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The role of conspecific social information on male mating decisions

"Documento Definitivo"

Doutoramento em Biologia
Especialidade Etologia

Inês Órfão Dias

Tese orientada por:
Professor Doutor Paulo Jorge Fonseca
Doutora Susana Araújo Marreiro Varela
Professora Doutora Anne Elizabeth Magurran

Documento especialmente elaborado para a obtenção do grau de doutor

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NOTA PRÉVIA

A presente tese contém capítulos já publicados (capítulo 4) e outros preparados para ser submetidos (capítulos 2, 3, e 6) de acordo com o Regulamento de Estudos Pós-Graduados da Universidade de Lisboa, E no Despacho N.º 4624/2012 do Diário da República II série nº65 de 30 de Março de 2012. A candidata realizou os trabalhos em colaboração, mas liderou e participou integralmente na concepção dos mesmos, desde a obtenção dos dados, análise estatística, discussão dos resultados e redação dos manuscritos.

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Inês Órfão Dias

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Que triste seria (...) se nós humanos viéssemos a perder a noção de mistério, todo o conceito de reverência. Se a parte esquerda do cérebro dominar um dia a direita, de forma a que a lógica e a razão triunfem sobre a intuição, o homem ficará totalmente alienado da sua mais profunda essência, do seu coração e da sua alma.

Janne Goodall

Em Motivo de Esperança

Abstract

Behavioural plasticity occurs when animals adjust their behaviour to current environmental conditions. Research suggests that this ability helps animals cope with changeable environments, especially in the social domain, where social information is highly variable and unpredictable. In this thesis I evaluated the contribution of the complexity of social information to: 1) male mating behaviours, 2) evolution of courtship display, and 3) evolution of dishonest communication.

For male mating behaviours, I performed experiments with guppies (*Poecilia reticulata*). I predicted that males would invest more on mating attempts if they spend more time without mating (chapter 2), and when there was a higher probability of mating success or fertilization success (chapters 3 and 4). I found that time between encounters with females (not time between actual mating opportunities) was determinant to male investment. Moreover, males did not avoid encountering competitors but invested more when they were the first to arrive near females (not the last), and when competing against more attractive males (but only for orange colouration). These evidences support that males produce complex plastic responses in face of diverse social information.

For the evolution of courtship display and dishonest communication, my hypothesis was that competitor (bystander) males use the courtship performance of other males as information about their competitive ability, and that displayer males adjust their behaviour accordingly. For this, I performed systematic reviews (chapter 5), where I found that male-male courtship display is, indeed, frequently associated with intrasexual competition, suggesting that courtship display has evolved a dual utility: attract females and intimidate competitors. A corollary of this, which I developed in a conceptual study (chapter 6), is that males can display dishonestly to deter competitors. If so, the interference of bystanders in communication systems could have a non-negligent role in the evolution of dishonest signalling.

Keywords: animal communication, behavioural plasticity, male mating behaviour, sexual selection, social information.

Resumo

A constatação de que animais de diversas espécies apresentam, em menor ou maior grau, a capacidade de ajustarem o seu comportamento face às condições ambientais encontradas (**plasticidade comportamental**) tem fascinado a comunidade científica. Consequentemente, vários estudos têm sido realizados no âmbito desta temática, nomeadamente partindo de três questões: como é que os indivíduos ajustam o seu comportamento face a diferentes componentes ambientais, se essa plasticidade comportamental traz benefícios para o seu *fitness* e quais as consequências evolutivas da plasticidade comportamental. Nesta tese pretendi abordar estes três pontos. Para tal considerei as decisões de machos no contexto de acasalamento e com base em informação social conspecífica (trasmitida por parceiros e competidores sexuais).

O **sucesso reprodutivo** de um indivíduo é parte determinante do seu *fitness*. Este sucesso está fortemente constrangido pelas **informações sociais** disponíveis durante um encontro de acasalamento, e os machos estão geralmente mais condicionados por este tipo de informação do que as fêmeas. Por exemplo, a probabilidade de um macho acasalar (**successo de acasalamento**) depende do interesse sexual da fêmea e da interferência de competidores; por sua vez, a probabilidade de um macho produzir descendência (**successo de fertilização**) depende de enfrentar ou não competição espermática e da escolha críptica da fêmea. Para além disso, a variância do sucesso reproductivo é maior nos machos do que nas fêmeas em espécies com o típico papel dos sexos (***sex roles***), pois está dependente do número de acasalamentos alcançados e, desta feita, das condições encontradas – abundância e receptividade de fêmeas e presença e qualidade de machos competidores. Assim, prevê-se que os machos ajustem o seu comportamento de acasalamento face a estes tipos de informação social de forma a aumentar a sua probabilidade de sucesso. De acordo com esta expectativa, muitos investigadores têm

