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Reproductive interference among spider mites: using behaviour and life-history effects to predict eco-evolutionary consequences

Doutoramento em Biodiversidade Genética e Evolução

Salomé Gomes Loureiro Hipólito Clemente

Tese orientada por:
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Documento especialmente elaborado para a obtenção do grau de doutor

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Documento especialmente elaborado para a obtenção do grau de doutor

Fundação para a Ciência e a Tecnologia (FCT)

2017

Acknowledgments

I would like to thank my supervisors, all people I am so glad to have met, and without whom this thesis would have not seen the light.

Thank you Sara, for chasing me with a PhD project, for pushing me (even if sometimes a bit hard) and in general for being you, an amazing scientist and person.

Jordi, thank you for showing me a more relaxed side of science, and how programming can be fun. Also for hosting me during my excursions to Almería (thank you also Eva for welcoming me and making me feel at home), and especially for your all the amazing discussions, about science, music and many other important subjects.

Thank you so much, Susana, for all your kindness and support, during my (sometimes rough) journey in science.

And thank you:

The entire Mighty mite group (the best group), for the all the help. Keep on rockin' guys, I expect great things from all of you!

Merci Flore pour tous les partages, les avis, les sessions bricolage et tous les minis et clopes à la fenêtre.

All my π friends. Who share the dream and each in their way make the world a better place: Ana Bastos, Ana Mestre, Vera, Rafa, and João.

All the companions in Rua da Saudade, past and present, for helping me keep some sanity.

All the people that shared a circus tent with me and helped me see the wonders of being upside down.

My oldest friends, Bruno and Igor, who are always there.

The three little critters who, even though unintentionally undermining my work, by chewing all my informatics material, kept me company, sleeping under my laptop, or climbing up my shoulder and making me laugh.

My mother, for the unconditional support, even if this whole work is a big mystery to her.

My father, even if not here, who showed me it is OK to make a mess. If you don't mess what is important someone will be along to help you clean up. And all those who showed him right.

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Abstract

Since specific recognition systems are often incomplete, heterospecific matings are likely to occur, and can have paramount implications to fitness. Reproductive interference refers to any interaction between two species during the process of mate acquisition that diminishes the fitness of at least one of them. Using *Tetranychus urticae* and *T. evansi,* a native and an invasive spider-mite species, respectively, co-occurring in the Mediterranean area, the main goal of this thesis was to assess species recognition, the reproductive interference consequences of its incompleteness, and how this may affect the exclusion dynamics between these species. First, I present a critical revision on the role of reproductive interference on biological invasions, which supports this hypothesis, although unequivocal demonstrations are still lacking. Next, I measured the behavioural component of *T. urticae* and *T. evansi* reproductive interactions, to determine the frequency of the opportunities for reproductive interference between them, and the behavioural consequences of reproductive interactions. Both low costs and low species discrimination were found in this system. Subsequently, the effects of heterospecific matings for both fecundity and offspring sex-ratio were investigated, and how these differ between the native-invasive *T. urticae*-*T. evansi* species pair and a pair of native species (*T. urticae*-*T. ludeni*). I found that the costs of these matings are contingent on the species involved, the order of heterospecific and conspecific matings and the time interval between them. Finally, I integrated these results in a model, which revealed that the effects of reproductive interference are dependent on its pattern, while also affected by the inclusion of genetic variance. Moreover, the evolutionary trajectories of reproductive interference were highly variable, although the ecological outcome was conserved. This thesis showed that reproductive interference effects in the species studied are context dependent and that its evolutionary trajectories are highly variable, despite a conserved ecological outcome.

Keywords

Reproductive interference, Tetranychidae, Biological invasions, Individual Based Model, Eco-Evolutionary dynamics, Mating behaviour.

Resumo

Devido a sistemas de reconhecimento específico incompletos, acasalamentos heteroespecíficos ocorrem frequentemente, podendo ter consequências na fitness dos indivíduos. Interferência reprodutiva refere-se a qualquer interacção entre indivíduos de duas espécies, durante o processo de acasalamento, que diminui a fitness de pelo menos um deles. Utilizando *Tetranychus urticae e T.evansi,* espécies de ácaro-aranha, uma residente e uma invasora na zona do mediterrâneo, o objectivo principal desta tese foi investigar o reconhecimento específico, as consequências, em termos de interferência reprodutiva, de este ser incompleto, e como isso pode afectar as dinâmicas de exclusão neste sistema. No segundo capítulo, apresenta-se uma revisão crítica do papel da interferência reprodutiva nas invasões biológicas, e como esta questão tem sido, até aqui, abordada na literatura. No capítulo seguinte é explorada a componente comportamental das interacções reprodutivas entre *T.urticae* e *T.evansi*, para determinar a frequência das oportunidades de interferência reprodutiva entre estas espécies, bem como as consequências destas interacções ao nível comportamental. Baixos custos e baixa descriminação foram encontrados neste sistema. No quarto capítulo, investigaram-se os efeitos de acasalamentos heterospecificos na fecundidade e no rácio sexual da descendência, e como estes diferem entre o par *T.urticae* e *T.evansi* e um par de espécies nativas (*T.urticae* e *T.ludeni*). Descobriu-se que os custos desses acasalamentos são contingents às espécies envolvidas, à ordem dos acasalamentos e ao intervalo entre estes. No quinto capítulo, estes resultados foram integrados num modelo eco-evolutivo. Verificou-se que os efeitos da interferência reprodutiva são dependentes do seu padrão, sendo também afectados pela inclusão de variância genética. As trajectórias evolutivas da interferência reprodutiva são altamente variáveis, embora o resultado ecológico seja conservado nas simulações. No seu todo, esta tese mostra que os efeitos da interferência reprodutiva, no sistema *T.urticae* - *T.evansi*, são dependentes do contexto, sendo o resultado ecológico conservado apesar de grande variação nas trajectórias evolutivas.

Palavras-chave

Interferência reprodutiva, Tetranychidae, Invasões biológicas, Modelo baseado no indivíduo, dinâmicas eco-evolutivas, comportamento sexual.

Resumo Alargado

Interacções heterospecíficas podem ter implicações importantes para a fitness dos indivíduos e populações. Frequentemente, a consequência dessas interacções é negativa, podendo estar implicada na determinação dos padrões de coexistência/exclusão de populações. Chama-se a este fenómeno interferência reprodutiva, e designa qualquer interacção entre indivíduos de espécies distintas, durante o processo de aquisição de parceiros sexuais, com um impacto negativo para a fitness de pelo menos um deles. A origem da interferência reprodutiva está possivelmente nos mecanismos de reconhecimento específico, que são muitas vezes incompletos, permitindo a ocorrência de acasalamentos heterospecíficos.

Utilizando *Tetranychus evansi* e *T. urticae* como espécies modelo, esta tese teve como objectivo principal o estudo dos efeitos da interferência reprodutiva nos padrões de coexistência e exclusão das duas espécies, que competem por recursos. *Tetranychus evansi* é um ácaro aranha haplodiplóide, fitófago, que expandiu recentemente a sua distribuição desde a América do Sul (onde é nativo) até ao Sul da Europa, África e Ásia (Boubou *et al.* 2012; Navajas et al. 2013). Nas áreas em que *T. evansi* é agora uma espécies invasora, é encontrado em simpatria com o congenérico *T. urticae*. Ambas as espécies são importantes pestes agrícolas, co-ocurrendo em várias espécies de plantas. As interacções competitivas entre *T. evansi* e *T. urticae* têm sido, recentemente, alvo de bastante atenção, nomeadamente as que ocorrem indirectamente, através da planta do tomate, tendo-se demonstrado que *T. evansi* é capaz de excluir *T. urticae*.

Estas interacções foram abordadas sob diversos pontos de vista, desde a influência do comportamento reprodutivo das duas espécies na probabilidade de ocorrência de interferência reprodutiva, às consequências destas interacções no comportamento reprodutivo subsequente das duas espécies e na fitness dos indivíduos envolvidos (fecundidade e proporção de fêmeas na descendência). Posteriormente, através do estudo, em paralelo, dos efeitos de interferência reprodutiva entre *T. urticae* e *T. ludeni* (uma espécie nativa, filogenéticamente próxima) pretendeu-se efectuar a comparação desses efeitos entre um par de espécies nativas e um par composto por uma espécie invasora e uma nativa.

Finalmente, através de modelação, investigou-se a influência de diferentes padrões de interferência reprodutiva e da existência de variabilidade genética e evolução rápida da interferência reprodutiva nos padrões de exclusão/coexistência.

A tese está estruturada em seis capítulos. O primeiro apresenta uma introdução geral da literatura e dos tópicos abordados nos capítulos subsequentes. O capítulo 2 consiste numa revisão crítica da literatura, enquanto os três capítulos seguintes reportam os resultados empíricos deste estudo. Finalmente, no capítulo 6, apresenta-se um sumário dos resultados obtidos, bem como uma discussão integrada destes e perspectivas futuras para o desenvolvimento dos vários tópicos abordados. O capítulo 3 está publicado, o capítulo 4 está submetido e os capítulos 2 e 5 encontram-se em processo de submissão.

No segundo capítulo, o principal objectivo da revisão crítica da literatura foi investigar até que ponto a interferência reprodutiva pode ser responsável pelo estabelecimento de espécies invasoras, e como esta questão tem sido até aqui abordada na literatura. Três questões principais foram abordadas: a) o que nos mostram os estudos realizados até à data sobre o papel da interferência reprodutiva nas invasões biológicas; b) como podem estudos futuros melhorar a nossa compreensão desse papel; e c) se devemos esperar impactos mais fortes de interferência reprodutiva envolvendo espécies invasoras. Verificou-se que é difícil demonstrar inequivocamente o papel da interferência reprodutiva nas invasões, embora muitos dos estudos revistos mostrem um forte efeito da interferência reprodutiva por parte das espécies nativas. Recomenda-se que estudos futuros incluam manipulação da interferência reprodutiva, e/ou incluam abordagens de modelação ou de meta-análise.

O terceiro capítulo desta tese pretendeu caracterizar os aspectos comportamentais das interacções reprodutivas entre *T. urticae* e *T. evansi*, com o objectivo de determinar a frequência das oportunidades para a ocorrência de interferência reprodutiva, investigando a primeira barreira para esta ocorrência: reconhecimento específico. Investigou-se, nomeadamente, o grau de discriminação específica de machos e fêmeas, através de experiências de escolha de parceiro sexual e análise da duração e tempos de latência dos acasalamentos entre estas espécies, bem como as consequências desses acasalamentos no comportamento sexual durante acasalamentos subsequentes. Verificou-se que estas espécies demonstram uma baixa discriminação de heterospecíficos e que, na medida em que foram avaliados, os custos destas interacções não são elevados.

No capítulo 4, foram investigados os custos dos acasalamentos heterospecíficos entre *T. urticae* e *T. evansi,* e entre *T. urticae* e *T. ludeni*, nomeadamente ao nível da fecundidade das fêmeas e proporção de fêmeas na descendência. O objectivo foi determinar os custos de acasalar com heterospecíficos uma vez quebrada a barreira de reconhecimento específico. *T. ludeni* é uma espécie nativa, filogenéticamente próxima de *T. urticae*. Com a sua inclusão neste estudo pretendeu-se efectuar a comparação dos efeitos de interferência reprodutiva entre um par de espécies nativas e um par composto por uma espécie invasora e uma nativa. Esperava-se que os acasalamentos com *T. evansi* tivessem custos para *T. urticae*, se a interferência reprodutiva tem de facto um papel importante na exclusão de *T. urticae*. A inclusão de *T. ludeni* permitiu a comparação dos efeitos de acasalamentos heterospecíficos entre espécies nativas (*T. urticae* e *T. ludeni*) e entre uma espécie nativa e uma invasora (*T. urticae* e *T. evansi*).

Os resultados obtidos mostraram que os acasalamentos hetrospecíficos podem acarretar custos para as três espécies envolvidas, através da redução da fecundidade das fêmeas, ou da proporção de fêmeas na descendência. Estes custos, no entanto, estão dependentes das espécies envolvidas, da ordem de ocoorrência dos acasalamentos conspecíficos e heterospecíficos e ainda do tempo decorrido entre estes acasalamentos. Surpreendentemente, alguns tipos de acasalamentos tiveram efeitos benéficos: aumento da fecundidade ou da proporção de fêmeas produzidas.

Estes resultados foram combinados numa meta-análise, com *T. urticae* como a espécie de referência, que permitiu determinar os efeitos globais das interacções. Verificou-se que os acasalamentos com a espécie nativa tiveram um efeito geral benéfico para *T. urticae*, enquanto os acasalamentos com a espécie invasora não resultaram em custos ou benefícios para esta espécie. Estes resultados confirmam a hipótese colocada, evidenciando uma diferença nos efeitos de acasalamentos com uma espécie nativa e uma invasora. No entanto, a direcção destes efeitos não foi a esperada, já que *T. urticae* não sofre custos com acasalamentos com *T. evansi*, e impõe custos a *T. ludeni*. A ocorrência de interferência reprodutiva não deverá então ser um factor para a exclusão de *T. urticae* por *T. evansi*.

No quinto capítulo apresenta-se um modelo eco-evolutivo baseado na interacção entre *T. urticae* e *T. evansi* na planta de tomate. Para a parametrização deste modelo foram utilizados dados da literatura, bem como os resultados obtidos nos capítulos precedentes. Com este modelo pretendeu-se testar se a) a simplificação do padrão de interfêrencia reprodutiva afecta o resultado da interacção; se b) a inclusão de variação genética na interferência reprodutiva affecta os padrões de coexistência; se c) há evolução da interferência reprodutiva, e até que ponto ocorre em cada espécie, e se d) a direcção da evolução afecta os padrões de exclusão.

As simulações efectuadas mostraram que diferenças no padrão de interferência reprodutiva entre *T. urticae* e *T. evansi* (um padrão baseado em dados empíricos e outro em que os efeitos da interferência estão distribuídos por todos os tipos de acasalamentos) alteram o resultado da interacção, bem como a inclusão de variação genética, que aumenta a probabilidade de extinção de *T. urticae*. Finalmente, verificou-se que a direcção das trajectórias evolutivas da interferência reprodutiva é altamente variável, embora o resultado em termos ecológicos seja bastante conservado (exclusão de *T. urticae*).

Globalmente, os resultados apresentados nesta tese mostraram que as consequências das interacções reprodutivas entre as espécies estudadas podem ser bastante diversos, sendo necessário considerar a frequência com que cada tipo de interacção ocorre na natureza e estudar os factores que afectam esses padrões e ocorrência.

Chapter 1

Introduction

1.1 Species interactions

All organisms are engaged in a constant interplay with their environment. They interact with their abiotic environment, but also with other organisms (either from the same or different species) in many different ways. Interactions between individuals can be broadly grouped into two categories: direct interactions, when organisms directly affect the fitness of others (e.g. predation), and indirect interactions, if the effects on fitness of one organism by another are wielded through direct interactions with a third party (e.g. exploitative competition) (Begon et al. 2009). Associations between two organisms, or two species, can combine multiple types of interactions, the effect of each being often hard to disentangle, given the complexity of the web of life (Bascompte 2009).

Interactions among males and females of different species, although pervasive, are not covered by most ecology textbooks. Indeed, these interactions are generally overlooked and considered mostly inconsequential by ecologists. The evolutionary effects of these reproductive interactions, in contrast, mainly in the cases where hybridization occurs, are widely addressed in the speciation literature, as they can lead to reinforcement of pre-mating barriers and reproductive character displacement (Butlin 1989, Servedio & Noor 2003, Gröning & Hochkirch 2008).

Recently, however, the ecological consequences of sexual interactions between different species have begun to capture the interest of researchers. Such interactions have been shown to lead to processes of species displacement – sexual exclusion (Kuno 1992, Hochkirch et al. 2007). Nonetheless, species pairs with heterospecific reproductive interactions can affect each other in other ways as well, for example via resource competition (Kishi et al. 2009). The outcome of the contact between species is thus often a combined result of the different interactions in which they engage – reproductive or non-reproductive – which can affect species' associations in the same or in opposite ways (Gröning & Hochkirch 2008, Kishi et al. 2009). However, clear demonstrations of the effect of reproductive interference in the wild are still scarce (Gröning & Hochkirch 2008).

Reproductive interactions among different species can be direct or indirect. Direct interactions may lead, for example, to infertile hybrid production or to a fecundity reduction in subsequent conspecific matings. Indirect interactions, as in the case of signal jamming during mate attraction, may lead to degradation of conspecific signals due to heterospecific signalling. Reproductive interference (RI) is the term most commonly used to define any interaction between heterospecifics associated with reproduction which leads to a fitness reduction of at least one of the individuals involved (Gröning & Hochkirch 2008).

Here, we will briefly describe the reproductive interactions between species and their possible consequences, both at short and long time scales, how they can play an important role during biological invasions and shortly review models developed so far to study their consequences at both ecological and evolutionary levels.

1.2 Mating interactions between heterospecifics - when do they happen and why

Species are classically portrayed as discrete breeding entities, and often considered one of the fundamental units of biology (Mayr 1963, Queiroz 2007). As such, the occurrence of reproductive interactions or competition for mates between individuals of different species is attributed to “mistakes”; that is, flaws in species recognition systems.

The occurrence of “mistaken” reproductive interactions is usually expected to occur between closely related species, and during secondary contact events, as species evolving separately will not be exposed to strong selective pressures for heterospecific discrimination (Gröning & Hochkirch 2008, Crowder et al. 2010b, Burdfield-Steel & Shuker 2011). They have also been described between species with overlapping ranges, but that do not necessarily co-occur at a local scale. In these species, the occurrence of reproductive interactions has been suggested as a factor promoting habitat, spatial or temporal segregation (Hochkirch et al. 2007, Noriyuki et al. 2012).

In some cases, however possibly rare, heterospecific matings can be beneficial. These are known as cases of adaptive hybridization, where under some ecological circumstances, hybrid production and subsequent introgression can be advantageous (Abbott et al., 2013). For example, under stressful environments, spadefoot toads benefit from hybrid production since hybrid individuals develop faster. This leads to increased offspring survival, thus counteracting effects of the lower hybrid fecundity (Pfennig 2007). Adaptive hybridization can also occur when it is a prerequisite for embryogenesis (in gynogenetic species) (Gumm & Gabor 2005), or when females receive nuptial gifts from heterospecific males (Vahed 1998, Costa-Schmidt & Machado 2012). Males can also benefit from such matings if they improve their attractiveness to conspecific females (heterospecific mate-choice copying) (Schlupp et al. 1994, Schlupp and Ryan 1996), or improve the quality of their courtship through experience (Magurran & Ramnarine 2004, Dukas 2005). In line with this, Mendelson & Shaw (2012) argue that the dichotomy between compatible conspecifics versus incompatible heterospecifics is not real and that compatibility should be considered as a continuous axis of variation in mate quality.

In addition to these potential beneficial effects of heterospecific matings, the existence of imperfect mate recognition can also be attributed to a trade-off between accurate discrimination and missing mating opportunities. Indeed, increased discrimination can lead to the rejection of otherwise compatible mates, thus reducing reproductive opportunities in high discriminating individuals. Thus, when there is within species variation for the signals involved in mate acquisition and in the discrimination ability, individuals with signals that deviate from the population average can be excluded from the mating pool by highly discriminating individuals. On the other hand, if an individual has lower discrimination, it can choose to mate with a heterospecific which emits signals that fall within the range of conspecific signals (Mendelson & Shaw 2012, Scharf & Martin 2013). Indeed, a recent study showed that the evolution of increased mate discrimination in yeast was linked to a reduction of matings with compatible (conspecific) individuals (Rogers et al. 2015).

1.3 The case of reproductive interference

Heterospecific matings, even when beneficial for some of the individuals involved, may also negatively impact at least one of the species involved. Reproductive interference (RI) is the term most commonly used to describe this scenario. It can occur at several levels: at the initial steps of the reproductive process, for instance as signal interference or misdirected courtship, which can affect the rate of conspecific encounters, or later on, with the occurrence of heterospecific matings, which may result in hybrid offspring. Although with gradual borders between them, several types of RI were described and more than one of these types can occur between a species pair (Gröning & Hochkirch 2008).

When heterospecific copulation occurs, it can lead to energy waste and increased predation risk. Furthermore, heterospecific copulations can directly decrease fecundity either through the production of inviable hybrids (Ben-David et al. 2009, Remnant et al. 2014) or, when no hybrids are produced, by affecting the outcome of previous or subsequent conspecific matings.

Mating with heterospecifics can lead to subsequent behavioural changes in males and females, which might affect future conspecific matings. In some species heterospecifics matings induce a period of reduced sexual receptivity, this state being usually induced by components of the seminal fluid following conspecific matings. This will occur if the components of the heterospecific male ejaculate are sufficiently similar to those of conspecific males, and thus capable of inducing this response in heterospecific females (Yamane & Miyatake 2010, Lima-Câmara et al. 2013).

Besides affecting the incidence of conspecific matings, heterospecific mating can also directly affect female fertility by increased sperm competition, obstructing conspecific fertilization or arresting embryonic development (Ribeiro & Spielman 1986). In some spider mite species, heterospecific matings have been shown to reduce the number of fertilized eggs resulting from subsequent conspecific matings (Boudreaux 1963**,** Takafuji 1986). Fertility can also be reduced due to the transfer of toxic substances or injuries to the reproductive organs during heterospecific matings (Sota & Kubota 1998, Kyogoku & Sota 2015).

Sperm precedence patterns (Parker 1970) can affect the outcome of interspecific reproductive interactions. In particular, the effects of heterospecific copulations on subsequent successful fertilizations by conspecific sperm is expected to be more severe in species with first male precedence, when the first mating occurs with a heterospecific male. In species with second male precedence, such effects are generally less conspicuous as females that mate with heterospecifics first can always compensate later on by mating with a conspecific (Gregory & Howard 1994, Price et al. 2000).

1.4 Long-term effects of heterospecific matings

By definition, reproductive interference leads to a reduction of fitness of individuals from at least one of the species involved. Displacement of one of the interacting species due to RI – sexual exclusion – is thus a possible outcome, which can be dictated either by differences in initial densities or asymmetry in the effects of one species on the other (Gröning & Hochkirk 2008, Kyogoku 2015).

A considerable amount of studies report the occurrence of RI among sympatric species, suggesting that coexistence among species incurring in RI is possible (Gröning & Hochkirk 2008). Several mechanisms can promote species coexistence, such as: habitat segregation at a local scale, either temporal or spatial (Fujimoto et al. 1996, Takafuji et al. 1997); resource partitioning (Kishi & Tsubaki 2014); or aggregation of conspecifics (Gröning et al. 2007). These ecological mechanisms of segregation and local exclusion patterns can dilute the effects of reproductive interference by reducing the frequency of heterospecific encounters (Kyogoku 2015). They can also prevent the evolution of pre-mating barriers. However, when reproductively interacting species coexist for enough time, the costs of reproductive interference can be reduced by adaptive evolution through reproductive character divergence. This process leads to a greater differentiation in mating-associated traits in areas of sympatry (Kameda et al. 2009, Bargielowski et al. 2013, Okamoto & Grether 2013).

Even though there are a growing number of studies in this area (Kyogoku 2015), the effects of RI are often difficult to disentangle from those of other interactions. This can lead to an underestimation of the importance of reproductive interference in both evolutionary and ecological processes. For instance, when species interact both through resource competition and reproductive interference, the effects of these interactions can be in the same direction - promoting the advantage of the same species (Kishi & Nakazawa 2013) - or act in opposite directions - one species is competitively superior but suffers higher fitness losses from reproductive interference (Kishi et al. 2009). Several studies on heterospecific competition were recently analysed by Kishi (2015) and the author suggests that the results found are consistent with the simultaneous occurrence of competition and RI, and points to the fact that the occurrence of reproductive interference and its impact on species coexistence patterns might have been severely overlooked in many other studies of heterospecific competition.

Another possible way in which the effects of reproductive interference are being overlooked is when a trait involved in mate recognition simultaneously affects resource use (e.g. body size). In this case, differences in the trait between allopatric and sympatric populations can be due to reproductive interference, if the intraspecific resource competition is more intense than interspecific resource competition, which means reproductive interference will most likely be the most prominent interspecific interaction. The resulting character displacement may thus be misinterpreted as ecological instead of reproductive – apparent ecological character displacement (Konuma & Chiba 2007).

1.5 Reproductive interference between invasive and native species

Due to global change and increased accidental human introductions, the incidence of invasive species has increased (Hänfling & Kollmann 2002, Crowder et al. 2010b). Such events of biological invasions can lead to secondary contact between species and are very likely to involve reproductive interference (Gröning & Hochkirch 2008).

When species have evolved for some time in sympatry, repeated contacts are expected to select for reinforcement, leading to strong prezygotic reproductive barriers, which limit the costs associated with reproductive interference (Servedio & Noor 2003). Given that invasive and native species have evolved mostly in allopatry, no such selection has occurred; hence reproductive interference is likely to be frequent among these species. Such “mistaken” reproductive interactions may influence the impact of invasions and the mechanisms behind coexistence/exclusion patterns (Fitzpatrick & Shaffer 2007, Kanbe et al. 2008, Crowder et al. 2010b).

Several cases of reproductive interference involving invasive species have been thus far reported. It is thus tempting to hypothesize that reproductive interference can be one of the mechanisms determining the outcome of biological invasions (Burdfield-Steel & Shuker 2011). The demonstration of the role of reproductive interference as one of the mechanisms determining the outcome of biological invasions requires the fulfilment of two requisites. First, the occurrence of reproductive interference has to be clearly demonstrated. This implies that reproductive contact should result in a fitness cost for at least one of the species involved (Gröning & Hochkirch 2008). Second, a causal link between such RI and the exclusion of one species needs to be established. In the case of native/invasive interactions, it is generally assumed that the fitness cost, or the largest costs, is incurred by the native species, resulting in its exclusion (Burdfield-Steel & Shuker 2011). Reproductive interactions in cases of successful invasion seem in general to entail a higher cost for native species. This suggests that successful invasive species, particularly those that have been successful worldwide, have a certain number of reproductive traits that give them a consistent reproductive advantage.

A major problem in assessing the prevalence of RI during biological invasions is that the exclusion of potentially invasive species by native ones is undetectable a posteriori. The cases in which the alien species is unsuccessful at invasion remain hidden to the researcher, thus potentially biasing the estimates on the proportion of alien and native species that are positively and negatively affected by RI. Furthermore, comparative studies of RI among native species and between native and invasive species in the same community are still notably lacking.

In addition, the effects of RI are often difficult to disentangle from those of other interactions. Although RI is thought to be common during biological invasions, its relative prevalence in communities undergoing biological invasions and in undisturbed natural communities remains to be determined (Kyogoku 2015).

The use of computational models including data gathered through exclusion experiments to try to dissect the effect of the factors potentially responsible for the outcome of these experiments is often a more viable alternative (Crowder et al. 2010b, Sun et al. 2014). Overall, even though in the past years there has been an increase of studies in this field, the role of RI in invasion events remains to be demonstrated.

1.6 Modelling reproductive interference

Since the effects of heterospecific reproductive interactions are often hard to disentangle from those of other heterospecific interactions, such as resource competition, mathematical modelling has been used as a valuable approach to study the effects of reproductive interference at both ecological and evolutionary levels. We reviewed the literature on ecological models, and the ones described below focus on the effects of reproductive interference on heterospecific population dynamics, namely exclusion/coexistence patterns.

The earlier mathematical models regarding reproductive interference use discrete, modified, Lotka-Volterra interspecific competition equations to describe the potential of such interactions on population dynamics (Ribeiro & Spielman 1986, Ribeiro 1988). In this model it is assumed that the interference between species occurs during reproduction, and the effects of interference are compared with the effects of resource competition, described through Lotka-Volterra equations. These population-dynamics models explore the effect of reproductive interactions with varying intrinsic growth rates and different assumptions regarding growth rates, male mating abilities, migration rates between populations and hybrid viability. They predict that even with large intrinsic growth rates for both species, reproductive interference affects population dynamics and that while in scenarios of high migration rates, the extinction of one of the species is likely, when migration is low, parapatry is expected even when their reproductive rates are highly asymmetric. Overall, these models find that RI interactions have stronger effects than interspecific competition in determining species exclusion patterns. Indeed, for the same carrying capacity, a much lower reproductive interference coefficient than interspecific competition coefficient is needed to preclude sympatry between two species.

