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EFEITOS DA SELEÇÃO SEXUAL SOBRE O PAREAMENTO
DEPENDENTE DE TAMANHO: PADRÕES GERAIS, VARIAÇÕES
ESPAÇO-TEMPORAIS E MECANISMOS SUBJACENTES

RAFAEL RIOS MOURA

UBERLÂNDIA

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Tese apresentada à Universidade Federal de
Uberlândia, como parte das exigências à defesa de
Doutorado do Programa de Pós-Graduação em
Ecologia e Conservação de Recursos Naturais

Orientador:
Prof. Dr. Marcelo de Oliveira Gonzaga

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UBERLÂNDIA
Fevereiro de 2018

I think



“Scientia amabilis”

Konrad Lorenz

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RESUMO GERAL

Moura, Rafael R. 2018. Efeitos da seleção sexual sobre o pareamento dependente de tamanho: padrões gerais, variações espaço-temporais e mecanismos subjacentes. Tese de Doutorado do Programa de Pós-Graduação em Ecologia e Conservação de Recursos Naturais. UFU. Uberlândia-MG. 107 p.

Pareamento dependente de tamanho (PDT) é um dos padrões mais estudados na ecologia comportamental porque afeta as variações de tamanho das populações e pode levar à especiação simpátrica. Alguns mecanismos foram propostos para explicar por que esse padrão é tão comum em animais e em que cenários ele deve ocorrer. Dentre eles, a hipótese de escolha de parceiros tem sido a mais estudada. Ela pressupõe que o pareamento deve ocorrer quando: (1) indivíduos grandes têm vantagens na competição intrassexual; (2) um ou ambos os sexos preferem parceiros grandes e (3) a variação na qualidade dos parceiros está associada ao seu tamanho. Apesar de ter suporte empírico, a escolha de parceiros pode ocorrer sem que isso resulte em PDT, provavelmente, porque outros fatores associados à competição por parceiros podem influenciar escolha de parceiros e mudar seus efeitos sobre o PDT, como a intensidade de competição intrassexual e a disponibilidade de parceiros. Por exemplo, altos custos de competição e a dificuldade de encontrar e avaliar parceiros podem enfraquecer a seleção de parceiros e o PDT. Além disso, essa hipótese permite explicar potenciais variações espaço-temporais. Nessa tese, analiso a influência desses fatores sobre os pareamentos em animais, usando duas espécies de artrópodes, como modelos experimentais, e uma metanálise. Com isso, descrevi os primeiros casos de PDT em formigas e percevejos e avaliei os padrões gerais e as variações espaciais e temporais do PDT em animais. Encontrei suporte empírico para a hipótese de intensidade de competição por parceiros, inaugurando uma nova linha de pesquisa com estudos prolíficos para avaliar os efeitos da seleção sexual sobre o PDT.

ABSTRACT

Moura, Rafael R. 2018. Effects of sexual selection on size-assortative mating: general patterns, spatio-temporal variation and underlying mechanisms. Thesis of the Programa de Pós-Graduação em Ecologia e Conservação de Recursos Naturais. UFU. Uberlândia-MG. 107 p.

Size-assortative mating (SAM) is one of the patterns more studied in behavioral ecology, because it affects size variation of populations and can promote sympatric speciation. Some mechanisms were proposed to explain why this pattern is common in animals and in which scenarios it may occur. Mate choice has been the most studied mechanism. This hypothesis proposes that SAM should occur when: (1) large individuals have advantages in intrasexual competition; (2) one or both sexes prefer large partners; and (3) there is variation in the mate quality related to body size. This hypothesis has clarified several cases in which SAM occurred. However, in some cases, SAM did not occur even when predictions of mate choice hypothesis were corroborated, probably, because factors related to mating competition may influence mate choice changing its effects on SAM, such as the intensity of intrasexual competition and mate availability. Excessive costs of intrasexual competition and the difficulty of finding a partner can weak mate choice, leading to random mating. Variations in mating competition and mate availability can be estimated using population parameters, such as sex ratio and population density. Furthermore, mating competition hypothesis may explain and predict spatio-temporal variation. In this thesis, I investigate effects of mating competition on SAM, using two arthropod species, as experimental models, and a meta-analytic approach. Thus, I describe the first cases of SAM in ants and stink bugs and evaluate general patterns and spatio-temporal variation of SAM in animals. I found empirical support to mating competition hypothesis, inaugurating a new line of research with prolific studies about effects of sexual selection on assortative mating.

INTRODUÇÃO GERAL

Um tópico de grande interesse nos estudos ecológicos e evolutivos é entender como a seleção sexual afeta a especiação. Para isso, precisamos primeiro investigar como os mecanismos da seleção sexual, como a escolha de parceiros e a competição intrasexual, afetam a formação de casais. Padrões de formação de casais são frequentemente observados em populações animais (Jiang *et al.* 2013). Um padrão de acasalamento comum é o pareamento baseado em características fenotípicas semelhantes (homotípicas) ou distintas (heterotípicas) dos parceiros (Burley 1983; Ridley 1983). Esse pareamento pode ser estimado a partir de correlações entre características fenotípicas dos parceiros durante a formação de casais ou o acasalamento, resultando em correlações positivas, quando o pareamento é homotípico, e negativas, quando ele é heterotípico (Jiang *et al.* 2013). O estudo dos efeitos da escolha de parceiros e da competição intrasexual sobre esses padrões de pareamento de machos e fêmeas por atributos fenotípicos pode nos ajudar a elucidar como a seleção sexual pode estar relacionada à origem de novas espécies.

Os primeiros estudos sobre seleção sexual

Desde a publicação do artigo clássico de Darwin e Wallace (1858) e da Origem das Espécies (Darwin 1859), pesquisadores têm se dedicado a entender como funcionam as dinâmicas evolutivas. Os primeiros trabalhos foram focados em atributos que afetam as chances de sobrevivência dos organismos. Variações nesses atributos levam a diferentes capacidades de deixar descendentes para a geração futura, de modo que indivíduos que apresentam atributos que aumentam suas chances de sobrevivências podem ter mais oportunidade de acasalar e deixar descendentes para a geração futura.

Desse modo, esses atributos se tornam cada vez mais comuns nos indivíduos da população. Esse processo é chamado de seleção natural. Apesar do importante papel desses atributos na evolução de populações e origem de novas espécies, o próprio Darwin percebeu que várias espécies apresentam atributos energeticamente dispendiosos e conspícuos, portanto, comprometendo o valor adaptativo de atributos relacionados à sobrevivência dos organismos, como a defesa imunológica contra patógenos e parasitas e a fuga de predadores. Além disso, ele percebeu que alguns desses atributos podiam variar de acordo com o sexo (i.e. dimorfismo sexual), como armamentos, ornamentos e sinalização química e vibratória (Andersson 1994). Darwin também notou que esses atributos garantiam vantagens na competição por oportunidades de acasalamento e na atração de parceiros do sexo oposto. Assim, ele propôs outro mecanismo para explicar a evolução de atributos sexuais, chamado de seleção sexual (Darwin 1871).

A síntese moderna da teoria evolutiva uniu os conceitos de seleção natural e sexual à genética de populações (Andersson 1994; Cornwallis & Uller 2010). Nesse contexto, seleção passou a ser definida como uma diferença consistente e não aleatória na sobrevivência e reprodução de entidades fenotipicamente (ou genotipicamente) diferentes entre gerações (Futuyma 2013; Moura & Peixoto 2013). A seleção sexual ocorre quando essa diferença está associada a atributos que afetam a escolha de parceiros ou o sucesso de acasalamento e de fertilização dos organismos (Cornwallis & Uller 2010; Moura & Peixoto 2013). Desde então, a seleção sexual passou a ser o principal mecanismo utilizado para testar hipóteses relacionadas à seleção de parceiros e à evolução de atributos sexuais adaptativos.

Machos competitivos e fêmeas exigentes: uma investigação sobre os papéis sexuais darwinianos

Darwin também previu que dois mecanismos têm importantes papéis na evolução de atributos sexuais: a competição entre machos e a escolha de parceiros pelas fêmeas (Darwin 1871). Por isso, esses comportamentos reprodutivos são normalmente chamados de papéis sexuais darwinianos. Eles receberam suporte empírico em experimentos clássicos usando moscas do gênero *Drosophila*, como modelo (Bateman 1948), e, mais recentemente, em uma metanálise que usou diferentes métricas da intensidade da seleção sexual (Janicke *et al.* 2016). Trivers (1972) argumentou que esses comportamentos são motivados pelo investimento na produção de cada gameta. Machos são aqueles que investem pouca energia na produção de cada gameta e, por isso, são capazes de produzir vários gametas por evento reprodutivo. Consequentemente, sua aptidão está associada ao número de óvulos que conseguem fertilizar. Fêmeas, por outro lado, são organismos que investem muita energia na produção de cada gameta, produzindo poucos gametas ao longo da vida. Portanto, sua aptidão está relacionada à fertilização dos óvulos por gametas advindos de machos de alta qualidade, o que aumentaria as chances de sobrevivência de sua prole.

Baseado nesses papéis sexuais, Trivers (1972) defendeu que a intensidade de competição entre os machos poderia ser estimada pela razão sexual operacional (RSO), que é tradicionalmente definida como o número de machos adultos dividido pelo número de fêmeas sexualmente receptivas de uma população (Emlen & Oring 1977; Moura & Peixoto 2013). Pode, no entanto, também ser definida como uma proporção de machos adultos em relação ao total de adultos de ambos os sexos em uma população (Jirotkul 1999). Quando a RSO apresenta desvio para machos, a intensidade de competição intrasexual aumenta, porque os machos passam a disputar por várias

oportunidades de acasalamento com um número proporcionalmente limitado de parceiras. Nesse cenário, machos com maior potencial de vencer disputas devem ter vantagens em conseguir parceiras. Assim, a seleção sexual deve ser mais intensa nos machos (Janicke & Morrow 2018) e, em um tempo evolutivo, pode favorecer a evolução de armamentos e ornamentos (Andersson 1994).

Trivers (1972) também argumentou que os papéis sexuais podem depender do investimento em cuidado parental. Devido ao alto investimento parental das fêmeas na produção de óvulos, elas tendem a ser o sexo que mais investe na proteção da prole de modo a aumentar as chances de sobrevivência do número limitado de prole que são capazes de produzir (Clutton-Brock 1991). Entretanto, machos também podem adotar essas estratégias parentais (e.g. Moura, Vasconcellos-Neto & Gonzaga 2017). Quando isso acontece, eles se tornam mais exigentes na escolha de parceiras (e.g. Moura & Gonzaga 2017) e as fêmeas podem passar a competir mais intensamente pelo acesso aos machos (e.g. Forsgren *et al.* 2004). Em casos mais raros, essas condições podem levar à inversão dos papéis sexuais darwinianos.

Quando machos brigam por fêmeas? Medidas da intensidade de competição por parceiros

Vários trabalhos mostraram limitações dos argumentos de Trivers (1972) (Wade & Shuster 2002; Kokko & Jennions 2008). Por exemplo, ele assume que desvios na RSO para machos devem estimulá-los a investir mais energia em disputas por parceiras. Entretanto, os custos energéticos e os riscos de sofrer injúrias aumentam à medida que aumenta a intensidade da competição por parceiras (Andersson 1994). Por isso, ao invés das disputas se tornarem mais intensas, os machos tendem a se tornar menos agressivos

e investir menos tempo e energia na corte e na guarda das parceiras (Weir, Grant & Hutchings 2011).

Outra possibilidade ainda pouco explorada é que aumentos no viés da RSO para machos podem favorecer machos grandes na disputa por parceiras, de modo que eles tenham um alto sucesso de acasalamento (Janicke *et al.* 2016), mas isso não garante que esses machos consigam as parceiras de melhor qualidade (i.e. mais férteis). Além disso, um alto sucesso de acasalamento não garante necessariamente uma maior aptidão para esses machos, porque as fêmeas podem exibir escolha críptica e não utilizar o esperma de machos que copularam com várias parceiras (Eberhard 1996). Competidores mais fracos nas disputas pré-copulatórias também pode ter maiores oportunidades de aumentar seu sucesso reprodutivos na competição espermática (i.e. pós-copulatória). Outro aspecto importante que pode afetar a evolução de atributos dos machos competidores é a sobrevivência da prole. Se a prole produzida depende de cuidado paternal para ter maiores chances de sobreviver até a idade reprodutiva, os atributos machos que cuidam da prole podem ter maiores chances de serem favorecidos pela seleção, independente dos atributos de competidores (e.g. Moura *et al.* 2017). Essas diferentes estratégias podem mudar as implicações da RSO sobre a aptidão de machos e fêmeas. Por isso, alguns estudos têm apresentado limitações da RSO como uma medida geral de competição por parceiros (Kokko & Rankin 2006; Klug *et al.* 2010; Kokko, Klug & Jennions 2012; Moura & Peixoto 2013).

Apesar das limitações, a RSO ainda pode ser usada como uma medida da intensidade de competição entre machos, mas a relação entre essas variáveis deve ser interpretada com cautela. Uma metanálise avaliou dados empíricos da relação entre a RSO e os comportamentos pré-copulatórios dos machos. Foi observado que comportamentos relacionados à competição entre os machos, como interações

agressivas, guarda da fêmea e duração da corte, tendem a se tornar mais frequentes e intensos quando não há desvio na RSO ou quando esta está levemente desviada para machos (até 2 machos:1 fêmea) (Weir *et al.* 2011). A intensidade de competição, portanto, aumenta quando a RSO se torna fortemente desviada para machos, mas isso leva a um aumento nos custos de competir por parceiras, tornando fêmeas de alta qualidade indefensáveis (Andersson 1994; Klug *et al.* 2010). Consequentemente, os machos investem menos energia na competição por parceiras. No outro extremo (i.e. RSO desviada para fêmeas), a competição entre machos tende a enfraquecer quando a disponibilidade de parceiras é alta, porque os machos podem abandonar a estratégia de disputar por fêmeas de alta qualidade e investir em copular com o maior número de parceiras possível (Crespi 1989; Andersson 1994). Portanto, os cenários competitivos ideais para que machos grandes possam exibir suas vantagens na disputa por parceiras são situações nas quais a RSO não tem viés ou tem um leve viés para machos.

Outra medida populacional que pode potencializar os efeitos da RSO sobre a competição entre machos é a densidade populacional. A taxa de encontro entre potenciais competidores e parceiros aumenta à medida que aumenta a densidade (Kokko & Rankin 2006; Muniz *et al.* 2017). Por exemplo, aumentos no viés da RSO para machos podem aumentar o assédio sexual das fêmeas e enfraquecer a escolha de parceiros (Fitze & Le Galliard 2008), mas esses efeitos devem ser potencializados à medida que aumenta a densidade populacional (Jirotkul 2000; Kokko & Rankin 2006). Por outro lado, à medida que a densidade diminui nesse cenário, a disponibilidade de fêmeas diminui. Assim, a dificuldade de encontrar uma parceira deve enfraquecer a competição e a escolha de parceiras (Barry & Kokko 2010), porque os machos não devem rejeitar parceiras que são raramente encontradas, independente da sua qualidade. Portanto, a RSO e a densidade devem ter efeitos sobre a competição por parceiras.

Especiação e competição entre machos

Como a seleção sexual tem o potencial de gerar inovações entre diferentes fenótipos de machos e fêmeas, é possível que ela seja um mecanismo conduzindo a origem de novas espécies tanto em alopatria (i.e. populações separadas por uma barreira geográfica) quanto em simpatria (i.e. fenótipos distintos em uma mesma população) (Dijkstra & Border 2018). Há um grande conjunto de evidências mostrando como a seleção por levar à especiação alopátrica, mas ainda há poucos estudos que mostram como esse mecanismo pode ocorrer em fenótipos simpátricos (Dijkstra & Border 2018). A escolha de parceiros tem sido explorada com um potencial mecanismo que pode levar à especiação simpátrica por seleção sexual (Safran *et al.* 2013). Entretanto, apenas a escolha de parceiros pode não ser suficiente para isolar o fluxo gênico entre fenótipos muito distintos dentro de um contínuo de variação de um atributo (i.e. fenótipos extremos), porque os fenótipos intermediários são capazes de copular com parceiros com fenótipos extremos. Mesmo uma frequência baixa dessa troca gênica é suficiente para evitar que a especiação ocorra (Kopp *et al.* 2018; Dijkstra & Border 2018; Tinghitella *et al.* 2018).

Um mecanismo que tem sido subestimado nos estudos de seleção sexual e especiação é a competição entre machos por parceiras sexualmente receptivas (Andersson 1994; Qvarnström, Vallin & Rudh 2012; Dijkstra & Border 2018; Tinghitella *et al.* 2018). A competição por parceiros pode levar à diversificação e rápida mudança evolutiva de atributos dos machos associados à atração e disputa por parceiros, como coloração, tamanho e armamentos (Andersson 1994; Dijkstra & Border 2018). Essa competição intrasexual também pode estabilizar a seleção disruptiva, porque fenótipos extremos podem ser mais agressivos com fenótipos intermediários, limitando

o fluxo gênico entre os fenótipos extremos (Dijkstra & Border 2018). Além disso, esse mecanismo também pode facilitar a coexistência de espécies irmãs em uma mesma área, evitando hibridização (Dijkstra & Border 2018). Essas condições favorecem a competição entre machos como um mecanismo importante, que pode levar à especiação simpátrica promovendo isolamento reprodutivo por seleção sexual (Andersson 1994; Qvarnström *et al.* 2012; Dijkstra & Border 2018; Tinghitella *et al.* 2018).

Formação de casais em animais: um passo antes da especiação simpátrica

A competição entre machos pode levar à especiação simpátrica, mas, antes que isso ocorra, é preciso que ela conduza à formação de casais no atributo sexualmente selecionado. Esse padrão de acasalamento que depende da seleção sexual é chamado de “pareamento” (alternativas a essa tradução podem ser “acasalamento associativos” ou “formação de casais”). Esses pareamentos podem ser medidos como uma correlação entre os atributos sexualmente selecionados de machos e fêmeas (Crespi 1989; Jiang *et al.* 2013). Pareamentos positivos (ou homotípicos), por exemplo, podem aumentar a variação gênica de uma população e levar à seleção disruptiva com o potencial de promover isolamento reprodutivo entre fenótipos extremos. Por outro lado, pareamentos negativos (ou heterotípicos) tendem a intensificar o fluxo gênico entre fenótipos extremos, reduzindo a variação gênica da população por seleção estabilizadora (Johannesson, Rolán-Alvarez & Ekendahl 1995; Jiang, Bolnick & Kirkpatrick 2013).

