not due to mispositioning of landmarks and that the influence of size on wing shape is significant but mild.

For *C. albiceps*, wing size was influenced by seasonality ($F = 56.81$, d.f. = 1, $p < 0.001$) (figure 2) but not by vegetation cover ($F = 1.97$, d.f. = 1, $p = 0.16$) and wing shape was also influenced by seasonality (Pillai tr = 0.46, d.f. = 20, $p < 0.001$) and by vegetation cover (Pillai tr = 0.15, d.f. = 20, $p = 0.001$ – but see discussion below) (figure 3).

For *C. idioidea*, wing size was influenced by seasonality ($F = 16.7$, d.f. = 1, $p < 0.001$) (figure 2) but not by vegetation cover ($F = 1.84$, d.f. = 1, $p = 0.18$) (table 1) and wing shape was not influenced by seasonality (Pillai tr. = 0.11, d.f. = 20, $p = 0.12$) neither by vegetation cover (Pillai tr. = 0.10, d.f. = 20, $p = 0.24$) (figure 3).

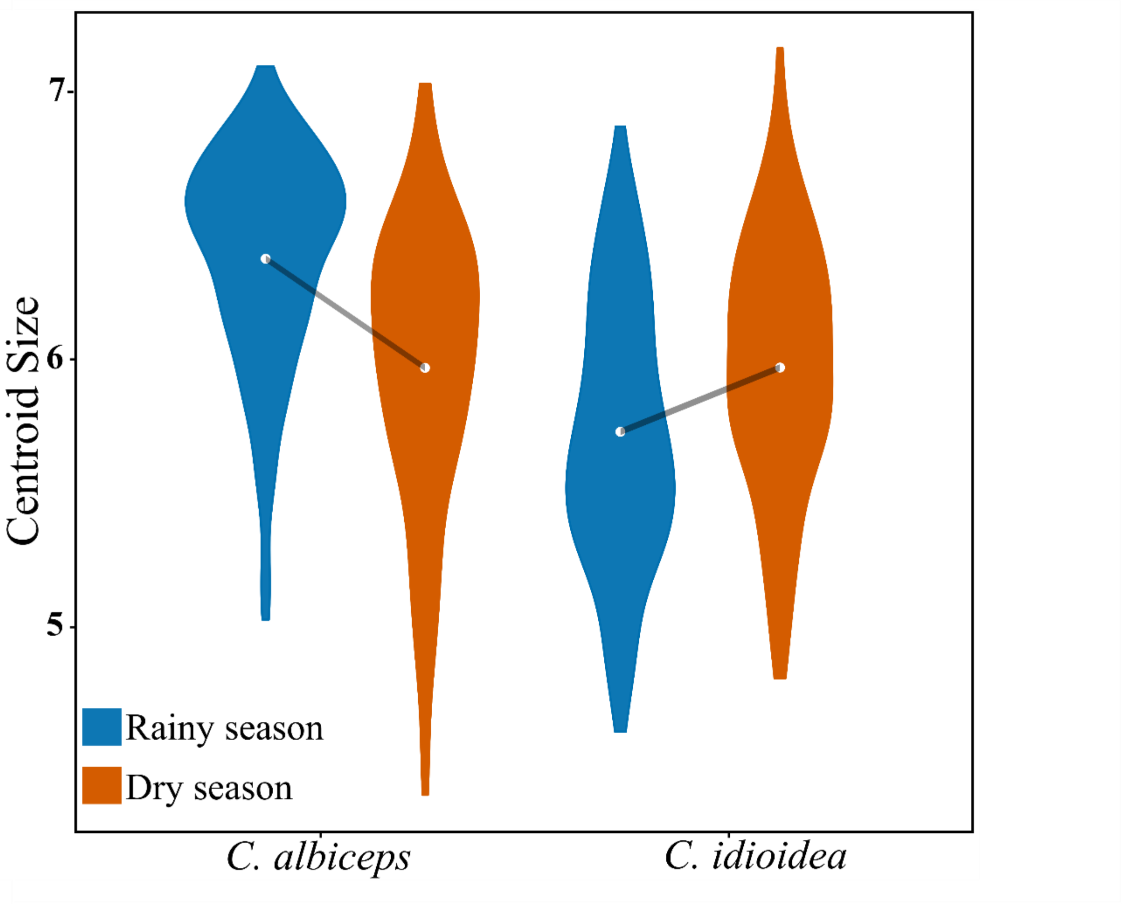

Figure 2. Wing size of *Chrysomya albiceps* (left) and *Chloroprocta idioidea* (right) captured in rainy (blue) and dry (orange) seasons. Violin plots show the probability densities for wing size in the population in each season. White dots show mean values. Gray lines show shrinkage or increase in populational values.

Chrysomya albiceps and *C. idioidea* differ in size in the overall dataset (F = 73.2, d.f. = 1, p <0.001). This difference is heightened in the rainy season, when *C. albiceps* is bigger than *C. idioidea* (F = 165.1, d.f. = 1, p <0.001), but is not detected in the dry season, when the species have no size difference at all (F = 0.008, d.f. = 1, p = 0.927). This disparity shows that, regarding wing size, *C. albiceps* and *C. idioidea* reacts in contrasting ways to the same set of environmental conditions (figure 2, table 1).