These early models were then extended and generalized by Kuno (1992). The main results are: 1) the outcome (exclusion of either species or coexistence) is always dependent on initial relative density; 2) the probability of stable coexistence increases with a lower interference coefficient, but, for the same coefficient values, the probability of coexistence is lower for RI than for resource competition; 3) the probability of stable coexistence depends on both species' reproductive rates, increasing with increasing birth rates or with decreasing death rates. Therefore, RI has a stronger effect on habitat segregation between species than resource competition, and species coexistence is primarily dependent on the ability to avoid interspecific mating interactions or the absence of interference effects from such matings.

Kuno's model was adapted by Takafuji et al. (1997) to describe the interactions between two *Panonychus* mite species and determine the role of reproductive interference in the observed geographical segregation patterns. In this model, the differential equations used include coefficients modulating both the intensity of reproductive interference and that of resource competition, and the assumptions and parameter values were based on empirical observations of the particular two-species system. They found that even when the effects of resource competition were removed from the model, stable coexistence was unlikely, due to stronger reproductive interference of one species (incidentally with a higher reproductive rate also) over the other. The distribution patterns of these species are thus at least partially attributable to reproductive interference. As described in Kuno (1992), when considering the effects of both resource competition and reproductive interference, initial density ratios greatly influence the outcome of the interactions: the species that suffers most deleterious effects from mating interactions and has lower reproductive rates can exclude the other one if it has sufficiently high initial densities at initialization.

Another model by Zeman & Lynen (2010) was used to specify the conditions that allow coexistence of two competing tick species that have a climate-dependent parapatric distribution. This model included Lotka-Volterra resource competition and reproductive interference. The simulations were run considering an environment where temperature and humidity varied spatially. Migration between different patches was population based and was assumed to be passive and density independent, but dependent on the presence of the other species, according to an avoidance index. In addition, the effects of cross-infection with shared pathogens through interspecific reproductive interactions were also included. Data from field surveys was used to validate the model. Simulations including competition, reproductive interference and mortality due to cross infections were compatible with the empirical distribution pattern of these species, suggesting that all these factors were involved in the distribution of these species in nature.

While previous models compared the effects of reproductive interference with those of resource competition on coexistence patterns, Kishi & Nakazawa (2013) investigated the joint effects of these interactions on species coexistence and exclusion. The model described in Kuno (1992) was used as a starting point, and it was assumed that net population growth rate ((births – deaths)/ time unit) decreases with increased population densities through resource competition and gross population growth rate (births/time unit) decreased with increasing heterospecific densities through reproductive interference. This work added new predictions to the analysis made by previous authors, showing the existence of synergistic effects of resource competition and reproductive interference when one species has both superior competitive resource and reproductive interference abilities, promoting the exclusion of the other species. Furthermore, results showed that a species that is superior in reproductive interference can coexist or exclude a species that is a superior resource competitor, which highlights the importance of the mechanism of reproductive interference in species interactions during biological invasions.

Crowder et al. (2010a) modelled the effects of behaviour in reproductive interactions and consequent sexual exclusion of haplodiploid whitefly biotypes. To determine the role of RI in the patterns of exclusion observed between different whitefly biotypes pairs, these models included developmental time and two behavioural components - female acceptance ratio and male propensity to court. Results from the simulations linked the observed patterns of sexual exclusion to the existence of behavioural plasticity in females of one of the biotypes: female acceptance rate of conspecific males increased with the increase of heterospecific densities. This change in acceptance rate can mean that these females become less selective when the probability of conspecific encounters is diminished. This leads to more stable sex-ratios (as virgin or heterospecifically mated females produce male offspring from unfertilized eggs), therefore precluding its exclusion. Later works used this model to investigate patterns of exclusion between other biotype pairs or other populations (Wang et al. 2012, Sun et al. 2014), also addressing the potential effect of other factors (Crowder et al. 2010b). Pesticide resistance, for instance, was shown to alter the exclusion patterns, otherwise predicted by the effects of RI.

Crowder et al. (2011) expanded this model to study the effects of several species traits, stochastic processes and niche partitioning on the coexistence patterns, using a spatially explicit framework. They found that natural stochastic processes and niche partitioning could promote coexistence, by reducing the frequency of interspecific encounters, thus mitigating the negative effects of RI.

While the above studies focused on the ecological consequences of RI, other models were developed that explore the evolutionary effects of these heterospecific interactions, namely concerning the emergence of character displacement. Resource competition can lead to ecological character displacement and reproductive interference, the latter having similar effects as competition, and having been shown to also be able to promote reproductive character displacement (Okuzaki et al. 2010, Yamaguchi & Iwasa 2013).

Konuma & Chiba (2007) built a model to investigate the role of reproductive interference in character displacement. This model considered the effects of both resource competition and reproductive interference and assumed the existence of a quantitative character associated with both interactions. Simulation results showed that reproductive interference can indeed be a major drive of character displacement if interspecific resource competition is less intense than intraspecific competition and the character associated with both interactions can lead to premating isolation. One example of this is character displacement in body size for some insects. In these cases, interspecific matings have negative effects on fitness. and differences in body size lead to premating isolation, as well as reduced interspecific resource competition.

Character displacement due to interspecific reproductive interactions (Reproductive Character Displacement, RCD) is expected to limit the occurrence of reproductive interference in sympatric populations, because it allows species to better avoid recognition mistakes during mating. In order to explore this hypothesis, the persistence of reproductive interference between sympatric species pairs was explored in a theoretical model (Takakura et al. 2015). The model was individual-based (IBM) and included the evolutionary dynamics of signalling traits (males) and mate recognition (females). It also investigated how the evolution of these traits could affect the frequency of interspecific courtship and mating. The model predicts the maintenance of interspecific reproductive interactions and, hence, reproductive interference, even after RCD, which goes against previous expectations. This result is linked to the existence of optimal recognition criteria, determined by balancing the costs of interspecific mating with those arising from the rejection of conspecific mates, which precludes the existence of complete reproductive isolation.

Drury et al. (2015) proposed a model linking reproductive interference and interspecific aggression. In this model, low interspecific divergence, due to ecological constraints on reproductive character displacement, would promote indiscriminate behaviour on the part of males, as lower species discrimination would be the best tactic for maximizing mating opportunities. Lower discrimination leads in turn to increased levels of reproductive interference, and to increased interspecific male-male aggression. In this model, reproductive interference caused by undiscriminating males is thus linked to either convergence or inhibited divergence in traits for competitor recognition.

To explore the relation between resource competition and reproductive interference, Nishida et al. (2015) developed individual based models (IBM) of host specialization evolution in herbivorous insects. For the construction of these models, a matrix was built, in which each of the individual cells corresponded to either one of two different host plants. The individual life cycle included 4 stages: mating, reproduction, competition and dispersal. It was assumed that there was no assortative mating (the herbivores mated randomly with another individual in the same cell, conspecific or heterospecific) and that fitness costs from reproductive interactions and resource competition were dependent on relative densities. Host preferences were genetically determined and evolvable, and host plant suitability was different for each species. The results from the simulations showed that intermediate levels of RI and resource competition lead to host specialization, while when RI was absent, this specialization rarely occurred, pointing to a potentially important role of RI in specialization.

The studies described in this section have shown how modelling approaches can be useful in the study of reproductive interference, both in a purely theoretical approach or in combination with empirical studies. These models predict that RI can have an important role in species exclusion, and even overcome the effects of other factors such as resource competition. Evolutionarily, RI can also be an important factor in character displacement and specialization. However, studies modelling the evolution of reproductive interference itself are still notably lacking.

1.7 The system

Spider mites (family Tetranychidae) are small (200 to 900 µm) phytophagous arthropods that feed on leaf cell contents. Spider mites are haplodiploid: virgin females lay unfertilized eggs that produce males, while fertilized eggs produce females. The life cycle of tetranychids includes the stages egg, larva, protonymph, deutonymph, and adult. The three immature stages are each followed by a quiescent stage: nymphochrysalis, deutochrysalis, and teleiochrysalis, respectively. The rate of development is dependent on temperature, humidity and host quality. For most species, at the optimum temperature, development duration ranges between 7 and 12 days (Boudreaux 1963, Van de Vrie et al. 1972). Many spider mite species produce a silken web over the leaves where they feed and lay eggs. This web possibly can act as a protection against abiotic elements and it is known to provide defence against predators, as many predator species are unable to penetrate the dense web produced by some spider mite species (Sabelis & Bakker 1992, Sarmento et al. 2011b).

The maximum number of eggs produced by females under optimal conditions can in some species be as high as 200 (Van de Vrie et al. 1972). The minute size, fast developmental rates, high reproductive capacity and remarkable ability to develop resistance to a wide range of chemicals are some of the characteristics that render as many as 100 species within the Tetranychidae the status of agricultural pests (Van de Vrie et al. 1972, Cranham & Helle 1985, Navajas et al. 2010). One of the species with major economic impact is *Tetranychus urticae*, a worldwide distributed generalist. It can feed on over 1.100 different host plants, many of which are economically important crops and quickly develops resistance to pesticides (Grbic et al. 2011, Dermauw et al. 2013).

Due to their small size and increased human trade rate, the number of spider mite species found outside their native range has been greatly increasing since the 1950's, including relevant invasion events (Navajas & Ochoa 2013). One of these species is *Tetranychus evansi*, a congeneric of *T. urticae*, specialized in Solanaceous plants, such as tomato (*Solanum lycopersicum*). It is endemic from South America and has expanded its range to North America, Africa, far-east Asia and the Mediterranean basin at a rapid pace (Boubou et al. 2012). In this last region, the invasion by *T. evansi* was shown to significantly reduce the absolute and relative abundance of the native *Tetranychus* species, including *T. urticae* (Ferragut et al. 2013).

Several hypotheses of competitive exclusion explain the displacement of native congeners by *T. evansi*. These include the absence of native natural enemies and the manipulation of the plant defences (Ferragut et al. 2013). *T. evansi* was recently found to down-regulate tomato defensive compounds, such as proteinase inhibitors, to lower levels than those of un-infested plants. This downregulation leads to a significant improvement in *T. evansi* performance on plants previously attacked by individuals of their own species, but also other herbivore species can benefit from this increase in nutrient availability (Sarmento et al. 2011a, Alba et al. 2015). However, the dense web produced by *T. evansi* is hypothesised to be involved in the exclusion of competitors (Sarmento et al. 2011b). Additionally, as found in several other spider-mite species (Collins & Margolies 1994, Takafuji et al. 1997, Ben-David et al. 2009), heterospecific matings have been observed between *T. urticae* and *T. evansi*. Moreover, a study manipulating the female mating status at the beginning of competition experiments, showed that the population growth of *T. urticae* and *T. evansi* is similar in the strong RI treatment (when the females introduced were virgin), while in the mild RI treatment (the females introduced had already mated with conspecifics), the population growth of *T. evansi* was inferior to that of *T. urticae*. Thus, RI seems to play a role in the outcome of population dynamics in these species (Sato et al. 2014).

Tetranychus ludeni is another congener of *T. urticae* and *T. evansi*. This spider mite species shares part of the host ranges of *T. urticae* and *T. evansi* and its distribution overlaps in several areas, namely in the Mediterranean basin, where *T. evansi* is invasive (Escudero & Ferragut 2005). Moreover, this species also downregulates tomato plant defences, although not as strongly as *T. evansi* (Godinho et al. 2016).

1.8 Goals and structure of the thesis

Using *T. urticae*, *T. evansi* and *T. ludeni* as model species, the main goal of this thesis was to study RI under a scenario of biological invasion. Knowing that in the sampled areas, *T. urticae* and *T. ludeni* naturally co-occur and *T. evansi* is an invader, the hypothesis tested is that the mechanisms of RI between *T. urticae* and *T. ludeni* should be different and less costly than RI between *T. urticae* and *T. evansi.*

With this purpose, we start by making a critical review (chapter 2) on the role of reproductive interference during biological invasions. While studies often report the occurrence of strong reproductive interference between invasive and native species, its role in the invasion process remains to be demonstrated. In order to establish an unequivocal link between reproductive interference and biological invasions, we propose potential methods to overcome the difficulty in discerning the effects of reproductive interference from those of other interactions and suggest future direction in this research field.

In order to test the main hypothesis, the third chapter of the thesis focuses on the behavioural aspects of *T. urticae* and *T. evansi* reproductive interactions, namely species discrimination by both males and females (Clemente et al. 2016, published in *Behavioral Ecology and Sociobiology*). Latency to copulation and copulation duration of heterospecific matings were recorded, and the effect of those matings on the mating behaviour on subsequent conspecific matings was also investigated. Our aim here was to determine how frequent the opportunities for RI are between these two species, based on the first barrier to RI, which is species recognition.

In the fourth part, the occurrence of reproductive interference between *T. urticae* and *T. evansi* and *T. urticae* and *T. ludeni* is investigated, namely the consequences of heterospecific matings on both female fecundity and offspring sex ratio (Clemente et al. 2017, submitted to *PCI EvolBiol*). Our aim was to determine the costs of mating with the wrong species when the recognition barrier is broken. Our prediction is that mating with *T. evansi* will have more severe effects on *T. urticae* than on *T. evansi* if RI plays indeed a role in *T. urticae* exclusion, as suggested by Sato et al.

(2014) results. The inclusion of *T. ludeni* allowed the comparison of the effects of heterospecific matings between a pair of native species (*T. urticae* and *T. ludeni*) and a pair of a native and an invasive species (*T. urticae* and *T. evansi*).

In the fifth and final chapter of this work, an individual-based model was developed. A novel feature of this model, relative to the existing literature on the subject, is the inclusion of genetic variance for reproductive interference, thus allowing for its evolution. To parameterize this model, we used data acquired in the literature and through the experiments of the two previous chapters, and aimed to test whether: a) simplification of the pattern of RI (e.g., occurring homogeneously regardless of the previous mating history of the female) changes the outcome of the interaction; b) genetic variance in RI affects the patterns of coexistence; c) there is evolution (or even coevolution) in reproductive interference and to what extent it occurs in each species; and d) the direction and extent of rapid evolution affect the patterns of exclusion/coexistence (eco-evolutionary dynamics).

This work, while focusing on the interactions between spider mite species, overall aimed at showing the importance of addressing reproductive interactions to understand both population dynamics and species evolution, using an integrative view, combining conceptual, empirical and modelling approaches.

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Chapter 2

Does reproductive interference play a role in species invasions?

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Abstract

The mechanisms underlying biological invasions are still under strong debate. Interspecific reproductive interactions often exert strong negative effects on species performance. Such reproductive interference may impact on species distribution and thereby determine invasion success. Here, we critically review studies addressing reproductive interference between invasive and native species, and ask whether reproductive interference may account for successful invasion. We find that studies often show high fitness costs for native, relative to the invasive species, resulting from reproductive interference, but its role in the invasion process remains to be demonstrated. Establishing this role may be problematic, given the methodological difficulty in singling out reproductive interference from other biotic interactions. We propose potential ways to accomplish this, and suggest future directions in this promising research topic.

Keywords

Interspecific reproductive interactions; biological invasions; competition;
competitive exclusion; population dynamics.

Introduction

Despite the remarkable increase in studies dealing with biological invasions, knowledge on the factors determining the establishment and spread of non-indigenous species are still a matter of intense debate (Lowry et al. 2013). Addressing this issue is key to understanding the invasion process, predict future invasions and potentially define programmes to control them.

Predicting the outcome of an invasion is still imprecise and risky. Several factors, possibly interacting, have been invoked to account for the success of invasions. First, aspects related to contingencies of a particular invasion event may be crucial. For example, the size of the propagule (Lockwood et al. 2005, Simberloff et al. 2009), or where it lands will severely affect whether a species will thrive in a new environment. Second, some biological traits enable species to invade new habitats (invasiveness). For instance, high genetic diversity and developmental plasticity allows rapid adaptation to novel environments (Lee 2002, West-Eberhard 2003). This capacity for adaptation increases flexibility for exploiting diverse local resources and reproductive performance and thus enables survival of the invader in a wide range of environmental conditions (Evans et al. 2011). Third, some habitat characteristics determine its susceptibility to the establishment and spread (invasibility) of non-natives species (Marco et al. 2002; Davis, 2009). An important example, concerning biotic habitat characteristics is enemy release (i.e., aliens thrive by escaping from natural enemies “left behind”; e.g., Colautti et al. 2004), which confers an advantage to the invasive species steaming from the lack of natural enemies in the invading habitat. . A fourth important aspect linking invasiveness and invasibility is the interaction among the invasive and native species, and their shared habitat. This interaction includes differences in species aggressiveness (Hudina et al. 2014), a trait associated to an array of mechanisms such as interference, exploitative competition, or intraguild predation (Soares et al. 2008; Grez et al, 2011).

More recently, it has been hypothesized that another type of heterospecific interaction, reproductive interference, can be an additional mechanism determining the success of invasions (Burdfield-Steel & Shuker 2011). Reproductive interference (RI) refers to interspecific reproductive interactions that result in a decrease in relative fitness in at least one of the two species involved (Gröning & Hochkirch

2008, Burdfield-Steel & Shuker 2011). Several studies have already reported the occurrence of reproductive interference between invasive and native species, and mathematical models predict that RI can sometimes be more effective than competition for resources at displacing other species (Ribeiro & Spielman 1986, Kuno 1992, Kishi & Nakazawa 2013).

The demonstration of the role of reproductive interference as a mechanism determining the outcome of biological invasions requires the fulfilment of two criteria. First, the occurrence of reproductive interference between an invasive and a native species has to be clearly demonstrated. This implies that reproductive contact – either through actual heterospecific matings or through impediments or interruptions of conspecific matings – should result in a fitness cost for one of the species involved (Gröning & Hochkirch 2008). Second, a causal link between reproductive interference and the exclusion of one species needs to be established. To verify this hypothesis, a critical review of existing studies is timely. Here, we perform such review as a preliminary assessment to understand whether a causal link between reproductive interference and invasion success can be established.

What do reproductive interference studies between invasive and native species show?

We have performed a search on the Web of Science (last accessed18 th of April 2017) for “reproductive interference” and “invasion” or “reproductive interference” and “invasive”. First, we critically assessed the list obtained of 126 articles, to ensure that at least one of the species involved was indeed invasive and that the occurrence of reproductive interference was tested, excluding also reviews and mathematical models (106 articles were excluded).We then complemented our list by adding more studies cited on the references and those who cite these articles, as well as occasional articles pointed out by colleagues (which comprised 10 added articles).

On Table 1, we present the list of 30 references that stemmed from this search. These studies have been performed in several animal and plant species, with a predominance of small arthropods. Studies investigated the effect of interspecific interactions on fitness through behavioural experiments and/or analysis of life history traits. Reproductive interference resulted, in most cases, from heterospecific matings and heterospecific male rivalry (i.e. heterospecific males are mistaken as conspecific competitors for mates, which can lead to time, energy, and nutrient waste; Sun et al. 2014, Crowder et al. 2010a,b, Liu et al. 2007, Luan & Liu 2012, Wang et al. 2012, Chapter 4, Manzano-Winkler et al. 2017), misdirected courtship and heterospecific mating attempts (Fea et al . 2013), or the induction of post-copulatory behavioural changes such as refractoriness to further matings (Tripet et al. 2011). Studies that analysed the effect of mating with heterospecifics on life history traits found effects on fecundity (Crowder et al. 2010a, Matsumoto et al. 2010, Luan & Liu 2012, Nishida et al. 2012, Takakura 2013, Martyniuk et al. 2014, Sato et al. 2014, Sun et al. 2014, Giatropolous et al. 2015, Clemente et al. 2016,. Chapter 4, Manzano-Winkler et al. 2017), hatching rate (Kanbe et al. 2008, Giatropolous et al. 2015), offspring sex ratio (Crowder et al. 2010a, Sun et al. 2014, Luan & Liu 2012, Clemente et al. 2016, Chapter 4) and survival (Fea et al. 2013).

Overall, reproductive interactions entail higher fitness costs for native, relative to the invasive species. However, this is not always the case: Lievens et al. (2016) reported negative effects on the fitness of the invasive species, and Clemente et al. (Chapter 4) found that while heterospecific matings can have negative effects on both invasive and native, the overall effect seems to be null. Furthermore, this apparent trend of higher costs for native species may be biased by the fact that observations are made on successful invasions only, and most invasions do not succeed (Lockwood et al. 2007). If failed invaders induce as much reproductive interference as the successful ones, then the correlation between this interaction and invasion success vanishes.

Although several studies reported the occurrence of reproductive interference, very few tested the link between this interaction and the exclusion of one of the species (Crowder et al. 2010b, Sun et al. 2014, Sato et al. 2014). Crowder et al. (2010b) compared the results from exclusion experiments with mathematical models including different factors, and found that the combined model, incorporating variation in both life history traits (development time, offspring sex ratio) and mating behaviour was the one that best fitted the experimental results. Sun et al. (2014) integrated laboratory population experiments, behavioural observations and simulation modelling, and found that interspecific asymmetric reproductive interference predicted the observed rate of species exclusion. Sato et al. (2014) performed exclusion experiments using either virgin or mated female spider mites. Because this group of mites has first male precedence (i.e. eggs are fertilized by the sperm from the first male, even when multiple matings occur), it was assumed that treatments involving virgin females would be more prone to reproductive interference. Although this reasoning seems sound, a later study (Chapter 4) showed that the strength of reproductive interference was not only contingent on the mating status of the female. Still, given that spider mites are more eager to mate when virgin (Clemente et al. 2016), reproductive interactions may be more frequent in treatment involving virgins. If this is the case, Sato et al.'s (2014) work would be the first direct test of reproductive interference. The results of this study showed that one species (*Tetranychus urticae*) had a similar population growth as the competitor species (*T. evansi*) on plants initially colonized by virgins, while in plants colonized by mated females, *T. urticae* population growth was higher than that of *T. evansi*

Three preliminary conclusions can be drawn from this survey. First, reproductive interference among native and invasive species seems to be frequent among the studied species (although there may be a publication bias, masking a number of species where reproductive interactions did not yield a measurable cost for any of the species involved). Second, reproductive interference affects the native species more often than the invasive species. Third, that reproductive interference may underlie successful invasions remains to be demonstrated.

Should we expect stronger impacts of reproductive interference from invasive species?

The studies of reproductive interference published so far (Table 1) show the existence of this mechanism in 15 species pairs and highlight the potential key role of reproductive interference in determining the success of biological invasions. However, why would invaders systematically induce more reproductive interference than natives?

Reproductive interference is a costly interaction, at least for one of the species involved. Therefore, one would expect selection against such interaction in species that have evolved in sympatry (Yamaguchi & Iwasa 2013, 2015, Nishida et al. 2015). That is, we expect strong prezygotic reproductive barriers among species that have

Table 1- Studies investigating the occurrence of reproductive interference between invasive and native species.

Organism	Type of study	Species involved	Traits	Observed effects	Outcome	References
Bemisia spp.	E^{d,f,}; E+M^{a,b,c,e,g}	1 invasive, 1/2^{c}/3^{d} native	Behaviour; Fecundity; Sex ratio; Insecticide resistance^{c}	↓ copulation frequency; ↓ female offspring (NAT);↑ copulation frequency (INV)	Native excluded (E^{a,c,d,g}, M^{a,b,c,g}, O^{d,e,f})	a) Sun et al. 2014, b) Crowder et al. 2010 a, c) Crowder et al. 2010b; d) Liu et al. 2007; e) Luan & Liu 2012; f) Luan et al. 2012; g) Wang et al. 2012
Aedes spp.	E	1 invasive, 1 native	Behaviour; Fecundity	↓ offspring (NAT);	Lower native densities (O^{e,f,g}) Native RCD(O^{a,b,c,d})	a)Bargielowski et al. 2013; b)Bargielowski & Lounibos 2014; c)Bargielowski et al. 2015a; d)Bargielowski et al. 2015b; e)Carrasquilla & Lounibos 2015; f) Giatropoulos et al. 2015; g) Tripet et al. 2011
Tetranychus spp.	E	1 invasive, 1/2^{d} native	Behaviour; Fecundity	↓ female offspring (NAT); ↓offspring(INV)	Native excluded (O^{a,b}); Native not excluded (E^{c,d})	a) Sato et al. 2014; b) Sato et al. 2016; c) Clemente et al. 2016; d) Chapter 4
Taraxacum spp.	E^{b,d}; E+M^{c}; E+O^{a}	1 invasive, 1 native	Fecundity	↓offspring (NAT)	Native excluded (O^{a,b,c}); Native not excluded (O^{d})	a)Takakura et al. 2009; b)Takakura et al. 2011; c)Matsumoto et al. 2010; d) Nishida et al. 2012
Veronica spp.	E+O	1 invasive, 1 native	Fecundity	↓offspring (NAT)	Native excluded (O)	Takakura 2013
Bombus spp.	E	1 invasive, 1 native	Fecundity	↓ offspring (total sterilization)(NAT)	Native excluded (O)	Kanbe et al. 2008
Miomantis caffra; Orthodera novaezealandiae	E	1 invasive, 1 native	Behaviour; Male survival	↑ male mortality (NAT)	Native excluded (O)	Fea et al. 2013

Organism	Type of study	Species involved	Traits	Observed effects	Outcome	References
Hemidactylus spp.	E	1 invasive, 1 native	Behaviour	None	Native excluded (O)	Dame & Petren 2006
Apis spp.	E+O	1 invasive, 1 native	Fecundity	↓ offspring (NAT)	Native fertility affected (O)	Remnant et al. 2014
Pseudotsuga menziesii; Austrocedrus chilensis	E+O	1 invasive, 1 native	Fecundity	↓ offspring (NAT)	Native fertility affected (O)	Martyniuk et al. 2015
Artemia spp.	E+O	1 invasive, 1 native	Sex ratio	↓ female offspring (INV)	Invasive sex ratio affected (O, E)	Lievens et al. 2016
Astacus astacus; Pacifastacus leniusculus	E+O	1 invasive, 1 native	Fecundity	↓ offspring (NAT)	Native excluded (O)	Westman et al. 2002
Drosophila subobscura; D. persimilis	E	1 invasive, 1 native	Fecundity	↓ offspring (NAT)	Native fertility affected (O)	Manzano-Winkler et al. 2017

Type of study: empirical (E), model (M), observations (O). Species involved: number of invasive/ native species studied; Observed effects in either the native (NAT) or the invasive species (INV). The Outcome column refers to either the observed in nature (O) or to the outcome of exclusion experiments and/or models (E).

evolved in sympatry (Yamaguchi & Iwasa 2013, 2015, Nishida et al. 2015). Such barriers are also expected when species have evolved in allopatry but subsequently co-occur in sympatry (i.e., reinforcement, Servedio & Noor 2003). Therefore, reproductive interactions are expected more often between invasive and native species than among natives, as the former have evolved in allopatry and the latter in sympatry.

But if reproductive interference is expected to provide benefits to the organisms that induce it, as they will negatively impact competing species, this implies that there should be selection not only for avoiding matings with interfering species, but also for inducing more reproductive interference if matings do occur, especially in those species evolving in sympatry with their competitors. This is likely in spatially very structured populations, in which males may get some inclusive fitness benefits from interfering (i.e., decreasing the reproductive output) with the other species. This could in turn select for more resistance in the species paying a cost in such interaction, eventually leading to an evolutionary arms race between competitors. If that is the case, we would expect that species evolving in sympatry induce strong reproductive interference, but also that sympatric species resist well to it. Invading species, on the other hand, should cause stronger reproductive interference on species of the invaded region, which have not coevolved with them and hence do not have the means to resist, than to species from the area of origin, assuming that the mechanisms are similar. The reverse is also true: native species should exert stronger reproductive interference on invasive species with which they have not been coevolving, than on other natives. These hypotheses remain to be tested. In fact, so far, no study has addressed the evolution of reproductive interference.

How can future studies improve our knowledge on the role of reproductive interference on invasions?

Moving beyond the two species paradigm

Clarifying the real impact of reproductive interference on the invasion process necessitates measuring the relative strength of interference between invasive-native species and that among native species. If reproductive interference induced by invasive species proves to be stronger than that between native species, then its role in invasion success may indeed be important. However, if reproductive interference induced by invasive species is of the same order of magnitude than that between native species, one may question whether invasive-native interactions do actually facilitate invasion. Most studies addressing reproductive interference in invasive species, however, concern a single heterospecific pair (*cf*. Table 1). In this thesis, we performed a study in spider mites occurring on tomato plants in the Mediterranean region (Chapter 4) by comparing two species pairs. The effects of the invasive *Tetranychus evansi* on the native *T. urticae* were stronger than those between the latter and *T. ludeni*, another native species. This finding suggests that reproductive interference can play a role in the observed displacement of *T. urticae* by *T. evansi* (Ferragut et al. 2013).