Pareamentos têm sido amplamente documentados em vários taxa animais (Crespi 1989; Jiang *et al.* 2013), como Annelida (Monroy, Aira & Velando 2005), Arthropoda (Brown 1993; Hernández *et al.* 2012; Moura & Gonzaga 2017), Chordata (Cuadrado 1999; Robart 2012; Seki, Yasuo & Aoki 2012; Sheppard *et al.* 2013; Green 2014), Mollusca (Clarke *et al.* 2013) e Platyhelminthes (Michiels & Streng 1998). Os

padrões de acasalamento podem depender de diferentes atributos dos parceiros, como condição corporal (Sharp & Agrawal 2009), comportamento (Kralj-Fišer *et al.* 2013), coloração (Pérez *et al.* 2012) e genótipo (García-Navas, Ortego & Sanz 2009). Os pareamentos podem ser explicados por outros processos além da seleção sexual, mas eles têm um suporte mais fraco na literatura (Crespi 1989; Jiang *et al.* 2013). Pode ser porque são raros ou por conta de um número limitado de estudos. Independente da razão, a seleção sexual por meio da escolha de parceiros e da competição intrasexual tem grande suporte de estudos empíricos e teóricos (Crespi 1989; Jiang *et al.* 2013).

Apesar da ampla variação de atributos que podem gerar padrões específicos de pareamento, o tipo mais comum em animais é uma correlação entre os tamanhos dos parceiros, chamado de “pareamento dependente de tamanho”, PDT (Jiang *et al.* 2013). Há suporte empírico na literatura de que esse padrão de pareamento que pode levar à especiação simpátrica associado a fatores ecológicos e comportamentais (Kirkpatrick & Ravigne 2002; Jones *et al.* 2003; Olafsdottir, Ritchie & Snorrason 2006; Ritchie 2007; Gauthey *et al.* 2016). Portanto, a medição desse processo e a identificação de seus mecanismos subjacentes podem ser importantes para entender como o tamanho dos indivíduos dentro e entre populações animais pode evoluir (Jiang *et al.* 2013).

Causas do pareamento dependente de tamanho em animais: escolha de parceiros e competição entre machos

Um trabalho clássico propôs três hipóteses, que são testadas até o presente, para explicar a ocorrência de PDT em animais (Crespi 1989). A hipótese de disponibilidade de parceiros pressupõe que os tamanhos de machos e fêmeas podem estar correlacionados no tempo (Miyashita 1994) e no espaço (Johannesson *et al.* 1995). A hipótese de restrição de acasalamento assume que as variações de tamanho entre os

parceiros podem gerar restrições físicas ou fisiológicas durante a corte ou a cópula (Robertson 1990; Brown 1993). Por fim, a hipótese de escolha de parceiros propõe que a vantagem na competição de indivíduos grandes associada à variação na qualidade dos parceiros e à preferência por parceiros grandes deve levar ao PDT (Hoefler 2007; Moura & Gonzaga 2017). Outra hipótese mais recente foi proposta para explicar alguns casos de PDT, a ‘escolha prudente de hábitat’ (Härdling & Kokko 2005). Ela assume que os indivíduos devem selecionar habitats com qualidades correspondente a suas habilidades competitivas e os indivíduos maiores podem monopolizar habitats de melhor qualidade. Consequentemente, o PDT ocorre pela escolha de habitat baseada nas habilidades competitivas dos machos e fêmeas, independentemente de haver escolha de parceiros ou não (Taborsky, Guyer & Demus 2014).

A escolha de parceiros é considerada como o principal mecanismo determinante dos padrões pareamento (Jiang *et al.* 2013). Muitos autores, entretanto, tem discutido limitações da ocorrência de pareamento por escolha de parceiros como um mecanismo que possa levar à especiação porque não impede a cópula dos fenótipos extremos com os fenótipos intermediários (Dijkstra & Border 2018; Tinghitella *et al.* 2018). Como discutido acima, esses autores defendem que a competição entre machos por parceiras é o mecanismo mais importante nesse processo (Andersson 1994; Qvarnström *et al.* 2012; Dijkstra & Border 2018; Tinghitella *et al.* 2018). Entretanto, é ignorado que a hipótese original de escolha de parceiros, formulada por Crespi (1989), incorpora um pressuposto de competição entre machos. É necessário que a preferência por fenótipos maiores esteja associada com as vantagens competitivas dos indivíduos maiores, de maneira que eles possam impedir que indivíduos menores obtenham cópulas com parceiros maiores.

Um equívoco da proposta de Crespi (1989) foi assumir que a existência de vantagem competitiva dos fenótipos maiores sobre os menores seja uma evidência de que a competição sempre vai ocorrer. Entretanto, a competição entre machos pode depender de variações em parâmetros populacionais, como RSO e densidade populacional, que são podem mudar no tempo e no espaço (Gosden & Svensson 2008; Aronsen *et al.* 2013; Moura & Gonzaga 2017). Assim, a escolha de parceiros só deve ter o potencial de gerar pareamento dependente de tamanho positivo quando a competição intrasexual for intensa. Em resumo, a hipótese tradicional de escolha de parceiros tem poder de previsão limitado sobre a ocorrência e a força dos pareamentos e precisa ser revisada.

Uma nova proposta: efeitos da intensidade de competição por parceiros sobre o pareamento dependente de tamanho

A RSO e densidade populacional podem ser boas medidas da competição por parceiros (Gosden & Svensson 2008; Weir *et al.* 2011; Aronsen *et al.* 2013; Moura & Gonzaga 2017). Aumentos no desvio da RSO para machos podem levar a um aumento na intensidade de competição por parceiras (Trivers 1972; Emlen & Oring 1977; Janicke & Morrow 2018). Entretanto, isso deve ocorrer apenas até um limiar, no qual comportamentos relacionados à competição intrasexual se tornam mais frequentes (até 2 machos: 1 fêmea) (Weir *et al.* 2011). Nessa situação, machos grandes podem ter vantagens competitivas e copular preferencialmente com as maiores fêmeas da população. Essa condição pode fortalecer o PDT (Moura & Gonzaga 2017). Entretanto, quando a RSO excede dois machos para uma fêmea, os altos custos da competição por parceiros dificultam o acesso a parceiras grandes, levando a uma redução na seletividade dos machos (Barry & Kokko 2010; Weir *et al.* 2011). Nesse cenário, a

intensa competição por parceiras pode enfraquecer o PDT, levando a acasalamentos aleatórios (Moura & Gonzaga 2017).

A densidade populacional também pode afetar a intensidade de competição entre machos (Jirotkul 2000; Kokko & Rankin 2006; Ribeiro, Daleo & Iribarne 2010) e o PDT (McLain 1982; McLain & Boromisa 1987; Moura & Gonzaga 2017). Os machos podem enfrentar uma forte intensidade de competição por parceiras à medida que aumenta a densidade. Assim, os custos crescentes de disputar fêmeas podem tornar os machos menos seletivos e leva-los a copular com parceiras independentemente do seu tamanho (Kokko & Rankin 2006; Barry & Kokko 2010). Por outro lado, em populações menos densas, a intensidade de competição por parceiros é mais fraca. Consequentemente, machos grandes podem monopolizar oportunidades de acasalamento com fêmeas grandes, levando à formação de PDT positivo (McLain 1982; McLain & Boromisa 1987; Moura & Gonzaga 2017). Também é importante considerar que densidades populacionais muito baixas deve diminuir as chances dos machos localizarem e avaliarem parceiras (Kokko & Rankin 2006; Rankin & Kokko 2007; Muniz *et al.* 2017). Esse acesso limitado a parceiras deve tornar os machos menos seletivos, de maneira que eles não devem rejeitar as oportunidades de cópula com fêmeas raramente encontradas, independentemente do seu tamanho (Kokko & Rankin 2006; Barry & Kokko 2010). Portanto, é importante identificar o limiar no qual a densidade não é muito baixa ao ponto de afetar negativamente a capacidade dos machos de localizar uma parceira.

Equívocos frequentes nas estimativas de pareamentos e suas implicações para a identificação de padrões gerais e variações espaço-temporais

A identificação de PDT em uma população nos ajuda a entender como o tamanho pode variar entre as gerações, ao ponto de levar à especiação entre fenótipos extremos. Entretanto, é importante analisar com cautela esse resultado, porque os pareamentos precisam ser consistentes no espaço e no tempo para influenciar o fluxo gênico de uma população (Schwartz 2013; Nonaka *et al.* 2014; Gauthey *et al.* 2016; Ng *et al.* 2016). Estudos empíricos encontraram evidências de que os pareamentos podem variar no tempo e no espaço (Miyashita 1994; Bel-Venner *et al.* 2008; Schwartz 2013; Ng *et al.* 2016; Moura & Gonzaga 2017). Infelizmente, pesquisadores frequentemente cometem dois equívocos ao medir os pareamentos. O primeiro deles é não avaliar as variações espaciais e temporais nos pareamentos. Ao invés disso, eles tendem considerar uma única medida de pareamento, em uma população, como um padrão para a espécie (Bourne 1993; Apanius & Nisbet 2006; Alcantara, Lima & Bastos 2007; Jiang *et al.* 2013). Esse equívoco pode levar a conclusões errôneas, porque fatores estocásticos ou peculiares de uma população ou de um momento da estação reprodutiva podem ter gerado o padrão observado (Rolán-Alvarez *et al.* 2015; Ng *et al.* 2016).

O segundo erro mais comum é agrupar medidas de pareamento de mais de uma população (Jones & Montgomerie 1991; Warkentin, James & Oliphant 1992; Forero *et al.* 2001) e/ou estação reprodutiva (Delestrade 2001; Christensen & Kleindorfer 2007; Bourgeois *et al.* 2014). Como é difícil acessar informações sobre casais em cópula na maioria das espécies de animais, pesquisadores tendem a agrupar essas medidas, visando adquirir maior poder estatístico nas análises (Rolán-Alvarez *et al.* 2015). Entretanto, um problema grave dessa abordagem é confundir a correlação entre os tamanhos dos indivíduos pareados com as variações de tamanho entre organismos de mais de uma população e de momentos distintos da estação reprodutiva ou dos períodos de coleta (Rolán-Alvarez *et al.* 2015; Moura & Gonzaga 2017). Esse fenômeno é

chamado de efeito da escolha da escala. Foi demonstrado empiricamente que pareamentos negativos observados em populações com indivíduos de tamanhos diferentes podem levar a estimativas de pareamento positivo quando os dados são agrupados em uma única análise (Rolán-Alvarez *et al.* 2015; Ng *et al.* 2016).

Um convite à leitura desta tese

Os estudos dos efeitos da competição entre machos sobre a especiação simpátrica tem se mostrado promissores nos anos mais recentes (Safran *et al.* 2013; Kopp *et al.* 2018; Dijkstra & Border 2018; Tinghitella *et al.* 2018). Entretanto, a tendência entre os pesquisadores que estão trabalhando nessa linha de pesquisa é usar apenas a competição como mecanismo para explicar a especiação. É possível que a competição só tenha efeitos fortes sobre o isolamento de fenótipos extremos quando associada à escolha de parceiros. Além disso, é difícil assumir que a competição entre machos leve a especiação simpátrica, mesmo atendendo a condições importantes para a explicar a evolução de atributos sexuais (Dijkstra & Border 2018), sem medir como a competição afeta um passo antes da especiação, o pareamento. Por isso, essa tese está focada em fornecer testes empíricos para avaliar a relação entre a intensidade de competição entre machos e o pareamento dependente de tamanho. Além disso, a intensidade de competição por parceiras, diferente das hipóteses tradicionais ou mais recentes (Crespi 1989; Härdling & Kokko 2005; Taborsky *et al.* 2014), é capaz de explicar e prever não apenas a ocorrência ou não de pareamento, mas as variações na força desse padrão de associação dentro das categorias normalmente descritas (positivo, negativo ou aleatório). Alguns estudos avaliaram como parâmetros populacionais, como RSO e densidade populacional, que são medidas da intensidade de competição por parceiras, afetam os pareamentos e podem promover variações espaço-temporais

(McLain 1982, 1985, 1988; McLain & Boromisa 1987; Larsson 1988; Miyashita 1994; Bel-Venner *et al.* 2008; Aronsen *et al.* 2013; Mobley, Abou Chakra & Jones 2014; Vojar *et al.* 2015; Moura & Gonzaga 2017). Entretanto, esses estudos estão desconectados entre si e não tem uma síntese teórica claramente definida, baseada na teoria moderna de seleção sexual. Por isso, também investigamos potenciais variações espaço-temporais no PDT e suas relação com a intensidade de competição entre machos e a escolha de parceiros.

Com esses objetivos em mente, nesta tese, avaliei potenciais variações espaço-temporais no PDT e como os pareamentos são afetados pela intensidade de competição por parceiros. Para isso, testei as previsões dessa hipótese usando duas espécies de artrópodes, a formiga *Tranopelta gilva* (Wheeler, 1922) (Myrmicinae) e o percevejo *Edessa contermina* (Pentatomidae), e dados metanalíticos de diversos grupos taxonômicos de animais. Cada uma dessas abordagens constitui um capítulo. Assim, propus uma nova hipótese que deve explicar e prever em quais cenários a seleção sexual, por meio da intensidade de competição por parceiros, pode influenciar o PDT.

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Capítulo 1

Sex ratio and density may affect temporal variation in size-assortative mating in a Neotropical ant species

Abstract

Size-assortative mating (SAM) is common in animals, especially in insects, owing to the considerable influence of body size on mate choice of both sexes. According to sexual selection theory, choosiness in mate selection may decrease when the costs of intrasexual competition and mate choice increase. Thus, scenarios with intense mating competition (i.e. with high population density and highly male-biased sex ratio) should weaken SAM. In this study, we tested this hypothesis in mating aggregations of the ant *Tranopelta gilva*. During mating swarm, population density and bias in sex ratios may increase with time, promoting changes in SAM. Considering this potential variation in SAM, we hypothesized that (a) *T. gilva* ants assortatively mate by size when mating competition is weak; (b) larger males win most intrasexual disputes; and (c) there is no significant variation in body size among individuals arriving earlier and later during mating aggregations. We attracted winged ants to open plastic arenas, placed under artificial light at night in a protected area of the Brazilian Savanna. Two intensities of mating competition were comparatively evaluated in the arenas: ‘weakly competitive’ (low ant density and a slightly male-biased sex ratio) and ‘intense competitive’ conditions (high ant density and strongly male-biased sex ratio). Assortative mating in *T. gilva* occurred only when mating competition was weak. Male size was not important in male-male competition and large males did not arrive first at mating aggregations. Because male-male competition did not explain variations in SAM, we suggest that

female choice at low densities and male harassment at high densities would explain these variations. In conclusion, mating competition may affect temporal variation in SAM of *T. gilva* and, potentially, of other animal species.

Key words: body size, intrasexual competition, temporal change, local density, OSR

Introduction

Assortative mating is mate assortment by similarity or dissimilarity of phenotypic or genotypic traits that occur more frequently than expected by chance (Burley 1983; Ridley 1983). When assortative mating is positive, this nonrandom mating pattern tends to increase population genetic variation for such traits through disruptive selection (Johannesson *et al.* 1995; Jiang *et al.* 2013), such as in the genetic variation responsible for the morphological variations among beaks of Darwin's finches (Grant & Grant 2008). A recent meta-analysis, using 269 species of five animal phyla, showed that positive assortative mating is the most common mating pattern (Jiang *et al.* 2013). Negative assortative mating, however, decreases population genetic variation through stabilizing selection (Johannesson *et al.* 1995; Jiang *et al.* 2013). Therefore, identifying patterns of assortative mating is a key step to better understand the direction and strength of sexual selection on phenotypic traits and its implications for genetic variation in animal populations.

Mate assortment may occur in response to various attributes of sexual partners, such as color (Summers *et al.* 1999), behavior (Kralj-Fišer *et al.* 2013), age (Cezilly, Boy & Johnson 1997), and size (Shine *et al.* 2001). However, size-assortative mating (SAM) is the most common and taxonomically widespread type of mate assortment (Jiang *et al.* 2013). To explain its causes, four hypotheses were proposed by Ridley

(1983), Crespi (1989) and Härdling and Kokko (2005): (i) mate-availability hypothesis (i.e., males and females of different sizes are separated in space (Ferrer & Penteriani 2003) and/or time (Miyashita 1994)); (ii) mating restriction hypothesis (i.e., size difference between mating couples physically and physiologically constrains courtship and/or copulation (Brown 1993)); (iii) prudent habitat choice hypothesis (i.e., weaker competitive individuals avoid high-quality habitats with intense competition, leading to habitat segregation based on body size (Taborsky *et al.* 2014)); and (iv) mate choice hypothesis (i.e., size assortment between mates due to choice of one or both mating partners (Shine *et al.* 2001)). When both males and females find themselves in the same space, but in different time, and there are no obvious physical and physiological restrictions for size assortment, temporal mate-availability and mate choice hypotheses would be causes of SAM.

The implications that temporal variation in population structure may have on the stability of assortative mating patterns have not been considered (Ng *et al.* 2016; Moura & Gonzaga 2017). Because of this, most studies have evaluated SAM at a single moment in the breeding season (Jiang *et al.* 2013). However, several important conditions favoring SAM's occurrence may vary in time (Montiglio *et al.* 2016; Moura & Gonzaga 2017). Mate choice hypothesis is frequently used to explain SAM's occurrence (references in Jiang *et al.*, 2013). However, mating can be random even when assumptions of this hypothesis are corroborated (Mobley *et al.* 2014; Moura & Gonzaga 2017). If mate choice is the main mechanism causing SAM, it is important to consider that population demography may affect its occurrence. According to the modern theory of sexual selection, mate choice may change over space and time, depending on variations in density and sex ratio (Forsgren *et al.* 2004; Weir *et al.* 2011; Aronsen *et al.* 2013; Vojar *et al.* 2015). Consequently, population demography may

affect variations on the strength and type of assortative mating throughout the breeding seasons (see Moura and Gonzaga, 2017). When the operational sex ratio (OSR: *sensu* Emlen and Oring, 1977; Rios Moura and Peixoto, 2013) becomes slightly biased toward males (close to two males for a female), aggression, mate guarding, and copulation duration also increase, but the frequency of these behaviors decrease when OSR becomes increasingly male-biased (Weir *et al.* 2011). In addition, males become less choosy in mate selection when OSR become highly male-biased, leading to a decrease in courtship rate (Weir *et al.* 2011). Therefore, variations in OSR would be correlated with the competition intensity by mates. An intensely competitive scenario would weaken male choice and, consequently, lead to random matings.