Table 1. Sample size (N), mean values of fluctuating asymmetry levels (FA), centroid size (CS) and standardized around the mean centroid size (Std CS) of *Chrysomya albiceps* and *Chloroprocta idioidea* collected in different seasons (rainy and dry) and vegetation cover (savannic grassland and gallery forest).

Species	Season	Vegetation Cover	(N)	FA	CS	Std CS
C. albiceps	rainy	grassland	(75)	0.0092	6.43	0.24
		forest	(74)	0.0097	6.34	0.15
	dry	grassland	(80)	0.0105	5.86	-0.33
		forest	(62)	0. 0093	6.12	-0.07
C. idioidea	rainy	grassland	(74)	0. 0091	5.68	-0.16
		forest	(76)	0. 0087	5.76	-0.08
	dry	grassland	(31)	0. 0093	5.90	0.06
		forest	(67)	0. 0082	6.01	0.17

Canonical Variate Analysis for *Chrysomya albiceps* revealed that the first CV contains 80.8% of the shape variance, and the second CV contains another 12.7%. Those shape changes were perceived as an elongation of the anterior-posterior axis during the rainy season, with no change in the proximal-distal axis (figure 3). For *C. idioidea*, the first CV contains 42.3% of the shape variance, and the second CV contains another 32.4% (figure 3). Confidence ellipses show displacement of the *C. albiceps* population from the rainy to the dry season in the first canonical variate axis while *C. idioidea* populations remain fairly unmoved in the graphical representation in both axis representing CV 1 and 2 (figure 3).