An approach that may corroborate role of reproductive interference during invasions is to document this interaction in several parts of the invasion range of a particular species. If reproductive interference is consistently found to imply a cost for the native species, this lends support to a relevant role of this interaction in the invasion process. Indeed, the negative impact of an invasive over a native species has been reported on different locations (Tripet et al. 2011, Giatropolous et al. 2015). For example, in the whitefly species complex, *Bemisia tabaci,* an invasive biotype, shown to negatively affect the reproductive success of other biotypes, has also been shown to displace several native biotypes (Liu et al. 2007) as well as one other invasive biotype (Crowder et al. 2011).Another example using more than two species is a series of studies on dandelions in Japan (Takakura et al. 2009, Matsumoto et al. 2010, Nishida et al. 2012), which have shown that the invasive species (*Taraxacum officinale*) exerts strong reproductive interference on one native species *T. japonica* (Takakura et al. 2009, Matsumoto et al. 2010), whereas the other native, *T. longeappendiculatum*, did not suffer significant fitness costs from heterospecific matings (Nishida et al. 2012). These results were corroborated with field observations showing that *T. longeappendiculatum* co-occurs with *T. officinale*, whereas *T. japonica* was displaced from its native habitats (Nishida et al. 2012). These results strongly suggest that reproductive interference may indeed foster invasions.

Finally, it would be important to assess the role of co-evolution on reproductive interference by comparing the magnitude of interference exerted by invasive species in their native *versus* their invasive range (Kyogoku 2015). Finding out how species interact among each other in their native range would help to timely identify potential candidates for future successful invasions.

More manipulative experiments and clearer predictions

Despite the fact that the studies presented above suggest a strong correlation between invasion success and reproductive interference, demonstrating the existence of a causal link requires stronger empirical evidence. Indeed, more experiments addressing invasion / exclusion in sets of populations that differ exclusively in the occurrence of reproductive interference are needed. Without such controlled experiments, disentangling the effects of reproductive interference from those of other interactions, namely competition for resources, may be problematic. Indeed, reproductive interference has many features in common with competition, such as reducing fitness, being density-dependent and, in most cases, asymmetric (Gröning & Hochkirch 2008). However, specifically manipulating reproductive interference while leaving other interactions, such as competition, intact, is a difficult task. A first challenge consists in clearly identifying at which stage reproductive interference is acting: if at the behavioural level, before mating, by interfering with courtship signals among conspecifics; if during mating attempts, by interrupting conspecific interactions or promoting heterospecific matings; or if after mating, by affecting female receptivity and components of fitness, such as hybrid production and viability, female fecundity, offspring sex ratio, among others. A second step would be to identify which type of resource competition occurs between the studied species. Third, it would be necessary to experimentally preclude one of those interactions, either resource competition or reproductive interference, to determine how likely the exclusion of one of the species is under the exclusive effects of each mechanism. The paucity of studies of this kind may be explained by the fact that such manipulation is often challenging. However, one may imagine cases in which it is conceivable to perform such experiments. For example, it may be feasible to prevent fertilization by alien pollen in plant systems, to ablate mating signal receptors in systems in which reproductive interference is expressed at this stage or to manipulate the densities of males of one species across several generations. Similarly, the strength of competition could be reduced by providing resources *ad libitum*. These experiments could furthermore be done using closely related species pairs, sufficiently segregated in space (i.e., biogeographically speaking), or that have never been previously in contact (at least as documented from the literature). The strength of reproductive interference in the species pairs that have not been previously in contact should be stronger than in native species pairs.

In systems where such manipulations are not practicable, the use of mathematical models may be an option. Indeed, such models can be built for the same system with and without reproductive interference, and parameterized with data from laboratory experiments. The predictions generated from these models can subsequently be tested against data from exclusion experiments. If a better match between experimental and model outcomes are found for models that include reproductive interference, it seems reasonable to conclude that this interaction affect the exclusion probability. This approach has been undertaken in several studies of reproductive interference (e.g., Takafuji 1997, Zeman & Lynen 2010), including in the system composed of invasive and native whitefly species (Crowder et al. 2010b, Sun et al. 2014). In the latter example, mathematical models confirmed reproductive interference as a driving force of exclusion of the native species.

Concluding remarks

Recent years have witnessed a strong increase in the number of studies dealing with reproductive interference in general, and with that between invasive and native species in particular. Still, although evidence points toward a potentially important role of reproductive interference in the outcome of biological invasions, unambiguous demonstrations are as yet lacking. Clearly, this promising research field will benefit from more empirical studies, meta-analyses, and more mathematical models. Also, the evolutionary consequences of reproductive interference in sympatry vs allopatry, modelled in some recent studies (Yamaguchi & Iwasa 2013, 2015, Nishida et al. 2015) remain to be tested.

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Chapter 3

Incomplete species recognition entails few costs in spider mites, despite first male precedence

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Clemente, S.H., Rodrigues, L.R., Ponce, R., Varela, S.A.V.M. & Magalhães, S. (2016) Incomplete species recognition entails few costs in spider mites, despite first-male precedence. *Behavioral Ecology and Sociobiology*, *70*, 1161-1170.
doi: 10.1007/s00265-016-2124-0

Behav Ecol Sociobiol
DOI 10.1007/s00265-016-2124-0

ORIGINAL ARTICLE

Incomplete species recognition entails few costs in spider mites, despite first-male precedence

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Received: 26 November 2015 / Revised: 6 April 2016 / Accepted: 15 April 2016

Abstract
The consequences of heterospecific matings may hinge on interspecies interactions, but also on characteristics of the intraspecies mating system, namely sperm precedence. Indeed, first-male precedence may entail costs of heterospecific matings that are usually overlooked in other systems, such as fertilization of oocytes that become unavailable to subsequent conspecific males or a decrease in female receptivity. Here, we used a system composed of two co-occurring haplodiploid spider-mite species with first-male precedence, *Tetranychus urticae* and *Tetranychus evansi*, to investigate (a) the potential costs of heterospecific matings and (b) whether mites avoid heterospecific mates. We found that heterospecific matings did not result in fertilized offspring (i.e. females). Moreover, fecundity (i.e. male offspring) of heterospecifically mated females did not differ from that of virgins, indicating that oocyte viability was not affected by heterospecific males. Furthermore, heterospecific matings did not trigger behavioural changes that typically derive from conspecific matings, namely reduced female receptivity for subsequent matings. In avoidance tests, we found that *T. evansi* females and *T. urticae* males mated as often with conspecifics as with heterospecifics, whereas *T. evansi* males and *T. urticae* females mated assortatively more often. Also, latency to copulation in virgin and mated females did not differ between conspecific and heterospecific matings, but matings between *T. urticae* individuals lasted longer than heterospecific matings. Therefore, heterospecific matings result in few costs despite first-male precedence and, concomitantly, species discrimination is low. Still, this study highlights the need to account for intraspecific mating systems in tests of the reproductive consequences of mating with heterospecifics.

Significance statement
In species where the first male fertilizes all the offspring (first-male precedence), mating with individuals from other species often yields few benefits and entails potential costs in terms of future mating events. Yet, several species exhibit incomplete recognition of conspecifics. We here show that this is the case among two spider-mite species that co-occur under natural conditions. However, we also demonstrate that the cost of mating with the 'wrong' species is low, even though they exhibit first-male precedence.

Keywords Tetranychidae · Species interactions · Mating behaviour · Sperm precedence · Species recognition

Introduction

The ability to recognize conspecifics may enable individuals to interpret heterospecific signals as coming from low-quality mates (or non-mates) and hence allow them to avoid engaging in heterospecific interactions that are often costly (Mendelson and Shaw 2012). Nonetheless, species recognition systems are

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Communicated by N. Wedell

Susana A. M. Varela and Sara Magalhães shared co-last authorship.

Electronic supplementary material The online version of this article (doi:10.1007/s00265-016-2124-0) contains supplementary material, which is available to authorized users.

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often incomplete, leading to the occurrence of interspecific copulations between individuals from closely related species (Burdfield-Steel and Shuker 2011).

Failures of recognition systems entailing the occurrence of interspecific mating events can be due to weak selective pressures for discriminating abilities when the overlap between species distributions is occasional or recent (Coyne and Orr 2004; delBarco-Trillo and Johnston 2010; Abbott et al. 2013). Additionally, even in primary sympatry, selection for discriminating ability might not suffice to prevent heterospecific interactions. Indeed, mistaken evaluation of the quality of potential mates can be maintained due to higher costs of missing reproductive opportunities than those of mating less optimally (Pfennig 2007; Mendelson and Shaw 2012; Scharf and Martin 2013; Burdfield-Steel et al. 2015). This suggests that when heterospecific matings result in high costs—in terms of female sexual receptivity, female fecundity and offspring viability—they should occur only rarely.

Heterospecific matings may modify the physiological status of females and consequently diminish the rate of subsequent conspecific matings (Mclain and Pratt 1999; Valero et al. 2008; Burdfield-Steel and Shuker 2011). However, this may not always be the case. For instance, sperm transfer by conspecifics can result in changes in female behaviour and attractiveness (Wirmer et al. 2010), but these post-mating effects may not be properly triggered by heterospecific sperm. Hence, females that have mated heterospecifically may be as receptive as virgins.

Within-species sperm precedence (Parker 1970) may also affect the outcome of the interaction between females and heterospecific males. In particular, in species with first-male precedence, if the first mating is heterospecific, it can hamper subsequent successful fertilizations by conspecific sperm, as well as female receptivity and attractiveness to conspecific males, entailing strong costs by reducing both the fertilization success and the fecundity of the involved individuals. Such effects are expected to be less conspicuous in species with second-male precedence, as females that mate with heterospecifics first can always compensate later on by mating with a conspecific. Therefore, if costs of mating first with heterospecifics in species with first-male precedence are expected to be higher, then this is likely to affect the behaviour of individuals engaging in such matings. If, however, the first mating occurs with a conspecific male, a second heterospecific mating might have negligible effects.

Here, we investigate the occurrence, characteristics and consequences of heterospecific matings between two haplodiploid phytophagous spider mite species: *Tetranychus urticae* and *Tetranychus evansi*. These species share part of their host range, with *T. urticae* being a generalist and *T. evansi* occurring mostly on solanaceous plants (Migeon et al. 2011; Sarmento et al. 2011). Moreover, a recent *T. evansi* expansion has led to new distribution overlaps, mainly in Europe (Boubou et al. 2012). In the field, they are frequently found on the same plant (Ferragut et al. 2013). First-male sperm precedence has been found in *T. urticae*, although dependent on the mating interval (Boudreaux 1963; Helle 1967): a second mating occurring within 24 h after the first can still produce some offspring. Moreover, heterospecific matings have been observed between *T. urticae* and *T. evansi*, and indirect evidence suggests that reproductive interference may affect the outcome of competitive dynamics between these species (Sato et al. 2014).

Given the abovementioned characteristics of these spider mites, they represent an ideal system to address the physiological/behavioural effects of heterospecific matings in species with first-male precedence. We hypothesize that, due to first-male precedence, spider mites pay a high cost of mating with heterospecifics. Specifically, we expect that females that mate with heterospecifics first will (a) have reduced offspring resulting from arrested development of oocytes and that (b) they will be less receptive to subsequent matings and will hence lose future mating opportunities. As a consequence, we expect that (c) spider mites avoid mating with heterospecifics.

Material and methods

Maintenance of populations

Populations of both mite species used in this study were collected in Carregado, Portugal (Quinta do Outeiro). A laboratory population of *T. urticae* was established in May 2010 from 300 adult females collected from tomato plants (*Solanum lycopersicum*). The population of *T. evansi* was established from 300 adult females collected from *Physalis* spp. in May 2012.

Even though adult females from each species are easily identifiable, species identity was further confirmed through polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) of the ITS2 region (Hurtado et al. 2008), on approximately 50 females of each population. Total genomic DNA was extracted from each individual spider mite using the Sigma-Aldrich GenElute™ Mammalian Genomic DNA Miniprep Kit. We followed the manufacturer instructions, except for the elution volume, which we set to 20 μL RNase free water (Qiagen NV, Venlo, The Netherlands), in order to increase the concentration of DNA obtained from this very small animal (c.a. 300 μm long).

One hundred adult females from each species were screened for *Wolbachia* using the primers *wsp* (*Wolbachia*-specific primers) 81F and 691R (Braig et al. 1998). PCR assay procedures were as described in Breeuwer (1997). Results were positive for *Wolbachia* infection, and spider mite populations were thus treated by placing adult females in detached bean leaves with tetracycline (0.025 % *w*/*v*) for three consecutive generations. Absence of *Wolbachia* was then confirmed using the same protocol as above. This was done to avoid potential effects of *Wolbachia* on the behaviour of their host (Vala et al. 2004). Since then, both populations have been screened for *Wolbachia* on a regular basis, always with negative results.

Spider mite populations were maintained on trays with six to ten 3-week-old bean (*Phaseolus vulgaris*) or tomato plants at 25 °C and with a 16L : 8D photoperiod. Plant trays were changed every 2 weeks, placing old leaves on top of the new plants. Bean plants were planted every week and grown in a herbivore-free greenhouse, being watered two to three times a week.

The developmental period from egg to adult of both spider mite species used is around 10 days under optimal conditions. The offspring sex ratio is female biased and varies between 2:1 and 3:1 (Oku 2014; Sato et al. 2014). Mating occurs when the male slips under the female from behind and holds her legs with his front legs (Oku 2014). Both cases of 'male choice' (Oku et al. 2005) and 'female choice' (Tien et al. 2011) have been reported (though they may also be the outcome of female and male competition, respectively).

Experimental procedure

All experiments were performed in an acclimatized room at approximately 25 °C. Blinded methods were used to minimize inadvertent observer biases (cf. below).

1) Are there reproductive costs resulting from mating with heterospecifics?

   1a) Does mating with a heterospecific male affect offspring viability?

   When females encounter heterospecific males and accept to mate with them, they may or not produce viable offspring. Given that, in haplodiploids, fertilized eggs produce females and unfertilized eggs result in males, and the degree of hybridization was assessed through the offspring sex ratio of heterospecific matings. If no female descendants are produced (in the absence of successful hybridization), heterospecific matings can still result in the aborted development of heterospecifically fertilized eggs, thus compromising the fertility of females that mated with a heterospecific male. To test this, we compared the fecundity of females that mated with a heterospecific male to that of virgin females and of females mated with a conspecific male.

   Females were collected from the stock populations, isolated in the quiescent stage that precedes their last moult before reaching adulthood and kept in groups of approximately 15 females in leaf discs until emergence, to ensure their virginity. Adult males were collected from the same populations and kept isolated in leaf discs for at least 24 h before the assay, to ensure sperm replenishment (Krainacker and Carey 1990). Females were placed with either a conspecific or a heterospecific male and observed continuously until copulation occurred. Subsequently, females were isolated on a leaf disc (2 cm^2) and transferred to a new disc every 3 days until the female's death. The number of eggs was recorded before transferring the female to a new leaf disc, and eggs were left to develop until adulthood such that offspring sex ratio could be determined. The observers were unaware of the treatment they were assessing, as leaf discs were numbered and the association between number and treatment had been performed by other colleagues while setting up the experiments.

   1b) Does mating first with a heterospecific male modify the behaviour of virgins?

   When females encounter heterospecific males and accept to mate with them, a second important question is whether these females modify their physiological status or if they are still receptive to subsequent matings with conspecifics.

   First, as a control, we measured the latency to copulation and copulation duration of virgin females with conspecific males (treatment A; see Table 1). For that, a virgin female and a sperm-replenished male were placed on a leaf fragment (1 cm^2) and their behaviour was continuously observed until a mating event was completed. The time elapsed until a first mating occurred (latency to copulation), as well as the duration of copulation, were measured with a stopwatch. These experiments had the maximum duration of 2 h. If no mating occurred within this time, latency time was considered maximum, 7200 s. We did not remove these females from the analysis to avoid the assumption that they will never mate, which is not necessarily true. Removing these females would also give the wrong impression that the latency times were shorter than they really were, and that the females were accepting to mate with all kinds of males much more easily than they actually did. By considering a latency of 100 %, we avoid making these assumptions and bring all observations to the reference of the 2 h of the experiment (but see more details in the "Statistical analysis" section below and in the "Results" section). On the other hand, when a mating did occur, only those that lasted at least 1 min were included in the analysis. This restriction has a biological meaning, as matings with less than 1 min are not effective in species of the genus *Tetranychus*, as described by Boudreaux (1963).

   Second, to assess the effect of heterospecific matings in the subsequent mating behaviour of females, we

Table 1 Type of matings included in the experimental design for the no-choice tests, their use in the questions addressed (cf. "Material and methods"), number of replicates for each and number of unmated females at the end of the 2-h observation period (maximum latency)

Treatment	First male	Second male	Remating interval	Question	Replicate numbers (number of unmated females)			
					T. evansi		*T. urticae*	
					Lat	Cop	Lat	Cop
A	Con	–	–	1b) and 2b)	53 (3)	49	56 (5)	50
B	Het	–	–	2b)	31 (5)	25	34 (5)	28
C	Het	Con	0 h	1b)	30 (3)	23	24 (2)	22
D	Het	Con	24 h	1b)	47 (6)	39	40 (9)	31
E	Con	Con	0 h	2c)	40 (16)	21	40 (11)	28
F	Con	Con	24 h	2c)	61 (42)	19	45 (33)	12
G	Con	Het	0 h	2c)	14 (5)	8	15 (6)	9
H	Con	Het	24 h	2c)	30 (17)	13	30 (16)	13

In the treatments with two mating events, behaviour was observed in the second

Con conspecific, *Het* heterospecific, *Lat* latency duration, *Cop* copulation duration

measured their latency to copulation and copulation duration in a mating with a conspecific male that followed a heterospecific mating. The interval between matings was of either 0 or 24 h (treatments C and D; see more details in Table 1). The observers were unaware of the treatment they were assessing, as leaf discs were numbered and the association between number and treatment had been performed by other colleagues while setting up the experiments.

2) Do spider mites avoid mating with heterospecifics?

2a) Mating outcomes in the presence of both species

When females and males encounter, at the same time, conspecific and heterospecific sexual partners, they may or may not reject mating with heterospecifics. Discrimination among species is predicted if there are costs to female fertility and receptivity. To test this, we performed experiments with one focal individual from each species, placed with a conspecific and a heterospecific mate. These individuals were placed in leaf fragments of circa 1 cm^2 and observed continuously until copulation occurred. In the experiments that involved a female from one species and a male from each species, these were previously dusted with powder of different colours (randomized between replicates). This dust does not affect mating outcomes (Magalhães et al. 2009). In the experiments that involved one male from one species and a female from each species, the dusting of the females was not necessary because they are easily distinguishable visually. In all experiments, if copulation did not take place within 2 h, the observations were interrupted and such individuals were not included in the final sample size. Fifty replicates for each sex and species were performed. Blinded methods were used again, as males were dusted by a different author than that performing the experiments. For the male choice, and because females are distinguishable by the eye, the species of the male that performed the choice was unknown to the observer.

2b) Does mating behaviour differ according to species identity?

In those cases where females encounter heterospecific males only, the question is how different their mating behaviour will be, when compared with single encounters with conspecific males. If discrimination exists, female receptivity should be higher in the presence of conspecific males. To test this, a virgin female was placed on a leaf disc with either a conspecific or a heterospecific male, and latency to copulation and copulation duration were recorded (treatments A and B; see more details in Table 1).

2c) Does species identity affect behaviour in second matings that are preceded by conspecific matings?

When females mate first with conspecifics, the question is whether they are still receptive to subsequent matings with heterospecifics. To assess this, we performed double matings where a conspecific mating was followed by a heterospecific one (treatments G and H; Table 1) and conspecific double matings (treatments E and F; Table 1) as a control. In both cases, the mating interval was of either 0 or 24 h.

In both 2b) and 2c), the observers were unaware of the treatment they were assessing, as leaf discs were numbered and the association between number and treatment had been performed by other colleagues while setting up the experiments.

Statistical analysis

All analyses were carried out using R (version 2.15.3, R Development Core Team 2013). Differences among the fecundity of virgin females and females mated conspecifically and heterospecifically (treatments A and B) were analyzed using linear models within each species, with the type of mating (conspecific or heterospecific) as a fixed factor (question 1a, cf. Table 1).

We present the data comparing the behaviour of heterospecifically mated females to that of virgins separately from that of the other no-choice tests, because it answers a different question (question 1b). However, we analyzed this data together with all no-choice tests in a single analysis, to minimize the familywise error rate. Hence, we will here present the analysis of 1b, 2b and 2c together.

Since the time interval was shown to affect the degree of sperm precedence (Helle 1967; Satoh et al. 2001), we compared C vs D, E vs F and G vs H to test if behaviour in double matings differed according to the mating interval. When this was the case, the subsequent comparisons were done among crosses with the same interval. When no effect of the mating interval was detected for any mating sequence involved in subsequent comparisons, we grouped the observations involving different mating intervals for each mating sequence. We then used linear models with the type of mating as a fixed factor. If the factor was significant, this analysis was followed by planned contrasts corrected for multiple comparisons using the sequential Bonferroni correction. Question 1b was addressed by comparing treatments A vs C + D. A comparison between A vs B tested differences in behaviour between single conspecific and heterospecific matings (question 2b). Finally, we compared double conspecific matings with double matings with a heterospecific as the second mating (question 2c). The compared treatments were E vs G and F vs H for the latency in both species, and E + F vs G + H for copulation duration. For latency to copulation and to investigate the effect of the inclusion of data with 100 % latency (when matings did not occur after the 2-h period of observation), we performed (a) the same analysis as described above in a data set without these data and (b) a survival regression analysis, in which data referring to 100 % latency are coded as censored. Censored observations provide an information on the status of the male and female (i.e. not mated) to the analysis between time 0 and the end of the observation period but they do not provide any information to the analysis beyond that moment (see Supplementary material). Mating outcome in the presence of both species was analyzed with chi-square tests (question 2a).

Results

1a) Do heterospecific matings affect offspring viability?

Heterospecific crosses resulted in 98 and 100 % male offspring for crosses involving *T. urticae* and *T. evansi* females, respectively. Hybrid production between these species is thus negligible (and the few hybrid females produced eggs that did not hatch). Moreover, the fecundity (i.e. male offspring) of females that mated heterospecifically was not significantly different from that of virgin females or from that of females mated with a conspecific male, (Fig. 1; $F_{2,57} = 1.249$, $p = 0.294$ for *T. urticae* and $F_{2,73} = 0.238$, $p = 0.789$, for *T. evansi*). Therefore, mating with heterospecifics does not result in the aborted fertilization of oocytes.

1b) Does mating with a heterospecific male modify the behaviour of virgins?

In the general model, we found a significant effect of the mating sequence in the latency to copulation in both species (*T. urticae*, $F_{7,\ 276} = 14.55$, $p < 0.0001$; *T. evansi*, $F_{7,\ 298} = 19.31$, $p < 0.0001$). For *T. urticae* females, copulation duration was also affected by the mating sequence ($F_{7,185} = 6.453$, $p < 0.0001$), whereas no effect was found for *T. evansi* females ($F_{7,189} = 1.76$, $p = 0.098$). Hence, for *T. evansi*, we did not perform planned comparisons for the latter trait.

The mating interval did not significantly affect latency to copulation with conspecific males following heterospecific matings (C vs D: $|t| = 0.547$, $p = 0.584$ for *T. urticae* and $|t| = 0.174$, $p = 0.862$ for *T. evansi*; Fig. 2a, c). Latency to copulation of the second conspecific mating was significantly lower when it occurred immediately after the first mating than 24 h later, for both species (E vs F, $|t| = 5.41$, $p < 0.0001$ for *T. urticae* and $|t| = 3.32$, $p = 0.001$ for *T. evansi*; Fig. 4a, c). For both species, latency to copulation with heterospecific males following conspecific matings did not differ significantly

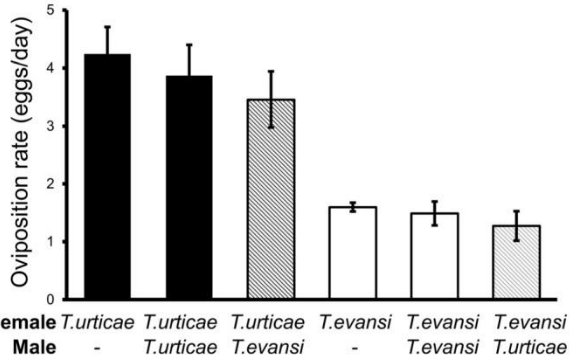

Fig. 1 Average daily fecundity of virgin, single conspecific and single heterospecifically mated *T. urticae* (*dark bars*) and *T. evansi* (*light bars*) females. *Full bars* correspond to conspecific matings and *striped bars* to heterospecific matings. *Error bars* represent the standard error of the mean

according to the mating interval (G vs H, $|t| = 0.841$, $p = 0.401$ for *T. urticae* and $|t| = 2.161$, $p = 0.0315$, $\alpha_c = 0.0083$ for *T. evansi*; Fig. 4a, c). Therefore, the mating interval affected latency in the second conspecific mating, but not in mating sequences involving heterospecific matings.

In all cases, the mating interval did not significantly affect copulation duration in *T. urticae* (second conspecific crosses, $|t| = 0.038$, $p = 0.970$; heterospecific crosses following conspecific ones, $|t| = 1.458$, $p = 0.147$; conspecific crosses following heterospecific ones, $|t| = 0.728$, $p = 0.467$; cf. general model for *T. evansi*; Figs. 2b, d and 4b, d).

In both species, the latency of conspecific matings following a heterospecific one was similar to that of single conspecific matings (A vs C + D, $|t| = 1.49$, $p = 0.137$ for *T. urticae* and $|t| = 0.232$, $p = 0.817$ for *T. evansi*; Fig. 2a, c). Similarly, there was no difference in the copulation duration of both species under the same conditions ($|t| = 1.548$, $p = 0.123$ for *T. urticae*; cf. general model for *T. evansi*; Fig. 2b, d). Therefore, females that mate with a heterospecific male behave as virgins in subsequent matings.

Overall, the additional analysis performed regarding latency to copulation did not yield different results from the one presented here, and are thus presented in the supplementary material (Table S1).

2a) Mating outcomes in the presence of both species

Four out of 54 *T. urticae* females failed to mate with any male after 2 h of observation; all of the 50 *T. evansi* females mated in this period. Females of *T. urticae* mated more often with conspecific than with heterospecific males ($\chi^2_1 = 3.92$, $p = 0.048$), whereas *T. evansi* females showed no preference ($\chi^2_1 = 1.285$, $p = 0.258$; Table 2). Seven out of 57 *T. urticae* males and 12 out of 62 *T. evansi* males did not mate after 2 h. While *T. urticae* males did not discriminate between females of the two species ($\chi^2_1 = 2$, $p = 0.157$), males of *T. evansi* mated assortatively ($\chi^2_1 = 3.92$, $p = 0.048$; Table 2).

2b) Does mating behaviour differ according to species identity?

Latency to copulation did not differ between single conspecific and heterospecific matings (A vs B, $|t| = 0.693$, $p = 0.489$ and $|t| = 0.694$, $p = 0.488$, for *T. urticae* and *T. evansi*, respectively; Fig. 3a). The duration of copulation was significantly longer in *T. urticae* conspecific matings than in heterospecific ones ($|t| = 4.217$, $p < 0.0001$; cf. general model for *T. evansi*; Fig. 3b). Therefore, mating behaviour of virgins toward conspecifics differs from that toward heterospecifics in *T. urticae*, but not in *T. evansi*.

2c) Does species identity affect behaviour in second matings that are preceded by conspecific matings?

In both time intervals, latencies of *T. urticae* conspecific and heterospecific second matings did not differ significantly (E vs G, $|t| = 1.13$, $p = 0.26$; F vs H, $|t| = 2.409$, $p = 0.017$, $\alpha_c = 0.0083$; for the 0- and 24-h intervals, respectively; Fig. 4a). Similarly, the latency to copulation of *T. evansi* heterospecific matings following conspecific ones did not differ significantly from that of conspecific double matings, for both mating intervals (E vs G, $|t| = 0.318$, $p = 0.751$ and F vs H, $|t| = 0.342$, $p = 0.732$ for the 0- and 24-h intervals, respectively; Fig. 4c).