The OSR, however, has no clear correlation with male mating success and some animal populations are naturally male-biased (Moura & Peixoto 2013). Density, in turn, may also lead to variations in SAM (McLain 1982; McLain & Boromisa 1987b; Crespi 1989; Aronsen *et al.* 2013; Moura & Gonzaga 2017). When OSR is strongly male-biased and density is high, the probability of finding potential mates is expected to be high (Kokko & Rankin 2006). However, males would be expected to expend substantial amounts of energy to copulate with highly preferred females due to the intense competition over mates, thus potentially increasing their risk of injury (Rankin & Kokko 2007; Kokko *et al.* 2012). Consequently, size advantages of males in contests may not be important for mate assortment, weakening SAM (McLain 1982; McLain & Boromisa 1987b; Moura & Gonzaga 2017). On the other hand, when OSR is slightly male-biased and density is lower, dominant males confront a less intense competition, thus monopolizing copulation with high-quality females (McLain 1982; McLain & Boromisa 1987b; Crespi 1989; Moura & Gonzaga 2017). This scenario would favor positive SAM whether male competitive ability and female quality are associated with

body size. The interaction of OSR and local density would explain the dynamics of competition over mates under these two scenarios. These consequences of bias in OSR and variation in density may conduct effect of mate choice on SAM. However, to evaluate whether male-male competition can explain variations in SAM relative to variations in OSR and local density, it is necessary to first evaluate the effects of male size relative to competition for mating opportunities.

In arthropods, sexual selection has strong implications for body size evolution, especially in species with sexual size dimorphism, in which sexual selection is supposed to be more intense (review: Janicke et al., 2016; Teder and Tammaru, 2005). Larger males usually have advantages in intrasexual competition (Vieira & Peixoto 2013). Moreover, female fecundity is frequently positively correlated with size (Honěk 1993). Larger females, therefore, may be more attractive to males (Crespi 1989; Moura & Gonzaga 2017). In ants, there is little information about mate choice and intrasexual competition. Most studies about reproductive behavior of ants have evaluated strategies of colony foundation and the optimal investment ratios in reproductive individuals of colonies (Abell et al., 1999; Peeters and Molet, 2010 and references therein). SAM is also understudied in ants, probably due to the difficulty in predicting the timing of mating swarms. However, some studies have found evidence for the importance of body size in mate choice. Larger males, for example, monopolize most mating opportunities in Myrmicinae ants, such as *Pogonomyrmex desertorum* and *P. barbatus* (Davidson 1982), *P. occidentalis* (Wiernasz, Yencharis & Cole 1995; Wiernasz et al. 2001; Abell et al. 1999), and *Hypoponera opacior* (Kureck, Nicolai & Foitzik 2013). Several studies have also provided data confirming that larger females are more fecund than smaller ones (Keller & Passera 1989; Wiernasz & Cole 2003; Clémencet et al. 2008). These

conditions would favor the occurrence of SAM in ants, as predicted by the mate choice hypothesis (Ridley 1983; Crespi 1989).

Some ant species mate in aggregations (Peeters & Molet 2010) that vary in OSR and local density, according to the time of arrival of individuals, as it occurs in *Tranopelta gilva* (Wheeler, 1922) (Myrmicinae). This special condition gives us the opportunity to study how variations in OSR and local density affect the strength of SAM. We can also evaluate if an individual's size affects its mating success owing to scramble competition during nuptial flights. Larger individuals may have an advantage in locating mating aggregations first than smaller ones because they possess larger energy reserves (Wiernasz *et al.* 1995, 2001). However, because this scramble strategy has not been observed in other Myrmicinae ants, such as *Leptothorax gredleri* (Oberstadt & Heinze 2003), SAM must not be a consequence of arrivals of males and females of different body sizes. Therefore, in this study, we hypothesized that (a) *T. gilva* ants assortatively mate by size when mating competition is weak; (b) larger males win most intrasexual disputes; and (c) there is no significant variation in body size among individuals arriving earlier and later during mating aggregations.

Materials and Methods

Study area

The study was conducted in November 1, 2015 in the Parque Estadual da Serra de Caldas Novas, PESCAN (17°46'03.0"S, 48°39'37.4"W; 755 m altitude), located between the cities of Caldas Novas and Rio Quente, southwest Goiás State, Brazil. The study area consists of several types of vegetation occurring in the Brazilian Savanna (i.e., Cerrado). The climate of this regions is Aw, according Köppen-Geiger classification (Peel, Finlayson & McMahon 2007). The dry season is from April to

October and rainy season is from November to March with average temperature is 26°C and average annual precipitation is 1049 mm (Aquino, Walter & Ribeiro 2007). Data collection was developed at the balcony of the research base of PESCAN. The balcony, overlooking an area with native Cerrado vegetation, has fluorescent lamps on the roof that attracted many insects at night.

Study species

Tranopelta gilva is a Neotropical subterranean ant that feeds on termites (Mertl *et al.* 2012) and mealybugs (Delabie & Fowler 1993). Their distribution ranges from Costa Rica to Brazil and Paraguay (Fernández, 2003; Ward, 2013). Workers are usually found under rocks in clay soil or in the leaf litter of forests (Fernández, 2003; Ward, 2013). However, the biology of this species is poorly understood and there is no published information about its reproductive behavior. Males and females are often attracted by artificial lights during nuptial flights (Wheeler 1922), creating the opportunity to study their mating behavior.

Data collection

We used six, open plastic arenas with white squared surfaces (50 cm × 50 cm) held by four supports, placed at 42 cm above ground. In order to attract winged ants at night, we placed the arenas 80 cm apart in an area under artificial illumination. Nuptial flights start around 10 p.m. Thus, we collected individuals in copula during 30 min. After one hour, we initiated new collections. We classified these two scenarios based on measures of ant density and OSR that varied according to the time of arrival of individuals in mating aggregations: “weakly competitive arenas”, when OSR ranged from two to three and the number of individuals ranged from three to 10, and “intensely

competitive arenas”, when OSR ranged from five to seven and ant density exceeded 30 individuals/arena. Mating swarms under weakly competitive conditions were a consequence of the first individuals to arrive early at arenas, while those under intensely competitive conditions were determined by individuals that arrived one hour later. We did not quantify precisely the number of individuals of each sex in the arenas because they may abandon arenas during observations, promoting slight variations in OSR and density. Therefore, we provided a range of these measures and compared the occurrence of SAM between two different scenarios.

During observations in the weakly competitive arenas, we also observed eight aggressive interactions between males by mating opportunities. At the end of the observed interactions, we collected eight males that had mated successfully with females (winners) and eight males that had lost the competition (losers). The winners were also included in the evaluation of SAM. We also described mating behaviors and contests based on focal observations.

We froze all collected individuals after observations and photographed them (with a length scale in the background) using a stereomicroscope Leica EZ4 W. Then we measured head width, wing length, and Weber’s length (diagonal length of mesosoma) using ImageJ software (National Institutes of Health). Since most of the allometric measures were correlated in both sexes (Table 1), we chose Weber’s length to represent body size of *T. gilva* because Weber’s length is usually correlated with body size in ants (Weiser & Kaspari 2006). We used an analysis of covariance (ANCOVA) to evaluate the relationship between Weber’s length of males and females and the effects of competition intensity on this relationship. We also evaluated male size advantages in intrasexual competition. We considered as contests those behaviors dedicated to interrupt other male’s mating attempts or to remove a female from other

males. Winners were those males that mated successfully with females. To test size differences between winners and losers of intrasexual contests (using head, wing and Weber's lengths), we used a paired t-test. Finally, we compared the Weber's length of males and females under weak and strong MCI using a Factorial ANOVA to evaluate potential advantages of large individuals in mating search. All data analyses were conducted using R software, version 3.3.2 (R Development Core Team 2016). We used the *yarr* package (version 0.1.2) to build a jitter plot (Phillips, 2016).

Ethical note

Field work complied with the current legal and ethical requirements for animal welfare. We collected some ants, freeze, preserved in alcohol and added to collection of Laboratório de Aracnologia, Universidade Federal de Uberlândia, MG, Brazil. We also deposited voucher specimens to identification in collection of Laboratório de Mirmecologia (Convênio UESC/CEPLAC, Centro de Pesquisas do Cacau, CEPLAC), Universidade Estadual do Sudoeste da Bahia, Itana, BA, Brazil, with registration number 5784 (curator J.H.C. Delabie). This study complies with the current laws of Brazil.

Results

We collected the first 14 mating pairs observed under a behavioral condition of weak competition and the first 21 mating pairs observed under intense competition. Females were, on average, 1.7 times larger than males (Tables 2, 4). Assortative mating in *T. gilva* depended on the OSR and density. Mate assortment by size was positive when mate competition was weak, but mate assortment did not occur when mate competition was intense (Table 3, Fig. 2).

Tranopelta gilva females mated with more than one male in the intensely competitive arena, but they abandoned mating arenas after their first mating when intrasexual competition was weaker. However, we did not know if these females returned to arenas when MCI became stronger. In addition, most males died after first mating by natural causes in both scenarios. Thus, females tended to be polyandrous, while males were monogamous. Supporting the generality of our field observations, mating swarms occurred in only a night. We observed only females in the followed seven days close to arenas, but we did not find other males or mating couples. During contests, males aggressively competed for females. However, these interactions started only after female approaching. In the 16 intrasexual disputes observed in the weakly competitive arenas, males started their mating attempts just after a female approaches a male. Prior to this, males made quick, short flights around the females before start mating attempts. No male successfully separated mating pairs in both scenarios, with distinct MCIs.

Male body size did not affect its success in intrasexual disputes (head length: $t_{1,7}=0.362$; $p=0.728$; wing length: $t_{1,7}=1.62$; $p=0.150$; Weber's length: $t_{1,7}=1.44$; $p=0.193$; Table 2). In addition, there was no relationship between the sizes of either sex relative to the timing of mating swarms (Fig. 3, Table 4). Therefore, we did not find evidence to support an individual's advantage in scramble competition during mating swarms.

Discussion

Most theoretical and empirical studies about reproductive output and sexual investment of ants have focused on the optimal investment ratios in reproductive individuals (Abell *et al.* 1999; Peeters & Molet 2010). However, those studies ignored

the possibility that mating success may vary among these individuals, thus affecting individual fitness. Therefore, mating patterns of reproductive individuals may change how one interprets optimal investment ratios of colonies, especially in polygamous species, because high-quality males and females may monopolize most mating opportunities (e.g. Abell et al., 1999; Davidson, 1982; Kureck et al., 2013; Wiernasz et al., 2001, 1995). In this study, we showed how an individual's time of arrival to mating aggregation might affect its mating success, depending on its size. That is, *T. gilva* individuals mated assortatively, by size, when mating competition was weak (i.e., under conditions of slightly male-biased OSR and low density). This mate assortment behavior was stronger ($r=0.60$) than SAM usually observed in other animal populations (Jiang *et al.* 2013).

Contrary to our expectation, however, intrasexual competition between males did not explain SAM, because winners were not larger in size than losers. Although we had evaluated a small sample of competing males, differences between winners and losers were very small. Therefore, male size advantages in intrasexual competition were not evident. As we expected, we did not find any evidence to support the hypothesis that behavioral strategies in scramble competition are based on body size, because male and female sizes were similar in distinct moments after the establishment of mating aggregations. This finding corroborates observations reported in studies of other ant species, such as *Leptothorax gredleri* (Myrmicinae) (Oberstadt and Heinze 2003). These results must be carefully analyzed because we compared size of males that arrived between two different moments. However, we did not assess body size of all males that have left the colonies. Thus, it is still possible that individuals' size would influence their ability to locate arenas during mating swarms.

To our knowledge, this is the first study of ants that showed a clear positive correlation between mate's size, and also evaluated its relation with OSR and density. Understanding better which conditions may affect the occurrence of assortative mating in ants is important because it may influence the genetic variation in ant colonies, thus affecting the direction of sexual selection (Kondrashov & Shpak 1998; Hawthorne & Via 2001). However, those effects may depend on the mating system of a given ant species. Females may copulate with one or several males and males may become facultatively polygamous or monogamous (Keller, Aron & Passera 1996; Yamauchi *et al.* 1996; Helms 1999; Fournier, Aron & Milinkovitch 2002; Fournier *et al.* 2003). In *T. gilva*, we find evidences that females would be polyandrous, while males were monogamous. These mating behaviors may have ecological and evolutionary implications for the genetic variation and viability of *T. gilva* colonies. In *P. occidentalis* (Myrmicinae), for example, colony growth was negatively correlated with intracolony relatedness (Wiernasz & Cole 2003). Indeed, there is robust evidence supporting benefits of polyandry to colony viability in Myrmicinae and other ants (Keller & Reeve 1994; Evison & Hughes 2011). Therefore, the viability of an ant colony may increase when queens mate multiple times, and so the first females arriving in mating aggregations would have an advantage in mate choice, thus selecting high-quality males. Consequently, early-arriving females may copulate with higher-quality (larger) males, which increases the viability of their progeny. After a while, they may increase the mixture of sperm when competition becomes increasingly stronger.

The mate choice hypothesis assumes that individuals copulate assortatively, by size, when the advantage in intrasexual competition of large individuals is associated with a sexual preference for high-quality partners (Ridley 1983; Crespi 1989). However, this favorable scenario of competition and mate choice preference for large individuals

may depend on population parameters, which it can be estimated by OSR and density (Weir *et al.* 2011; Aronsen *et al.* 2013). Several authors have reported effects of OSR and density on SAM (McLain 1982; McLain & Boromisa 1987b; Aronsen *et al.* 2013; Vojar *et al.* 2015; Moura & Gonzaga 2017). In *T. gilva*, large males accessed more mating opportunities with large females when OSR was slightly male-biased and density was lower, thus strengthening SAM. Small individuals, in turn, had more mating opportunities with those females when OSR was strongly male-biased and density was higher, leading to random mating. These findings provide evidences that OSR and density may affect mate choice and, consequently, influence SAM.

Effects of OSR and density on mate choice and SAM are still unclear. For instance, when female availability is low or the access to mates is sequential, and not simultaneous (e.g. in populations with male-biased OSR and very low densities), males should not avoid mating opportunities with low-quality females (Kokko & Rankin 2006; Barry & Kokko 2010; Edward & Chapman 2011), inducing random matings. The mate choice hypothesis depends on the ability of one sex to find partners, to evaluate them and choosing a mate. In *T. gilva*, however, there was no temporal variation of individuals' size in mating aggregations, and males presented similar capacities to displace competitors. They also find several conspecifics in mating aggregations, leading to a situation in which the access to partners is relatively easy and simultaneous. Mating aggregations, therefore, are an effective strategy to increase partners evaluation, which give opportunities for females filtering information about male quality (Muniz *et al.* 2017). This strategy also favors male-male competition as a potential mechanism driving mate choice (Rankin & Kokko 2007).

Male-male competition did not explain the promotion of assortative mating in the weakly competitive condition of *T. gilva*. Therefore, we propose other hypotheses to

explain variations in SAM, such as female choice and male harassment. Other studies of ants found that larger males had greater mating success relative to smaller ones (Davidson 1982; Wiernasz *et al.* 1995, 2001; Abell *et al.* 1999; Kureck *et al.* 2013). Despite this, males in copula of *T. gilva* were not larger than their mating rivals in the weakly competitive condition; however, larger males mated with larger females more frequently than expected by chance. Therefore, it is possible that female choice was more important to mate assortment than male choice and male-male competition, and it would be an important mechanism conducting assortative mating in animal populations (Servedio 2015). In support of this argument, we observed male courtship behaviors and mating attempts only after a female approaches a male. Therefore, females could evaluate partners and thus choose larger males over smaller ones. Even when undesirable males were able to get close to females, females could reject copulation with these males. In *Pogonomyrmex occidentalis* (Myrmicinae), for example, females are able to interrupt copulations by biting males (Wiernasz *et al.* 2001). Therefore, female choice would strengthen SAM when mating competition is weak, as we observed in this study.

High densities may, indeed, increase opportunities to females evaluate more partners before choosing a mate (Rankin & Kokko 2007; Muniz *et al.* 2017). However, the costs of rejection of undesirable males may become higher to females, due to intense harassment from males (Kokko & Rankin 2006; Fitze & Le Galliard 2008). Consequently, females may become less selective, in that they accept copulation with males regardless of size. Thus, intense sexual competition may favor random mating and weak SAM, as we also observed in this study. In the lizard, *Lacerta vivipara*, for instance, females became less choosy (due to intense harassment from males) when sex ratios became more skewed towards males (Fitze & Le Galliard 2008). This pattern was

also observed in the stream water strider *Aquarius remiges* (Hemiptera, Gerridae). In the mosquito *Aedes aegypti*, the costs of resistance to forced copulation attempts were high, because experimental increases in population density lead to a reduced female longevity and a low reproductive rate (Helinski & Harrington 2012). Other studies have reported similar results of OSR and density on male harassment (Sih & Krupa 1995; Jirotkul 1999; Wigby & Chapman 2004; Smith & Sargent 2006). Likewise, in *T. gilva*, male harassment was also intense when OSR was strongly male-biased and density was higher. Consequently, small males may explore these hot spot areas to increase their mating opportunities (Krupa & Sih 1993). We observed from three to six males harassing a single female when sexual competition was intense, while only two males harassed a female when mating competition was weaker. Therefore, in the intensely competitive arenas, smaller males are capable to access mating opportunities with large females due to intense harassment and get its fecundity benefits, leading to random mating. We hypothesized that females (regardless of size) may copulate with more males when mating competition is stronger compared to weakly competitive scenarios, but this hypothesis must be tested with further study.

In conclusion, we have shown how variations in OSR and density, through the timing of an individual's arrival in artificial mating aggregations, may affect SAM in *T. gilva*. Consequently, scramble competition during nuptial flight may have behavioral and evolutionary implications on male and female fitness and viability of colonies, despite the fact that we did not identify any size relationship between sexes and the relative time of arrival in mating aggregations. Our findings are consistent with current sexual selection theory, which assumes that intense competition by mates should reduce mate choosiness (Bonduriansky 2001; Jennions & Petrie 2007) and, consequently, weaken SAM (McLain 1982; McLain & Boromisa 1987b; Crespi 1989; Aronsen *et al.*

2013; Moura & Gonzaga 2017). Further studies may test predictions of the hypotheses proposed here, by experimentally evaluating effects of female choice and male harassment on SAM according to variations in OSR and density.