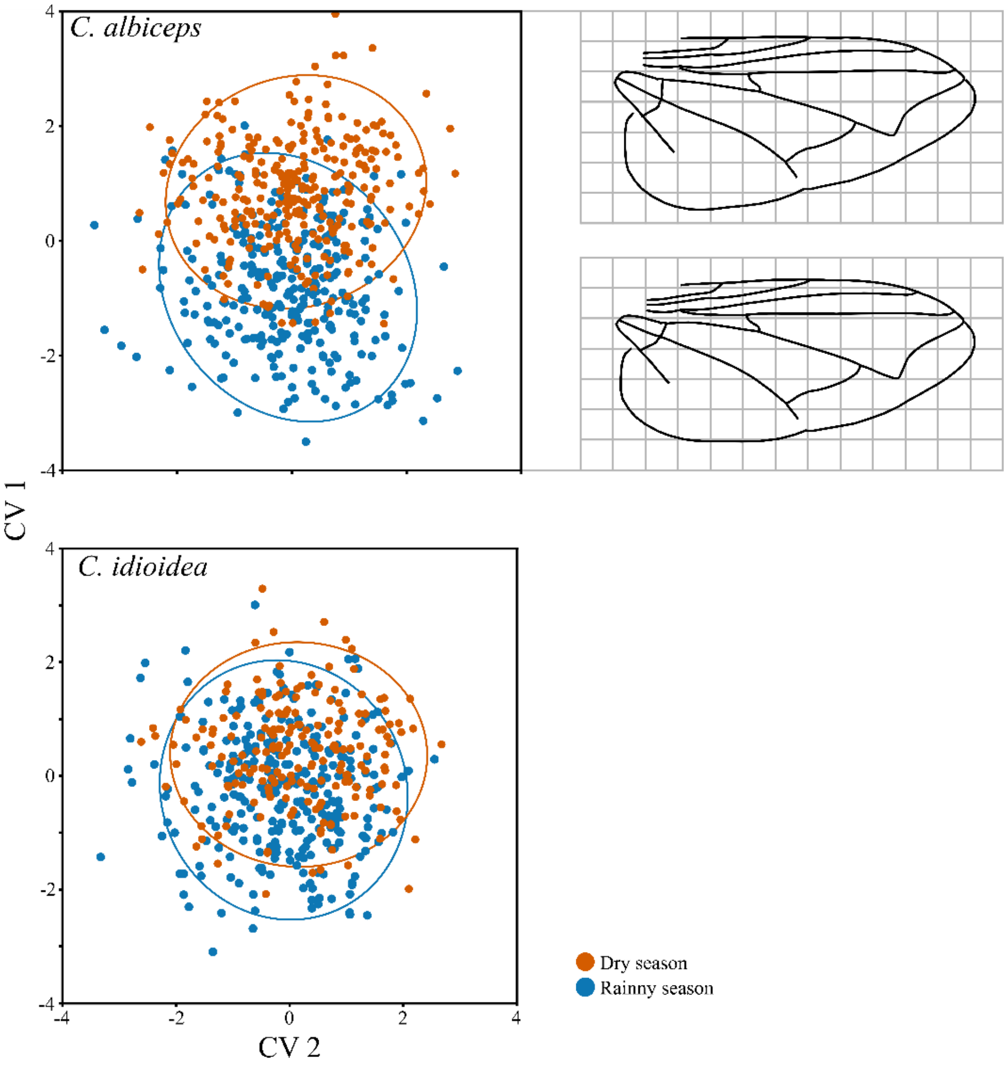

Figure 3. Scatterplot of the first canonical variates (CV1 and CV2) of *Chrysomya albiceps* (upper left) and *Chloroprocta idioidea* (lower left) specimens in the rainy season (blue) and dry season (orange). In the upper-right there is a visual representation of shape change detected on CV2 for *C. albiceps* specimens.

Fluctuating asymmetry was not influenced by seasonality for *C. albiceps* (F = 2.26, d.f. = 1, p = 0.13) neither for *C. idioidea* (F = 0.75, d.f. = 1, p = 0.39). Also, fluctuating asymmetry was not influenced by vegetation cover for *C. albiceps* (F = 2.57, d.f. = 1, p = 0.11) neither for *C. idioidea* (F = 0.86, d.f. = 1, p = 0.35).

Discussion

General findings

In this study of two blowfly species, we observed contrasting responses to seasonality between the native *Chloroprocta idioidea* and the invasive *Chrysomya albiceps*. Wing size was affected by seasonality in both species, but while *C. idioidea* increases its wing size from rainy season to dry season, *C. albiceps* reacts in the opposite direction, decreasing its wing size in the dry season. Calliphorids are known to reduce size due to high temperature, food scarcity and increased density (Charabidze et al. 2011, Macedo et al. 2017), in fact, this is a general trend among all insect groups. Previous studies found stable trajectories, with no significant difference between seasons in wing size for *C. albiceps* (Riback and Godoy 2008) and *Chrysomya megacephala* (Reigada and Godoy 2005) in the Cerrado. This is the first record of a factor (seasonality) resulting in contrasting responses in size. Interestingly, the contrasting trends between *C. albiceps* and *C. idioidea* size in response to seasonality result in significant size differences between species in the rainy season but no difference at all in the dry season.