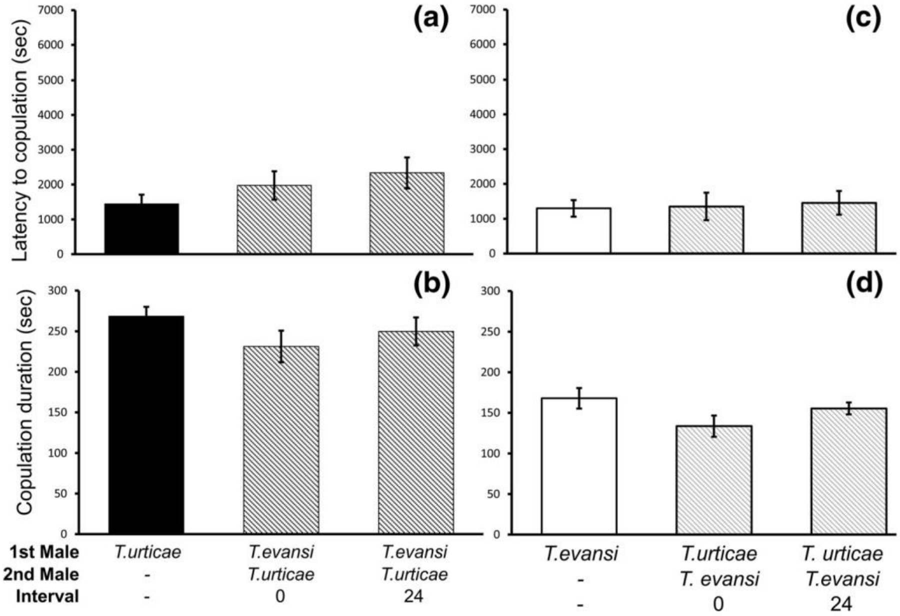

Fig. 2 Latency to copulation (**a**, **c**) and copulation duration (**b**, **d**) of single conspecific and double heterospecific matings of *T. urticae* (**a**, **b**) and *T. evansi* females (**c**, **d**). *Full bars* correspond to conspecific matings and *striped bars* to treatments in which the first mating was heterospecific and observations were made on the second, conspecific, mating. *Error bars* represent the standard error of the mean

Table 2 Mate choice experiments

Chosen mate	Female choice		Male choice	
	T. urticae	*T. evansi*	*T. urticae*	*T. evansi*
Conspecific	32	29	30	32
Heterospecific	18	21	20	18

Number of conspecific and heterospecific mates chosen by individuals after having been introduced in a leaf arena with one *T. urticae* and one *T. evansi* individuals of the opposite sex

No differences were found in copulation duration between conspecific and heterospecific matings that follow conspecific ones (*T. urticae*, EF vs GH $|t| = 1.271$, $p = 0.205$; cf. general model for *T. evansi*; Fig. 4b, d). Therefore, overall, species identity did not affect the mating behaviour of females that have previously mated with a conspecific.

Discussion

We investigated the occurrence of species recognition and its consequences for fertilization and mating behaviour in two spider mite species, with first-male sperm precedence. We found that heterospecific matings resulted in very few female offspring, confirming earlier results in this system (Sato et al. 2014). In spider mites, the production of females seems to be more costly than that of males (Macke et al. 2012). Hence, the production of male-only offspring removes the cost of producing potentially inviable females. Furthermore, since virgins and heterospecifically mated females produce the same number of unfertilized eggs (i.e. males), it seems unlikely that heterospecific sperm fertilizes oocytes, resulting in their arrested development. Therefore, our findings indicate that heterospecific crosses result in weak, if not absent, costs in terms of egg fertilization. Moreover, females that have mated with a heterospecific male remain as receptive to conspecific males as virgins (i.e. there were no significant differences in latency and copulation duration between these females). Hence, costs in terms of lowering the likelihood of future matings with conspecific sexual partners are also absent.

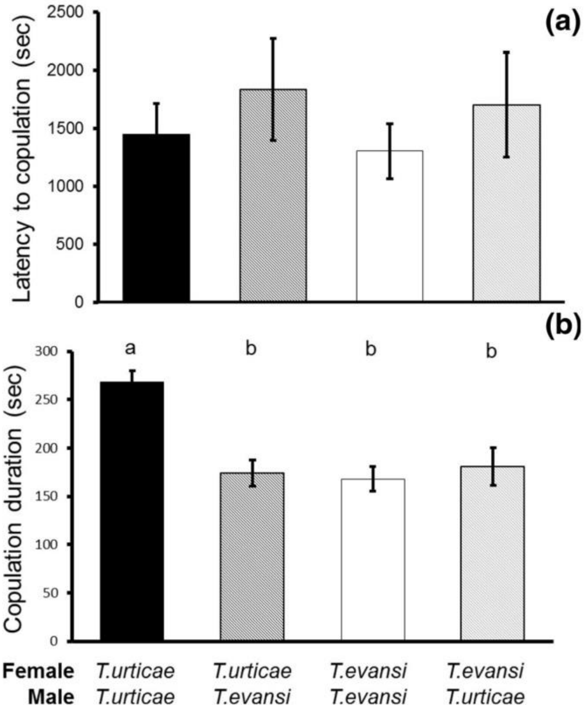

Fig. 3 Latency to copulation (**a**) and copulation duration (**b**) of single conspecific and single heterospecific matings of *T. urticae* (*dark bars*) and *T. evansi* females (*light bars*). *Full bars* correspond to conspecific matings and *striped bars* to heterospecific matings. *Error bars* represent the standard error of the mean

Given these reduced costs, we predict weak discrimination among these spider mite species. Indeed, mate-choice tests revealed that *T. urticae* males and *T. evansi* females did not discriminate between conspecific and heterospecific mates. Moreover, even *T. evansi* males and *T. urticae* females, which did mate assortatively, often chose to mate with heterospecifics. It must be noted that the experiments performed here (as most experiments on mate choice) do not allow discrimination between the effects of female preference and male–male competition on female mate choices (Wagner 1998). However, this does not affect the conclusion that heterospecific matings are likely to be common whenever populations of these two species co-occur. Furthermore, latency to copulation was similar for conspecific and heterospecific single matings in both species. *T. urticae* conspecific single matings, however, lasted longer than heterospecific single matings, which, in agreement with the mate choice results, points to some degree of discrimination in *T. urticae* females. These results do not fully reproduce the observation that both *T. urticae* and *T. evansi* males prefer *T. urticae* females (Sato et al. 2014). This difference can be due to variations in the protocols used or reflect variability among populations for this trait. In any case, the weak discrimination observed could result from the fact that the two species were only recently in contact, as the populations used were collected in areas where *T. evansi* has only recently invaded (Boubou et al. 2012) Possibly, this weak discrimination is due to a lack of specificity of chemical compounds that act as sexual attractants in each species, as shown to occur in arachnids (Gasket 2007), or to an inability of mates to distinguish between these attractants. It should be noted, however, that spider mites do perceive intraspecific differences and act accordingly. For example, *T. urticae* males show a clear preference for virgin over mated females (Oku 2010, 2014).

Despite the fact that virgins are equally receptive to males of both species, their remating behaviour may still be affected

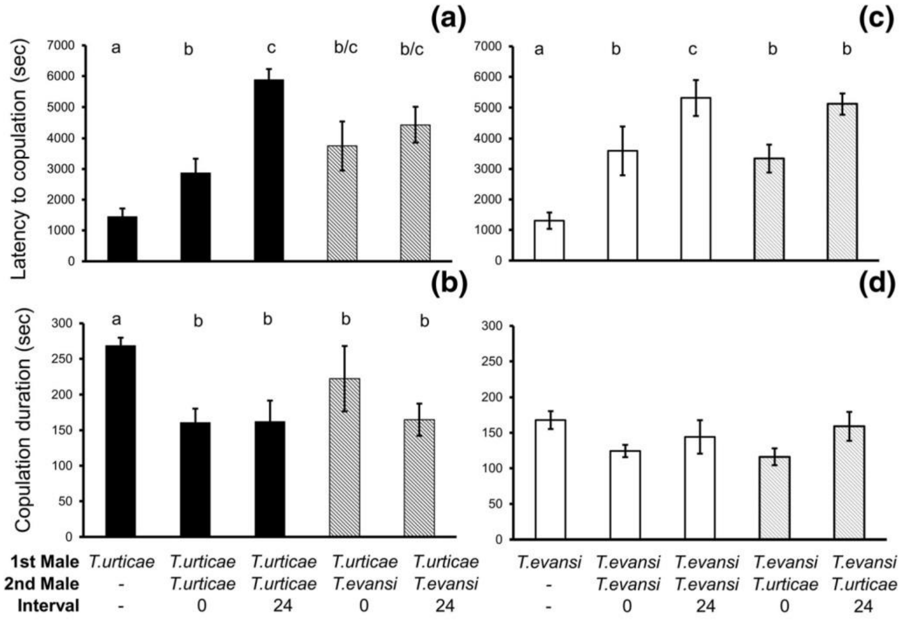

Fig. 4 Latency to copulation (**a**, **c**) and copulation duration (**b**, **d**) of single conspecific, double conspecific and double heterospecific matings of *T. urticae* (**a**, **b**) and *T. evansi* females (**c**, **d**). In treatments involving two males, *bars* represent matings by the second male. *Full bars* correspond to conspecific matings and *striped bars* to heterospecific matings. *Error bars* represent the standard error of the mean

if females modify their physiological status after a first mating. Given the occurrence of first-male precedence in *T. urticae*, once mated, females are expected to decrease their receptivity to males, and this may even be exacerbated if such males are heterospecific. Indeed, in both species, when females mate with a heterospecific male following a conspecific mating, latency to copulation in the second mating is higher than that in matings involving virgins. Furthermore, copulation duration is lower in the second conspecific mating than in the first, in *T. urticae*. These results are congruent with the occurrence of first-male precedence in *T. urticae* (Helle 1967) and lend support to the hypothesis that this is also the case in *T. evansi*, as suggested by Sato et al. (2014).

Moreover, when females mate with a heterospecific male following a conspecific mating, the behavioural traits observed are not different from those of females that mate twice with conspecific males. Hence, experience with conspecifics does not affect mate discrimination between conspecifics and heterospecifics. Furthermore, when the first mating is heterospecific, latency and copulation duration of second matings are similar to those of single conspecific matings. This finding is in agreement with studies on wolf spiders (Rutledge and Uetz 2014) and sticklebacks (Kozak et al. 2013), in which previous experience with either con- or heterospecific males did not modify female receptivity. These results suggest the existence of a cue informing about the success/failure of the first mating and that the first mating with a heterospecific might be perceived as an unsuccessful one. This putative cue may also underlie differences in the aerial dispersal of *T. urticae* females, according to their mating history. Indeed, this behaviour has been shown to occur with the highest frequency in conspecifically mated females, lowest in virgin females and with intermediary values in females that mated with a heterospecific male (Collins and Margolies 1991).

Together, these results indicate that heterospecific matings fail to trigger changes in behaviour that are normally induced by conspecific matings. Possibly, heterospecific males do not transfer sperm, or this sperm does not reach the oocytes. In any case, this may result in a lower selection pressure for traits involved in species recognition.

In more ecologically realistic environments, a cost of misrecognition may arise. In fact, Macke et al. (2012) showed that, in *T. urticae*, females that mate multiple times had a lower total reproductive investment. Possibly, mating with several heterospecific males may also entail some cost. In line with this possibility, Sato et al. (2014) found that virgin females placed on plants with conspecific and heterospecific males had lower fecundity than females that had mated conspecifically before being placed on such plants. This suggests that (a) mated females, being less receptive, mated less often with heterospecific males than virgins, and that (b) repeatedly mating with heterospecific males entails a cost. Hence, the question remains as to why discrimination has not evolved. It could be that these species rarely encounter each other in the field. However, field data show that this is not the case (Ferragut et al. 2013). Still, given that invasion of Europe by *T. evansi* is recent (Boubou et al. 2012), it may be that contact has not occurred for sufficient time for adaptation to the presence of the competitor to occur. Once both species meet in the field, severe costs resulting from the low species discrimination level in this system should only be expected when a virgin female arrives to a patch where conspecific density is very low, where she will mate indiscriminately with heterospecifics, as in Sato et al. (2014). This situation, however, is probably not very common in nature, as in these species, female dispersal occurs mainly after a mating event (Mitchell 1970; Collins and Margolies 1991).

In sum, our results indicate that an accurate assessment of the potential costs of mating with heterospecifics necessitates knowledge on the intraspecific mating system, namely sperm precedence. This information, corroborated with knowledge on the ecology and evolutionary history of the spider mites species studied here, allowed us to conclude that mating with heterospecifics is expected to result in low, if any, costs. Therefore, the lack of discrimination we also find here is not surprising. Whether costs will be low in other spider mite species is unknown, but one could speculate that they may be higher in species that are more closely related to each other and hence produce hybrids. Indeed, many examples of cryptic speciation exist in mites, among which isolation is not complete (Skoracka et al. 2015). In such cases, mating with a heterospecific may modify the future receptivity of females, especially in the case of first-male precedence. Moreover, being haplodiploid allows addressing the costs of heterospecific matings even in the absence of fertilization, given the production of haploid males from unfertilized eggs. Spider mites can, thus, be seen as good models to study the evolution of reproductive isolation, regarding both pre- and post-mating barriers.

Acknowledgments The authors wish to thank Flore Zélé and Inês Santos for helping with the maintenance of mite cultures and improving experimental infrastructures, Jordi Moya-Laraño for suggestions concerning statistical analyses, and FZ, IS, Yukie Sato and Maurice W. Sabelis for their helpful discussions. These experiments were funded by the Portuguese National Funds through an FCT-ANR project (FCT-ANR//BIA-EVF/0013/2012) to SM and Isabelle Olivieri. SHC and LRR had PhD fellowships funded by FCT (SFRH/ BD/ 90156/2012 and SFRH/BD/87628/2012, respectively). SAMV and RP had post-doctoral fellowships from FCT, (SFRH/BPD/66042/2009 and SFRH/BPD/42801/2008), respectively. We also thank Margarida Santos Reis for her support in the initial phase of this project.

Author contributions SM conceived the study; SM and SAMV designed the experiments; SHC, LRR and RP collected the data; SHC and SAMV analyzed the data; and SHC, SAMV and SM wrote the manuscript, with significant input from all authors.

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Table S1 Alternative analysis of latency to copulation in questions 1b, 2b and 2c (cf. Material and Methods). Without 100% lat: the original analysis as in the manuscript, but excluding females that had not mated at the end of the observation period. Survival regression: survival regression analysis of latency to copulation, in which data referring to 100% latency are included, but coded as censored. n.s. αc = non-significant after accounting for the Bonferroni sequential correction.

Species	Contrast	Question	Analysis			
			Without 100% lat		**Survival regression**	
			|t|	P	|t|	P
T. urticae	C vs D	1b)	1.90	0.17	0.44	0.96
	E vs F	1b)	2.82	0.02	5.80	< 0.0001
	G vs H	1b)	0.36	0.99	0.99	0.75
	A vs CD	1b)	1.54	0.33	1.49	0.36
	A vs B	2b)	0.09	1.00	0.17	1.00
	E vs G	2c)	0.49	0.97	0.73	0.89
	F vs H	2c)	2.19	0.11	2.94	0.013 n.s. αc
T. evansi	C vs D	1b)	0.36	0.98	0.55	0.93
	E vs F	1b)	0.12	1.00	3.42	0.00
	G vs H	1b)	2.714	0.02 n.s. αc	2.03	0.15
	A vs CD	1b)	1.55	0.32	0.73	0.85
	A vs B	2b)	1.23	0.58	0.52	0.96
	E vs G	2c)	0.03	1.00	0.03	1.00
	F vs H	2c)	3.40	0.00	0.26	1.00

Chapter 4

Despite reproductive interference, the net outcome of reproductive interactions among spider mite species is not necessarily costly

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Abstract

Reproductive interference is considered a strong ecological force, potentially leading to species exclusion. This supposes that the net effect of reproductive interactions is strongly negative for one of the species involved. Testing this requires a comprehensive analysis of interspecific reproductive interactions, accounting for the order and timing of mating events, and for their effects on either fertility or fecundity. To this aim, we measured reproductive interactions between a focal species, *Tetranychus urticae*, and an invasive (*T.evansi*) and a native (*T. ludeni*) species, varying the mating sequence and interval, and measuring the effect of such crosses on fecundity and offspring sex ratio (a measure of fertility, as these species are haplodiploid). We found that mating with heterospecifics affected fecundity and sex ratio negatively, but also positively, depending on the species involved, and on the order and timing of mating events. To assess the net effect of these interactions on *T. urticae*, we performed a meta-analysis on the data obtained. This revealed that the net effect of the interaction *T. urticae* / *T. evansi* was neutral, whereas that between *T. urticae* and *T. ludeni* was slightly positive for *T. urticae*. Therefore, the net effect of such interactions may be weak despite strong effects of particular events. In natural situations the outcome of reproductive interactions will thus hinge upon the frequency of each event.

Keywords

Biological invasions, sperm precedence, *Tetranychus*, reproductive interactions, mating, meta-analysis.

Introduction

Reproductive interference refers to any kind of sexual interaction between two species that diminishes the fitness of at least one of them (Gröning & Hochkirch 2008, Kishi et al. 2009, Burdfield-Steel & Shuker 2011). It can occur at different levels: overlapping or masking conspecific sexual signals (signal jamming), interrupting conspecific sexual interactions, or promoting heterospecific matings, thereby reducing the frequency or outcome of conspecific matings, or inducing hybridization, leading to a lower offspring fitness (Gröning & Hochkirch 2008). Given these negative effects, reproductive interference may lead to the exclusion of one of the species involved (Gröning & Hochkirch 2008; Kishi et al. 2009). Indeed, theory predicts that reproductive interference may contribute to species exclusion more often than resource competition (Kishi et al. 2009, Kishi & Nakazawa 2013). For example, it has been posited that reproductive interference may underlie the success of some invasive species (e.g. Nishida et al. 2012), if it is stronger between invasive and natives than among natives.

The bulk of studies of reproductive interference concerns the fitness outcome of interspecific matings of two species that do not produce viable hybrids (Gröning & Hochkirch 2008). In this case, the reproductive effects of the interspecific interaction will be expressed only when organisms mate with both conspecifics and heterospecifics (as mating with heterospecifics alone will yield no offspring). Moreover, clearly evaluating the effects of reproductive interference on species exclusion necessitates measuring all possible combinations of mating order (i.e., whether heterospecific matings occur before or after conspecific ones) and timing (i.e., the interval between mating events) between pairs of species. Moreover, it is important to test whether reproductive interactions affect fecundity (egg production) or fertility (egg fertilization). This information can then be integrated to predict the net outcome of reproductive interactions between species. Despite the many studies on reproductive interference, none has yet applied this approach. Indeed, some studies attempt to predict how reproductive interference affects species exclusion, but do so while not measuring all possible effects of this interaction. For example, Takafuji (1997) used a Lotka-Volterra modified model to predict the effect of reproductive interference between two *Panonychus* mite species from Japan (*Panonychus citri* and *P. mori*) on species exclusion. However, only one possible combination of mating interactions between these two species (a female mating first with a heterospecific then with a conspecific) was tested. In contrast, other studies consider different orders of mating events (e.g. Kyogoku & Nishida 2013), but do not integrate this information to generate a prediction concerning the net effect of reproductive interactions on species distributions.

Here, we aimed at testing how the outcome of different mating events among spider mite species can be integrated into a net measure of the effect of reproductive interactions on a focal species. Spider mites are haplodiploid, hence the distinction between fecundity and fertilization effects can be made given that fertilized eggs result in female offspring and unfertilized eggs in male offspring. Thus, fertilization failures can be detected by a reduction in the proportion of female offspring, whereas impairment of egg production is detected by a reduction in the total number of offspring. Moreover, reproductive interference has been frequently observed in this group (Collins & Margolies 1994, Takafuji et al. 1997, Ben-David et al. 2009, Sato et al. 2014).

We studied the outcome of reproductive interactions in a system composed of one focal species – the spider mite *Tetranychus urticae* – in sexual heterospecific interactions with another native species, *T. ludeni*, and an invasive species, *T. evansi*. These three herbivorous species co-occur in the Mediterranean region and are often found on the same host plants (Escudero & Ferragut 2005, Boubou et al. 2012, Godinho et al. 2016). Whereas *T. urticae* and *T. ludeni* are native species, *T. evansi* has only recently invaded the European continent (Boubou et al. 2012). We used *T. urticae* as the focal species because it is the spider-mite species for which most information is available. Indeed, it has been shown that this species exhibits first male sperm precedence, with second matings being sometimes effective if they occur within the 24 hours following the first (Helle 1967). However, females that mate multiple times with conspecific males, after the 24h interval, produce fewer fertilized offspring (i.e., females) (Macke et al. 2012), suggesting that sperm displacement after 24h is possible. Hence, we hypothesize that mating order and the mating interval will affect the outcome of reproductive interference in *T. urticae*. Whereas information on the interaction between *T. urticae* and *T. ludeni* is as yet lacking, heterospecific matings have been observed between *T. urticae* and *T. evansi* (Sato et al. 2014, 2016, Clemente et al. 2016). Moreover, *T. evansi* has been shown to exclude *T. urticae* on tomato plants (Sarmento et al. 2011a), a result that correlates with field observations (Ferragut et al. 2013). Finally, a recent study has shown that, in competition with *T. evansi*, the population growth of *T. urticae* is more severely affected when plants are colonized by virgin females than when plants are colonized by mated females, suggesting that reproductive interference may be responsible for the species distribution patterns observed (Sato et al. 2014).

Material and Methods

Stock Cultures

The mite species used in this study were collected in Carregado (39.022260, -8.966566), Portugal, and all laboratory populations were established from an initial pool of 300 mated females. The laboratory population of *T. urticae* was collected on tomato plants (*Solanum lycopersicum)* in May 2010, that of *T. evansi* on *Physalis angulata* in May 2012 and that of *T. ludeni* on tomato in September 2012. The populations of *T. evansi* and *T. ludeni* became extinct in August 2012 and May 2013, respectively, being subsequently replaced with populations from the same location, both collected in *Datura stramonium* plants. Both populations of *T. evansi* and *T. ludeni* were used in the experiments.

Species identity was confirmed through polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) of the ITS2 region (Hurtado et al. 2008), on approximately 50 females of each population. Total genomic DNA was extracted from each individual spider mite using the Sigma-Aldrich GenEluteTM Mammalian Genomic DNA Miniprep Kit, following manufacturer´s instructions, except for the elution volume, which we set to 20μL of RNase free water (Qiagen NV, Venlo, The Netherlands) to increase the concentration of DNA obtained from this very small animal (c.a. 300μm long).

Adult females from populations used in this experiment were screened for *Wolbachia* using the primers *wsp* (*Wolbachia*-specific primers) 81F and 691R (Braig et al. 1998). We did this to avoid potential cytoplasmic incompatibility as a confounding factor in our measurements. PCR assay procedures were as described in Breeuwer (1997). Results were positive for *Wolbachia* infection and spider mite populations were thus treated by placing adult females in detached bean leaves with tetracycline (0.025% w/v) for three consecutive generations, then absence of *Wolbachia* was confirmed using the same protocol as above. Other endosymbionts tested (Arsenophorous, Rickettsia, Spiroplasma and Cardinium) were absent from these populations.

Bean (*Phaseolus vulgaris*) and tomato (*Solanum lycopersicum*) plants were planted every week and grown in an herbivore-free greenhouse, being watered two to three times a week. *T. urticae* populations were maintained on trays with 6-10 bean plants whereas those of *T. evansi* and *T. ludeni* were kept on tomato plants at 25°C, both with a 16 L: 8D photoperiod. Plant trays were changed every two weeks, placing old leaves on top of uninfested plants. Cultures were kept inside plastic boxes (28x39x28 cm), with an opening of 25x15 cm polyamide fabric (80 µm mesh width).

Experimental procedure

Experiments were done on the plant species from which the female tested had been cultured. As in the literature there was no information on whether hybridization is possible between *T. urticae* and *T. ludeni*, we studied the outcome of a single heterospecific mating between these two species (the same analysis for *T. urticae* and *T. evansi* was performed in a previous experiment (Clemente et al. 2016)). Subsequently, we set out to study the heterospecific interactions between *T. urticae* and the invasive *T. evansi* and the native *T. ludeni* species for which we analysed the outcome of mating with a heterospecific male before or after a conspecific male. Since we focused on interactions with *T. urticae* (the focal species of our study), we performed crosses between *T. urticae* males or females and *T. evansi* or *T. ludeni* males or females, but not between the two latter species. All experiments were performed in an acclimatized room at approximately 25°C.

a) The outcome of a single heterospecific mating between *T. urticae* and *T. ludeni*

To determine whether hybridization occurred between *T. urticae* and *T. ludeni*, we measured the offspring sex-ratio resulting from single heterospecific matings. Given that only females develop from fertilized eggs, a whole-male offspring would mean unsuccessful hybridization. However, even in the absence of viable hybrids, heterospecific matings could result in aborted development of heterospecifically-fertilized eggs, meaning that females would produce fewer eggs. To test this, we compared the fecundity of *T. urticae* and *T. ludeni* females that mated with a heterospecific male to that of virgin females and of females mated with a conspecific male.

Females were collected from the stock populations, isolated at the quiescent deutonymph stage (which precedes their last moult before reaching adulthood), and kept in groups of approximately 15 females on bean (*Phaseolus vulgaris*) leaf discs (2 cm^2) until emergence, to ensure their virginity. Adult males were collected from the same stock populations and kept isolated in leaf discs (2 cm^2) for at least 24 hours before the assay, to ensure sperm replenishment. Females were placed individually in leaf discs (1 cm^2) with either a conspecific or a heterospecific male and observed continuously until copulation occurred. Only matings that lasted at least 1 minute were considered effective (Boudreaux 1963). These experiments had the maximum duration of 2 hours. If no mating occurred within this time, individuals were discarded. Subsequently, females were isolated in a leaf disc (2 cm^2), then transferred to a new disc every three days until the female's death. The number of eggs laid was registered after female transfer to a new leaf disc. Eggs were left to develop until adulthood when offspring sex-ratio could be determined. With this data, we tested whether heterospecific matings affected (a) the mean daily fecundity and (b) offspring sex ratio (hence the proportion of fertilized offspring).

b) The outcome of heterospecific matings that precede or follow conspecific ones

To determine the outcome of mating with a heterospecific male before or after a conspecific male between *T. urticae* and the other two species, we compared the fecundity and offspring sex ratio of those crosses to that of females that mated with two conspecific males. The experimental procedure was as described above, except that we let females mate with a conspecific or a heterospecific male, then placed the focal females with another male. We created the following mating sequences: conspecific-conspecific, conspecific-heterospecific and heterospecific-conspecific. The second mating occurred either immediately after the first mating (0 hours treatment) or 24 hours later. If no mating was observed within 2 hours, the females were discarded. We used the 0h and 24h mating intervals because the time interval was shown to affect the degree of sperm precedence in spider mites (Helle 1967).

Statistical analysis

All analyses were carried out using R (version 3.3.2, R Development Core Team 2016). To analyse female fecundity within each species (*T. urticae*, *T. evansi* and *T. ludeni*), we used linear models (LM procedure), considering the mean number of eggs per day as the response variable (oviposition rate). To analyse offspring sex ratio within each species, we used generalized linear models (GLM procedure) with a quasi-binomial distribution – due to overdispersion of the data –, considering the number of female and male offspring produced by each focal female as the response variables (analysed together with the function cbind).

For both types of analyses, we used as fixed factors the mating order (with three levels: the control treatment, where a female mated twice with conspecific males; an experimental treatment where the heterospecific male was the first to mate with the female; and another experimental treatment where the heterospecific male was the second to mate with the female) and the mating interval (with two levels: either 0h or 24h interval between matings). We also tested the interaction among these fixed factors. If the interaction was non-significant, a backward stepwise procedure was used to find the best simplified fitted model. We performed independent analyses for each species within each species pair (i.e. for *T. urticae* and *T. evansi* females in *T.*

urticae versus T. evansi crosses; and for *T. urticae* and *T. ludeni* females in *T. urticae versus T. ludeni* crosses), as shown in Table 1.

We did a first block of experiments with the populations of *T. evansi* and *T. ludeni* collected in 2012 (block 1). For question b) we also did a second block of experiments with populations of those species from 2013 (block 2). In block 2 we did not repeat all treatments, but only the crosses that were not complete before the extinction of block 1 populations, as well as their respective controls – hence, there were no treatments that were only performed in block 2. Because of that, instead of including the factor block in the statistical models as a covariate, we did all the statistical analyses with block 1 only and with block 1 and block 2 together. Since the results were qualitatively similar, here we present the results from the analysis with block 1 and block 2 together.