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Figures

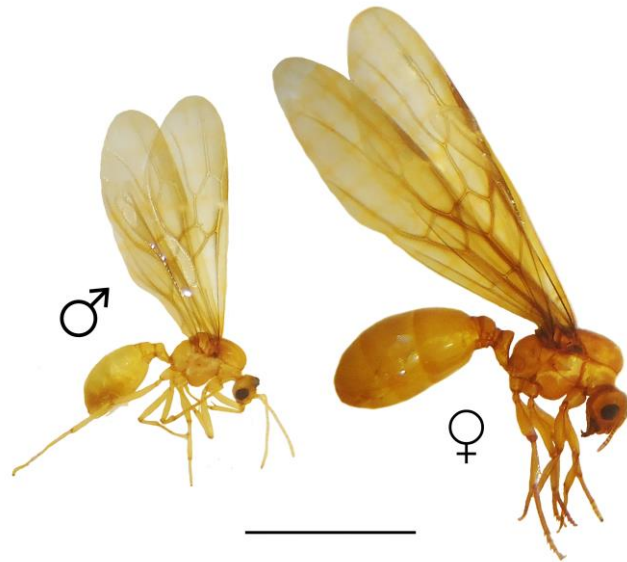


Figure 1. Male and female of *Tranopelta gilva*. Scale: 5 mm.

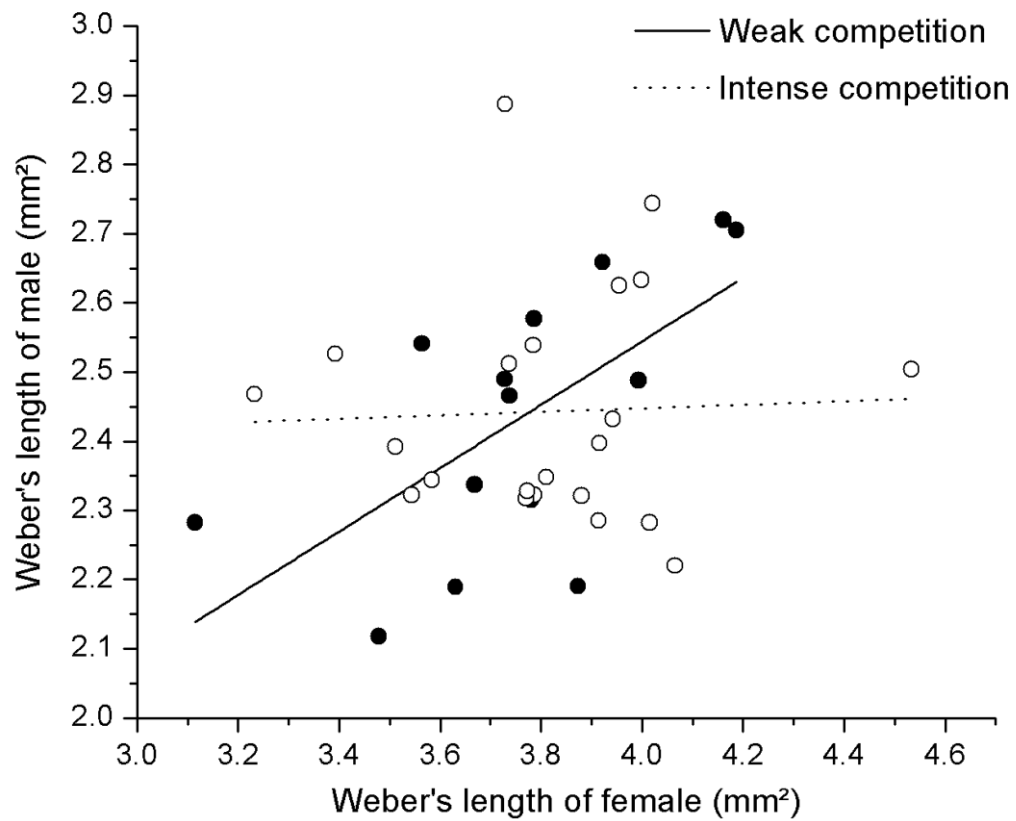


Figure 2. Relationship between Weber's length of male and female *Tranopelta gilva* according to mating competition (weak and intense).

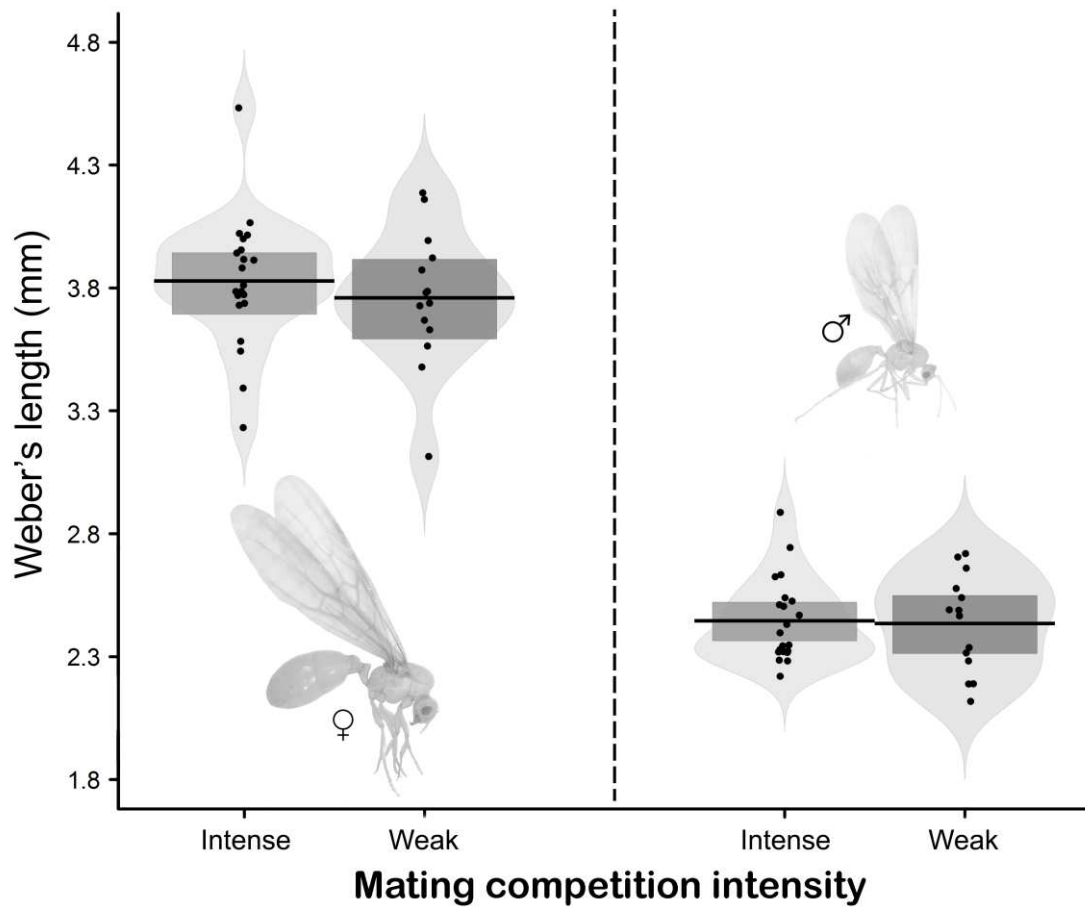


Figure 3. Comparison males and females in copula of *Tranopelta gilva* (Weber's length) according to mating competition (weak and intense). Dots, central lines and boxes for each category correspond to size measures, means and standard errors.

Tables

Table 1. Pearson's correlations of allometric measures of *Tranopelta gilva* males and females.

Sex	Variables	r-value	N	p-value
Male	Head width x Wing length	0.353	36	0.035
	Head width x Weber's length	0.150	36	0.381
	Wing length x Weber's length	0.503	36	<0.001
Female	Head width x Wing length	0.528	36	0.001
	Head width x Weber's length	0.378	36	0.023
	Wing length x Weber's length	0.464	36	0.004

Table 2. Measures of body size of males and females of *Tranopelta gilva*, winners and losers of intrasexual competition. Values correspond to mean \pm standard error (sample size in parentheses).

Measures/Individuals	Head width (mm)	Wing length (mm)	Weber's length (mm)
Male	1.19 \pm 0.02 (36)	8.03 \pm 0.08 (36)	2.43 \pm 0.03 (36)
Female	2.12 \pm 0.02 (36)	13.49 \pm 0.15 (36)	3.79 \pm 0.05 (36)
Winner	1.16 \pm 0.03 (8)	8.25 \pm 0.15 (8)	2.40 \pm 0.07 (8)
Loser	1.14 \pm 0.05 (8)	7.89 \pm 0.19 (8)	2.24 \pm 0.07 (8)

Table 3. Results of ANCOVA, testing effects of mating competition on the relationship between Weber's length of males and females of *Tranopelta gilva* ($r^2=0.194$).

Source of variation	D.f.	Sum of squares	F-ratio	P-value
Size-assortative mating	1	0.0962	3.3992	0.07
Mating competition	1	>0.001	0.0014	0.97
Interaction	1	0.1148	4.0526	0.05
Residuals	31	0.8779		
Total	34	1.0890		

Table 4. Values of factorial ANOVA using Weber's length of *Tranopelta gilva* individuals, as the response variable, and sexes and mating competition, as explanatory variables.

Variables	Sum of Squares	D.f.	F-value	p-value
Sex	32.333	1	609.260	<0.0001
Mating competition	0.027	1	0.510	0.478
Interaction	0.014	1	0.260	0.612
Residuals	3.503	66		
Total	35.877	69		

Capítulo 2

Spatial variation in sex ratio and density explain subtle changes in the strength of size-assortative mating in *Edessa contermina* (Hemiptera: Pentatomidae)

Abstract

Size-assortative mating (SAM) is usually explained by the mate-choice hypothesis. However, mate choice depends on an ideal scenario to have an influence on SAM. For examples, in populations with unbiased or slightly male-biased operational sex ratio (OSR) and low density, large individuals will have more access to mating opportunities with large, preferred females (i.e. mating competition hypothesis). We investigated effects of mating competition and mate choice on the strength of SAM. We also described the mating system and sexual size dimorphism of *Edessa contermina* (Hemiptera: Pentatomidae) in a conservation area of the Brazilian savannah. The mating system of *E. contermina* was promiscuous, and multiple copulations occurred, solely on *Byrsonima verbascifolia* (Malpighiaceae) plants. Sexual size dimorphism was female-biased. SAM was observed in individuals regardless of the mating competition, but it was stronger under intensely competitive conditions. Thus, mating competition hypothesis explained the fine-grained variation in the strength of SAM in *E. contermina*. Males exhibited SAM in both first and second matings, while females randomly mated regardless of size. Male mating patterns, however, were not explained by intrasexual competition. In addition, male and female size did not determine the probability of mating with at least two partners. Therefore, we found evidence to support SAM in males mating with at least two partners. In conclusion, we propose a

new hypothesis based on the modern theory of sexual selection that may explain the fine-grained variation in SAM.

Key-words: Pentatomidae, OSR, population density, assortative mating, mating competition intensity

Introduction

Size-assortative mating (SAM) in animals is a positive or negative correlation between the body sizes of mating pairs (Burley 1983; Ridley 1983). SAM may be a result of the following mechanisms: (1) ‘mate choice’, where preference for a specific phenotype influences the establishment of mating pairs; (2) ‘mate constraints’, where there are physical and/or physiological restrictions for mating between individuals with different sizes; (3) ‘mate availability’, where there is spatial or temporal overlapping of males and females with similar body sizes (Ridley 1983; Crespi 1989); or (4) ‘prudent habitat choice’, a situation in which small individuals with weaker competitive ability ignore high-quality habitat patches with strong competition for mating (Härdling & Kokko 2005; Taborsky *et al.* 2014).

Mate choice is often assumed to be the main mechanism influencing SAM (Crespi 1989; Hoefler 2007; Baldauf *et al.* 2009; Jiang *et al.* 2013). This hypothesis predicts that SAM depends on mate preference by large partners in one or both sexes, large individuals’ advantage in intrasexual competition, their preference for large partners, and variations in mates’ quality related to their size (Crespi 1989; Moura & Gonzaga 2017). However, according to the sexual selection theory, mate choice may vary according the intensity of competition by mating opportunities (Arak 1983; Baur 1992; Harari *et al.* 1999; Aronsen *et al.* 2013; Moura & Gonzaga 2017). When mating

competition increases, male contests for high-quality partners may become intense, reducing the competitive advantages of large individuals and the potential of mate monopolisation (Forsgren *et al.* 2004; Weir *et al.* 2011; Aronsen *et al.* 2013; Moura & Gonzaga 2017). Females would also be subject to intense harassment from males, thus decreasing their choosiness and leading them to accept mating attempts from males, regardless of their size (Krupa & Sih 1993; Smith 2007; Fitze & Le Galliard 2008).

The scenario of intense competition weakens SAM and leads to random mating (Moura & Gonzaga 2017). However, when mating competition decreases, large males may win more intrasexual contests, monopolising mating opportunities with large, high-quality females (Weir *et al.* 2011; Moura & Gonzaga 2017). Females may also reject the mating attempts of unattractive males (Fitze & Le Galliard 2008). This alternative scenario would favour positive SAM (Moura & Gonzaga 2017). Thus, mating competition should act as a complementary mechanism, creating ideal conditions to mate choice affects SAM.

Mating competition can be measured using population parameters, such as operational sex ratio (OSR; i.e. the number of adult males per sexually receptive females) (Moura & Peixoto 2013; Vojar *et al.* 2015), and density (Arak 1983; Baur 1992; Krupa & Sih 1993; Harari *et al.* 1999; Aronsen *et al.* 2013; Moura & Gonzaga 2017). In populations with slightly male-biased or unbiased OSR, mating competition should be weak, and thus large males may monopolise most mating opportunities with high-quality females (Weir *et al.* 2011; Moura & Gonzaga 2017). Therefore, SAM would be positive. However, in populations with highly male-biased OSR, the mating competition increases, thus increasing the costs of competition (Weir *et al.* 2011; Moura & Gonzaga 2017). As a result, males would be less choosy and copulate with females

regardless of size to avoid intrasexual competition, thus weakening SAM and leading to random mating (Crespi 1989; Moura & Gonzaga 2017).

Additionally, variations in population density may also influence SAM. At low population densities, males may have difficulty in finding females (Kokko & Rankin 2006; Muniz *et al.* 2017). Consequently, they may not reject mating opportunities with females regardless of their size, weakening SAM (Moura & Gonzaga 2017). At high densities, on the other hand, the encounter rate of individuals increases and, consequently, males may evaluate more partners, thus investing more energy in competition for high-quality partners (Kokko & Rankin 2006; Muniz *et al.* 2017). This scenario would favour a positive SAM (Moura & Gonzaga 2017). However, in highly dense populations, the costs associated with intrasexual competition and male mating harassment of females may increase, reducing mate choosiness (Rankin & Kokko 2007; Kokko *et al.* 2012) and weakening SAM (Moura & Gonzaga 2017). Population density, therefore, may have a threshold where the mating competition is low enough to favour the occurrence of a positive SAM. Several empirical studies have reported the effects of density on SAM (McLain 1982, 1988, McLain & Boromisa 1987a; b; Crespi 1989; Moura & Gonzaga 2017).

In arthropods, large individuals' advantage in contests (Vieira & Peixoto 2013) and the positive correlation between female fecundity and body size (Honěk 1993) may lead to mating preferences between large individuals (Andersson 1994) and the potential occurrence of SAM (Crespi 1989; Jiang *et al.* 2013). These effects would be stronger in species in which the sexes experience varied selection pressures, leading to sexual size dimorphism (Teder & Tammaru 2005; Janicke *et al.* 2016).

In stink bugs, body size is an important, inheritable attribute that influences mate choice (McLain 1987). Intrasexual competition is frequently related to courtship

efficiency, copulation duration, and mating disputes, including physical contact between males (McLain 1981; Capone 1995; Pereira *et al.* 2017). Large females lay more viable eggs (McLain & Marsh 1990; Capone 1995) and a larger number of eggs per clutch (Capone 1995). The number of eggs laid is also correlated to the body size of the male, probably due to the nutritional content of male ejaculation (McLain 1980; McLain *et al.* 1990; McLain and Marsh 1990 – but see Pereira *et al.* 2017). Consequently, individuals may prefer to copulate with larger partners rather than smaller ones (McLain 1987; McLain *et al.* 1990; Capone 1995).

The conditions observed in stink bugs may favour the occurrence of positive assortative mating. However, there are no published studies that show a positive correlation between body size of mating pairs in pentatomid species. To our knowledge, there are only discussions about implications of mate choice to SAM in *Acrosternum hilare* (Capone 1995), and about non-random mating based on size in *Nezara viridula* (McLain *et al.* 1990). Therefore, SAM is poorly understood in Pentatomidae, despite the family having conditions that favour its occurrence. In addition, stink bugs are good models to test potential mechanisms influencing SAM, because mating pairs and sexes are easy to observe, handle, mark and recapture.

Here, we used *Edessa contermina* Walker, 1868 (Hemiptera: Pentatomidae) as a model species to evaluate the effects of mating competition and mate choice on SAM. This phytophagous insect mates and feeds on plants of *Byrsonima verbascifolia* (L.) Rich (Malpighiaceae) in the Brazilian savannah (Moura *et al.* 2017). Each plant acts as an arena to attract individuals, where they compete and court sexual partners (Moura *et al.* 2017). This scenario creates a fine-grained variation in OSR and population density, and provides an ideal scenario to evaluate the effects of (1) mating competition and (2) mate choice as potential mechanisms conducting SAM. For this approach, we marked

and monitored *E. contermina* individuals over five days to describe their mating system and mating strategies. Then, we tested the following predictions: (1) body size correlation between the sexes is stronger when the population density is lower and the OSR is less skewed to males; (2a) the large competing males win the contests for females; (2b) large individuals have a higher probability of mating with a new partner; (2c) both sexes consistently mate with partners of similar size in the first and second observed copulations.