On the other hand, seasonality effects on wing shape were limited to *C. albiceps*. The shift from rainy to dry season induced elongation of the anterior-posterior axis, with no change in the proximal-distal axis, leading to a more rounded (close to half an ellipse) shape. That shape change has been reported for drosophilids, and it is possibly associated with colonization of patches in a source-sink meta-population dynamics (personal communication), characterized by population decline with local extinctions in the dry season and expansion and recolonization in the rainy season (Dias 1996). Furthermore, source-sink dynamics in patchy landscapes regulates populations of the tsetse fly (Cecilia et al. 2018), enables coexistence between competing species

(Amarasekare and Nisbet 2015) and allows invasive species to persist in new territories (Dauphinais et al. 2018).

Despite significant statistical values for *C. albiceps* shape differences between vegetation covers, we prefer the conservative approach, that is to analyze these values comparatively. We are convinced that our sample size and the multivariate statistics framework of morphometric analysis indicate extremely subtle differences in wing shape. In that sense, Procrustes ANOVA values show that the preponderant factor for shape changes was temporal rather than spatial heterogeneity.

Is the morphological change of Chrysomya albiceps adaptive?

Our results show us that *C. albiceps* change its wing shape and size from the rainy to the dry season. Visualization of shape changes (figure 3, both in the scatterplot and the grid that represents the shape changes) reveal a seemingly subtle change in wing shape. Nevertheless, insect flight is of great importance throughout the insect Order (Nicholson et al. 2014), and the adaptive value of the wing is integrated with several other structures and mechanisms in the thorax, constraining the variation on these structures, preventing abrupt changes in wing structure (Deora et al. 2017).

In insects, wing shape influences flight capacities and can relate to behavioral and competitive features of a population, affecting populational performance as well. Rounded wings, close to a half ellipse shape, lowers induced drag and lift-to-drag ratio on the wing structure, reducing energy costs for flight. This conformation may have a significant impact on the organism’s lifestyle (Hoffmann et al. 2005). Colonization of novel territories poses an environmental change in a population perspective, and have been shown to rapidly modify wing morphology (Laparie et al. 2016). Thus, *C. albiceps* may undergo selection for wings types favoring a specific flight in response to seasonality to facilitate propagule establishment in lower quality patches, enabling persistence of a meta-population throughout time.

Sources of variability in wing morphology

The mechanisms underlying changes in wing phenotype in *C. albiceps* response to seasonality are not well understood. Selection acting on genetic variability is a well-known evolutionary force (Pfennig and Pfennig 2010, Abrams and Cortez 2015) but two issues arise with that possibility: 1- *C. albiceps* is an invasive species and it is not expected do display high genetic diversity due to founder or bottleneck effects and 2- *C. albiceps* and *C. idioidea* are both necrophagous Calliphoridae and display considerable niche overlap. Thus, we expect similar reactions to seasonality unless we are dealing with a scenario where the regional equilibrium relies on the trade-off between competitive and dispersal capacities.

On the other hand, phenotypic plasticity is also an important force driving community dynamics (Berg & Ellers 2010) because it allows rapid phenotypic change in the absence of genetic diversity. This phenomenon has attracted the attention of evolutionary biologists for over a century (Baldwin 1896, Waddington 1942, Bradshaw 1965, Pigliucci 2005, West-Eberhard 2005, Beldade et al. 2011), and more recently it has been associated with biological invasions (Peacor et al. 2006b). Through rapid phenotypic change, invader populations can increase invasiveness (Richards et al. 2006, Pichancourt and van Klinken 2012), whereas native species rely on their plasticity to resist those newly arrived challengers (Berthon 2015).