With the outputs from these analyses, we further compared the general net effects of reproductive interference from the invasive and native species on *T. urticae* with a meta-analysis procedure (Borenstein et al. 2009; Nakagawa & Poulin 2012). This procedure allowed us to test which species, within each species pair, exerts the strongest effect on the other; and whether, between species pairs, invasive-native heterospecific sexual interactions are more severe than native-native interactions. For that we calculated the effect sizes of the statistical results obtained from the LM and GLM analyses described above and shown in Table 1, converting p-values and sampling sizes into the Fishers' z transformation of the correlation coefficient (Z_r) and its corresponding variance (Var_{Zr}). The correlation coefficient varies between -1 and 1 and can be interpreted as the strength of female response with respect to oviposition rate and offspring sex ratio: the more significant the p-values obtained from the LM and GLM models the greater the departure from a random response, and so the "stronger" the effect of reproductive interference of *T. evansi* and *T. ludeni* on *T. urticae* and vice versa.

We used the p-values from the contrasts between the control and the two experimental treatments. However, to avoid duplicating the contribution of the control to the effect sizes (Borenstein et al. 2009), we did two independent analysis, one for when a female's first mating was with a heterospecific male and a second analysis for when a female's second mating was with a heterospecific male. The effect sizes are shown in Table S1 from the Supplementary Material. Additionally, because each female contributed with two data outputs (oviposition rate and offspring sex ratio), and to avoid redundancy in our data again, we calculated a synthetic effect size that was defined as the mean between oviposition rate and offspring sex ratio and their variance (Borenstein et al. 2009). To calculate the variance of the mean, we had to calculate a correlation between outcomes (Borenstein et al. 2009). We did this using a Pearson correlation, and obtained 0.18 (shown in Table S2 from the Supplementary Material).

The effect sizes could be either positive or negative, depending on whether the interactions of *T. urticae* with the other species were beneficial or costly to *T. urticae*: positive effects occurred when oviposition rate and offspring sex ratio increased in *T. urticae* females or decreased in *T. evansi* and *T. ludeni* females; negative effects occurred in the opposite way.

We used the Compute.es package (Del Re 2013) to convert p-values and sample sizes into Z_r and Var_{Zr} (see Tables S1 and S2 in Supplementary Material) and the Metafor package v1.9-8 (Viechtbauer 2010) for the meta-analysis (Table S3 in Supplementary Material). We used a meta-analytic fixed-effects linear model (using the rma.uni function in Metafor) with the interfering species (Invasive versus Native) as the explanatory variable.

Results

a) The outcome of a single heterospecific mating between *T. urticae* and *T. ludeni*

Crosses between *T. ludeni* and *T. urticae* resulted in 100% male offspring, indicating that hybrid production between these species is inexistent. The fecundity of *T. urticae* females that mated heterospecifically was not significantly different from that of virgin females or from that of females mated with a conspecific male (Figure 1 and Table 1). On the other hand, the fecundity of *T. ludeni* females that mated with conspecifics or heterospecifics was significantly higher than that of virgin females (Figure 1 and Table 1). Therefore, mating with heterospecific males does not result in the aborted fertilization of oocytes for *T. urticae* and *T. ludeni* females.

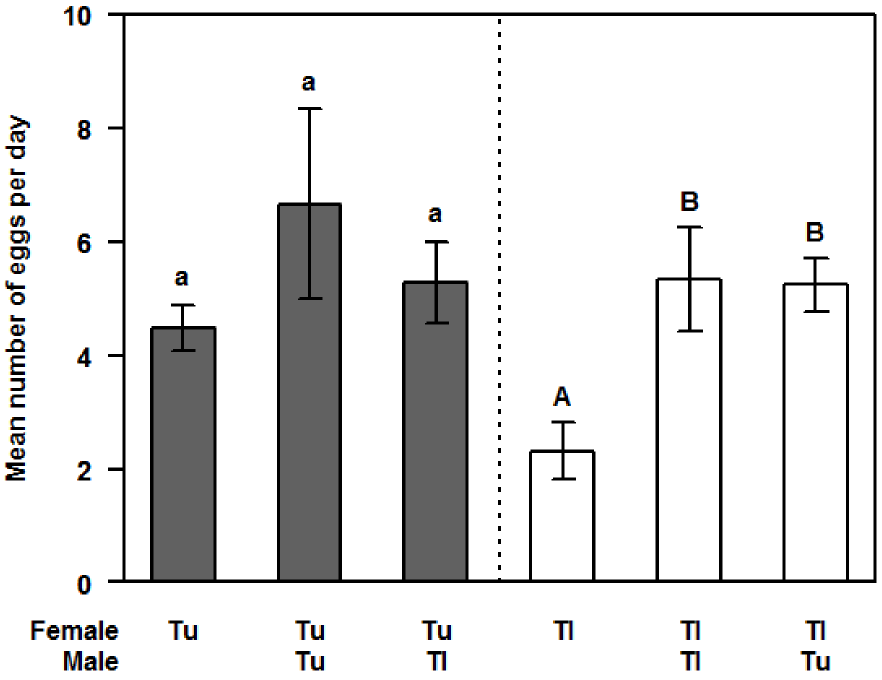

Figure 1 - Average daily fecundity of virgin females, and of females that have mated with a conspecific or a heterospecific male. Tu: *T. urticae* males or females; Tl: *T. ludeni* males or females. Grey bars: matings involving *T. urticae* females; white bars: matings involving *T. ludeni* females. Error bars represent the standard errors of the mean.

b) The outcome of heterospecific matings that precede or follow conspecific ones

(i) T. urticae vs T. evansi

The oviposition rate of *T. urticae* females that mated with either a conspecific and a heterospecific or with two conspecific mates varied significantly according to mating order in interaction with mating interval (Table 1). Specifically, it was higher for *T. urticae* females that mated with *T. evansi* males just before mating than for any other cross at 0h mating interval (Fig. 2a, Table 1). At the 24h interval, however, mating combinations did not affect this trait. The proportion of fertilized offspring (*i.e.*, daughters) of females *T. urticae* also varied significantly according to mating order in interaction with mating interval (Table 1). But in contrast to the oviposition rate, this trait was affected at the 24h interval only, in which mating with a *T. evansi* male after mating with a conspecific male resulted in a decrease in the proportion of fertilized offspring of *T. urticae* females, relative to other mating sequences (Fig. 2b, Table 1).

The mating order also affected differentially the oviposition rate of *T. evansi* females, depending on the interval between matings. *T. evansi* females that mated with *T. urticae* males immediately after conspecific mates had reduced oviposition rate relative to other mating sequences at this time interval (Fig. 2c; Table 1); however, if the heterospecific cross occurred 24 hours before the conspecific cross, the oviposition rate of *T. evansi* females increased relative to other mating sequences at this time interval (Fig. 2c; Table 1). These crosses did not significantly affect sex ratio (Fig. 2d; Table 1).

(i) T. urticae vs T. ludeni

In crosses with the native species (*T. ludeni*), the oviposition rate of *T. urticae* females varied significantly according to mating order in interaction with mating interval (Table 1). Specifically, we found no effect of the mating order at 0h interval, but at 24h interval the oviposition rate of females that mated first with a conspecific then with a heterospecific male was lower than that of other crosses at this time interval. (Fig. 3a; Table 1). *T. urticae* females suffered no significant changes in offspring sex ratio from matings with *T. ludeni* males (Figure 3b; Table 1).

In *T. ludeni* females, the oviposition rate and the proportion of fertilized offspring varied significantly according to mating order in interaction with the mating interval (Table 1). Compared to the control treatment, *T. ludeni* females had lower oviposition rate when mating with *T. urticae* males immediately before conspecifics males, or when hetero- and conspecific matings had 24h interval, irrespective of the mating order (Fig. 3c, table 1). Additionally, when *T. ludeni* females mated with *T. urticae* males 24h after conspecific matings, the proportion of fertilized offspring was significantly lower than that of other crosses at this time interval (Figure 3d; Table 1). The mating sequence had no effect on the sex ratio at the 0h interval.

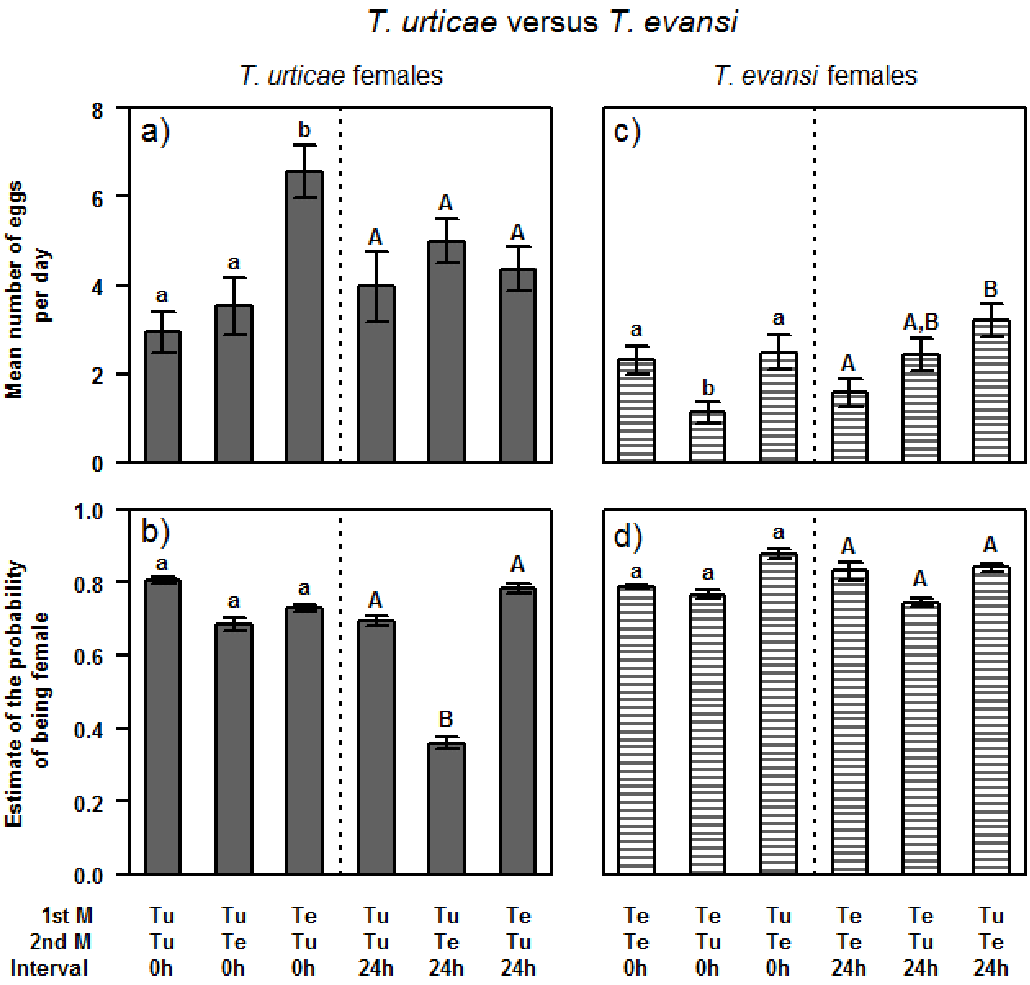

Figure 2 - Average daily fecundity and estimated offspring sex ratio resulting from interactions between *T. urticae* (a, b; grey solid bars) and *T. evansi* (c,d; striped bars) females with conspecific and heterospecific males. In each plot, bars on the left side of the dotted straight line correspond to treatments where second matings occurred immediately (0h) after the first one; bars on the right side correspond to treatments where second matings occurred 24h after the first one. "1st M": first male that mated with the female; "2nd M": second male. The interval indicates the time of occurrence of the second mating, i.e., if immediately after the first mating (0h) or 24h later. "Tu": *T. urticae* males; "Te": *T. evansi* males. Letters above the bars indicate significant differences among treatments (small letters: among crosses occurring with a 0h interval; capital letters: among crosses occurring with a 24h interval). Error bars represent the standard errors of the mean. For offspring sex ratio, we obtained the estimates of the probability of being female and correspondent standard errors of the mean from the statistical GLM models. This takes into account sex ratio variation among females, as well as the quasi-binomial correction for overdispersion of the data.

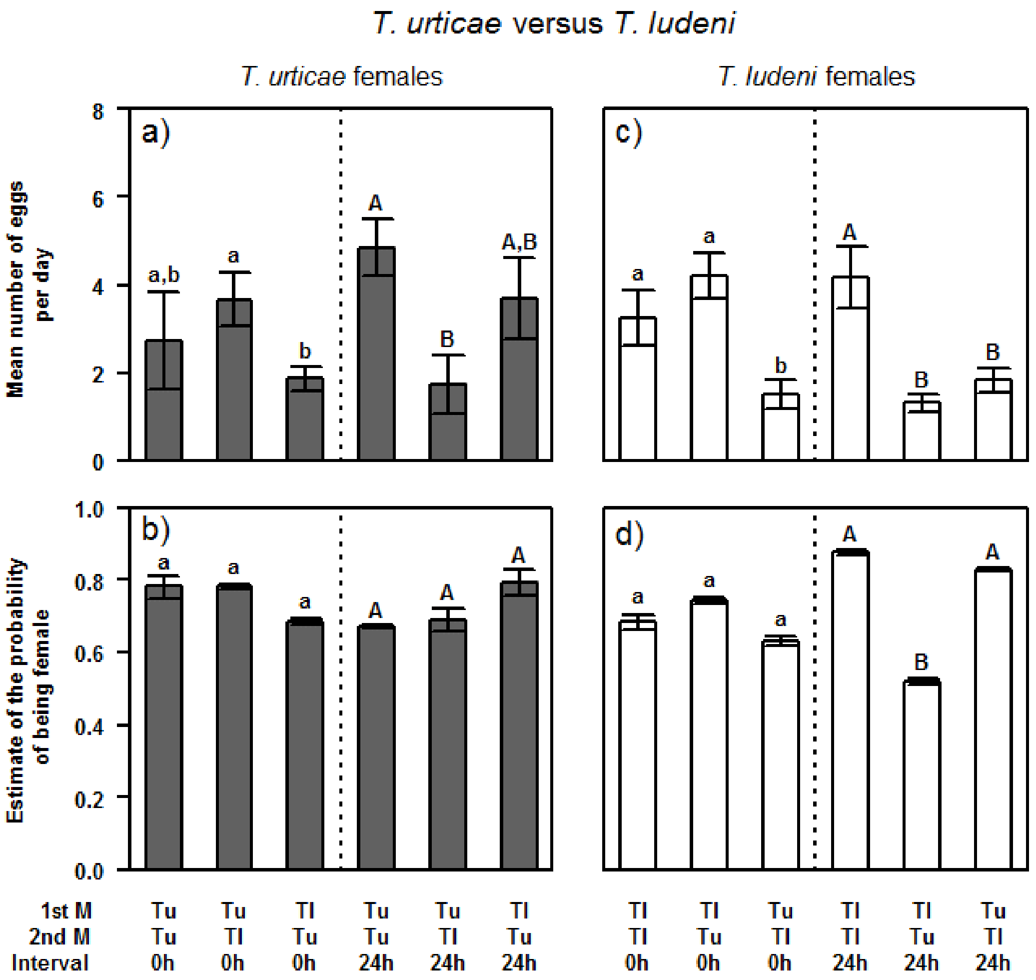

Figure 3 - Average daily fecundity and estimated offspring sex ratio resulting from interactions between *T. urticae* (plots a, b; grey bars) and *T. ludeni* (plots c, d; white bars) females with conspecific and heterospecific males. In each plot, bars on the left side of the dotted line correspond to treatments where second matings occurred immediately (0h) after the first one; bars on the right side correspond to treatments where second matings occurred 24h after the first one. "1st M": first male that mated with the female; "2nd M": second male. The interval indicates the time of occurrence of the second mating, i.e., if immediately after the first mating (0h) or 24h later. "Tu": *T. urticae* males; "Tl": *T. ludeni* males. Letters above the bars indicate the significant differences between treatments (small letters: among crosses occurring with a 0h interval; capital letters: among crosses occurring with a 24h interval. Error bars represent the standard errors of the mean. For offspring sex ratio, we obtained the estimates of the probability of being female and correspondent standard errors of the mean from the statistical GLM models. This takes into account sex ratio variation among females, as well as the quasi-binomial correction for overdispersion of the data.

Meta-analysis on the effects of mating with heterospecifics

Because the effects of mating with heterospecifics were contingent upon the species involved, but also the order and timing of mating events, we performed a meta-analysis on these results to obtain the net effect of each interaction (Figure 4 and Tables S1, S2 and S3).

The overall effect of mating with heterospecifics was neutral, though slightly positive, for *T. urticae*, both when the female's first and second matings were with a heterospecific male (first male heterospecific: $Z_r = 1.088$, $P = 0.277$; second male heterospecific: $Z_r = 1.439$, $P = 0.150$). Matings involving the invasive species did not result in overall net costs or benefits for *T. urticae* (first male heterospecific: $Z_r = -0.460$, $P = 0.646$; second male heterospecific: $Z_r = 0.087$, $P = 0.931$). Matings with the native species, on the other hand, were mainly beneficial, both for first ($Z_r = 1.878$, $P = 0.060$, marginally significant) and second matings with a heterospecific male ($Z_r = 1.989$, $P = 0.047$). The difference between matings with the invasive and the native species was, however, non-significant (first male heterospecific: $Z_r = 1.598$, $P = 0.110$; second male heterospecific: $Z_r = 1.376$, $P = 0.169$).

Concerning the effect of the mating interval, when matings occurred at the 0h interval, the net effect for *T. urticae* from both invasive (first male heterospecific: $Z_r = 0.080$, $P = 0.936$; second male heterospecific: $Z_r = 1.234$, $P = 0.217$) and native (first male heterospecific: $Z_r = 0.497$, $P = 0.619$; second male heterospecific: $Z_r = -0.671$, $P = 0.502$) species was mainly neutral, with no significant differences between the net effect from the invasive and the native species (first male heterospecific: $Z_r = -0.279$, $P = 0.781$; second male heterospecific: $Z_r = 1.310$, $P = 0.190$). When matings occurred at the 24h interval, the net effect for *T. urticae* from matings with the invasive species was again neutral (first male heterospecific: $Z_r = -0.787$, $P = 0.431$; second male heterospecific: $Z_r = -1.237$, $P = 0.216$). Contrastingly, however, the net effect for *T. urticae* from matings with the native species was significantly positive for both first ($Z_r = 2.219$, $P = 0.027$) and second matings ($Z_r = 3.223$, $P = 0.001$) with heterospecifics. Additionally, there were significant differences between the invasive and native species (first male heterospecific: $Z_r = -2.051$, $P = 0.040$; second male heterospecific: $Z_r = -3.099$, $P = 0.002$).

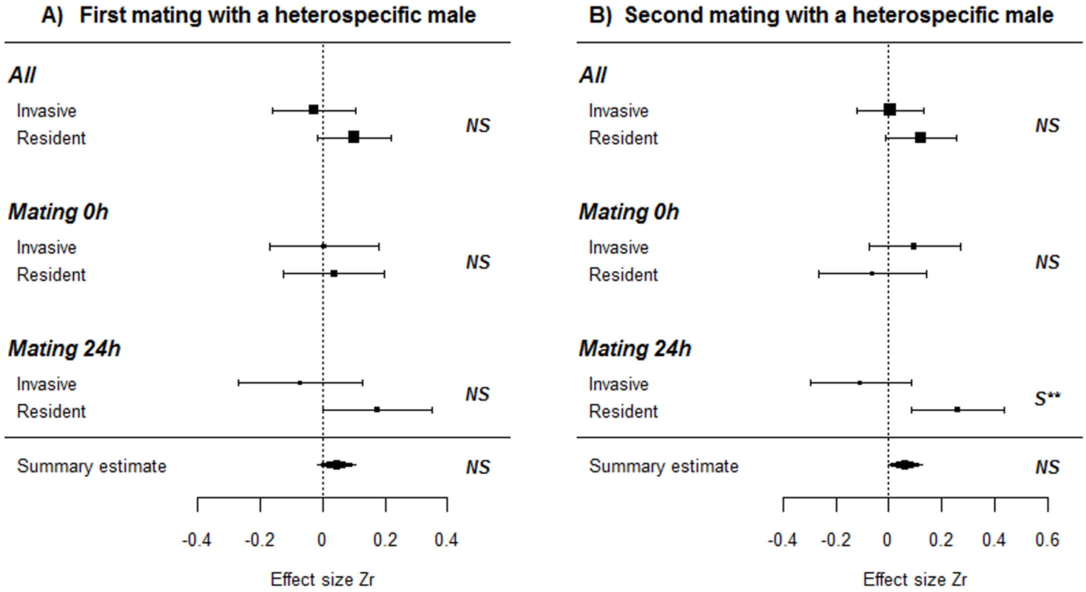

Figure 4 - Mean strength of reproductive interference by the invasive (*T. evansi*) and native (*T. ludeni*) species on *T. urticae*, when a female's first (A) or second (B) mating is heterospecific. Squares show the mean effect size estimates derived from the meta-analytic models; the squares' size represent the weights given to the observed effects during the model fitting; and the bars show the 95% confidence intervals (CI) around the mean effect size estimates. Negative or positive effects towards *T. urticae* are significant when the effect size and both anchors of the CI fall below or above zero. The results of the meta-analytic models testing the effect of the interfering species (invasive versus native) on all effect sizes and for each subgroup of explanatory variables (Mating intervcal with a first male at 0h or 24h) are shown with the "NS", "S*" and "S**" symbols: "NS" for non-significant differences ($p>0.05$); "S*" for significant differences ($p<0.05$); and "S**" for significant differences ($p<0.01$). At the bottom is a summary effect size representing pooled effect sizes. The effect sizes were defined as the mean between female fecundity and offspring sex ratio and their variance. To obtain the variance of the mean, we calculated a correlation between outcomes, which was 0.18.

Table 1 - Statistical tests and contrasts for the comparisons of fecundity and offspring sex ratio in crosses between con- and heterospecific males and females.

	Matings			**Fecundity (F-test)**	**Sex-ratio (F-test)**
a) Single mated females					
T. urticae* vs *T. ludeni					
With *T. urticae* females					
Mating order				$F_{2,78}$ = 1.886, P = 0.1585	
Contrasts	No mating	*vs*	Tu	|t| = 0.922; P = 0.3595	
		vs	Tl	|t| = 1.885; P = 0.0631	
	Tu	*vs*	Tl	|t| = 1.083; P = 0.2822	
With *T. ludeni* females					
Mating order	-			$F_{2,66}$ = 5.636, P = 0.0055	
Contrasts	No mating	*vs*	Tl	|t| = 2.621; P = 0.0109	
		vs	Tu	|t| = 3.240; P = 0.0019	
	Tl	*vs*	Tu	|t| = 0.105; P = 0.9170	
b) Matings with an invasive species					
T. urticae* vs *T. evansi					
With *T. urticae* females					
Mating order				$F_{2,136}$ = 7.919, P = 0.0006	$F_{2,109}$ = 16.371, P < 0.0001
Mating interval	-			$F_{1,136}$ = 0.039, P = 0.8440	$F_{1,108}$ = 6.878, P = 0.0100
Mating order x Mating interval				$F_{2,136}$ = 6.026, P = 0.0031	$F_{2,106}$ = 4.963, P = 0.0087
Planned contrasts					
Mating interval 0h:	TuTu	*vs*	TuTe	|t| = 0.712; P = 0.4719	|t| = 1.430; P = 0.1556
		vs	TeTu	|t| = 4.964; P < 0.0001	|t| = 1.116; P = 0.2670
	TuTe	*vs*	TeTu	|t| = 3.288; P = 0.0009	|t| = 0.552; P = 0.5819
Mating interval 24h:	TuTu	*vs*	TuTe	|t| = 1.044; P = 0.2984	|t| = 5.362; P < 0.0001
		vs	TeTu	|t| = 0.406; P = 0.6852	|t| = 1.419; P = 0.1587
	TuTe	*vs*	TeTu	|t| = 0.848; P = 0.3980	|t| = 5.103; P < 0.0001
With *T. evansi* females					
Mating order				$F_{2,187}$ = 4.680, P = 0.0104	$F_{2,114}$ = 2.462, P = 0.0898
Mating interval	-			$F_{1,187}$ = 2.555, P = 0.1116	$F_{1,113}$ = 0.045, P = 0.8320
Mating order x Mating interval				$F_{2,187}$ = 4.977, P = 0.0078	$F_{2,111}$ = 0.368, P = 0.6931
Planned contrasts					
Mating interval 0h:	TeTe	*vs*	TeTu	|t| = 2.841; P = 0.0050	|t| = 0.295; P = 0.7680

		vs	TuTe	$\lvert t \rvert = 0.348$; P = 0.7281	$\lvert t \rvert = 1.327$; P = 0.1870
	TeTu	*vs*	TuTe	$\lvert t \rvert = 2.692$; P = 0.0078	$\lvert t \rvert = 1.377$; P = 0.1714
Mating interval 24h:	TeTe	*vs*	TeTu	$\lvert t \rvert = 1.682$; P = 0.0943	$\lvert t \rvert = 1.016$; P = 0.3118
		vs	TuTe	$\lvert t \rvert = 2.948$; P = 0.0036	$\lvert t \rvert = 0.101$; P = 0.9199
	TeTu	*vs*	TuTe	$\lvert t \rvert = 1.561$; P = 0.1203	$\lvert t \rvert = 1.689$; P = 0.0940
c) Matings with a native species					
T. urticae* vs *T. ludeni					
With *T. urticae* females					
Mating order				$F_{2,144} = 6.997$, P = 0.0013	$F_{2,102} = 2.516$, P = 0.0858
Mating interval	-			$F_{1,144} = 2.598$, P = 0.1092	$F_{1,101} = 0.654$, P = 0.4206
Mating order x Mating interval				$F_{2,144} = 3.694$, P = 0.0273	$F_{2,99} = 1.141$, P = 0.3237
<u>Planned contrasts</u>					
Mating interval 0h:	TuTu	*vs*	TuTl	$\lvert t \rvert = 0.859$; P = 0.3915	$\lvert t \rvert = 0.005$; P = 0.9957
		vs	TlTu	$\lvert t \rvert = 0.857$; P = 0.3931	$\lvert t \rvert = 1.016$; P = 0.3119
	TuTl	*vs*	TlTu	$\lvert t \rvert = 2.736$; P = 0.0070	$\lvert t \rvert = 1.895$; P = 0.0610
Mating interval 24h:	TuTu	*vs*	TuTl	$\lvert t \rvert = 2.505$; P = 0.0134	$\lvert t \rvert = 0.164$; P = 0.8700
		vs	TlTu	$\lvert t \rvert = 1.115$; P = 0.2501	$\lvert t \rvert = 0.964$; P = 0.3370
	TuTl	*vs*	TlTu	$\lvert t \rvert = 1.382$; P = 0.1692	$\lvert t \rvert = 0.640$; P = 0.5230
With *T. ludeni* females					
Mating order				$F_{2,248} = 10.534$, P < 0.0001	$F_{2,155} = 2.147$, P = 0.1204
Mating interval	-			$F_{1,248} = 5.180$, P = 0.0237	$F_{1,154} = 2.567$, P = 0.1112
Mating order x Mating interval				$F_{2,248} = 14.098$, P < 0.0001	$F_{2,152} = 10.1064$, P < 0.0001
<u>Planned contrasts</u>					
Mating interval 0h:	TlTl	*vs*	TlTu	$\lvert t \rvert = 1.297$; P = 0.1957	$\lvert t \rvert = 0.853$; P = 0.3952
		vs	TuTl	$\lvert t \rvert = 2.605$; P = 0.0097	$\lvert t \rvert = 0.631$; P = 0.5292
	TlTu	*vs*	TuTl	$\lvert t \rvert = 5.141$; P < 0.0001	$\lvert t \rvert = 1.619$; P = 0.1075
Mating interval 24h:	TlTl	*vs*	TlTu	$\lvert t \rvert = 4.646$; P < 0.0001	$\lvert t \rvert = 4.084$; P < 0.0001
		vs	TuTl	$\lvert t \rvert = 3.805$; P = 0.0002	$\lvert t \rvert = 0.841$; P = 0.4018
	TlTu	*vs*	TuTl	$\lvert t \rvert = 0.401$; P = 0.2020	$\lvert t \rvert = 3.586$; P = 0.0005

Legend: "Tu": matings involving *T. urticae* males. "Te": matings with *T. evansi* males. "Tl": matings with *T. ludeni* males. "0h" and "24h" indicate the time of occurrence of the second mating, i.e., if immediately after the first mating (0h) or 24h later. TuTu means that both mating events were with a *T. urticae* male. TuTe means that the first mating was with a *T. urticae* male and the second with a *T. evansi* male. The same logic applies to TeTe, TeTu, TlTl, TlTu and TuTl.