Materials and Methods

General information

Edessa contermina is a Neotropical stink bug species of the subfamily Edessinae (Schuh & Slater 1995; Grazia *et al.* 2015). Individuals feed on plant sap from the inflorescences of *B. verbascifolia* (Fig. 1). Males compete against other males by courting and copulating with females on these inflorescences (Moura *et al.* 2017). *Edessa contermina* also exhibit feeding behaviour during copulation, suggesting that the exudate may affect the reproductive performance of individuals (Moura *et al.* 2017). There is no parental care, because egg masses were not under the protection of parents. Mating pairs also avoid plants with higher number of nymphs, probably, to reduce competition between these nymphs and their own progeny (Moura *et al.* 2017).

We collected, marked and recorded mating pairs in less warm times of the day (7:30 and 12:00 a.m. and 3 p.m. to 6:30 p.m.) between November 11 and 15, 2015. We also observed cases of two males courting a female between October 23 and November 06, 2016 and November 06 and 10, 2017, totalizing 135 hours of field observations. We undertook data collections in the Parque Estadual da Serra de Caldas Novas (17°46'03.0"SE, 48°39'37.4"W), located between the cities of Caldas Novas and Rio

Quente, southwestern Goiás State, Brazil. The study area was composed of cerrado *sensu stricto* vegetation (Batalha, Mantovani & Mesquita Júnior 2001; Silva *et al.* 2002). The climate of this regions is Aw, according Köppen-Geiger classification. The dry season is from April to October and rainy season is from November to March (Aquino *et al.* 2007).

Data collection

We marked 27 plants of *B. verbacifolia* with inflorescences in the cerrado area close to a trail (100 m × 10 m study area). Then, we searched daily for mating pairs of *Edessa contermina* in the inflorescence of the marked plants, for a period of five days. We also recorded the number of males and females in each plant, to estimate OSR and local population density, and the number of inflorescences as an estimation of plant size.

When we found a mating pair, we collected the couple with a pot of 100 ml, containing a size scale on the bottom. Then, we photographed the mating pairs, and marked individuals with a single colour combination using acrylic paint. In the following days, when we found marked individuals mating with a new partner, we also photographed them using the same procedure, and marked them with a new colour combination. We processed the photographs using ImageJ software (National Institute of Health, USA) to measure length (l), from the anterior tip of the pronotum to the posterior tip of abdomen, and width (w), from the widest part of abdomen, of each individual. Finally, we estimated the body dorsal area of individuals as an ellipse ($S = \pi \times 0.25 \times l \times w$).

During the data collection period in 2016 and 2017, we noticed some instances where two males were courting a female. After a while, one of these males initiated the

copulation. In those cases, we collected both males, to photograph them alongside a scale for size, and to measure their body area, as described above. We measured size from 16 males (eight pairs) in 2016 and eight males (four pairs) in 2017. We used these measurements to assess whether large males had an advantage in intrasexual contests. We also tried to conduct experiments in laboratory manipulating OSR and density with sexually receptive adults of *E. contermina*. However, individuals did not survive or copulate in 48 h of experiment, probably, because they depend on a source of nutrients, such as inflorescences.

Data collection complied with the current legal and ethical requirements for animal welfare. We collected some stink bugs, freeze, preserved in alcohol and added to collection of the Laboratório de Aracnologia, Instituto de Biologia, Uberlândia, Minas Gerais, Brazil (M. O. Gonzaga, curator). The species was identified by Prof. José A. M. Fernandes from the Instituto de Ciências Biológicas, Universidade Federal do Pará, Pará, Brazil.

Statistical analysis

Population density exhibited bimodal distribution and was positively correlated with the OSR (Spearman test: $r_s = 0.627$, $n = 27$, $p = 0.0005$, Fig. 2). Thus, we classified mating competition into two conditions: “weakly competitive” (density: 3.31 ± 0.95 , 2–5; OSR: 1.02 ± 0.45 , 0.3–2), and “intensely competitive” (density: 7.55 ± 1.21 , 7–10; OSR: 1.7 ± 0.56 , 1.3–2.5). OSR was more skewed toward males under the intensely competitive condition, according to the assumptions of the mating competition hypothesis (Kruskal–Wallis test: $U = 14.581$, $df = 1$, $p = 0.0001$).

In response to our hypotheses, (1) to evaluate the occurrence of SAM and its association with mating competition, we conducted a General Linear Mixed Model

(GLMM) using the restricted maximum likelihood (REML) and normal distribution from residuals. We used the male body area as the response variable, the female body area as the covariate and mating competition categories as the predictor. We also control for potential sources of bias by including plant identity and sampling day as random variables and number of inflorescences as covariable. Due to limitations of GLMM, we were not able to calculate a R^2 for each straight. Therefore, we estimate R^2 values using simple linear models. We also calculate values of $R^2_{\text{GLMM(m)}}$ as a measure of goodness fit of predictors and $R^2_{\text{GLMM(c)}}$ as a measure of goodness fit of all model, including random variables (Nakagawa & Schielzeth 2013).

We included only the first mating of each individual in this analysis to avoid pseudoreplication. (2a) To evaluate the potential size advantage for large males in mating competition, we performed another GLMM with REML using male size as response variable, the status of copulation (copulating or non-copulating) as predictor variable, and the pair of males competing for a female as random factor. (2b) We compared the probability of males and females mating again according to their body size using logistic regressions. We considered the condition in which males and females mated once as “0”, and the condition in which individuals mated twice as “1”. (2c) We conducted two ANCOVA for males and females that mated twice. We used male size as response variable, female size as covariate, and the mating order (first and second mating) for each sex as predictor. We performed all data analyses using R software version 3.4.1 (R Development Core Team 2017).

Results

Population demography and mating system of E. contermina

We found a mean of 44 individuals and 11 mating pairs per day of observation (Table 1). The mean OSR and population density were 1.1 and 1.93 individuals by plant, respectively (Table 1). We marked 61 individuals and recaptured 31 (50.8%). Among these, 14 individuals (seven males and seven females) were mating with an additional partner (45.16%). One male and one female copulated with three partners. Therefore, the mating system of *E. contermina* can be considered as promiscuous. We also observed two marked couples mating for more than 24 hours. We found 10 marked individuals located on the same plants as they were initially found (three males and seven females), 10 marked individuals on different plants located less than 10 m from their initial plant (eight males and two females), and six marked individuals on plants located more than 10 m from their initial location (two males and four females). Plants of *B. verbacifolia* presented, on average, 5.3 ± 0.7 , 1-12 (mean \pm se, min.-max.) inflorescence stems.

Mating competition and size-assortative mating

We found 27 mating couples: 16 couples under the weakly competitive condition and 11 under the intensely competitive condition. Females of *E. conterminal* were, on average, 1.22 times larger than males (Table 1). Male body area was equal to $61.80 \pm 1.16 \text{ mm}^2$ (mean \pm standard error) and female body area was equal to $75.12 \pm 1.54 \text{ mm}^2$. SAM was positive regardless of mating competition categories (Table 2). Assortative mating was stronger in the condition of intense competition ($R^2=0.39$) than in the condition of weak competition ($R^2=0.28$) (Fig. 3). Most the variance was explained by our full model ($R^2_{\text{GLMM}(c)}=0.92$), but we found a less fit for our predictors ($R^2_{\text{GLMM}(m)}=0.28$).

Mate-choice hypothesis

Males positively assortatively mated based on size in the first and second matings, while females randomly copulated by size in both matings (Fig. 4, Table 3). SAM in the first mating of males was stronger ($r^2 = 0.62$) than that in the second mating ($r^2 = 0.23$). However, we found no evidence to support the size advantage of large males in intrasexual disputes ($F_{1,11} = 0.893$, $p < 0.001$). Most the variance was explained by the predictors ($R^2_{\text{GLMM(m)}}=0.37$) compared to the full model ($R^2_{\text{GLMM(c)}}=0.37$). In addition, non-mating males ($55.49 \pm 1.15 \text{ mm}^2$, ranging from 50.89 to 63.38 mm^2) were similar in size to mating males ($53.95 \pm 1.15 \text{ mm}^2$, ranging from 46.24 to 60.40 mm^2).

We observed males surrounding a female, shaking their abdomens and touching the female with the antennae during courtship. After a couple initiate the copulation, non-mating males continued to surround it, touching both individuals with their antennae. However, no take-over attempts were observed. Additionally, male and female size did not influence the probability of copulation occurring once or twice (females: $\chi^2 = 0.006$, $df = 29$, $p = 0.936$; males: $\chi^2 = 0.304$, $df = 30$, $p = 0.581$). Males that mated only once ($62.19 \pm 1.36 \text{ mm}^2$; range from 44.41 to 75.66 mm^2) were similar in size to males that mated twice ($60.69 \pm 1.98 \text{ mm}^2$; range from 52.65 to 67.62 mm^2). Females that copulated once ($74.79 \pm 1.66 \text{ mm}^2$; range from 63.83 to 94.47 mm^2) also had a similar average size to females that mated twice ($74.53 \pm 2.58 \text{ mm}^2$; range from 65.55 to 83.05 mm^2).

Discussion

Mate-choice hypothesis is often assumed to be the main mechanism affecting the strength and direction of SAM (Crespi 1989; Jiang *et al.* 2013). However, mate choice requires a suitable scenario, with strong sexual selection intensity on body size,

to affect SAM (Arak 1983; Baur 1992; Harari *et al.* 1999; Aronsen *et al.* 2013; Moura & Gonzaga 2017). We proposed that such scenario depend on mating competition, based on effects of OSR and population density. Higher biases toward males in OSR and high densities in a population should intensify male-male competition, increasing the cost of competing for a high-quality mate. Therefore, males should avoid conflicts or be unable to evaluate potential partners and choose a high-quality mate to dispute (Weir *et al.* 2011). Consequently, SAM would be weakened. We also tested mate choice predictions on promotion of SAM. *Edessa contermina* individuals assortatively mated by size in both scenarios of mating competition intensity (Table 2). However, SAM had stronger effects on the assortment of individuals with similar size under the intensely competitive condition. Males were choosier than females and assortatively mated based on size in both first and second matings, while females randomly copulated based on size (Table 3). The male mating patterns, however, were not explained by intrasexual competition, because large and small males had similar mating opportunities with a female during contests. Associated with the support for mate choice, the mating competition promoted a fine-grained variation in the strength of SAM. This corroborates the fact that the *E. contermina* individuals experienced a suitable scenario for sexual selection to influence SAM.

Almost half of the individuals (45.16%) mated with at least an additional partner, while 54.84% of *E. contermina* mated with only one partner during five days of observation. Therefore, the mating system of *E. contermina* was monogamous and promiscuous. However, the frequency of promiscuity may be higher in longer-term samplings, because both sexes frequently mate with several partners in other pentatomid species (McLain *et al.* 1990; Capone 1995; Pereira *et al.* 2017). Although *E. contermina* individuals are often promiscuous, some individuals of both sexes may adopt a

monogamous strategy. However, we did not find support to this hypothesis because there was no difference in size between individuals that copulated once and those who copulated twice.

Edessa contermina individuals were able to move between all plants in the study area, but they tended to search for mates in plants that were located close to the position where they were originally found. Additionally, large females did not reject copulation with small males, while large males preferred to mate with large females, leading to positive SAM in the first and second matings. Male choice, therefore, was more important than female choice in inducing SAM. In addition, *E. contermina* males did not aggressively fight for females. Mating competition was based in the intensity of courtship. Males surrounded a female touching her with their antennae and vibrating their abdomens. However, male size did not affect its mating success. Males were also unable to dislodge a mating pair.

Pentatomid males usually compete by adjusting copulation time (McLain 1981), or focusing on virgin females (Capone 1995). Copulation duration, for example, may increase when the OSR is slightly male-biased (4:2 male/female) than female-biased (2:4 male/female) (McLain 1981). Therefore, a male-biased OSR may increase mating competition and the investment in copulation, consequently, strengthen SAM. In *E. contermina*, positive SAM was stronger when the mating competition was intense (OSR = 1.7 ± 0.56 ; density = 7.55 ± 1.21). However, decreases in OSR weakened mate assortment, but it still led to a positive SAM. Extreme increases in mating competition, otherwise, may lead to random mating. In the ant *Tranopelta gilva*, for example, SAM was positive when the OSR ranged from two to three and population density varied among 2-5 individuals/m² in weakly competitive conditions, but mating was random

when the OSR ranged from five to seven and population density reached more than 20 individuals/m² in intensely competitive conditions (Moura and Gonzaga, in prep.).

In conclusion, we found evidences supporting the male mate choice hypothesis in *E. contermina*. Males that mated with at least two females were particular about size, leading to positive SAM in first and second observed mating. We also found evidence to support our predictions of fine-grained variation in SAM, explained by the mating competition hypothesis. Males and females assortatively mated based on size in both scenarios of mating competition. However, assortative mating was stronger under weakly competitive conditions compared to that under intensely competitive conditions. Thus, we developed empirical support for a new hypothesis that provides a basis to identify ideal scenarios in which sexual selection effects conduct mate choice and its fine-grained effects on SAM. To our knowledge, this is also the first study reporting SAM in a Neotropical pentatomid species. On the other hand, we expect that other stink bug species may also assortatively mate based on size and provide opportunities to test predictions of the mating competition hypothesis.

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Figures

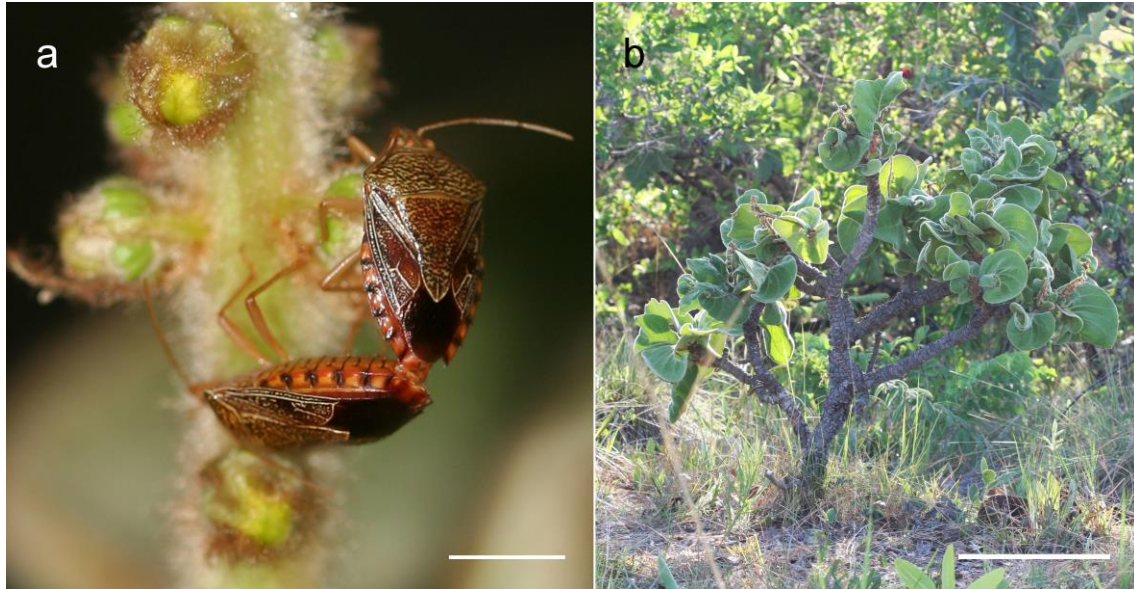


Figure 1. (A) *Edessa contermina* individuals copulating in an inflorescence of a *B. verbascifolia* plant. Scale: 10 mm. (B) A *Byrsonima verbascifolia* plant from Parque Estadual da Serra de Caldas Novas, Goiás, Brazil. Scale: 50 cm.

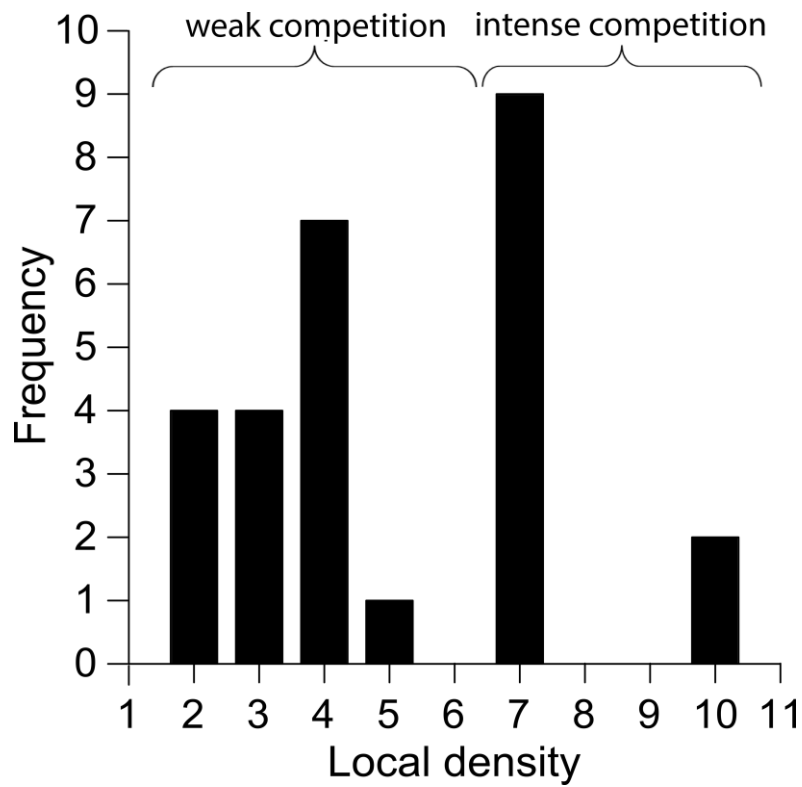


Figure 2. Categories of mating competition intensity according to density.