Even though divergent selection and phenotypic plasticity can both lead to ecological character displacement under different environmental conditions (Rice and Pfennig 2007), it is not expected that invasive species display higher genetic diversity than the native one. Therefore, further studies of phenotypic plasticity in the constitution of wing shape seem to be vital to understand how *C. albiceps* individuals respond to environmental changes.

Practical implications

Many blowfly species are used as biological indicators, especially in forensic practice to indicate minimum post mortem interval (PMI_{min}) because they lay eggs on the victim's body shortly after death and, within hours, larvae hatch and grow at predictable rates (Amendt et al. 2011). Size is the most used phenotype to estimate larval age, and size variance of a population implies error in PMI_{min} estimations (Tomberlin, Benbow, et al. 2011). Thus, it is imperious to forensic practice to understand variation in natural populations and especially the ecological and evolutionary features that drive them (Tomberlin, Mohr, et al. 2011).

In previous studies, we demonstrated that different species of blowflies react differently to intraspecific competition and that males and females of the same species react differently to that same stimulus (Macedo et al. 2018). Here we show that different species of Calliphoridae react in opposite ways regarding size and shape in the influence of seasonality. This evinces that we cannot assume a general trend among blowfly species and should develop independent models for each species in every environment in which we wish to make predictions.

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CONSIDERAÇÕES FINAIS E CONCLUSÕES

Considerações finais

A presente tese abordou a variação fenotípica em espécies de califorídeos, apontando implicações desta variação para a prática forense. Inicialmente, avaliamos a distribuição dos fenótipos em função do dimorfismo sexual em três espécies de califorídeos necrófagos (capítulo 1). Em seguida, avaliamos como essas espécies reagem a situações de competição intra- e interespecífica, sofrendo alterações nas suas morfologias alares (capítulo 2).

Em sequência, caracterizamos a estrutura da comunidade de califorídeos necrófagos em uma área protegida de Cerrado no Distrito Federal. Avaliamos como a estrutura da comunidade responde à sazonalidade (estação seca x chuvosa), bem como ela se distribui espacialmente em função da cobertura vegetal (mata de galeria x cerrado campo sujo). Avaliamos ainda se a variação fenotípica acompanha os índices de diversidade da comunidade e se essa variação difere entre estações (heterogeneidade temporal) e coberturas vegetais (heterogeneidade espacial) (capítulo 3).

Por fim, no capítulo 4, avaliamos a variação na estrutura alar e os níveis de assimetria flutuante em duas espécies (uma nativa e uma exótica) de califorídeos necrófagos no Cerrado em função da sazonalidade.

Inicialmente, demonstramos que a o fenótipo tamanho se distribui de forma desigual nas populações de *Calliphora vicina*, *Calliphora vomitoria* e *Lucilia sericata*, com fêmeas maiores que machos nas três espécies. Da mesma forma, fêmeas e machos reagem de formas distintas ao aumento de densidade bem como as diferentes espécies reagem de forma diversa a esse mesmo fator. Apesar de bem documentado entre insetos, o dimorfismo sexual não é incorporado em modelos preditivos na prática forense e a formulação de protocolos para sexagem de evidências forenses pode reduzir os níveis de incerteza associados ao cálculo do intervalo pós morte.

Em sequência mostramos que essas três espécies reagem de formas distintas a competidores intraespecíficos e interespecíficos alterando sua morfologia alar em função da identidade dos competidores – se da mesma espécies ou não. Enquanto *C. vicina* apresenta maior espalhamento no morfoespaço na presença de outras espécies, *C. vomitoria* não sofre alterações morfológicas e *L. sericata* apresenta uma mudança direcional na forma de sua estrutura alar. Isso nos permite concluir que essas espécies podem apresentar diferentes estratégias de evitamento de competição e, por isso a composição da comunidade de califorídeos em um recurso pontual (carcaça) pode alterar a constituição fenotípica das populações presentes.