Discussion

In this study, we investigated the consequences of mating with heterospecifics for the fertilization success and offspring viability in a system composed of three spider-mite species. We found that heterospecific matings between *T. urticae* and *T. ludeni* did not result in fertilized offspring (i.e., females), nor did it have any negative effects on egg viability, as shown for matings between *T. urticae* and *T. evansi* (Sato et al. 2014, Clemente et al. 2016). In fact, a *T. ludeni* female that mates with a *T. urticae* male will produce more male offspring than a virgin *T. ludeni* female. Second, the effects of heterospecific matings on the outcome of previous or subsequent matings with conspecifics were highly dependent on the species pair involved, on the trait measured and on the timing and order of mating events. Despite strong effects of particular mating sequences, our meta-analysis for the net effect of reproductive interactions on *T. urticae* revealed a neutral net effect of the interaction with *T. evansi,* and a positive net effect of the interaction with *T. ludeni*.

Positive effects of interspecific reproductive interactions were found for fecundity. This can be due to a stimulation of oogenesis by the sperm of heterospecific males, increasing the availability of oocytes to subsequent matings with conspecifics. Indeed, oogenesis is stimulated by conspecific sperm in several species (Qazi et al. 2003, Xu & Wang 2011). This could also be the case with heterospecific sperm. If so, it could explain the higher fecundity found in crosses between *T. urticae* and *T. evansi*. In fact, earlier studies have documented that interactions with heterospecific males are not always negative. In some gynogenetic species, heterospecific mating is a prerequisite for embryogenesis (Gumm & Gabor 2005, Schlupp 2010). Moreover, in some invertebrate species, females receive nuptial gifts from heterospecific males (Vahed 1998, Costa-Schmidt & Machado 2012). However, to our knowledge, this is the first time that an increase in fecundity following a heterospecific mating is described in the literature. Such effects may thus be rare. Still, earlier studies may have overlooked them because they have not examined the roles of the order of mating in the outcome of heterospecific mating interactions.

Nonetheless, we also detected several negative effects of mating with heterospecifics, as found in most studies of reproductive interference (Gröning & Hochkirch 2008, Kishi 2015). We found both a reduction in the number of eggs laid and a decrease in fertilization success (i.e., offspring sex ratio). However, the incidence of these two effects varied according to the species involved, the order of matings and the time interval. Whereas effects on fecundity were found in several mating sequences, an effect on fertilization success was found only when the heterospecific male mated with the female 24 hours after the conspecific male. This is at odds with expectations stemming from findings on conspecific matings, which show (a) first-male precedence and (b) exceptions to this rule only if the second male mates immediately after the first. Therefore, the mechanisms underlying sperm displacement by heterospecific males in spider mites should be investigated.

The meta-analysis confirmed this finding, showing that effects were stronger at the 24h interval. Also, it showed that effects were similar irrespective of the order of the mating events. In fact, in some cases, effects of mating with heterospecifics are stronger if such matings follow conspecific ones. This suggests that first male precedence found in conspecific matings cannot be extrapolated to matings involving heterospecific sperm. This contrasts with the recent finding that effects of heterospecific matings in *Drosophila* could be predicted from the harmful effects of conspecific mates (Yassin & David 2015), and that genes involved in conspecific male precedence also affect sperm precedence in multiple matings involving heterospecifics (Civetta & Finn 2014). This indicates that the equivalence of effects of conspecific and heterospecific sperm on the outcome of conspecific matings is dependent on the type of effect and/or the species involved in the interaction.

Despite the fact that many interactions have a negative outcome, the meta-analysis also revealed that the overall effect of mating with heterospecifics is neutral for *T. urticae*. This is because the negative impact of mating with heterospecifics is compensated by the negative impact that *T. urticae* males have on fertility and fecundity of the other species. This leads to the prediction that selection for species discrimination should be low in *T. urticae*, as the net outcome of interspecific reproductive interactions is not costly. Indeed, it has been shown that both males and females of *T. urticae* show weak, if not absent, discrimination between conspecifics and *T. evansi* mates (Sato et al. 2014, 2016, Clemente et al. 2016). However, it may be possible that costs are found if matings with heterospecifics become very frequent.

Since effects of heterospecific matings depend on the order and timing of occurrence, the outcome of these interactions will depend on the frequency with which those different types of matings occur in nature. In the species studied here, conspecific males tend to guard quiescent females (i.e, last larval stage before becoming adult female), to ensure mating immediately after emergence. If males guard preferentially conspecific females, as has been shown in other spider mite species pairs (Collins et al. 1993, Takafuji et al. 1997), heterospecific matings will occur more often after rather than before conspecific ones. If this is the case, the effects of *T. evansi* and of *T. ludeni* on the offspring of *T. urticae* females will not be the same. Indeed, whereas mating with *T. evansi* males after a conspecific male leads to a reduction in offspring fertilization in *T. urticae*, *T. ludeni* matings that follow conspecific ones have no effect on the offspring of *T. urticae* females. Moreover, we have shown that females become less receptive to both conspecific and heterospecific matings if the first mating has occurred 24h before the second (Clemente et al. 2016). This leads to the prediction that the most common mating sequence among these species will be a heterospecific mating immediately following a conspecific one. If this is the case, then we predict that the effect of heterospecific matings in *T. urticae* will be relatively mild.

The meta-analysis also showed that the net effect of mating with *T. ludeni*, the native species, was positive, whereas that of mating with *T. evansi*, the invasive species, was neutral. Therefore, our hypothesis that reproductive interference could be more costly (or less beneficial) between native and invasive species than between natives is confirmed by our results. However, as the net outcome of the native-invasive interaction was neutral, reproductive interference cannot be invoked to explain the exclusion of *T. urticae* in habitats with *T. evansi* (Ferragut et al. 2013, Sarmento et al. 2011b). Other factors may contribute to this exclusion, as the production of a dense web by *T. evansi*, which prevents heterospecifics from accessing the surface of the leaves to feed and oviposit (Sarmento et al. 2011b). Importantly, however, we show that the occurrence and strength of reproductive interference cannot be assessed with a partial evaluation of the outcome of reproductive interactions. Indeed, the order and interval between matings have great influence on the outcome of heterospecific interactions. Therefore, the net outcome will hinge on the frequency of such events. This confirms the importance of using complete experimental designs on the detection and characterization of reproductive interference.

Acknowledgements

The authors wish to thank Yukie Sato and Maurice W. Sabelis for helpful discussions during the early stages of this work. These experiments and Inês Santos were funded by Portuguese National Funds through an FCT-ANR project (FCT-ANR//BIA-EVF/0013/2012) to SM and Isabelle Olivieri. SHC and LRR had PhD fellowships funded by FCT (SFRH/ BD/ 90156/2012 and SFRH/BD/87628/2012, respectively). SAMV and RP had Post-doctoral fellowships from FCT, (SFRH/BPD/66042/2009) and (SFRH / BPD / 42801 /2008) respectively. We also thank Margarida Santos Reis for her support during the initial phase of this project. The authors declare no conflict of interest.

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Table S1 - All effect sizes extracted for the meta-analyses. We extracted the effect sizes from the statistical results shown in Table 1. For that we used the planned contrasts between the control and the experimental treatments. For each effect size, we converted the p-value and corresponding sample sizes to the Fishers' z transform of the correlation coefficient (Zr) and the corresponding sampling variances, using the Compute.es package in R. Abbreviations: 0h and 24h = the second mating occurred 0h or 24h after the first; 1st male = first male that mated with the female; 2nd male = second mate that mated with the female; 2nd Hetero = the female's second mating was with a Heterospecific male; 1st Hetero = the females's first mating was with a Heterospecific male; Tu = T. urticae, Te = T. envansi, Tl = T. ludeni; Var = variance.

Focal species	**Interfering species**	**Females**	**Trait**	**Mating interval**	**Control treatment (1st male; 2nd male)**	***N* control treatment**	**Experi-mental treatment (1st male; 2nd male)**	**Mating order (exp.treatment)**	***N* exp. treatment**	**P-value**	**Effect on *T. urticae***	**Effect Size Zr**	**Var$_{Zr}$**
T. urticae	Invasive (*T. evansi*)	*T. urticae*	Fecundity	0h	TuTu	31	TuTe	2nd Hetero	7	0,4719	positive	0,118	0,029
					TuTu	31	TeTu	1st Hetero	21	0,0001	positive	0,557	0,02
				24h	TuTu	11	TuTe	2nd Hetero	26	0,2984	positive	0,173	0,029
					TuTu	11	TeTu	1st Hetero	28	0,6852	positive	0,065	0,028
			Offspring sex ratio	0h	TuTu	17	TuTe	2nd Hetero	11	0,1556	negative	-0,273	0,04
					TuTu	17	TeTu	1st Hetero	17	0,2670	negative	-0,193	0,032
				24h	TuTu	29	TuTe	2nd Hetero	24	0,0001	negative	-0,551	0,02
					TuTu	29	TeTu	1st Hetero	14	0,1587	positive	0,217	0,025
		T. envansi	Fecundity	0h	TeTe	49	TeTu	2nd Hetero	37	0,0050	positive	0,306	0,012
					TeTe	49	TuTe	1st Hetero	24	0,7281	negative	-0,041	0,014
				24h	TeTe	23	TeTu	2nd Hetero	36	0,0943	negative	-0,22	0,018
					TeTe	23	TuTe	1st Hetero	24	0,0036	negative	-0,434	0,023
			Offspring	0h	TeTe	37	TeTu	2nd Hetero	21	0,7680	positive	0,039	0,018

		sex ratio		TeTe	37	TuTe	1st Hetero	9	0,1870	negative	-0,196	0,023
			24h	TeTe	12	TeTu	2nd Hetero	19	0,3118	positive	0,184	0,036
				TeTe	12	TuTe	1st Hetero	19	0,9199	negative	-0,18	0,036
Native (*T. ludeni*)	*T. urticae*	Fecundity	0h	TuTu	10	TuTl	2nd Hetero	31	0,3915	positive	0,135	0,026
				TuTu	10	TlTu	1st Hetero	60	0,3931	negative	-0,103	0,015
			24h	TuTu	30	TuTl	2nd Hetero	7	0,0134	negative	-0,416	0,029
				TuTu	30	TlTu	1st Hetero	12	0,2501	negative	-0,179	0,026
		Offspring sex ratio	0h	TuTu	8	TuTl	2nd Hetero	21	0,9957	negative	-0,001	0,038
				TuTu	8	TlTu	1st Hetero	40	0,3119	negative	-0,147	0,022
			24h	TuTu	26	TuTl	2nd Hetero	6	0,8700	positive	0,029	0,034
				TuTu	26	TlTu	1st Hetero	4	0,3370	positive	0,177	0,037
	T. ludeni	Fecundity	0h	TlTl	15	TlTu	2nd Hetero	29	0,1957	negative	-0,197	0,024
				TlTl	15	TuTl	1st Hetero	59	0,0097	positive	0,304	0,014
			24h	TlTl	18	TlTu	2nd Hetero	67	0,0001	positive	0,43	0,012
				TlTl	18	TuTl	1st Hetero	66	0,0002	positive	0,413	0,012
		Offspring sex ratio	0h	TlTl	13	TlTu	2nd Hetero	24	0,3952	negative	-0,141	0,029
				TlTl	13	TuTl	1st Hetero	34	0,5292	positive	0,092	0,023
			24h	TlTl	15	TlTu	2nd Hetero	34	0,0001	positive	0,575	0,022
				TlTl	15	TuTl	1st Hetero	38	0,4018	positive	0,116	0,02

Table S2 - Effect sizes used in the meta-analyses. For each pair of effect sizes (shown in Table S1) that corresponded to the same treatment comparisons for the outputs Fec and SR, and to avoid redundancy in our data, we calculated a synthetic effect size that was defined as the mean between Fec and SR and their variance. To calculate the mean variance, we assumed a correlation between outcomes of 0.50 and 0.75. Abbreviations: Fec = Fecundity; SR = offspring Sex Ratio; 0h and 24h = the second mating occurred 0h or 24h after the first; 2nd Hetero = the female's second mating was with a Heterospecific male; 1st Hetero = the female's first mating was with a Heterospecific male; Tu = T. urticae, Te = T. envansi, Tl = T. ludeni; Var = variance.

Focal species	**Interfering species**	**Females**	**Mating interval**	**Mating order**		**Effect on *T. urticae***	**Effect Size *Zr***	**Var$_{Zr}$**
T. urticae	Invasive	Tu	0h	1st Hetero	TeTu	positive	0,182	0,0193246
	(*T. evansi*)	Tu	24h		TeTu	positive	0,141	0,0198644
		Te	0h		TuTe	negative	-0,1185	0,0137361
		Te	24h		TuTe	negative	-0,307	0,0219437
	Native	Tu	0h		TlTu	negative	-0,125	0,0137915
	(*T. ludeni*)	Tu	24h		TlTu	negative	-0,001	0,023504
		Tl	0h		TuTl	positive	0,198	0,0137361
		Tl	24h		TuTl	positive	0,2645	0,011873
	Invasive	Tu	0h	2nd Hetero	TuTe	negative	-0,0775	0,0257647
	(*T. evansi*)	Tu	24h		TuTe	negative	-0,189	0,0182708
		Te	0h		TeTu	positive	0,1725	0,0111742
		Te	24h		TeTu	negative	-0,018	0,019864
	Native	Tu	0h		TuTl	positive	0,067	0,0238581
	(*T. ludeni*)	Tu	24h		TuTl	negative	-0,1935	0,0236002
		Tl	0h		TlTu	negative	-0,169	0,0198455
		Tl	24h		TlTu	positive	0,5025	0,012562

Table S3 - Mean effect size estimates resulting from the meta-analysis. Output of the meta-analytic models performed from the mean between Fec and SR outcomes (with a correlation of 0.50), showing the mean strength of reproductive interference (Fisher's z transform of the correlation coefficient r) from the invasive (T. evansi) and native (T. ludeni) species on T. urticae. Analyses were made with grouping variables (Interfering species and Mating interval at 0h and 24h) and a summary estimate. Abbreviations: Fec = Fecundity; SR = offspring Sex Ratio; 0h and 24h = the second mating occurred 0h or 24h after the first; 2nd Hetero = the female's second mating was with a Heterospecific male; 1st Hetero = the female's first mating was with a Heterospecific male.

Grouping variables	**Focal species**	**Interfering species**	**Mating order**	**Mean effect size Z_r**	**Standard deviation**	**Variance**	**Z-value**	**P-value**	**Lower 95% CI**	**Upper 95% CI**	**Meta-analyses (rma.uni funtion in Metafor)**
All effect sizes	*T. urticae*	Invasive	1st Hetero	-0,0277	0,0673	0,004529	-0,4109	0,6811	-0,1597	0,1043	Z_r= 1.4243, P = 0.1544, 95% CI [-0.0485, 0.3067]
		Native		0,1014	0,0606	0,003672	1,6724	0,0944	-0,0174	0,2202	
Mating 0h		Invasive		0,0064	0,0896	0,008028	0,0709	0,9435	-0,1693	0,182	Z_r= 0.2496, P = 0.8029, 95% CI [-0.2089, 0.2698]
		Native		0,0368	0,083	0,006889	0,4439	0,6571	-0,1258	0,1994	
Mating 24h		Invasive		-0,0719	0,1021	0,010424	-0,7038	0,4816	-0,272	0,1283	Z_r= 1.8270, P = 0.0677, 95% CI [-0.0180, 0.5125]
		Native		0,1754	0,0888	0,007885	1,9748	0,0483	0,0013	0,3495	
Summary estimate		All		0,0436	0,0451	0,002034	0,9681	0,333	-0,0447	0,1319	Z_r= 0.9681, P = 0.3330, 95% CI [-0.0447, 0.1319]

All effect sizes	*T. urticae*	Invasive	2nd Hetero	0,0051	0,0655	0,00429	0,0772	0,9385	-0,1232	0,1333	Z_r= 1.2337, P = 0.2173, 95% CI [-0.0687, 0.3022]
		Native		0,1218	0,0683	0,004665	1,7826	0,0746	-0,0121	0,2557	
Mating 0h		Invasive		0,0969	0,0883	0,007797	1,0973	0,2725	-0,0762	0,2699	Z_r= -1.1628, P = 0.2449, 95% CI [-0.4262, 0.1088]
		Native		-0,0618	0,1041	0,010837	-0,5941	0,5525	-0,2658	0,1422	
Mating 24h		Invasive		-0,1071	0,0976	0,009526	-1,0976	0,2724	-0,2983	0,0841	Z_r= 2.7633, P = 0.0057, 95% CI [0.1069, 0.6287]
		Native		0,2607	0,0905	0,00819	2,8795	0,004	0,0833	0,4382	
Summary estimate		All		0,0609	0,0473	0,002237	1,289	0,1974	-0,0317	0,1536	Z_r= 1.2890, P = 0.1974, 95% CI [-0.0317, 0.1536]

Chapter 5

How does evolution of reproductive interference affect coexistence of competitors? An Individual Based Model

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Abstract

Heterospecific reproductive interactions may play an important role in determining species exclusion if they exert a negative impact on the life-history traits of the species involved. We developed an Individual Based Model to help disentangle these effects from those of resource competition, which is seldom an easy task. As Evolutionary processes can operate on ecological timescales, and can be particularly relevant for species interactions, in our model, reproductive interference was modeled as a trait with genetic variance, and thus evolvable. This model was based on the system composed of two spider mite species competing on tomato plants (*Tetranychus urticae* and *T. evansi*). In this system, both competition for resources and reproductive interference have been documented. First, we analysed how species exclusion was affected by different types of reproductive interference (the "scattered" scenario, where the reproductive interference effects are distributed among all the possible types of reproductive interactions and the "empirical" scenario, where the patterns and reproductive interference effects follow those of empirical observations). Second, we investigated how the presence of genetic variance in reproductive interference affected species coexistence. Finally, we analysed how the strength and direction of the evolutionary response of the reproductive interference trait varied depending on the two types of reproductive interference mentioned above. We found that the type of reproductive interference affected both the probability of species exclusion and the rate at which it occurs, while the inclusion of genetic variance affected only the probability of exclusion. We also found that the direction of the evolutionary trajectories was unpredictable, and that no coevolution between the two species occurred. Our findings thus show that reproductive interference and eco-evolutionary processes severely affect the outcome of interspecific interactions.

Key words

Competition, Rapid evolution, Tetranychidae, species interactions

Introduction

Reproductive interference (RI) is the term most commonly used to describe the situation when heterospecific sexual interactions have negative consequences for the fitness of at least one of the species involved (Gröning & Hochkirch 2008, Burdfield- Steel & Shuker 2011). If strong enough, this interaction may result in the exclusion of one of the species involved (Gröning & Hochkirch 2008).

Species exclusion is often attributed to interspecific resource competition. However, the potential of reproductive interference to also play a part in species exclusion has increasingly being given more attention (Kishi et al. 2009). Kishi (2015) reviewed the results of several laboratorial population dynamics experiments, in which potential effect of reproductive interference was being overlooked. The outcomes of such experiments turned out not to be consistent with theoretical predictions based on resource competition alone. In fact, these outcomes were more reasonably interpreted as being shaped by the joint effects of both reproductive interference and resource competition.

Disentangling the role of either competition or reproductive interference in species can prove difficult. Indeed, in most systems, it is not possible to manipulate the occurrence of reproductive interference while leaving competition for resources intact, and vice versa. In fact, most studies so far have not succeeded in doing so. For example, a recent study has attempted to do so using the fact that spider mites exhibit complete first male precedence (Sato et al 2014). The authors predicted that reproductive interference would be stronger in treatments with virgin females, as heterospecific matings involving mated females were not expected to yield any outcome. However, in Chapter 4, we showed subsequently that the outcome of interspecific reproductive interactions did not follow the intraspecific first-male precedence pattern. That is, the strength of reproductive interference was not contingent on the mating status of the female.

Given the difficulty in designing experiments to test the effect of reproductive interference on population dynamics, computational models can be a powerful tool to address this issue (Crowder at al. 2010, Kishi & Nakazawa 2013, Sun et al. 2014). Several models have been developed so far to study the effects of reproductive interference on species coexistence patterns. Some models compare the effect of reproductive interference with that of resource competition (Ribeiro & Spielman 1986, Ribeiro 1988, Kuno et al. 1992, Takafuji et al. 1997, Zeman & Lynen 2010, Kishi & Nakazawa 2013). These models predict that reproductive interference may promote species exclusion, and that this effect can in some cases be stronger than that of resource competition. In contrast, a recent model has shown that reproductive interference triggers dispersal, which leads to aggregated species distributions and thus spatial segregation between species, thereby facilitating coexistence (Ruokolainen & Hanski 2016). However, none of these models tackles the evolution of species traits, including that of reproductive interference.

Another set of models analyse the evolutionary effects of reproductive interference, namely its effect on character displacement (Yamaguchi & Iwasa 2013, Drury et al. 2015, Nishida et al. 2015, Takakura et al. 2015). These models showed that reproductive interference can induce reproductive character displacement (Yamaguchi & Iwasa 2013), but also inhibit the divergence of traits involved in competitor recognition (Drury et al. 2015). Moreover, it can promote host specialization (Nishida et al. 2015), and be maintained even after the occurrence of reproductive character displacement (Takakura et al. 2015). Even though these models include genetic variance for some traits, such as female preference and the associated male trait (Yamaguchi & Iwasa 2013), competitor recognition traits (Drury et al. 2015), host preference (Nishida et al. 2015), and mate recognition and signal traits (Takakura et al. 2015), none has addressed the potential for rapid evolution of reproductive interference itself and its potential effects on the patterns of exclusion/coexistence.

The predictions generated by mathematical models that include genetic variance for traits involved in species interactions differ significantly from those of purely ecological models (Moore et al. 1997, Wolf et al. 1998). For example, the inclusion of genetic variance for competitive ability may a) promote the evolution of trait values leading to coexistence through ecological processes, and b) enable coexistence through the establishment of competitive cycles, which ecological processes alone (i.e., without evolution) would not allow (Vasseur et al. 2011).

Here we investigate how genetic variance for reproductive interference affects species coexistence. Our model is inspired in the system composed of two spider mite species competing on tomato plants (*Tetranychus urticae* and *T. evansi*). In this system, both competition for resources (Sarmento et al. 2011) and reproductive interference (Sato 2014, Chapter 4) have been documented. We developed an Individual Based Model (IBM), parameterized with data from this system. As a novelty to previous models, this model includes genetic variance for reproductive interference (following Moya-Laraño et al. 2012; 2014), thus allowing to test how reproductive interactions affect the evolution of reproductive interference itself, and how this evolution may affect the patterns of exclusion/coexistence. The model is spatially-explicit, and recently mated females may migrate from patches when food resources are close to depletion. We explore *in silico* whether: i) simplification of the pattern of reproductive interference (e.g., occurring homogeneously regardless of the previous mating history of the female) changes the outcome of the interaction, ii) whether genetic variance in reproductive interference affects the patterns of coexistence, iii) if reproductive interference evolves and, if so, in which direction, and iv) how the direction and extent of rapid evolution affect the patterns of exclusion/coexistence.

The Model

The model has been fully parameterized with data available for two species of haplodiploid spider mites, *Tetranychus urticae* and *T. evansi* and adheres to the ODD (Overview, Design Concepts and Designs) protocol for IBMs (Grimm et al. 2006). Due to the relative complexity of the model, needing extended detailed explanations of the algorithms, most of the details are in the Supplementary Material.

The study system

Tetranychus urticae and *T. evansi* are two haplodiploid, phytophagous spider mite species. These species share plant hosts, and the study of their interaction has gained special relevance since *T. evansi* has invaded several areas, in particular the Mediterranean region (Boubou et al. 2012). In these invaded areas, the native spider mite community composition has changed, with a strong effect on the distribution of *T. urticae* (Ferragut et al. 2013). These observations are congruent with laboratory experiments, which have shown that *T. evansi* excludes *T. urticae* (Sarmento et al. 2011). A few studies investigated the potential impact of reproductive interference in this exclusion (Sato et al. 2014, Clemente et al. 2016, Chapter 4). In the present model, we used the results of the behavioural experiments in Clemente et al. (2016) to parameterize the probability of occurrence of the different types of matings between the two species (order of occurrence of conspecific and heterospecific copulations, and the time interval between those copulations). The results of the Chapter 4 were used to parameterize the effects on fitness of those different types of matings. The authors found that heterospecific matings can affect the offspring sex ratio of *T. urticae* females and the fecundity of *T. evansi* females, but these effects are contingent on the order and time interval between conspecific and heterospecific mating events (Chapter 4).

General description of the model

We simulated the contact between two spider mite species, interacting through both asymmetric reproductive interference and symmetric resource competition.

We included a haplodiploid sexually-reproducing system with continuous generations. Traits had a multidimensional multilocus genetic system, in which genetic correlations between traits were allowed (Moya-Laraño et al. 2012; 2014). As a novelty to previous studies that have used this same genetic framework, we included dominance, therefore considering true sexual differences instead of just hermaphroditism. Thus, haploid males expressed their entire genome, whereas for diploid females we considered dominant-recessive gene expression. The life history of the spider mites consisted of four phases: mating, oviposition, competition, and dispersal (Fig. S1). The model was spatially-explicit, with a spatial unit being a plant and all plants being arranged in a row. Each step in the main loop of the simulation corresponds to one day in the mites' life.

The code written for the model allows incorporating genetic variation for four functional traits (see Supplementary Material). However, here we only explored the inclusion of genetic variation for reproductive interference. We defined the RI trait as the among-species detrimental effect from RI. This trait is thus expressed in males but it affects reproductive performance of females. The costs of heterospecific matings incorporated in the model are based on empirical data (Chapter 4). Traits for which we did not include genetic variation (i.e., assimilation efficiency, dispersal propensity and sex ratio) were fixed to mean population values for all individuals.

Males of both species could mate up to 10 times (Krainacker & Carey 1989) and females up to 3 times (Magalhães pers. obs.) per day. The probability of each mating, in females, depended on her previous mating experiences with con- and heterospecifics males and were calculated according to data in the literature regarding latency to copulation (Clemente et al. 2016). Oviposition depended on the amount of resources assimilated by each female per day, and the latter depended on the growth rates of *T. urticae*, as documented in the literature (no such information was found for *T. evansi*, thus the same values were used for both species) (Mitchell 1973), and data on assimilation efficiencies for both species (Kant et al. 2004, Oliveira et al. 2015; see Supplementary Material). Since in spider mites dispersal is mostly done by mated females, the only individuals allowed to disperse in the model were females, and they did so within the first day after mating with a conspecific male (Collins & Margolies 1991, Li & Margolies 1993).

Simulation scenarios

The first aim of our model was to explore the conditions leading to exclusion of one species. In these simulations, populations were allowed to grow based on plant resources, facing both competitive and reproductive interference interactions until one of the two species became extinct. We set three possible scenarios: 1) the patterns of RI and their effects followed those from empirical observations (“empirical”, Chapter 4); 2) the patterns of RI and their effects were equally scattered across the period of female receptivity (“scattered”), and 3) reproductive interference was absent, which served as a null model (*i.e.*, only competitive effects were at play – “null”).

Subsequently, the scenarios 1 ("empirical") and 2 ("scattered") were replicated in simulations in which the presence of genetic variation in both species allowed for rapid evolution ("var"), whereas in other simulations there was no genetic variation ("no var"). The "null" simulations did not include genetic variation, as the reproductive interference trait did not effectively exist. Each scenario had the following number of simulation run replicates: "empirical var", N=94; "empirical no var", N=69; "scattered var", N=70; "scattered no var", N=71; "null", N=82.

Statistical analyses

All statistical analyses were performed in R 3.2.2. To test for differences in the proportion of replicates in which one species or the other were excluded, we used a binomial test. To compare exclusion outputs (whether *T. urticae* or *T. evansi* were excluded) among simulation scenarios we used Generalized Linear Models (GLM) with a binomial distribution and a logit link function. The generation at which exclusion occurred (the dependent variable) was compared among scenarios using a GLM with normal error distributions and an identity link function. Data were box-cox transformed to meet the normality assumption of the residuals.