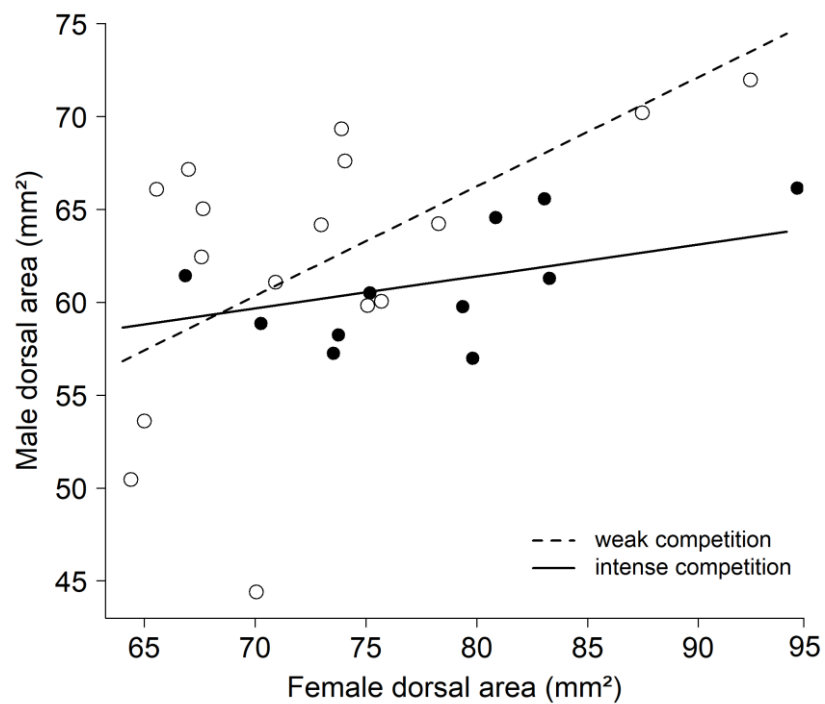


Figure 3. Size-assortative mating in *Edessa contermina* according to mating competition intensity groups (weak and strong).

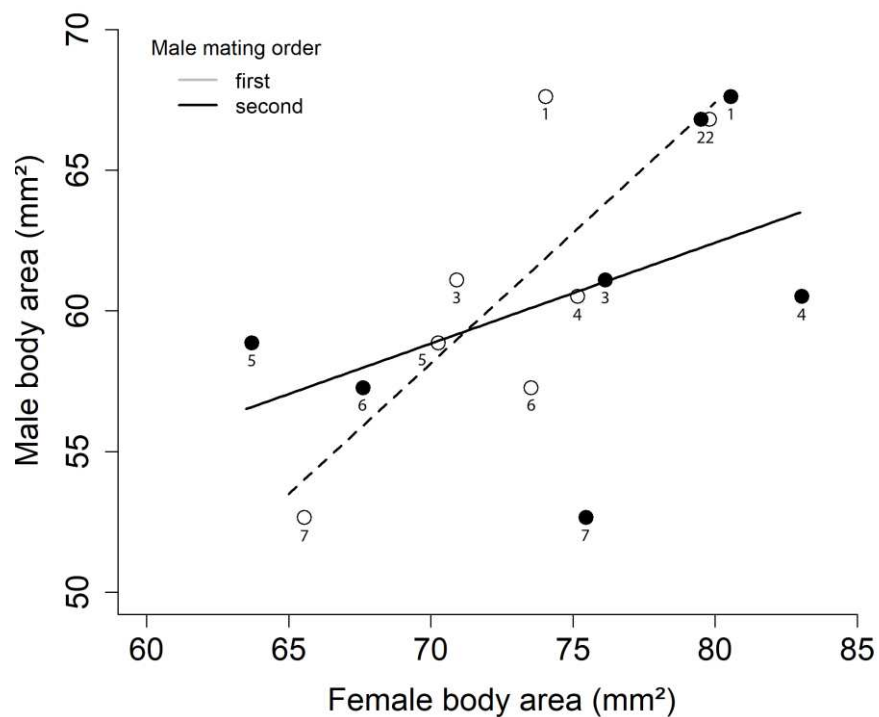


Figure 4. Size-assortative mating in *Edessa contermina* according to male mating order (first and second matings).

Tables

Table 1. Population of *Edessa contermina* observed on 27 *Byrsonima verbascifolia* plants during November 2015 in the Parque Estadual da Serra de Caldas Novas, GO, Brazil. In estimation of OSR, we included plants with at least a male and a female.

Day	Nº individuals	Nº mating pairs	Nº males	Nº females	OSR	Density (individuals/plant)
1º	44	10	23	21	1.12±0.12 (12)	2.32±0.42
2º	54	16	29	25	1.17±0.24 (10)	2.25±0.60
3º	45	12	24	21	1.47±0.22 (10)	1.96±0.49
4º	44	11	23	21	1.30±0.14 (11)	1.63±0.33
5º	34	5	17	17	1.10±0.16 (7)	1.48±0.33

Table 2. Results from a GLMM male body area of *E. contermina*, as response variable, female body area, mating competition categories and their interaction, as predictors, and plant identity and sampling day as random variables. * represents values of $p < 0.05$.

Source of variation	<i>df</i>	<i>F</i>	<i>P</i>
Intercept	15	2131.07	<0.001*
Female body area (FBA)	5	18.87	0.007*
Mating competition categories (MCC)	2	0.68	0.495
FBA * MCC	5	8.85	0.031*
N° of inflorescences	5	0.35	0.582

Table 3. Results from two ANCOVA using male body area as response variable, female body area as covariate and mating order (first and second mating) for each sex as predictor. * represents values of $p < 0.05$.

Source of variation	<i>df</i>	Sum of squares	<i>F</i>	<i>P</i>
<i>Male remating</i> (model: $r^2=0.427$)				
Size-assortative mating	1	108.29	5.710	0.038*
Mating order	1	5.21	0.274	0.612
Interaction	1	27.63	1.457	0.255
Residuals	10	189.63		
<i>Female remating</i> (model: $r^2=0.128$)				
Size-assortative mating	1	27.97	0.823	0.385
Mating order	1	0.01	0.0002	0.988
Interaction	1	57.31	1.692	0.223
Residuals	10	338.78		

Capítulo 3

Effects of mating competition on size-assortative mating: a meta-analysis

Abstract

Sexual selection is supposed to promote sympatric speciation because of mate choice, however, levels of male-male competition and female availability may affect mate assortments. Intense intrasexual competition may increase costs of copulation with high-quality partners, weakening size-assortative mating (SAM). Likewise, low female availability may have equivalent effects on SAM, because males should not reject found that are found only rarely partners regardless of their size. Conditions of low male-male competition and high female availability may favor SAM, such as when operational sex ratio (OSR) is female-biased and populational density is high. In this study, we used a meta-analytical approach to investigate general patterns of SAM in animals and potential spatio-temporal variation in its occurrence and strength. We also evaluate effects of mating competition on SAM, controlling scale-of-choice effects. We then hypothesized that SAM benefits from decreasing mating competition intensity. Thus, we tested the following predictions: (1) effect size of SAM will be negatively correlated with OSR; (2) density will be positively correlated with effect size; and (3) OSR and density will explain variations in effect sizes. We found that average assortative mating was positive, weak, and consistent throughout space and time. The OSR was negatively related with SAM, but the strength of mate assortments was higher in populations with unbiased OSR. Density, on the other hand, did not explain variation in mate assortment, and had no association with OSR. We also found weak bias of scale-of-choice effects. The meta-analytical models presented robust evidence that sexual selection has effects

on SAM across a wide range of taxonomic groups. We also discuss potential implications of these results with insights into sexual selection effects on sympatric speciation and future research.

Key-words: mate assortment, mating opportunities, male-male competition, scale-of-choice effect, spatio-temporal variation

Introduction

Assortative mating is defined as a positive or negative correlation between phenotypic (or genotypic) traits of mating pairs (Burley 1983; Ridley 1983). Mate assortment has been largely documented across several taxa (Crespi 1989; Jiang *et al.* 2013), such as Annelida (Monroy *et al.* 2005), Arthropoda (Brown 1993; Hernández *et al.* 2012; Moura & Gonzaga 2017), Chordata (Cuadrado 1999; Robart 2012; Seki *et al.* 2012; Sheppard *et al.* 2013; Green 2014), Mollusca (Clarke *et al.* 2013), and Platyhelminthes (Michiels & Streng 1998). It may depend on different partners' traits associated with mate quality, such as age (Friedl & Klump 2005), behavior (Kralj-Fišer *et al.* 2013), body condition (Sharp & Agrawal 2009), and genotype (García-Navas *et al.* 2009). However, the most common pattern of mate assortment in animals is a correlation between body sizes of mating pairs, called size-assortative mating (SAM) (Burley 1983; Jiang *et al.* 2013).

Identifying patterns of SAM is pivotal for evolutionary, ecological, and conservation studies, because they may direct the evolution of body size and promote reproductive isolation and, consequently, sympatric speciation (Kirkpatrick & Ravigne 2002; Jones *et al.* 2003; Olafsdottir *et al.* 2006; Ritchie 2007; Gauthey *et al.* 2016; Kopp *et al.* 2018). Positive assortments, for example, may lead to disruptive selection

on body size, favoring sympatric speciation, while negative assortment should increase gene flow among different phenotypes (Jiang *et al.* 2013). Assortative mating, however, needs to be consistent in space and time to affect gene flow of a population (Johannesson *et al.* 1995; Schwartz 2013; Nonaka *et al.* 2014; Gauthey *et al.* 2016; Ng *et al.* 2016). Most studies, however, did not evaluate this variation and took conclusions based on a single measure of mate assortment in a population (Bourne 1993; Apanius & Nisbet 2006; Alcantara *et al.* 2007; Jiang *et al.* 2013). This approach may provide a biased scenario of SAM because a single measure may represent a pattern of assortative mating determined by conditions of a specific moment of the breeding season or exceptional conditions in which that population was submitted. Therefore, it may underestimate SAM implications on body size variation in animal species (Johannesson *et al.* 1995; Ng *et al.* 2016). Indeed, empirical studies have reported evidence that the occurrence of mate assortments and its strength may change over space and time (Miyashita 1994; Bel-Venner *et al.* 2008; Schwartz 2013; Ng *et al.* 2016; Moura & Gonzaga 2017).

Observations of mating pairs and the evaluation of the size of pairing individuals in the field are available for a few species (Jiang *et al.* 2013; Ng *et al.* 2016). Consequently, researchers may incur in a second mistake presenting estimations of SAM. To increase statistical power, several measures of SAM of different populations (Jones & Montgomerie 1991; Warkentin *et al.* 1992; Forero *et al.* 2001) and/or more than one breeding season (Delestrade 2001; Christensen & Kleindorfer 2007; Bourgeois *et al.* 2014) are usually pooled. However, individuals may naturally vary in size among populations and breeding seasons (Johannesson *et al.* 1995; Ng *et al.* 2016; Moura & Gonzaga 2017). Consequently, there is a bias to positive assortative mating in pooled data; SAM can be confused with this variation in body size among populations and

throughout time, which it is called a scale-of-choice effect (Johannesson *et al.* 1995; Rolán-Alvarez *et al.* 2015; Ng *et al.* 2016). Thus, estimates of SAM can be a consequence of scale-of-choice effects and not reflect mating patterns of the species.

Another important mechanism influencing sympatric speciation is male-male competition (Jones *et al.* 2003; Lackey & Boughman 2013; Keagy, Lettieri & Boughman 2016; Tinghitella *et al.* 2018). Males are usually the competitive sex, while females are the choosier sex (Darwin 1871; Bateman 1948; Andersson 1994). Although parental care and other alternative reproductive tactics may influence sexual behaviors and even invert sex roles (Andersson 1994; Forsgren *et al.* 2004), there is strong evidence that Darwinian sex roles are phylogenetically widespread (Janicke *et al.* 2016; Janicke & Morrow 2018). Based on these sex roles, male-male competition by mating opportunities can have strong effects on mate choice and would affect SAM (Vojar *et al.* 2015; Moura & Gonzaga 2017; Tinghitella *et al.* 2018). However, the intensity of competition may also depend on female availability, because females are the disputed resource (Andersson 1994; Moura & Gonzaga 2017). Effects of intrasexual competition and female availability on animals are usually estimated by using sex ratio and population density (Gosden & Svensson 2008; Weir *et al.* 2011; Aronsen *et al.* 2013; Janicke & Morrow 2018).

Classical theory of sexual selection assumes that selection should be stronger when the operational sex ratio (OSR), defined here as the number of sexually active males divided by the total number of sexually active adults of both sexes (Jirotkul 1999), is biased towards males (Trivers 1972; Emlen & Oring 1977). This approach has received support recently, because there is new evidence that few males are able to monopolize most mating opportunities as OSR becomes increasingly biased toward males (Janicke & Morrow 2018). In such scenarios, highly competitive males may be

able to copulate with more females than weaker ones, but they may not necessarily copulate with high-quality females. Scenarios in which OSR is highly biased towards males reduce aggressive interactions, mate guarding, and copulation duration (Weir *et al.* 2011), suggesting that males invest less energy by mating. They also become less choosy in that case, decreasing courtship rate (Weir *et al.* 2011), probably, selecting females regardless their size. Therefore, large males may copulate with more females than smaller ones, but they are unable to evaluate females before choosing a mate. This condition should weaken mate choice (Barry & Kokko 2010) and, consequently, SAM. Higher bias towards females may have similar effects. Owing to the high availability of females, males may invest more energy in copulation with higher number of partners than competing for larger females, increasing their fitness (Andersson 1994; Weir *et al.* 2011; Moura & Gonzaga 2017). Contrary to classical assumptions, intense mating competition was observed in populations with unbiased or slightly male-biased OSR (i.e., 2 males: 1 female) (Weir *et al.* 2011). Therefore, these scenarios can provide ideal conditions for males to evaluate female size and compete for large partners, favoring mate choice (Barry & Kokko 2010) and positive assortative mating (Moura & Gonzaga 2017).

Some studies had called attention to restrictions of OSR to estimate sexual selection intensity (Kokko & Johnstone 2002; Klug *et al.* 2010; Kokko *et al.* 2012; Moura & Peixoto 2013). However, prediction power of OSR can be increased when combined with population density because mating competition also may change because of variations in density (McLain 1982; McLain & Boromisa 1987a; de Jong *et al.* 2009). For example, high density may increase mating competition when OSR is highly skewed towards males and weaken it when OSR female-biased. Considering populations with unbiased OSR, we may also expect variations in competition

according to changes in density. Increases in population density may favor encounters and evaluation of potential partners, favoring mate choice (Kokko & Rankin 2006; Barry & Kokko 2010; Muniz *et al.* 2017). In highly dense populations, however, males may confront an intense competition for mating opportunities with larger females. Therefore, they can become less choosy and copulate with females regardless of their size. Those females, in turn, can be intensely harassed by males, reducing their requirements for partners (Kokko & Rankin 2006; Rankin & Kokko 2007). In such scenarios, mating would be random. At very low population densities, males may have difficulty in finding females and, consequently, they may not reject mating opportunities with females that are rare to find, regardless of their size (Kokko & Rankin 2006; Barry & Kokko 2010), weakening SAM. In situations of intermediate population density, otherwise, mating competition can be relatively weak and, consequently, large males can evaluate partners and monopolize mating opportunities with large, high-quality females. This situation can lead to positive SAM (Moura & Gonzaga 2017). Corroborating this hypothesis, some studies have reported effects of OSR and density on SAM (McLain 1982; McLain & Boromisa 1987a; b; Crespi 1989; Miyashita 1994; Bel-Venner *et al.* 2008; Aronsen *et al.* 2013; Mobley *et al.* 2014; Vojar *et al.* 2015; Moura & Gonzaga 2017).

We used a meta-analytic approach to investigate general patterns of SAM and its potential spatio-temporal variations. We also evaluated effects of mating competition on general patterns of SAM, controlling for scale-of-choice effects. We hypothesized that SAM decreases in response to increases in mating competition intensity. Thus, we tested the following predictions: (1) effect size of SAM is negatively correlated with OSR; (2) density will be negatively related to effect sizes; and (3) the interaction between OSR and density will explain variations in the effect sizes.

Material and methods

Eligibility criteria and information sources

We conducted a meta-analysis following the Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA; Liberati *et al.* 2009). We searched for studies providing information about mates' size correlations in one or more populations or breeding seasons in animal species (Crespi 1989; Jiang *et al.* 2013). We also looked-for information about OSR and density related with size-assortative mating. We used the data set published by Jiang *et al.* (2013) as a start point (225 publications). Their last search of papers was undertook in November 10, 2011 (see Appendix A from Jiang *et al.* 2013). Subsequently, we searched for more publications with information of interest between 2011 and 2015. We conducted the literature search using Google Scholar, JSTOR, and ISI Web of Knowledge databases between July and August 2015.

Search, study selection, and data collection process

We used “size assortative” and “size disassortative” as key words to search for publications in Google Scholar (13,200 and 1,970 results, respectively), JSTOR (541 and 69 results, respectively), and ISI Web of Knowledge (202 and 23 results, respectively). Our last search was conducted in August 20, 2015. We also included publications cited by studies reporting data of interest.

First, we searched for information about measures of SAM in the title, abstract, and key-words (375 results). In the second stage, we read methods and results of each study to collect the following information: species name, raw correlation coefficient, number of samples, OSR (number of males/total number of adults), and density (individuals/m²). We also tried to separate SAM measures according population and

breeding season. Finally, we obtained data for 315 publications, including the database of Jiang et al. (2013).

Effect size extraction/calculation

We chose Pearson's correlation coefficient (r) as the measure of effect size, however, the raw values are not normally distributed (Borenstein *et al.* 2009). Thus, we used a normalization of Pearson's correlation coefficient using Fisher's z (Z_r) transformation and calculated associated standard errors based on numbers of samples. These measures allow the comparison among studies with different samples (Borenstein *et al.* 2009). When authors reported measures of SAM using different statistical approaches, we performed transformations of the reported statistics (R^2 , F , χ^2) into Z_r (see Borenstein *et al.* 2009) using the effect size calculator in MetaWin, version 2 (Rosenthal 1994). We maintained the signs of all original statistics (indicating positive or negative relationships). When a study provided data only in figures, we used the WebPlotDigitizer software (Rohatgi 2012) to estimate and extract raw values of mates' size and calculate Pearson's correlation coefficient. When some relevant information was unavailable in the paper, we contacted the authors for clarification.