No terceiro capítulo, constatamos que a comunidade de califorídeos necrófagos está estruturada tanto no tempo quanto no espaço. Nos meses que compreendem a estação chuvosa, a espécie nativa *Chloroprocta idioidea* dominou a comunidade, enquanto a espécie exótica *Chrysomya albiceps* foi a mais abundante no período da seca. Essa dualidade se mantem quando comparamos diferentes coberturas vegetais; *C. idioidea* domina a comunidade em ambientes de mata de galeria, enquanto *C. albiceps* é a espécie mais abundante em áreas de campo sujo. Todas as espécies nativas apresentaram preferência por algum ambiente, geralmente mata de galeria, mas somente *Hemilucilia semidiaphana* foi exclusiva desse ambiente. Por isso, consideramos necessária uma rigorosa validação de dados na prática forense para a utilização de espécies como indicadoras de ambiente. Os valores de variação morfológica para ambas as espécies não apresentam correlação com nenhum dos índices de diversidade de comunidade utilizados, o que nos permite concluir que a variação dos fenótipos nas populações dessas espécies é independente dos níveis de diversidade da comunidade.

Por fim, demonstramos que essas duas espécies reagem de forma contrastante em seus tamanhos entre os meses da estação seca e da chuvosa; enquanto a população de *C. albiceps* reduz seus tamanhos de asa da estação chuvosa para a seca, populações de *C. idioidea* tem trajetória inversa. É a primeira vez que registramos essas espécies de interesse forense reagindo de forma oposta ao mesmo estímulo (sazonalidade). Dessa forma, não é recomendável extrapolar dados de uma espécie de califorídeos para as demais. É importante que cada espécies seja avaliada individualmente para validação forense.

À luz das teorias sobre formação de comunidades, concluímos que para a comunidade de insetos necrófagos, as espécies, que são todas do mesmo nível trófico, não se comportam de maneira equivalente. As espécies estudadas apresentaram preferências diversas por habitats, bem como reagiram de forma contrastante ao mesmo estímulo ambiental (i.e., sazonalidade e competição). A espécie nativa *Cochliomyia macellaria* foi a única apresentar preferência por habitat coincidente com a exótica *C. albiceps*, e os relatos de exclusão competitiva corroboram nossas observações.

Em suma, as espécies de califorídeos apresentam níveis de variação morfológica em resposta às variáveis ambientais bióticas (e.g., competição) e abióticas (i.e., sazonalidade). Esses fenótipos não se distribuem de forma homogênea nas populações e a variação regular de um indicador forense é tido como fonte de incerteza em análises preditivas. Por isso é importante delimitar com cautela a população a que pertence um determinado indivíduo, possibilitando a estimativa de algum fato com base em um fenótipo com o menor grau de incerteza possível.

Conclusões

O fenótipo tamanho se distribui de forma desigual entre os sexos em *Calliphora vicina*, *Calliphora vomitoria* e *Lucilia sericata*

Calliphora vicina, *Calliphora vomitoria* e *Lucilia sericata* reagem de forma distinta à competição interespecífica

A comunidade de califorídeos necrófagos no Cerrado está espacial e temporalmente estruturada, *Chrysomya albiceps* e *Chloroprocta idioidea* são as espécies dominantes nessas comunidades

Hemilucilia semidiaphana foi coletada apenas em ambientes de floresta e é possível que seja um indicador desse tipo de local.

Variação morfológica não está ligada à diversidade da comunidade para as populações de *Chrysomya albiceps* e *Chloroprocta idioidea*.

Chrysomya albiceps e *Chloroprocta idioidea* tem reações contrastantes à heterogeneidade temporal.

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