To standardize trait values, we calculated the mean and standard deviation of the initial trait values (for the 150 individuals of each species), then standardized all trait values in the simulation (for all the individuals born during it and those at initialization) according to this mean and standard deviations. In this way we could estimate the evolutionary responses in a common scale, therefore allowing a comparison of responses among simulation runs. Rather than using the per-generation means of the responses for analysis, we used a GLM with normal distribution of errors and an identity link function to fit a spline (function "bs" in the R-package *splines*) around generation number (the independent variable treated as ordinal). The fitted model was then used to predict the response variable in the standardized units (i.e., the value of the standardized evolutionary response in the last generation of the simulation). For further statistical analyses, as described below, we used the responses predicted from the GLM, which were extracted using the *effects* package. The evolutionary responses were tested in different ways. We first analyzed whether there was a significant predictable direction of evolution by testing whether the mean evolutionary response across replicates within each scenario differed significantly from zero, for which we used conventional t-tests. Coevolutionary responses between the two species were tested by correlating the estimated evolutionary responses between the two species in the pair across simulation runs (Pearson correlation coefficients): antagonistic coevolution would entail a significant negative correlation between the trajectories. We finally ran GLM analyses (normal error distributions and identity link functions) to test whether the evolutionary response of one species affected the probability of exclusion of the other. The latter test addresses the following question: Is there evidence that rapid evolution is involved in the patterns of exclusion? In other words, is there evidence for eco-evolutionary dynamics?

Results

Patterns of exclusion

T. urticae was excluded in 77% of all simulations, which is well above the 50% predicted by chance (N=385, binomial test: Z=10.1, P<0.0001). However, this proportion changed depending on whether genetic variation for reproductive interference was present. Indeed, when genetic variation was allowed, *T. urticae* was about 1.2X more likely to be excluded (binomial GLM, χ^2=8.0, d.f.=1, P=0.005; Fig. 1A). Also, there were significant differences in the probability of *T. urticae* exclusion depending on the type of reproductive interference (i.e.; whether "scattered" or "empirical"; binomial GLM, χ^2=22.2, d.f.=2, P<0.0001), with *T. urticae* being 1.3X more likely to be excluded in the "scattered" scenario relative to the "empirical" one (Z=-4.3, P<0.0001, Fig. 1B) and 1.2X more likely to be excluded in the "null" scenario relatively to the "empirical" one (Z=2.6, P=0.029). No differences were found between "scattered" and "null" scenarios (Z=-1.3, P=0.385). Note that since in simulations with no reproductive interference there is systematically no genetic variation, we could not test for an interaction between genetic variation and the occurrence of reproductive interference.

The existence of genetic variation had no significant effect in the generation of exclusion (binomial GLM, χ^2=0.02, d.f.=1, P=0.875; Fig. 2A). The effect of reproductive interference was significant (binomial GLM, χ^2=12.6, d.f.=2, P=0.002; Fig. 2B), with exclusion occurring at 1.2X later generations in "null" scenarios as compared with "scattered" scenarios (Z=3.5, P=0.001). No other significant differences between scenarios were revealed. Also, there were significant differences depending on the species that was excluded, with simulations in which *T. evansi* was excluded lasting 1.2X longer than those in which *T. urticae* was excluded (binomial GLM, χ^2=20.1, d.f.=2, P<0.0001; Fig. 2C).

Evolutionary response of reproductive interference

The mean evolutionary response among replicates was zero in all simulations with genetic variation, for both *T. urticae* and *T. evansi* (t-test for whether the mean differs from 0, all P>0.35). However, the range of evolutionary responses was quite large: "empirical", *T. urticae* (min=-1.7, max=1.7), *T. evansi* (min=-1.1, max=1.8), N=94; "scattered": *T. urticae* (min=-1.3, max=2.0), *T. evansi* (min=-0.7, max=1.0), N=70. There was no evidence of coevolution, as the evolutionary responses of *T. urticae* and *T. evansi* were not correlated in any simulation scenario (t-tests on Pearson correlation, both *P*>0.9). There was no evidence for eco-evolutionary dynamics in the "empirical" scenario, as the evolutionary responses of neither species explained the probability of exclusion of the other species (*T. urticae*: binomial GLM, χ^2=0, d.f.=1, P=0.986; *T. evansi*: binomial GLM, χ^2=0.59, d.f.=1, P=0.442). In the "scattered" scenario, on the other hand, we found evidence for eco-evolutionary dynamics, as the stronger the evolutionary response of *T. evansi* the higher the probability that *T. urticae* was excluded (binomial GLM, estimate = 5.5, χ^2=14.9, d.f.=1, P=0.0001; Fig. 3). The evolutionary response of *T. urticae*, however, had no effect on its own exclusion probability (binomial GLM, χ^2=1.57, d.f.=1, P=0.210).

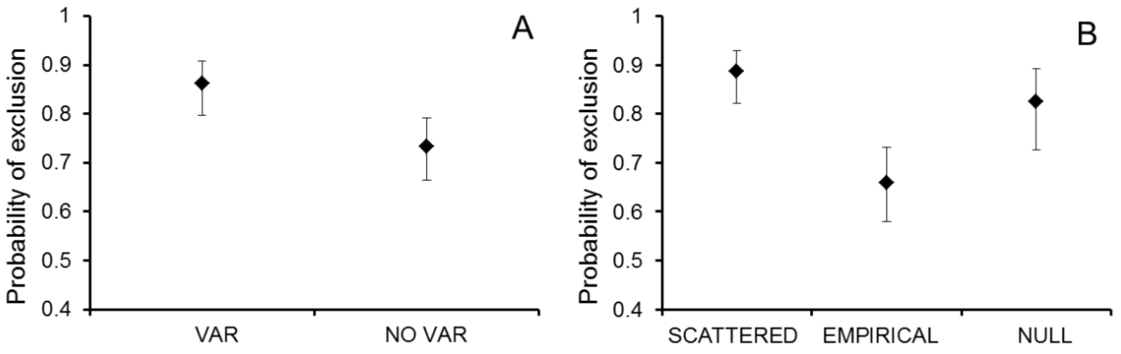

Figure 1 - Probability that *T. urticae* (coded as 1) or *T. evansi* (coded as 0) is excluded, depending on A), the presence (VAR) or absence (NO VAR) of genetic variance for reproductive interference, and B) the different simulation scenarios. Bars correspond to 95% confidence intervals.

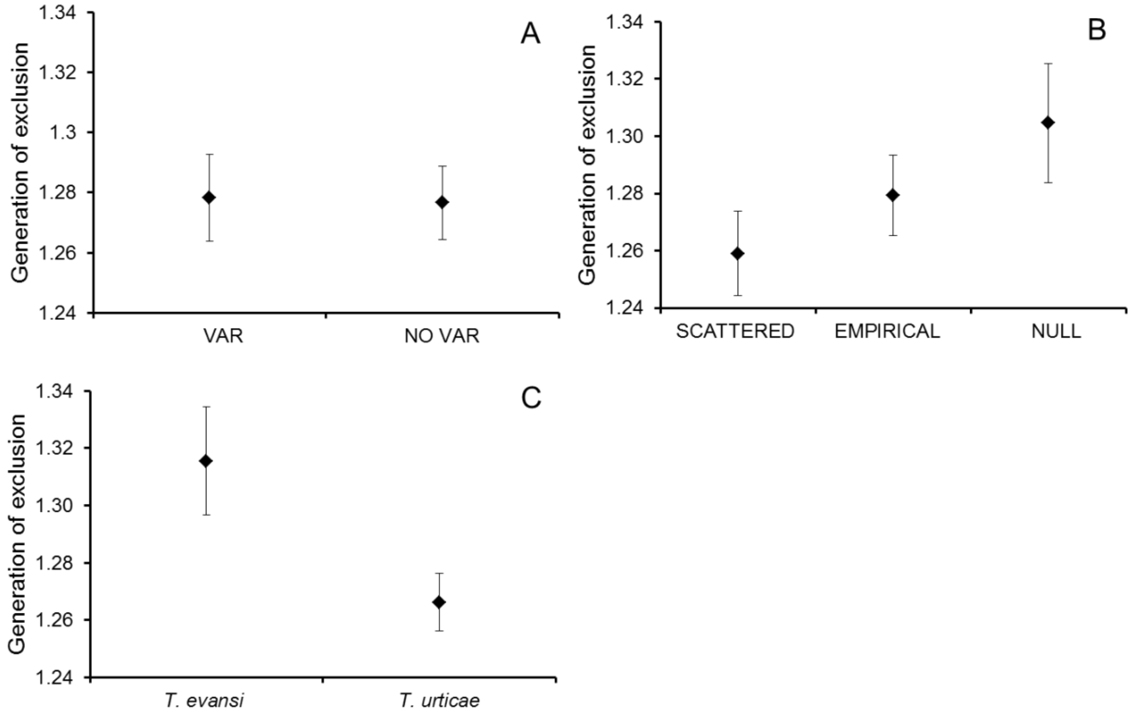

Figure 2 - Timing (generation) of exclusion (box-cox transformed), depending on A, the presence (VAR) or absence (NO VAR) of genetic variation for reproductive interference; B, the different simulation scenarios and C, the species excluded. Bars correspond to 95% confidence intervals.

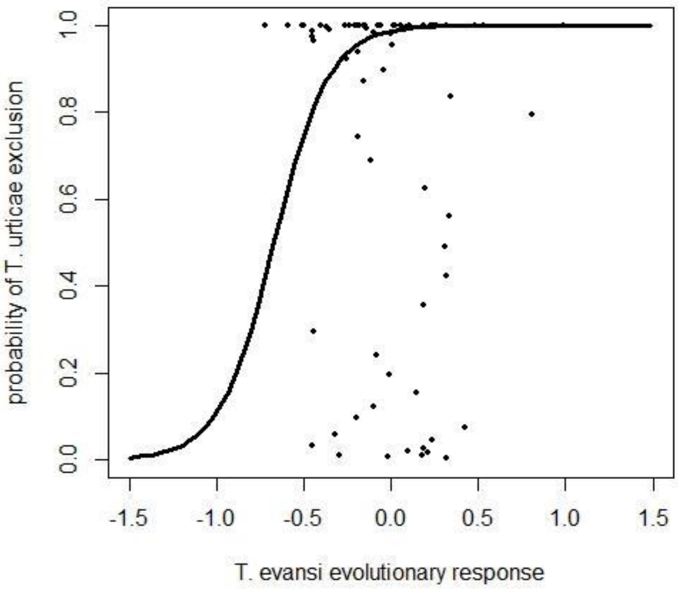

Figure 3 - Probability of *T. urticae* exclusion as a function of the evolutionary response shown by *T. evansi* in "scattered" scenarios.

Discussion

Overall, our results show that *T. urticae* is more likely to be excluded than *T. evansi*, and that exclusion is faster when the former species is excluded. Additionally, we found that, regardless of the scenario, genetic variance affects species exclusion, as in simulations with genetic variance *T. urticae* is more likely to be excluded, even though this does not affect the rate (generation) of exclusion. The pattern of reproductive interference also influences the outcome of the interaction between the two species. When the effects of reproductive interference were scattered among all the possible types of reproductive interactions (scattered scenario), the probability of exclusion of *T. urticae* was higher than when these effects were modelled based on empirical data (empirical scenario). Similarly, the pace of exclusion was affected by the pattern of reproductive interference, exclusion taking longer in the scattered simulations than in empirical ones. Also, the mean evolutionary response of the simulations with genetic variance was 0, but the range of responses was large, which means that even though reproductive interference evolves, the direction is unpredictable. Furthermore, no evidence was found for coevolution, as the evolutionary trajectories of the two species were uncorrelated across replicates. In the scattered scenario, we found that the evolutionary responses of *T. evansi* could explain the probability of *T. urticae* exclusion, indicating eco-evolutionary dynamics.

In all simulations, *T. urticae* was more likely to be excluded than *T. evansi*, both in empirical and scattered scenarios of reproductive interference. Moreover, even in the simulations in which *T. evansi* was excluded, this occurred at a slower pace than for *T. urticae*. These results are consistent with field observations (Ferragut et al. 2013) and laboratory experiments (Sarmento et al. 2011). In the latter, it is argued that the web produced by *T. evansi* may contribute to such exclusion. However, our model was parameterized with data from environments without web. This suggests that the web is not necessary to ensure the exclusion of *T. urticae.* This does not mean that the web does not play a role in species exclusion in this system. In fact, if the effects of the web were to be included in the model, the probability of *T. urticae* exclusion would probably be higher than that observed in the present results.

The higher probability of exclusion of *T. urticae* in all scenarios, even when no specific male reproductive interference effects are included (i.e.; the "null" scenario in which males do not inflict any specific damage to the female), is most probably due to the fact that *T. urticae* females are less likely to mate with a conspecific male after heterospecific matings than *T. evansi* females (table S5, pp. 119 , Clemente et al. 2016). However, Clemente *et al*. (2016) did not find significant differences between species in the latency to mate with a conspecific after first mating with a heterospecific. And these non-significant differences are here the cause of exclusion of *T. urticae* in our model. Since the probability values were calculated based on the above empirical latency to copulation times, and no sensitivity analysis was performed on these parameters, these results must be considered with caution. Further research is needed to know if increasing the sample size relatively to Clemente *et al.* (2016) could end with significant differences between the two species, as we have assumed in the simulations.

When genetic variability for reproductive interference was included in the simulations, we found that the direction of evolution in this trait was unpredictable.

This can be attributed to the fact that reproductive interference, here modelled as a male damage trait, is an interacting trait. The term interacting phenotype was coined to describe traits that require or are influenced by interactions with conspecifics (Moore et al. 1997). Reproductive interference, although involving heterospecific interactions, can easily be included in this definition, being in part determined by an evolvable environment; i.e., traits of individuals which have a genetic basis and that by the biotic interaction modify the genetic expression of other individuals (indirect genetic effects). These traits are predicted to have faster evolutionary responses than non-interacting traits (Moore et al. 1997, Wolf et al. 1998) and have been shown empirically to have unpredictable evolutionary trajectories (Bacigalupe et al. 2008, Bárbaro et al. 2015). However, despite this unpredictability, we found that the inclusion of genetic variance in the reproductive interference trait, regardless of the reproductive interference scenario, resulted in a higher exclusion probability of *T. urticae*. Since this pattern is independent on the direction of the evolutionary trajectories (as these are unpredictable), the outcome is likely due to the increased spread of the ecological effect of one species on another (i.e., the increase in phenotypic variation of reproductive interference traits). More detailed investigation is needed (e.g., programing the IBM to have detailed outputs and running additional simulations with different parameters) to understand this emerging pattern. For example, it would be interesting to determine how increased variability in the *T. evansi* effect on the sex ratio of *T. urticae* increases the success of the former species over the latter. Alternatively, variation in the damage on fecundity of *T. urticae* over *T. evansi* could also, although less intuitively, affect the outcomes in favor of the latter species.

Exclusion of *T. urticae* occurred more rarely when real data from empirical reproductive interference patterns were used than without such interaction (competition only) or in a hypothetical scenario of reproductive interference effects randomly scattered around female mating history. This is not in agreement with the meta-analysis performed in Chapter 4, which predicted no overall effect of reproductive interference on *T. urticae* from matings with *T. evansi.* These contradictory results may be due to the fact that the combination of conspecific and heterospecific mating frequencies and patterns that negatively affects the fitness of

T. urticae, as experimentally induced in Chapter 4, is different in nature, and this was accounted for in our model by including the “scattered” scenario, but not in the meta-analysis. Moreover, the meta-analysis, although integrating all the data observed, is not sufficient to predict the effects of reproductive interference across generations, as no single study had addressed this question, which would imply an experimental evolution study. An effect of reproductive interference on the sex ratio, as that observed on the offspring of *T. urticae*, will generate more *T. urticae* males, which in the next generation will interfere with the fecundity of *T. evansi* females. This may provide an advantage of reproductive interference to *T. urticae* leading to the exclusion (or lack of invasion) in *T. evansi*, a pattern which may not arise easily in single-generation studies. The fact that *T. urticae* was more likely to be excluded from reproductive interference in the “scattered” than in the “empirical” scenario is in agreement with the interpretation that the changes induced by *T. evansi* on the sex ratio of *T. urticae* in the “empirical” scenario may help preventing the invasion of the former, and reiterates the importance of the details of the mating interaction order and the strength of the associated RI effects in determining the fate of the system (Chapter 4).

Reproductive interference is modeled here as a trait in males that lead to either a decrease in fecundity, or a decrease in the probability of laying fertilized (female) eggs. The addition of a female defense trait could be expected to modify the evolutionary responses here observed. Besides this, three other traits are already included in the model, although in our simulations, they did not have genetic variance: dispersal, assimilation efficiency and sex ratio (probability of laying female eggs). The inclusion of genetic variance for these traits can allow us to investigate, in the future, whether the evolution in RI affects the evolution of these traits, and vice-versa. Rogers et al. (2015), showed that the evolution of increased mate discrimination can lead to a reduction of matings with compatible (conspecific) individuals. It would thus be also interesting to include, in future versions of this model, genetic variability for signalling and mate discrimination.

It has become increasingly clear that evolutionary processes can operate on ecological timescales, and that these can be particularly relevant for species interactions (Bolnick 2001, Yoshida et al. 2003, Carrol et al. 2007, Schoener 2011).

Such rapid changes in genetic frequencies can then affect ecological processes which will in turn generate new selective pressures involving new rapid changes in genetic frequencies, if what we know as eco-evolutionary feedback loops (Schoener 2011). Indeed, we have shown here that including genetic variation for one trait; i.e., reproductive interference, affects the ecological outcome of the interaction between competitors, and that although the evolutionary response is unpredictable, the magnitude and sign of it explains the ecological output; i.e., the probability of exclusion of *T. urticae*, which is consistent with eco-evolutionary dynamics.

In conclusion, even though the model developed here has not been yet been explored to its full potential, we showed here the utility of eco-evolutionary modeling to unravel the intricacies of reproductive interactions among species, and that small reproductive differences between species; e.g., probability of mating with a conspecific after first mating with a heterospecific, can have profound effects on crucial ecological outcomes such as coexistence versus exclusion.

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Description of the model following the ODD protocol (Grimm et al. 2006)

Purpose

This IBM aims at testing whether: i) patterns of RI can affect whether two species symmetrically competing for a common resource may coexist, ii) whether genetic variance in RI affects the patterns of coexistence, iii) there is evolution (or even coevolution) in reproductive interference and to what extend it occurs in each species, and iv) the direction and extend of rapid evolution affect the patterns of exclusion/coexistence. The model has been fully parameterized with data available for two species of haplodiploid spider mites, *Tetranychus urticae* and *T. evansi*.

The code is available for download at: https://github.com/salomehc/Rhea-code

1) State variables and scales

1.1 Individual state variables

Sex – male or female.

Instar – Individual instar: egg (0), larva (I), protonymph (II), deutonymph (III), adult (IV).

Position – plant on which the individual inhabits at a given day in the simulation.

Day food (µg) – Amount of food ingested by one individual in one day.

Mass (µg) – The total mass of an individual.

Quiescence period (days) – Days of inactivity, either because the animal is still in embryonic development (egg) or because it is about to molt or in the process of molting (instars other than egg or adult).

Active period (days) – Days of activity within each instar (once the quiescence period has been subtracted).

Generation – generation number since the beginning of the simulation.

Growth_mass – Mass difference between contiguous instars (M_I-M_{I-1}), where M is body mass and I is instar. Growth mass is the target mass to achieve in order to molt to the next instar.

Mass_day - The fraction of energy acquired every day to meet the energy requirements for molting to the next instar, or as an adult (Growth_mass$_I$/Active period).

Matings per day – Maximum number of matings achieved during each day of either a male or a female life.

Adult male lifespan – Maximum age of males living as adults.

Adult female lifespan – Maximum age of females living as adults.

1.2 Genetics

Genetic contribution per allele – a number within the range 0-1 drawn from a Uniform distribution.

Number of alleles per locus – Number of alleles present in the population for each locus.

Number of loci per trait – each trait takes its value from the sum of a number of loci in the genome of each individual. For simplicity, the number of loci in one chromosome is the same as the number of loci determining each trait (number of loci per correlosome), and the number of chromosomes equals the number of traits. A correlosome (Moya-Laraño et al. 2014) is an array in which all the loci determining the genetic value of a trait are located next to each other as to allow the implementation of additive genetic correlations (rho1, rho2). The loci in correlosomes are randomly shuffled across all the correlosomes before recombination to mimic real chromosomes. Therefore, each correlosome has the information for one trait, but before recombination the loci for each trait are scattered across the genome (across chromosomes). After recombination, the loci are back to their original correlosome positions.

Number of chiasmas per chromosome – each chromosome has only one single chiasma for crossover.

Number of traits – total number of functional traits (i.e., traits that have a genetic basis and can respond to natural selection).

Number of modules – number of sets of traits that may be or may be not genetically correlated (additive genetic correlation) to each other.

Number of traits per module – number of traits that compose a module and which may be more or less, positively or negatively correlated within that module (Moya-Laraño et al. 2012; 2014).

Genetic values – The sum of all genetic contributions of all alleles for a particular trait and individual.

Drift trait – A neutral trait which has the same number of loci and initial alleles as the functional traits above but is neutral in the sense that although subject to recombination, it is not functional; i.e., not subject to natural selection because it is not linked to any phenotype. Therefore, it has genetic values but not phenotypic values.

1.3 Individual traits

Functional traits - Traits determined by functional genes which are therefore susceptible to respond to natural selection. Furthermore, the action on the environment (e.g., resource depletion) may be highly variable due to these genetic differences among individuals.

Currently 4 functional traits are implemented:

1) Assimilation efficiency – mass gained divided by mass ingested.

2) Among-species detrimental effect from RI (expresses in males) – This is based on the literature (Chapter 4) and the damage is asymmetric. When *T. urticae* males mate with *T. evansi* females, females are negatively affected in their fecundity.

When *T. evansi* males mate with *T. urticae* females, they affect the sex ratio of the females, decreasing the probability of laying female eggs.

3) Dispersal propensity (expresses in females) – dispersal decisions are weighed according to the available resources and the number of future competitors present.

4) Sex ratio (expresses in females) – the probability that a laid egg is a female.

Trait phenotypic ranges (L_x, U_x) – Maximum range of phenotypic values for each trait.

Phenotypic values – Values of the traits after genes are mapped into phenotypes.

φ – Modulates the range of phenotypic variation determined by functional genes (Moya-Laraño et al. 2012; 2014).

rho1 = amount of genetic correlation between dispersal propensity and assimilation efficiency.

rho2 = amount of genetic correlation between RI effects and sex ratio.

mod_type = whether the correlation between traits is positive or negative (i.e.; a genetic trade-off).

1.4 Species specific state variables

pred_damage – Is the necessary amount of damage inflicted to a plant (in µg) by one individual in order to grow from egg to adult. Is the sum of growth masses across instars divided by the assimilation efficiency of each species during coexistence.

p_matings – Probability for a female of mating with a courting conspecific or a heterospecific male depending on what all her previous mating experiences with co- and heterospecifics were before.

1.5 Habitat state variables

Spatial structure – The spatial unit is a plant and all plants are arranged in a row.

Temporal structure – Each step in the main loop of the simulation is one day.

Number of plants – number of plants potentially infested during the simulation.

Crowdedness – All plants are "uncrowded" at the time they are infested. Once a plant has been infested for more than 16 days, the density of mites is sufficiently high (Mitchell 1973) that resource depletion starts being noticed and animals start growing at smaller sizes (Mitchell 1973), changing the plant to "crowded" status.

Plant age – Days from infestation (arrival of the very first female).

Infestation date – Day in which the very first fertilized female arrives to a plant.

K_date – Day in which R becomes 0. Carrying capacity (K) has been reached.

P – At a given time, it is the forecasted plant biomass which will be needed to build the secondary productivity according to the number of individuals present in a plant and their predicted growth. It is the sum of all the future damage that will be caused by all the individuals in the plant until they become adults divided by the assimilation efficiency. This parameter is used for dispersal and it is assumed that females can perfectly assess it.

R – Amount of resources (in μg) provided by a given plant (we assume no plant growth during the simulation).

2) Process overview and scheduling

The main processes involved in the model are: animal feeding, mating, RI effects, recombination, offspring production, molting and dispersal. The scheduling of such processes can be seen in the flowchart of Fig. S1. Each day, each active individual on a plant feeds and if it has the right instar (adult) and age will mate. Then RI effects, recombination and offspring production takes place. After that, all the state variables for the animals on that particular plant are updated. Then, all non-adult individuals which have accrued enough mass will molt to the next instar. Finally, recently mated females will make a decision of whether to disperse to the next plant. Animals disperse from plant to plant in a stepping-stone pattern.

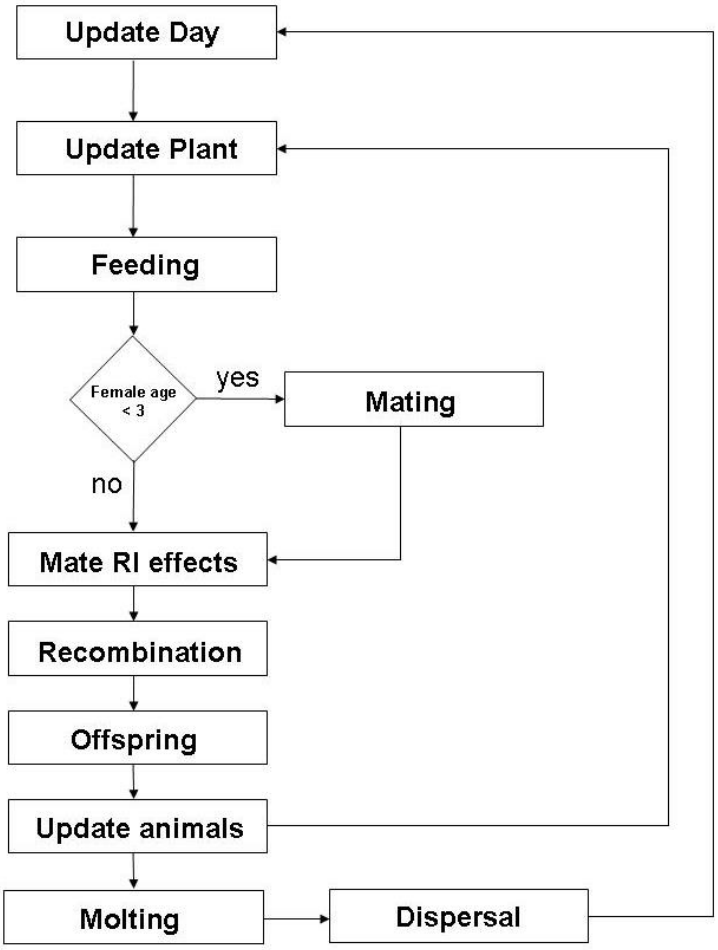

Figure S1 - Flowchart showing the processes involved in the simulations.

3) Design concepts

Emergence: from genes that determine functional traits in two populations (population level) the aim is to know the patterns of exclusion from competition and reproductive interference (community level), and also the patterns of adaptive evolution for traits such as those related to RI.

Adaptation: Adaptive traits are those for which quantitative genetic variation has been explicitly modeled: assimilation efficiency, among-species reproductive detrimental effect from RI, dispersal propensity and sex ratio.

Fitness: Fitness is implicitly modeled, meaning that female traits have a genetic basis and the link between the phenotypic value of the trait and fitness comes through the effects of the environment acting on those genes.

Prediction: Adult females are able to accurately predict the future secondary production (P) of the plant they are living on (both from the current number of animals and from predicting the entire amount of mass taken from the system for them to grow to adulthood).

Sensing: Adult females sense the number of animals on the plant they live in and are able to assess the entire joint amount of mass they will consume from that plant. They can therefore, accurately assess whether P will soon surpass R (i.e., the reaching of the carrying capacity of the system).

Interaction: The two species interact both through exploitative competition and through RI. Species recognition during mating occurs based on empirical data, summarized in *p_matings*.

Stochasticity: The model is completely stochastic and no seeds have been fixed for random number generation. We rely on replicates for a better understanding of the robustness of the patterns found.