Building phylogenies

We updated the names of species using the data set from Open Tree of Life (tree.opentreeoflife.org/ - Hinchliff et al. 2015). Further, we classified these species according to the following taxonomic groups: "Teleostei," "Aves," "Amphibia," "Chelicerata," "Crustacea," "Squamata," "Insecta," "Lophotrochozoa," and "Mammalia." We used these taxonomic categories to identify potential bias and to provide a broader description of SAM patterns. Subsequently, we controlled

phylogenetic non-independence in our meta-analytical models (Lajeunesse, Rosenberg & Jennions 2013). For this approach, we first downloaded the supertree of Metazoa in the TRE format from Open Tree of Life in April 2016. Second, we pruned the supertree and kept only species of interest. Finally, we calculated a phylogenetic correlation matrix to include in the meta-analysis. To do these procedures, we used *ape* (Paradis, Claude & Strimmer 2004), *adehylo* (Jombart, Balloux & Dray 2010), *picante* (Kembel *et al.* 2010), *betapart* (Baselga *et al.* 2017), *vegan* (Oksanen *et al.* 2017), *fossil* (Vavrek 2011), and *expm* packages (Goulet *et al.* 2017) in R software, version 3.4.4 (R Development Core Team 2017). When a species was absent in the supertree of Metazoa, we used the name of a closely related species of the same genus or family to build the phylogeny.

Heterogeneity and publication bias

We searched for publication bias by visually inspecting funnel plots with 95% confidence intervals. Subsequently, we conducted a modified version of Egger's test (Egger *et al.* 1997) to evaluate potential funnel plot asymmetries, in which we considered Zr values as response variables and the standard error of the Zr values as the predictor variable (e.g. Leal & Peixoto 2017). Following recommendations of Senior *et al.* (2016), we estimated the heterogeneity among effect sizes using τ (i.e., the sum of variance components in the meta-analytic model that represents a standard deviation of observed effect sizes) with its significance test (Q_E) and I^2 (Borenstein *et al.* 2009; Viechtbauer 2010; Nakagawa & Santos 2012). The I^2 statistics is the percentage of variation between effect size that cannot be attributed to sampling error, which varies from 0–100% (Higgins *et al.* 2003; Rosenberg 2013). Values of 25%, 50%, and 75% were classified as small, medium and high, respectively (Higgins *et al.* 2003).

Sensitivity analysis

To identify potential missing values in our data set, we visually inspected ‘contour-enhanced’ funnel plots, which are funnel plots that have been displayed with areas of statistical significance (90%, 95%, 99% CI). This visual analysis is a better approach than common funnel plots to distinguish strong publication bias related with other causes of asymmetry, such as study quality (Peters *et al.* 2008; Nakagawa & Santos 2012). We also used the trim and fill method to estimate missing values and evaluate their effect on our estimation of average effect size. For this procedure, we ran a random-effects meta-analysis (REMA) without random variable using *rma* function of package *metafor*. Subsequently, to evaluate if the mean effect size of SAM was different from zero, we conducted a REMA using random variables described below and compared its results with estimated missing values of the model using trim and fill method.

Meta-analyses

All analyses were conducted in R software, version 3.4.0 (R Development Core Team 2017), using *metafor* (Viechtbauer 2010) and *devtools* packages (Wickham & Chang 2017). We ran meta-analytic models using general linear mixed models (Koricheva *et al.* 2013), considering the Z_r values as the response variable and the inverse of standard error as the weight (Borenstein *et al.* 2009; Zaykin 2011). This approach allows us to control for non-independence in the data arising because of multiple effect sizes originating from the same study, the same species, taxonomic groups, shared ancestry among species (i.e., phylogenetic relationship), potential bias in effect sizes according publication year and whether the study was conducted under field

or laboratory conditions. Thus, we included study identity, species identity, taxonomic group, phylogeny, publication year, and the design of the study into our models as random factors. Subsequently, we ran the full model and analyzed the τ^2 values. We removed variables with lower τ^2 values one by one and compared the new models with the null model using Akaike Index Criteria (AIC) and Likelihood test (LRT) of the *lme4* package. Finally, we chose the best model as the model showing lower AIC compared with the null model and fewer variables.

The OSR is usually defined as the number of adult males divided by the number of sexually receptive females (Emlen & Oring 1977). However, we used a different definition to normalize its variation between zero and one and to minimize bias of species with higher male-biased OSR (e.g. Vojar *et al.* 2015). We defined OSR as the proportion of adult males divided by the number of adult sexes (*sensu* Jirotkul 1999). To test effects of OSR on SAM, we performed two separate multi-level meta-analyses (MLMA) using OSR (continuous and categoric measures) as explanatory variable. The OSR categories was classified as female-biased (0 to 0.449), unbiased (0.450 to 0.549), slightly male-biased (0.550 to 0.749) and strongly male-biased (0.750 to 1.000). We analyzed potential effects of density on SAM by testing the relationship between effect sizes, as response, and density (individuals/m²) as predictor. We also control for taxonomic groups (Amphibian and Arthropoda) by including it as random variable. Finally, we tested the effect of potential association between density and OSR on effect size. To control potential bias of scale-of-choice effects on average effect size, we removed values of pooled data from two or more populations or breeding seasons and ran all data analyses again. In the results, we report correlation coefficients (r) \pm confidence interval (CI), $Zr \pm$ confidence interval and z-score value and its statistical

significance (P -value). When necessary, we back-transformed Z_r values to raw correlation coefficients.

Spatio-temporal analyses

We calculated standard errors for spatial (SE_{spatial}) and temporal (SE_{temporal}) replicates of SAM, separately, using raw values of Pearson's correlation coefficient. We selected data from studies that estimated SAM for more than one population or breeding season. When studies presented data from more than one breeding season for the same population, we calculated the mean r to estimate SE_{spatial} between populations. We used the same procedure to estimate SE_{temporal} between breeding seasons. Following these calculations, we used bootstrap random sampling to build a null model (with 10,000 random samplings), and compared the average SE according to a uni-causal 95% confidence interval because we expected SE to be greater than values above this interval. We used this approach for three data sets for each spatial and temporal data: (1) full data; (2) data without pseudoreplication (i.e., we kept r values with more replicates and sort other data from the same species); and (3) data without pseudoreplication and values with only two replicates.

Results

Model selection

Our full model containing six random factors (i.e., study identity, species identity, taxonomic group, phylogeny, publication year, and the design of the study) was better than the null model ($AIC_{\text{six factors}}: 975.225$; $AIC_{\text{null model}}: 1118.496$; $LRT = 153.271$; $P < 0.001$). However, after removing taxonomic groups, publication year, and the design of the study (all $\tau^2 < 0.01$), AIC estimation improved ($AIC_{\text{three factors}}: 970.251$;

AIC_{null model}: 1118.496; LRT = 152.245; $P < 0.001$). Further, we built meta-analytic models using study identity, species identity, and phylogeny as random factors.

Heterogeneity and publication bias

We obtained 1108 effect sizes from 315 studies using 298 species (Fig. 1, Table 1S). We found evidences of publication bias in our data set (Egger's test: $\beta = 0.318$; CI = 0.152–0.484; $z = 3.763$; $P < 0.001$, Fig. 1S). However, heterogeneity of our data set was small ($I^2 = 12.18\%$; $\tau = 0.335$; $Q_E = 985.950$; $df = 1106$; $P = 0.996$). Subsequently, we excluded potential values that represented inferior quality estimations of effect sizes (i.e., outliers outside the 95% interval confidence of the funnel plot and the highest values of standard error (Figure 1S)). We removed a total of 46 values, which left 1062 effect sizes from 286 species (Fig. 2S). Consequently, heterogeneity increased substantially in our model, but did not affect our estimation of average effect size ($I^2 = 90.34\%$; $\tau = 0.126$; $Q_E = 541.139$; $df = 1030$; $P = 1.000$), and Egger's test still identified a publication bias ($\beta = 0.247$, CI = 0.139–0.356, $P < 0.001$). Because the removal of outliers did not affect the result of Egger's test, the bias observed could be a consequence of true bias on average effect size of SAM. Because of these results, we used our full data set in the meta-analyses and removed outliers (i.e., values outside 95% CI) when it was necessary to evaluate whether they affected our conclusions.

Sensitivity analysis and general patterns of SAM in animals

We found more values in the left side of the contour-enhanced funnel plot inside the 99% CI (Fig. 2S). Therefore, potential missing values on the right side may increase bias. We estimated missing values using a trim and fill method ($r = 0.263$; CI = 0.242–0.284; $Zr = 0.269$; CI = 0.247–0.292; $z = 23.27$; $P < 0.001$). The average effect size of

SAM in animals also was greater than zero ($r = 0.324$; $CI = 0.172\text{--}0.461$; $Zr = 0.336$; $CI = 0.174\text{--}0.499$; $z = 4.054$; $P < 0.001$). Heterogeneity of this model was low ($I^2 = 11.72\%$; $\tau = 0.338$; $Q_E = 990.907$; $df = 1107$; $P = 0.995$). The estimations of average effect sizes in both models were similar, suggesting that missing values had a small effect on the estimation of average effect size. Thus, the bias in our data set can be a consequence of a true biological effects favoring positive assortative mating in animals. The removal of pooled data from two or more populations and breeding seasons also had a weak bias on our estimation of average effect size ($r = 0.300$; $CI = 0.189\text{--}0.428$; $Zr = 0.310$; $CI = 0.191\text{--}0.428$; $z = 5.121$; $P < 0.001$). Heterogeneity of this model was moderate ($I^2 = 44.12\%$; $\tau = 0.289$; $Q_E = 597.434$; $df = 861$; $P = 1.000$). In this approach, we retained 862 effect sizes from 216 studies using 213 species.

Effects of OSR and density on SAM

We evaluated 546 effect sizes from 168 studies using 163 species. We found a negative relationship between OSR and effect size ($Zr = 0.293$; $CI = 0.071\text{--}0.520$; $z = 2.754$; $P = 0.006$, Fig. 2). The heterogeneity of our model was very small ($I^2 = 5.13\%$; $\tau = 0.333$; $Q_E = 523.175$; $df = 550$; $P = 0.789$). After removing effect sizes of pooled data from two or more populations and/or breeding seasons, we used 421 effect sizes from 119 studies using 119 species. Likewise, OSR was negatively related to effect size ($Zr = 0.426$; $CI = 0.229\text{--}0.622$; $z = 4.253$; $P < 0.001$). The heterogeneity of this model was moderate ($I^2 = 37.97\%$; $\tau = 0.335$; $Q_E = 304.408$; $df = 420$; $P = 1.000$).

We included more studies that reported only categoric levels of OSR (female-biased, unbiased, slightly male-biased and highly male-biased) to build a MLMA with 595 effect sizes from 187 studies involving 178 species. We found average effect sizes greater than zero for all OSR categories (female-biased: $Zr = 0.224$; $CI = 0.045\text{--}0.404$; z

= 2.447; $p = 0.014$; unbiased: $Z_r = 0.369$; $CI = 0.205-0.533$; $z = 4.403$; $P < 0.001$; slightly male-biased: $Z_r = 0.306$; $CI = 0.140-0.473$; $z = 3.605$; $P < 0.001$; highly male-biased: $Z_r = 0.266$; $CI = 0.073-0.459$; $z = 2.702$; $P = 0.007$). The effect size was stronger in population with unbiased OSR (Fig. 3). The heterogeneity of this model was low ($I^2 = 6.82\%$; $\tau = 0.297$; $Q_E = 553.245$; $df = 591$; $P = 0.865$). After the removal of effect sizes pooled from more than one population and/or breeding season, we retained 455 effect sizes from 129 studies using 128 species. However, we found no bias of scale-of-choice effects in our conclusions (female-biased: $Z_r = 0.207$; $CI = 0.042-0.372$; $z = 2.455$; $P = 0.014$; unbiased: $Z_r = 0.327$; $CI = 0.181-0.473$; $z = 4.391$; $P < 0.001$; slightly male-biased: $Z_r = 0.296$; $CI = 0.149-0.443$; $z = 3.948$; $P < 0.001$; highly male-biased: $Z_r = 0.292$; $CI = 0.107-0.477$; $z = 3.098$; $P = 0.002$). The heterogeneity of this model was moderate ($I^2 = 41.52\%$; $\tau = 0.239$; $Q_E = 318.69$; $df = 451$; $P = 1.000$).

We evaluated 79 effect sizes from 19 studies using 22 species. We used the squared root of effect size plus one and its standard error in this model to obtain homogeneity of residual variance. We found no relationship between density and effect size ($Z_r = 0.0001$; $CI = -0.0003-0.0004$; $z = 0.368$; $P = 0.713$). The heterogeneity of our model was very low ($I^2 = 3.87\%$; $\tau = 0.701$; $Q_E = 75.090$; $df = 78$; $P = 0.572$). After removing effect sizes of pooled data from two or more populations or breeding seasons, we used 65 effect sizes from 15 studies using 18 species. This approach did not change our conclusions ($Z_r = 0.00001$; $CI = -0.00011-0.00013$; $z = 0.100$; $P = 0.921$). The heterogeneity of this model was low ($I^2 = 14.20\%$; $\tau = 0.203$; $Q_E = 56.040$; $df = 64$; $P = 0.750$).

We also did not find an effect of density or its association with continuous values of OSR (density: $Z_r = -0.0004$; $CI = -0.0010-0.0003$; $z = -1.109$; $P = 0.268$; interaction: $Z_r = 0.001$; $CI = -0.001-0.002$; $z = 1.102$; $P = 0.270$). However, the OSR

was positively related to effect size ($Z_r = 0.292$; $CI = 0.039\text{--}0.546$; $z = 2.262$; $P = 0.024$). We log-transformed effect size plus one and its standard error in this model to obtain homogeneity of residual variance. We evaluated 73 effect sizes from 16 studies using 18 species. The heterogeneity of this models was low ($I^2 = 8.74\%$; $\tau = 0.248$; $Q_E = 76.705$; $df = 70$; $P = 0.272$). The removal of effect size of pooled data did not affect our results (OSR: $Z_r = 0.317$; $CI = 0.046\text{--}0.588$; $z = 2.293$; $P = 0.022$; density: $Z_r = -0.0003$; $CI = -0.0009\text{--}0.0004$; $z = -0.876$; $P = 0.381$; interaction: $Z_r = 0.0006$; $CI = -0.0007\text{--}0.0018$; $z = 0.880$; $P = 0.379$). The heterogeneity of this models was moderate ($I^2 = 24.87\%$; $\tau = 0.190$; $Q_E = 44.846$; $df = 56$; $P = 0.858$). We retained 59 effect sizes from 12 studies using 14 species in this model.

Spatial and temporal variation on SAM

We obtained 89 values of SE for 53 species from 56 studies and 107 values of SE for 52 species from 50 studies for spatial and temporal variations of SAM, respectively. After random selection of one of repeated species, we retained 52 results from 46 studies of 52 species in temporal analyses. We did not find spatio-temporal variation in the SE calculated from raw values of Pearson's correlation coefficient ($SE_{\text{spatial}} = 0.128$; $CI = > 0.150$; $P = 0.489$; $SE_{\text{temporal}} = 0.114$; $CI = > 0.132$; $P = 0.468$). Our results did not change when we excluded more than one SE for the same species ($SE_{\text{spatial}} = 0.104$; $CI = > 0.123$; $P = 0.475$; $SE_{\text{temporal}} = 0.804$; $CI = > 0.092$; $P = 0.501$) and SE calculated only two replicates ($SE_{\text{spatial}} = 0.096$; $CI = > 0.115$; $P = 0.487$; $SE_{\text{temporal}} = 0.087$; $CI = > 0.100$; $P = 0.489$).

Discussion

In this study, we used a meta-analytical approach to evaluate general patterns and potential spatio-temporal variation in SAM, with an aim of investigating how population parameters related to the intensity of sexual selection and mating competition can influence SAM in animals. Assortative mating was positive, weak ($r = 0.32$), and consistent throughout space and time. Another meta-analysis about general patterns of assortative mating also identified an average positive and weak correlation between the size of individuals in mating pairs (Jiang *et al.* 2013). There also was no support for negative assortative mating (Jiang *et al.* 2013). The OSR was negatively related to SAM, but the strength of mate assortments was higher in populations with unbiased OSR. Density, on the other hand, did not explain variation in mate assortments. There also was no association between both population parameters. We found low heterogeneity (I^2) in most models, indicating that our results are conserved across animal species (Senior *et al.* 2016). We also conducted a sensitivity analysis that corroborated our results. Furthermore, scale-of-choice effects were expected to bias interpretation of pooled measures of SAM from more than one population or breeding season, because this mate assortment may be confused with size variation of different populations or seasons (Rolán-Alvarez *et al.* 2015; Ng *et al.* 2016). However, this bias may occur only in specific cases (Ng *et al.* 2016) because there is no strong bias from the general conclusions of our meta-analytic models. Thus, we presented robust empirical evidence that mating competition has phylogenetically widespread effects on SAM.

Sympatric speciation by assortative mating has been a hot topic of discussion recently (Servedio 2015; Servedio & Bürger 2015; Servedio & Boughman 2017; Kopp *et al.* 2018). Most researchers are investigating mechanisms underlying conducting assortative mating and in which scenarios mate assortment may lead to sexual isolation

(Kopp *et al.* 2018). Ecological factors may intensify the strength of mate assortments to promote sympatric speciation (Dieckmann & Doebeli 1999; Kirkpatrick & Ravigne 2002; Ritchie 2007), but the emergence of this pattern may depend on sexual selection. Sexual selection is supposed to be the main mechanism driving assortative mating by mate choice (Crespi 1989; Andersson 1994). However, there is conflicting evidence supporting assortative mating in the absence or presence of mate choice (e.g. Arnqvist *et al.* 1996; Taborsky *et al.* 2009; Mobley *et al.* 2014; Moura & Gonzaga 2017). This conflicting result may occur because authors may underestimate the role of male-male competition and female availability on SAM that vary according to population parameters, such as OSR and population density (McLain 1982; McLain & Boromisa 1987b; Aronsen *et al.* 2013; Wacker & Mobley 2014; Moura & Gonzaga 2017). It is important to highlight that preferences for larger individuals are associated with advantages of large individuals in competition (see predictions of mate choice hypothesis in Crespi 1989), but some authors tend to ignore this assumption (Kopp *et al.* 2018; Dijkstra & Border 2018; Tinghitella *et al.* 2018). Although the mate choice hypothesis provides a robust theoretical background based in sexual selection theory, its creators assumed that advantages of large males in competition for large, desirable females are conserved in any scenario (Ridley 1983; Crespi 1989). However, sexual selection intensity may change according to population parameters affecting mating competition and mate choice (Kokko & Johnstone 2002; Gosden & Svensson 2008; Barry & Kokko 2010; Aronsen *et al.* 2013). Consequently, some authors have found limitations of the mate choice hypothesis to explain SAM even when its assumptions are corroborated (Mobley *et al.* 2014; Moura & Gonzaga 2017). Here, we provided evidence that mating competition can indeed explain variations in SAM, but its effects vary according to OSR.