Observation: The outputs studied are the generation at which one or another species is excluded, and which species is actually excluded; and we also study the responses to natural selection, for which we apply B-splines using the R library “splines” and then “effects” to plot and visualize the patterns of responses for each trait (Moya-Laraño et al. 2014). As to make them comparable, responses are documented in terms of number of standard deviation units, for which we estimated the initial mean and standard deviation for the trait (at initialization) and then subtracted the above mean from all the trait values in the simulation and then divided the result by the above standard deviation.

4) Initialization

Population sizes: *T. urticae* = 150; *T. evansi* = 150

Instar: randomly assigned to each individual

Sex ratio = 0.75 (based on Chapter 4)

Position = All individuals start in plant 1.

mass_ini = we assumed identical masses between the two species and assigned the mass of each instar following Mitchell (1973) for *T. urticae*. Table S1 shows the initial masses for each instar depending on whether the plant is "crowded" or "uncrowded".

Table S1- Initial body mass of each instar and each sex, measured in crowded (C) or uncrowded (U) conditions.

Instar	**Live weight (µg)**			
	Female C	**Female U**	**Male C**	**Male U**
Egg	1.23	1.23	1.23	1.23
Larva	1.72	1.72	1.72	1.72
Protonymph	3.83	3.83	3.83	3.83
Deutonymph	5.32	10.95	4.05	3.18
Maturation	8.16	13.41	4.16	3.28
Adult gain	20.95	24.50	4.72	3.81
Adult mass per day	1.58	1.37	0.07	0.06

Quiescence period = Instars others than eggs were initialized as active. Eggs were randomly assigned a quiescence period between 1 and 7 (from a Uniform distribution), based on the known developmental time at 21 -23ºC (Mitchell 1973).

Generation = 1

Matings per day = Fixed amount of matings per day allowed and identical for both species. Based on previous observations (Krainacker & Carey 1989) we assumed that mating occurs only in the first 2 days of age in females (3 on day one and 1 on day two) and during the entire lifespan for males (10 days) but with a decreasing probability; i.e. from age 1 to age 10 the maximum number of matings per day was: 15, 10 ,9 ,8 ,7 ,6 ,5 ,4 ,3 ,3.

Adult male lifespan = 10 days.

Adult female lifespan = 30 days.

Genetics

Genetic contribution per allele – a number within the range 0-1 drawn from a Uniform distribution.

Number of alleles per locus = 10

Number of loci per trait (correlosome) and chromosome = 20

Number of chiasmas per chromosome = 1

Number of traits = 4

Number of modules = 2

Trait phenotypic ranges (L_x, U_x) – Maximum range of phenotypic values for each trait.

Assimilation efficiency: the range of assimilation efficiencies depended on whether the species where alone, in coinfection, one species arrived to a clean plant or one species arrived to a plant previously infested by the other (Table S2). In this set of simulations we only used the coinfection scenarios.

Table S2 - Ranges of phenotypic values, lower (Lx) and upper (Ux) values, for assimilation efficiency for both *T. urticae* and *T. evansi*, in plants with no competitors (clean), in plants with competitors (coinfection) or in plants previously occupied by either conspecific or heterospecifics (*T. urticae*, *T. evansi*).

Species	Plant state	Lx	Ux
T. evansi	coinfection	0.414	0.714
	clean	0.465	0.765
	T. urticae	0.331	0.631
	T. evansi	0.574	0.874
T. urticae	coinfection	0.427	0.727
	clean	0.478	0.778
	T. evansi	0.558	0.858
	T. urticae	0.3	0.6

5) RI effect

For simulations based on empirical data on matings

T. urticae effect on *T. evansi* (fecundity): L_x = 0.31, U_x = 0.41

T. evansi effect on *T. urticae* (sex ratio): L_x = 0.43, U_x = 0.63

For simulations in which RI effects are scattered regardless of mating order

T. urticae effect on *T. evansi* (fecundity): L_x = 0.08, U_x = 0.18

T. evansi effect on *T. urticae* (sex ratio): L_x = 0.2, U_x = 0.3

Dispersal propensity (R_factor): L_x =0 , U_x = 0.5

Sex ratio: L_x = 0.6, U_x = 0.9

Phenotypic values – The initial phenotypic values for each trait were obtained by linear interpolation of the genetic values. As input range (minimum and maximum values) for interpolation, we used the range obtained for 50000 individuals having 20 loci per trait (min=7.12, max=13.68). As output range we used the result of imputing the minimum and maximum values of the trait phenotypic ranges in the following equation (Moya-Laraño et al. 2012; 2014):

$$l_X = L_X + \varphi\left(\frac{U_X - L_X}{2}\right) \qquad \text{eq 1}$$

$$u_X = U_X - \varphi\left(\frac{U_X - L_X}{2}\right) \qquad \text{eq 2}$$

where l_x and u_x define respectively the lower and upper limits of the range used for trait X in the simulation, L_x and U_x define standard lower and upper limits for the trait (the min and max of the trait phenotypic ranges above) and φ is a coefficient (range 0-1) which determines what proportion of the distance from the standard limits to the mid-point between them is used to calculate the final trait range (l_x, u_x). Thus, a higher φ involves lower trait variability.

φ = 0.01 for simulations with genetic variation in RI, and φ =1.0 for simulations without genetic variation in RI. φ was kept at 1 for the remaining traits (i.e., we assume not genetic variation and thus no evolution for assimilation efficiency, dispersal propensity and sex ratio).

rho1 = 0

rho2 = 0

mod_type = negative.

R = 40644 µg

6) Input

Quiescence, active periods, growth mass and mass_day (for both “crowded” and “uncrowded” scenarios) by instar were obtained from Mitchell (1973) (Tables S1 and S3). For each potential infection scenario: clean plants, *T. urticae* only, *T. evansi* only and coinfection, we calculated assimilation efficiencies for each species transforming the oviposition data in Oliveira et al. (2015) and Kant et al. (2004), (Table 4). For the present simulations we used the coinfection data only. The among-species detrimental effect from RI was obtained from Chapter 4. We estimated that *T. urticae* fecundity effects on *T. evansi* were of the order of decreasing on average by 36% per mating, and occurred only if the female had previously mated with a conspecific. For simulations in which male damage was scattered regardless of mating order, we estimated that fecundity would decrease by 13% per mating on average. We also estimated that *T. evansi* sex ratio effects on *T. urticae* were of the order of decreasing on average by 53% per mating, and occurred only if the female had previously mated with a conspecific and the heterospecific mating occurred when the female was 24 hours old. For simulations in which male damage was scattered regardless of mating order, we estimated that sex ratio would decrease by 25% per mating on average. To parameterize pred_damage, we used the total amount of energy (**μ**g) necessary to grow from a given instar to adult (Mitchell 1973) and then divided this value by the assimilation efficiency under the coinfection scenario (Table S4). p_matings were obtained from Clemente et al. (2016) by converting the latencies to mate in each different conspecific-heterospecific combination to probabilities (i.e., assigning to the longest latency p=0 and changing all other latencies accordingly, Table S5). R was calculated by preliminary simulations until we obtained about 5000-8000 individuals living and growing on each plant before all resources were depleted.

Table S3 - Number of active and quiescent (inactive) days for each sex and developmental stage.

Instar	**Active days**		**Quiescent days**	
	Female	**Male**	**Female**	**Male**
Egg	-	-	7	7
Larva	2.1	2.1	1.6	1.6
Protonymph	1.5	1.5	1.5	1.5
Deutonymph	1.8	1.6	1.7	1.5

Table S4 - Assimilation efficiencies (proportion of nutrients ingested available for growth or reproduction) for individuals of each species, in each infection scenario: clean plants (Clean), both introduced simultaneously (Coinfection) and introduction after previous infestation with *T. urticae* or *T. evansi* individuals.

Species	**Assimilation Efficiency**			
	Coinfection	**Clean**	***T. urticae***	***T. evansi***
T. evansi	0.564	0.615	0.481	0.724
T. urticae	0.577	0.628	0.450	0.708

7) Submodels

7.1 Reordering - All individuals enter each turn of the loop in a random order

7.2 Plant updates- Each day, each plant with alive animals is checked for age and its status maintained ("uncrowded") or changed ("crowded") beyond certain age after infection.

7.3 Feeding algorithm

All active (non-quiescent) animals will feed on the plant. The amount of food taken from the plant will be determined by the ratio mass_day/assimilation efficiency. Then, by multiplying the above value by assimilation efficiency the total mass to be added to the individual mass is obtained, allowing the animal to grow in mass. If R reaches 0 or a lower value, all the animals in the plant but the females that can still disperse (under 2 days of age) are automatically killed.

7.4 Mating algorithm

All the individuals that can mate on that particular day are randomly paired. After that, these pairs are submitted to the "reassign" function, which decides whether those pairs truly mate based on the "p_matings" probabilities (Table 5), which for decision are compared against a random number drawn from an Uniform distribution: U(0,1).

reassign – This function decides whether an encounter with a male actually ends in mating. To that end, uses the observed *p_matings* (Table 7) depending on the identity of the mates and the previous mating experience and to reach a decision contrasts these values against a value drawn from a Uniform distribution in the range 0-1.

Table S5 - Mating probabilities of the several types of second matings, depending on the order of conspecific and heterospecific first and second matings, and the time interval between both mating events (either 0 or 24 hours). Second matings with the highest latency to mate values were attributed 0 and the probabilities of the other were calculated according to that baseline. Values in bold indicate combinations which are associated to traits affected by RI (sex ratio in *T. urticae* females and fecundity in *T. evansi* females).

Female	**1st male**	**2nd male**	**Mating probability (p)**	
			0 hours	**24 hours**
T. urticae	*T. urticae*	*T. urticae*	0.48	0.23
	T. urticae	*T. evansi*	0.39	**0.33**
	T. evansi	*T. urticae*	0.7	0.59
	T. evansi	*T. evansi*	0	0
T. evansi	*T. evansi*	*T. evansi*	0.38	0.26
	T. evansi	*T. urticae*	**0.41**	0.27
	T. urticae	*T. evansi*	1	0.94
	T. urticae	*T. urticae*	0	0

7.4 Reproduction algorithm

This algorithm calls to the relevant functions to set dominance, crossover and mating effects, both RI and genetic. Below we describe each of the functions involved.

mate_effect - the mating effect algorithm has implemented in it all the mating effects (both RI and genetic). Depending on the combination of matings (con- or heterospecific) and the order (first, second mating) the RI effect may be different

(Chapter 4). When a *T. evansi* female fist mated with a conspecific male, mates subsequently with a *T. urticae* male (both within the female first day of adult life), she suffers an average 36% reduction in fecundity. When a *T. urticae* female first mates with a heterospecific male (in the first 24 hours of her adult life), and mates subsequently with a *T. evansi* male (after she is 24 hours old), she suffers an average 53% reduction in sex ratio, understood as the probability that an offspring is a female; thus, highly increasing the chances of producing male offspring. At least in one of the species there is first male sperm precedence (Helle 1967); and we assume this is the case in both of them. Thus, the genetic of all the diploid offspring (females) come from the first conspecific male with which the female. When females are not able to mate with any male, they will lay haploid (male) eggs. The same occurs if the female first mates with a heterospecific male. In order to determine the male RI and genetic effects in the first 12 hours of a female's adult life (when they mate with up to 3 mates), this algorithm divides the number of eggs laid that first day accordingly in a period of 12 hours randomly split among the three males with which she has mated. For instance if the female is *T. urticae* and she mates with *T. evansi* first, and then with a *T. urticae* male and finally with a *T. evansi* male again, and the random sequence of timing is 3, 7, 8, she will lay a fraction 3/12 of the eggs as males (as she has not mated yet), then (7-3)/12 of males (because she has mated with a heterospecific first), then (8-7)/12 eggs both male and female eggs according to her genetically-determined sex ratio and finally (12-8)/12 will be both male and female eggs but the sex ratio will be affected by RI according to the male genotype for that trait. The fecundity of females is calculated heuristically according to the amount of resources acquired by each female each day, which has been obtained from the literature (see Mass_day above). The *mate_effect* function, calculates the number of eggs and the sex associated to each egg and calls in turn to the *reproduce* function.

reproduce – this function takes the number of eggs that a female will lay and builds the gametes with the genes and alleles of each offspring. To that end it first calls to *crlsm_to_chrom*, which transforms a correlosome to a chromosome to allow a true crossover and returns the female gametes. Then the function calls to the function *male_genetics*, which builds male gametes without recombination. After collecting the male and female gametes, the function calls to *make_both* which builds the new offspring with the new genotypes and genotypic values. Finally, this function assigns initialization values to all the state variables in the new individuals and calls to the function *phenotypes* to assign phenotypic values to the individuals.

crlsm_to_chrom – This function uses the information generated in *chrom* for each species to reorder the loci, and then calls to the function *crossover* to generate gametes with true recombination. After recombination has been generated, all loci in chromosomes are returned to the original positions, regenerating the correlosomes, necessary to induce the wanted genetic correlations (rho1, rho2) among traits. Returns the female gametes.

chrom – At initialization, all the loci involved in a trait are embedded in a correlosome. This function then takes all the loci for all the traits and randomly shuffles them across the genome, as to mimic the real distribution of loci across the entire genome, originating one chromosome for each trait (for simplification). This is a necessary step before crossover, allowing the mimicking of true recombination. Although the positions for shuffling are random, the random position is established at the beginning of the simulation and the same for all individuals in a species, mimicking a real genome.

crossover – this function establishes a single chiasma point for each chromosome and implements true recombination across the *number of loci per trait*.

male_genetics – this function merely collects the genotypes of the sires and builds the male gametes.

make_both – This function collects the male and female genotypes and builds the genotypes of the offspring. To assign genotypic values to the trait modules in males, it calls the function *male_func* and for females the *chrom_expres* and *female_func* functions. For single traits calls the functions *male_func_one_trait*, *chrom_expres_trait* and *female_func_one_trait*.

male_func – Function for males only. This function adds the values for all loci involving the two traits in a module and assigns genetic correlations (rho1, rho2), returning the genotypic values for each trait.. For a single trait the function is called *male_func_one_trait*.

chrom_expres – builds dominance effects for two trait modules in females. To that end, the two allele names are ordered alphanumerically and the dominance established according to increasing alphanumerical order; i.e., the allele with the higher alphanumerical rank expresses. For a single trait the function is called *chrom_expres_trait*.

female_func – Function for females only, implemented after *chrom_express*. This function adds the values for all loci involving the two traits in a module and assigns genetic correlations (rho1, rho2), returning the genotypic values for each trait. For a single trait the function is called *female_func_one_trait*.

phenotypes – this function assigns phenotype values to individuals by using the genotypic values generated in the above functions and equations 1 and 2.

7.5 Molting algorithm

When juvenile instars reach a body mass beyond that necessary for the next instar they molt to a new instar. The body masses to decide molting are the same as for mass_ini (Table 1).

7.6 Dispersal algorithm

Recently mated females (in their first day of adult age) disperse when:

*R-P <= R_factor*R*
where *R_factor* is the trait value.

P is calculated in the *P_function.*

P_function – with all the information of how many individuals of each instar are present in the plant, uses the information from *growth_mass* and of assimilation efficiency to forecast how much biomass will be used from the plant to build the secondary productivity necessary for all these individuals to grow to maturation.

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Chapter 6

Final Considerations

6.1 Summary

Individuals are inserted in a web of biotic interactions, the complexity of which has only been addressed recently. Heterospecific reproductive interactions have been increasingly investigated in the last decades, although due to the difficulty to isolate its effects from other interspecific interactions, namely resource competition, the ecological and evolutionary consequences of these interactions remain somewhat elusive.

Due to the increasing awareness of distribution shifts associated with climate change and the introduction, either deliberate or accidental, of non-indigenous species, the interest of biological invasions has been increasing. However, the factors determining the establishment and spread of non-indigenous species are still matter of intense debate, being key to understand the invasion process, predict future invasions and potentially define control programmes.

The purpose of this thesis was to investigate the existence and the eco-evolutionary consequences of reproductive interactions between spider mite species, including those related to biological invasions. In order to do this, we used several methodologies. First, we performed a critical review of existing studies. Next, we experimentally assessed the effects of reproductive interference on behaviour and life-history traits in a system composed of invasive and native spider mites. Finally, we constructed an eco-evolutionary model to investigate how the evolution of reproductive interference affects species exclusion patterns. Each chapter presents the main outcomes of the corresponding topic addressed. Still, it is worth summarizing here the main findings.

In Chapter 2 we critically reviewed studies of reproductive interference between invasive and native species. The main goal was to investigate to which extent reproductive interference can be responsible for the establishment of invasive species, and how this has been addressed so far. Three main questions were addressed: a) What do reproductive interference studies between invasive and native species natives show us? b) How can future studies improve our knowledge on the role of reproductive interference on invasion? And c) should we expect stronger impacts of reproductive interference from invasive species, relative to that occurring between two native species?

We concluded that the importance of reproductive interference in the outcome of biological invasions still has to be unequivocally demonstrated, although studies often show strong asymmetrical reproductive interference, with the native species paying the highest cost in most cases. Also, we recommend that future studies should address this issue, measuring the relative strength of interference between invasive and native species, and among native species, and also documenting the reproductive interactions in several locations of the invasive range of a species. We further recommend that the assessment of reproductive interference between species should be performed preferably by manipulating reproductive interference (when possible), and/or through the use of meta-analyses or modelling approaches. In Chapter 3, we assessed empirically the degree of species discrimination of *T. urticae* and *T. evansi*, as well as the behavioural consequences of reproductive interactions between these species. We found that the degree of species discrimination is low in these species: a) only males of one species and females of the other mated assortatively; b) latency to copulation was not different between single conspecific and heterospecific matings; and c) copulation duration differed only between single conspecific and single heterospecific matings with *T. urticae* females. Hybrid production was negligible and single heterospecific matings seem not to affect female fecundity (i.e., male offspring produced by virgins). Additionally, we verified that after mating with a heterospecific male, latency to copulation was the same as in matings with virgin females. Thus, heterospecific matings did not aggravate the reduced mating receptivity that is seen after conspecific matings.

The effects of heterospecific matings between *T. urticae* and *T. evansi* on female fecundity and offspring sex ratio were investigated in Chapter 4. Moreover, we investigated the effects of heterospecific matings between *T. urticae* and another native species, *T. ludeni.* The inclusion of this third species allowed us to compare the strength and direction of reproductive interference between a pair of native species, and a pair of a native and an invasive species. We subjected the females of each species to double matings with conspecific and heterospecific males, with the second mating occurring either immediately or 24 hours after the first one.

Although we found no significant costs of heterospecific matings and low species discrimination for *T. urticae* and *T. evansi* in chapter 3, we saw in chapter 4 that heterospecific matings can be costly for those species, as well as in the *T. urticae* / *T. ludeni* pair, by reducing either female fecundity, or the proportion of female offspring. However, in both species pairs this was dependent on whether heterospecific matings occurred before or after conspecific ones, and on the time elapsed between matings. Surprisingly, also a beneficial effect of heterospecific matings was found, namely an increase in fecundity of *T. urticae* females that mated with a *T. evansi* male and, immediately after, with a conspecific male..

To assess the net outcome of heterospecific reproductive interactions, we combined all these results in a meta-analysis, with *T. urticae* as the reference species. This allowed us to assess the net effect of the interaction with each species. We saw that the overall effect of mating with a native species was positive for *T. urticae*, while matings involving the invasive species did not result in any cost or benefit for *T. urticae*. These results confirmed our hypothesis that the effects of reproductive interference differ between the invasive and native species, although not in the direction predicted. While not generalizable, this result is a good indicator of the importance of assessing the effects of reproductive interference between the different species occurring in the same habitat, as opposed to the classical single species pair approach. This is so because the effects of reproductive interference may differ between the native-native and native-invasive species pairs. However, for reproductive interference to have a role in facilitating biological invasions, the overall effect of RI between native-invasive pairs has to be larger than that between native species pairs. Indeed, because of this comparative approach, our results suggest that reproductive interference does not seem to be contributing to the exclusion of *T. urticae* by *T. evansi*, contradicting previous findings suggesting that reproductive interference between these species can be implicated in the displacement of *T. urticae* by *T. evansi* (Sato et al. 2014)

Finally, we built an eco-evolutionary Individual-Based Model to explore the role of reproductive interference in coexistence/ exclusion dynamics of *T. urticae* and *T. evansi* (Chapter 5). In this model, reproductive interference was modelled as a trait with genetic variance and thus allowed to evolve.

The result of our simulations showed that the simplification (i.e. scattering the reproductive interference effects among all types of matings) of the pattern of reproductive interference, changes the outcome of the interaction increasing the probability of exclusion of *T. urticae* when compared with to the “empirical” scenario (where reproducitve interference effects where restricted to some of the types of matings). The inclusion of genetic variance for reproductive interference also lead to higher probability of exclusion of *T. urticae,* compared to the simulations without genetic variance. We also saw that there is evolution of reproductive interference, although the evolutionary trajectories were quite variable, despite the fact that the ecological outcome was more conserved (higher exclusion probability of *T. urticae* in all scenarios). Finally, the possibility of complex eco-evolutinary dynamics was documented, as under simplified reproductive interference the magnitude and direction of the evolutionary response in *T. evansi* explained the probability of exclusion of *T. urticae*.

6.2 Perspectives

In Chapter 2 we found that studies often suggest high fitness costs for native, relative to the invasive species, resulting from reproductive interference. These results are compatible with reproductive interference fostering invasion. However, they are biased by the fact that only successful invasions are documented. A potential way to reach a more solid conclusion concerning the role of reproductive interference in biological invasions is to perform a meta-analysis comparing reproductive interference between invasive and native species, and that found between native species, to assess the relative strength of reproductive interference in scenarios involving biological invasions or only native species.

The results obtained in chapters 3-5 allowed us to obtain a clearer picture of the role of reproductive interference in the exclusion dynamics of *T. urticae* and *T. evansi*. These results highlight the complexity of reproductive interactions between these species and the importance of tackling them from diverse perspectives.

Results from Chapter 3 showed no considerable costs, low specific recognition, and that the occurrence of multiple matings should be relatively low in nature, decreasing the probability of reproductive interference. Results from chapter 4 showed that costs can be present, though the meta-analysis showed no overall effects of reproductive interference in the interactions between *T. urticae* and *T. evansi*. In our simulations (Chapter 5), the probability of *T. urticae* exclusion was lower in the "empirical" scenario (which included the observed effects of reproductive interference) than in the scenarios with no reproductive interference ("null"), or with effects scattered over the different order and timing of matings ("scattered"), suggesting that the actual pattern of reproductive interference benefits rather than harms *T. urticae*, This points to competition, instead of RI, potentially underlying the observed exclusion patterns. Nonetheless, the fact that the probability of exclusion differed between scenarios reiterates the importance of the details of the mating interaction in determining the fate of the system. The lower probability of *T. urticae* exclusion in the "empirical" scenario would lead us to expect that *T. urticae* females do not avoid mating with *T. evansi*, whereas *T. evansi* females should avoid mating with *T. urticae* males, since the "null" and "empirical" scenarios differ only in the existence, or absence, of consequences from heterospecific matings. If in the "empirical" scenario the probability of exclusion is lowered for *T. urticae,* this suggests that mating with heterospecifics could be advantageous for this species, but not for *T. evansi.* However, our results from Chapter 3 are not in accordance with this expectation: *T. urticae* females choose more often conspecific mates than *T. evansi* females, which show no preference, and while *T. urticae* males do not show a preference for conspecifics, *T. evansi* males mate more often with conspecific females. This apparently non-adaptive behaviour may be due to a yet short common evolutionary history between the two spider-mite species. Different results were obtained by Sato et al. (2014). The authors performed only male choice experiments, and their results showed that both *T. urticae* and *T. evansi* males prefer *T. urticae* females. This discrepancy can be due to differences in the experimental protocol, but also to the fact that the individuals used in the experiments originated from different populations. The latter suggests that each population underwent an idiosyncratic evolutionary trajectory, which may account for the discrepancies observed.

Thus, it is pertinent to compare our results with those of this earlier study addressing reproductive interference in this system (Sato et al. 2014). In their study the authors found higher relative numbers of *T. urticae* in “low” reproductive interference treatments, compared to “strong” reproductive interference treatments. In the “low” reproductive interference treatments, females of each species were placed together with both conspecific and heterospecific males, after having mated with a conspecific male, while in the “strong” reproductive interference treatment, the females introduced were virgin. These two treatments being quoted “low” and “strong” reproductive interference is based on the fact that *T. urticae* has first male precedence, thus it is assumed that mated females would be less affected by matings with heterospecific males than virgin females. However, we found that virgin females are not subjected to higher reproductive interference levels than mated females. In fact, our results (Chapter 4) show that *T. urticae* females mating with a heterospecific before mating with a conspecific male (a situation only possible in the “strong” reproductive interference treatments) have increased fecundity, while no effects were shown for *T. evansi* virgin females. Furthermore, *T. evansi* females mating with a conspecific before mating with a heterospecific male (possible in the “low” RI treatment) have reduced fecundity, while *T. urticae* females with the same mating history have a lower proportion of females in their offspring. This results in an increased proportion of *T. urticae* males in the next generation, which may be an advantage rather than a cost, as those males can increase the negative effects of *T. urticae* on *T. evansi*, while increasing the probability of conspecific matings for *T. urticae* females. The results of our model corroborate these predictions, as they showed that incorporating the observed effects of heterospecific matings in the model reduced the probability of extinction for *T. urticae,* relative to a scenario without reproductive interference. Our results are thus consistent with those of Sato et al. (2014) concerning the extinction probability of *T. urticae*. However, the interpretation suggested by our model differs from that of Sato. Indeed, our simulations suggest that coexistence is due to effects of heterospecific matings on sex-ratio, rather than weaker effects of heterospecific matings due to the mating status of the females. However, a sensitivity analysis of our model is needed to provide robustness to this prediction.

Most chapters of this thesis focus on the interactions between *T. urticae* and *T. evansi*. In chapter 4, we introduced a third spider mite species, *T. ludeni*, which has not been given as much attention in the literature. Further experiments involving *T. ludeni* would be pertinent. On the one hand, the behavioural aspects of the interaction between *T. urticae* and *T. ludeni* remain to be evaluated. We should expect *T. ludeni* to avoid mating with *T. urticae*, as they suffer costs from heterospecific matings. On the other hand, the possible existence of reproductive interference between *T. evansi* and *T. ludeni* could also be assessed, as these species share hosts and are likely to interact in the field (Migeon et al. 2011, Ferragut et al. 2013).

We tested for the presence of endosymbionts in our experimental populations, as it is routinely done in studies involving species where these can have effects on reproduction (Sato et al. 2014, Ben-David et al. 2009, Sun et al. 2011). Nevertheless, since the prevalence of *Wolbachia* and other endosymbionts can be very elevated in natural populations (Nakamura et al. 2009, Zhang et al. 2013), it could be worth in the future, to investigate potential effects of endosymbiont presence on reproductive interference patterns. In fact, mate choice in *T. urticae* has been found to be affected by *Wolbachia* infection status, with uninfected females mating preferentially with uninfected males (Vala et al. 2004), although studies in our lab showed that this ability is population-dependent (Rodrigues et al. *in prep*). It could be hypothesised that differences in endosymbiont infection, (different strains or species) could lead to higher specific discrimination,

The model presented in this thesis has not yet been fully explored. Three additional traits are already included in the model: dispersal propensity, assimilation efficiency and sex ratio. However, to date, we did not include genetic variance for these traits in our simulations. This would be a natural extension for running simulations with the current model. Also, the downregulation/ upregulation of tomato plant defences, by *T. evansi* and *T. urticae*, respectively, would be relevant traits to include in future versions. It would also be worth to include genetic variability in signalling and mate discrimination, as the evolution of increased mate discrimination was recently shown to be linked to a reduction of mating opportunities with conspecific individuals in yeast (Rogers et al. 2015). The inclusion of these traits would allow a more precise characterization of the interactions between these two species, and to unravel the effect of short-term evolutionary changes in the outcome of these interactions.

Additionally, this model could be further explored in conjunction with experimental evolution assays. Such experiments could be designed with the aid of the model results, while the experimental results could, in turn, be used to further parameterize the model, thus creating an *in silico*-*in vivo* loop (Moya-Laraño et al. 2014). This integration could allow overcoming limitations of both modelling and experimental approaches.

While the questions addressed and raised by this work deserve further attention, the results obtained shed some light on the complexity of reproductive interactions, and also of the effects that these can have in the coexistence/exclusion patterns between species. From an applied perspective, the present and future results in this system should be taken into account both in pest management and invasion control programs.

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