Traditional theory proposed that sexual selection should be stronger in the more abundant sex. Consequently, stronger bias in OSR towards males, associated with higher density, should increase sexual selection intensity on males, leading to evolution of attributes that increase male advantages in intrasexual competition, such as body size (Emlen & Oring 1977; Andersson 1994). Recent studies have identified several limitations in OSR to estimate mate monopolization and mating success in males (Klug *et al.* 2010; Kokko *et al.* 2012; Moura & Peixoto 2013; but see Janicke & Morrow 2018). A meta-analysis also showed that male-male competition may become more intense according to increases in OSR, but just until a condition of two males for each female is reached (Weir *et al.* 2011). Higher bias toward males had negative effects in mating competition. We found similar effects of OSR on SAM. The OSR was negatively related with SAM, but mate assortments were stronger when OSR was unbiased. Therefore, OSR can still be a good predictor of mating competition and mate availability, but its effects should be interpreted in a different way from what is typical. Higher bias in OSR toward males may weaken SAM because of the intense male-male competition and higher costs to monopolize mating opportunities. The difficulty of finding a female and intense female harassment by males may also weaken mate choice in such scenarios (Fitze & Le Galliard 2008; Barry & Kokko 2010). Higher bias in OSR toward females had similar effects, probably, because high mate availability may change males' strategy of fighting for high quality partners to fertilize as many females as possible and, consequently, weaken mate choice (Andersson 1994; Barry & Kokko 2010). In both conditions, mate choice should not evolve or be reinforced (Barry & Kokko 2010), leading to a weak SAM.

Density could also affect SAM, as was found in other studies (McLain 1982, 1985, 1988, McLain & Boromisa 1987a; b; Kokko & Rankin 2006). Increased density

can enhance the chance of finding a partner and, consequently, favor mate choice (Kokko & Rankin 2006; Barry & Kokko 2010; Muniz *et al.* 2017). This scenario may strengthen SAM (Moura & Gonzaga 2017; Muniz *et al.* 2017). In highly dense populations, otherwise, intense male harassment may weaken mate choice because of the high costs of rejection of undesirable partners by females (Kokko & Rankin 2006), weakening SAM. Low densities also may weaken SAM, owing to the low probability of finding a mate and, consequently, both sexes would not reject a partner that is found only rarely (Kokko & Rankin 2006; Moura & Gonzaga 2017; Muniz *et al.* 2017). Contrary to our expectations, however, density or its association with OSR did not affect SAM. These results also can be a consequence of the difficult to access comparable measures of density across published studies. We standardized our estimates of density (individuals/m²) and used only two taxonomic groups (Arthropoda and Amphibia) in our models. The scarcity of such data for other taxonomic groups may bias our conclusions. Further steps in studies involving sexual selection effects on SAM may concentrate efforts in poorly studied taxonomic groups and provide comparable measures of density associated with OSR.

Other factors also may affect mating competition and mate choice in addition to population parameters, such as variations in locomotion ability and body size of each species (Andersson 1994; Schwagmeyer 1995; Kelly, Bussière & Gwynne 2008). For example, very mobile or large species may find and evaluate partners more easily than species with little mobility or small size. However, OSR and population density also may proximally and distally influence individuals' mobility (Moorhouse & Macdonald 2008; Kelly *et al.* 2008) and body size (Cox & Calsbeek 2010). Therefore, these parameters may covariate with OSR and density to predict variation in SAM. Thus, they

should be considered in more complex models. Besides potential effects of other factors, we detected a general relationship between OSR and SAM.

In conclusion, we provided empirical evidence that SAM in animals is positive, weak, and consistent throughout space and time. We also found that a higher bias in OSR towards males or females resulted in weak SAM, but density did not affect mate assortments. These results support the general assumption that mating competition may affect SAM. Ideal scenarios of mating competition may favor SAM (i.e., populations with unbiased OSR). Thus, mating competition may not be the main mechanism driving sympatric speciation, but it has effects on the size variation of animal populations by affecting assortative mating. Further studies may provide empirical and theoretical information about the combined effects of OSR and density on SAM. They also may provide comparable measures of density across species to test the extension of our conclusions.

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Figures

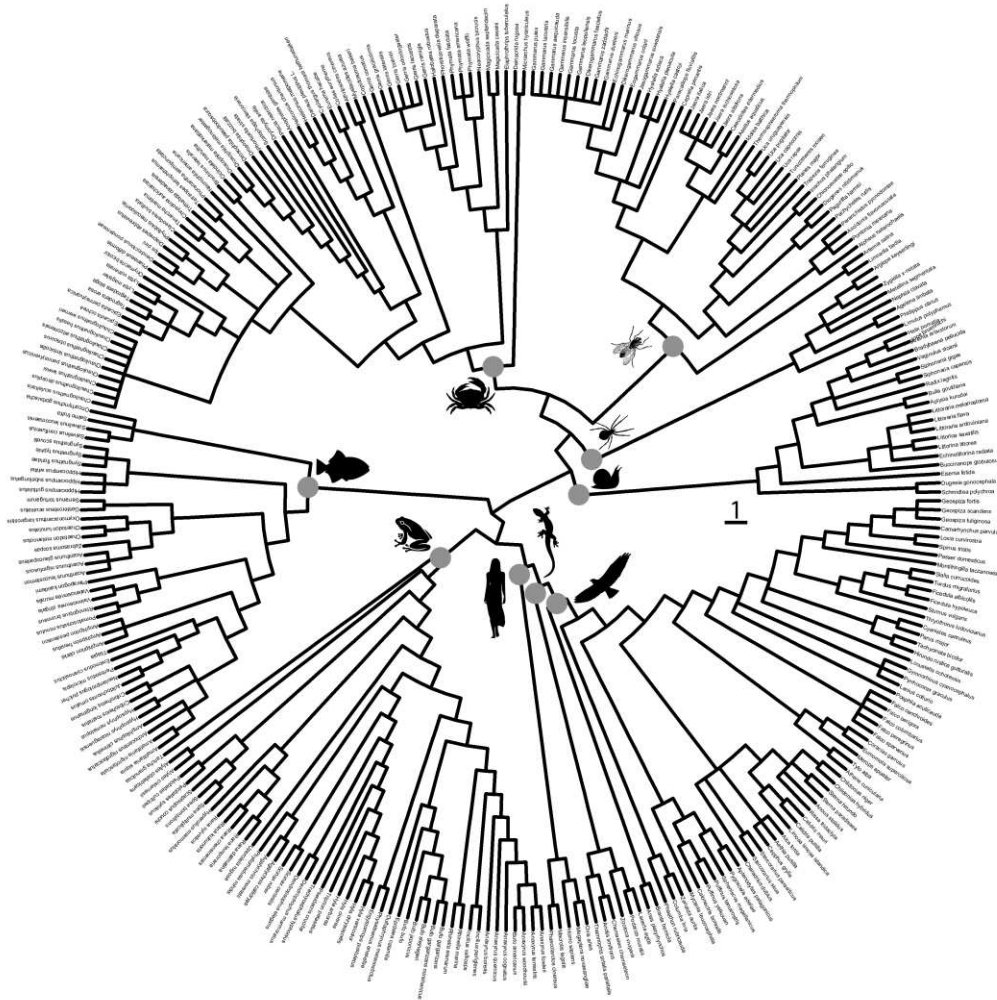


Figure 1. Phylogeny of animal species involved in the meta-analysis built from data base of *Open Tree of Life*. The nodes corresponding to the taxonomic groups that we used in our meta-analytical models are highlighted by gray points and images (Teleostei, Amphibia, Mammalia, Squamata, Aves, Lophotrochozoa, Chelicerata and Crustacea). The bar in the legend corresponds to phylogenetic distance estimate equal 1.

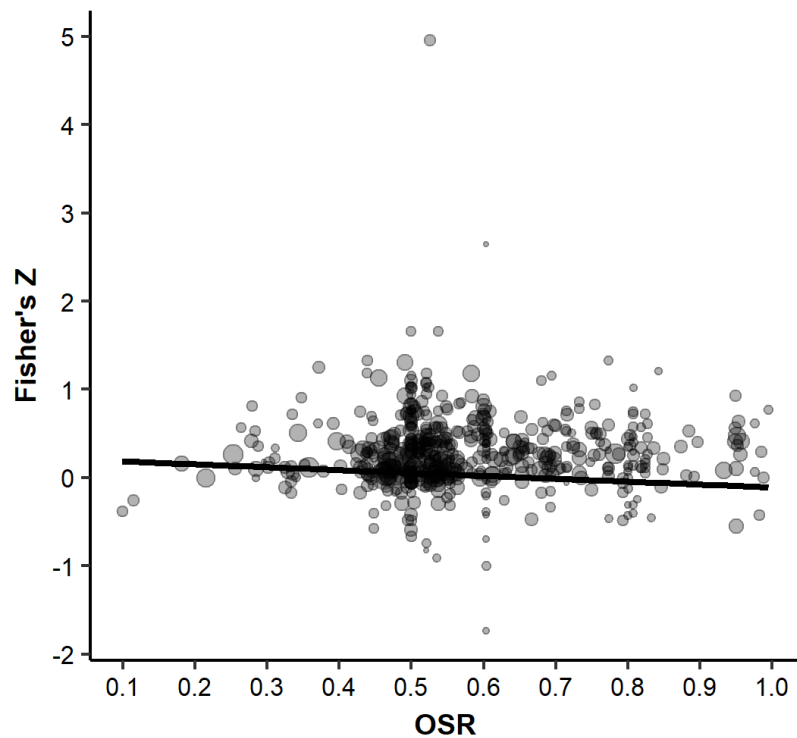


Figure 2. Relationship between OSR and effect size (Fisher's Z) estimated from raw values of Pearson's correlation. The size scale corresponds to variation of weight (i.e. inverse of standard error) in which smaller points are lower weights and larger ones are stronger weights.

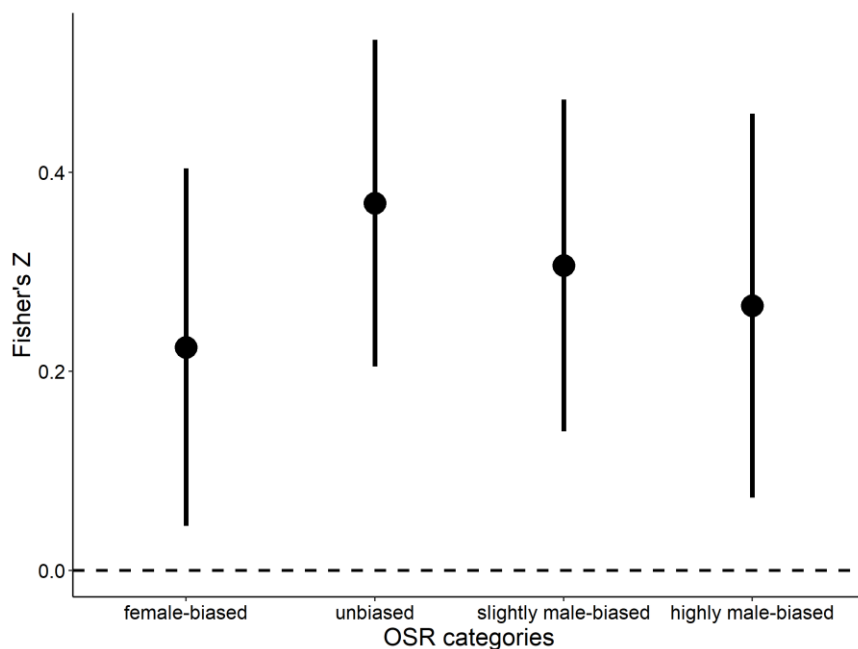


Figure 3. Differences in effect size (Fisher's Z) between OSR categories. The OSR categories was classified as female-biased (0 to 0.449), unbiased (0.450 to 0.549), slightly male-biased (0.550 to 0.749) and highly male-biased (0.750 to 1.000). The points and bars correspond to means and 95% confidence interval.

Supplementary information

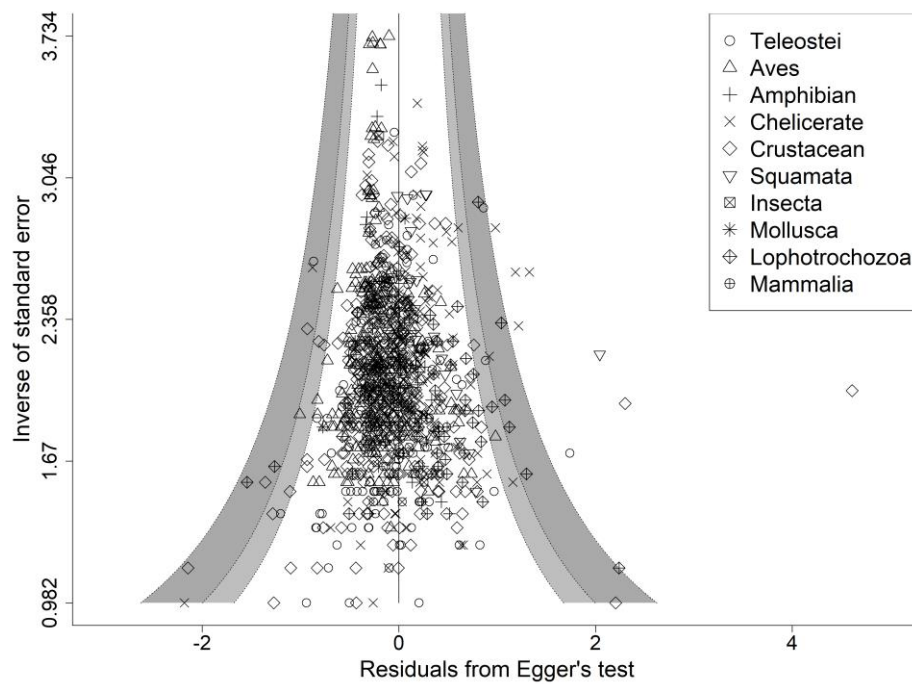


Figure 1S. Funnel plot of the relationship between residuals from Egger's test and the weights (i.e. inverse of standard error) of all data set.

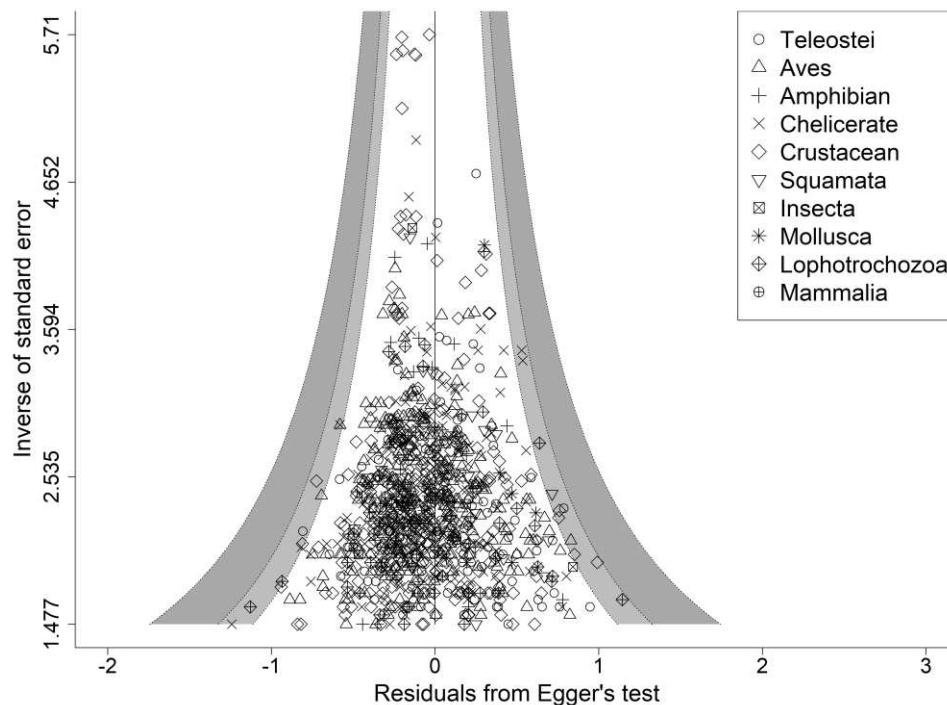


Figure 2S. Funnel plot of the relationship between residuals from Egger's test and the weights (i.e. inverse of standard error) of data without values within the 95% confidence interval.

CONSIDERAÇÕES FINAIS

Com esses três capítulos, oferecemos suportes empíricos e teóricos, consistentes com os pressupostos mais recentes da Teoria Moderna de Seleção Sexual, para a relação entre a competição por parceiros e o PDT. Além disso, enquanto a maioria das hipóteses tradicionais explica apenas a ocorrência ou não de pareamento, a hipótese de competição por parceiros pode explicar variações espaciais e temporais na ocorrência e na força do PDT em animais. Investigamos essa hipótese e apresentamos novos casos de PDT em grupos de organismos em que os padrões de pareamentos ainda não haviam sido identificados, como formigas e percevejos. Isso indica que outras espécies desses grupos podem apresentar padrões semelhantes e se tornarem modelos experimentais importantes para testar as previsões avaliadas nos capítulos desta tese.

O capítulo 1, que apresenta efeitos da competição por parceiros sobre variações temporais no PDT da formiga *Tranopelta gilva*, foi submetido em outubro de 2017 para revisão no periódico *Zoologischer Anzeiger*. Após a defesa, os demais capítulos serão submetidos para revistas de alto impacto, visando divulgar, da melhor maneira possível, os resultados do nosso trabalho. Os próximos passos das nossas pesquisas são avaliar como a relação entre a OSR e o PDT se comporta quando a hipótese de escolha de parceiros é corroborada em comparação a situações em que ela não teve suporte. Depois, vamos investigar como o PDT pode ser afetado por diferentes parâmetros sujeitos à seleção sexual, como o sistema de acasalamento (poligâmico, monogâmico e hermafrodita), dimorfismo sexual de tamanho (desviado para machos, fêmeas e sem viés) e sucesso de acasalamento dependendo das diferenças de tamanho de indivíduos acasalando em relação a indivíduos que não conseguiram parceiros (em machos e fêmeas). Por fim, pretendemos avaliar potenciais padrões macroecológicos nos pareamentos e nos efeitos da seleção sexual dependendo de regiões globais (como zonas tropicais e temperadas). Em resumo, esse é o começo de uma linha de pesquisa frutífera em estudos de seleção sexual e comportamento reprodutivo em animais.