estudado interações sociais entre dois ou três indivíduos e os **sinais** (e.g. **traços sexuais secundários**) transmitidos nessas interacções. Estes estudos têm-se sobretudo focado em interações macho-fêmea no âmbito da escolha da fêmea e da sua correlação com os ornamentos dos machos, e nas interações macho-macho no âmbito de lutas pelo acesso às fêmeas e a sua correlação com armas. As interacções no âmbito de grupos sociais maiores, assim como o papel das **pistas sociais** (que constituem informação social transmitida inadvertidamente) têm sido, geralmente, menos estudadas. Nesta tese as interacções sociais no âmbito de grupos complexos e a contribuição de sinais e pistas foram estudados em conjunto, partindo do reconhecimento de que os animais comunicam em rede (***communication network***). Em particular, considerei o papel de indivíduos espectadores (***bystanders***) na evolução de comportamentos de acasalamento; isto é, de indivíduos que não são o alvo direto de um sinal, mas que os conseguem detetar (dizendo-se, por isso, que fazem ***eavesdropping***).

Primeiro realizei estudos empíricos para avaliar a contribuição da informação social conspecífica nas decisões de acasalamento dos machos (capítulos 2 a 4). Nestes estudos usei o guppy como modelo (*Poecilia reticulata*). Esta espécie é muito utilizada em ciência, e o seu comportamento é amplamente conhecido. Observei machos focais em sistemas de mesocosmos, que permitem estudar o comportamento de grupos em ambientes naturalizados. Especificamente, avaliei as decisões de machos focais face à 1) disponibilidade de parceiras sexuais – tempo à procura de fêmeas e tempo entre acasalamentos – e ao 2) risco de competição – presença e atractividade de machos competidores, e ordem de aproximação à fêmea. As decisões consideradas foram: aproximação à(s) fêmea(s), tempo a seguí-la(s), tática de acasalamento realizada (côrte ou tentativas não-solicitadas) e sequência destes comportamentos. Estas decisões têm diferentes custos e benefícios para os machos. Enquanto através da tentativa de acasalamento não-solicitada um macho guppy pode acasalar mais rapidamente uma vez que não necessita do consentimento da fêmea, ao acasalar com a fêmea após realizar côrte o macho produz, geralmente, mais descendentes. Assim, diferentes estratégias podem ser benéficas em diferentes contextos. Por exemplo, quando a fêmea não está recetiva os machos beneficiam em investir menos tempo a seguí-la e em acasalar realizando a tática não-solicitada, podendo assim produzir pelo menos alguns descendentes. A previsão geral era que os machos deveriam escolher a estratégia que mais benefícios tivesse para o seu sucesso reproductivo face às condições encontradas.

No capítulo 2, onde testei o efeito do tempo à procura de fêmeas, esperava que os machos que passassem mais tempo entre encontros com fêmeas investissem mais a tentar acasalar quando colocados numa parcela com fêmeas, e que iriam realizar mais côrte. De facto, observei que os machos seguiam as fêmeas por mais tempo se tinham estado mais tempo sem encontrar nenhuma, mas realizavam mais tentativas não-solicitadas. Para além disso, esse esforço era maior do que quando passavam mais tempo entre acasalamentos propriamente ditos. Este resultado demonstra que quando passam mais tempo sem fêmeas os machos arriscam menos realizando a tática de acasalamento que não exige a cooperação da fêmea. Para além disso, mais do que a oportunidade de acasalamento em si, é a presença ou ausência de fêmeas no ambiente que determina as decisões dos machos. Assim, a abundância de fêmeas é provavelmente uma pista social que informa sobre as oportunidades de acasalamento futuras, determinando o melhor investimento a fazer no presente.

Nos capítulos 3 e 4 testei se machos focais optavam por estratégias diferentes dependendo se havia ou não um competidor a seguir a fêmea, uma vez que o competidor pode comprometer o acasalamento do focal (por eventual preferência da fêmea ou através da interferência sexual) e indica uma maior probabilidade de ocorrer competição espermática (podendo também ocorrer escolha críptica da fêmea). De facto, a frequência de côrte era maior quando os machos focais não tinham competição e, pelo contrário, a frequência de tentativas não-solicitadas era maior quando havia um competidor (capítulo 3). Contudo, no capítulo 4 onde testei se os machos evitavam o contexto de competição, observei que não o faziam. Este resultado está provavelmente relacionado com a alta probabilidade de machos guppies enfrentarem competição espermática na natureza, uma vez que apresentam um elevado nível de promiscuidade. Para além disso, os resultados mostram que a preferência por uma ou outra tática de acasalamento está dependente de outros fatores relacionados com o competidor – atratividade e ordem de aproximação à fêmea (capítulos 3 e 4, respetivamente).

Relativamente a estes dois pontos, verifiquei no capítulo 3 que os machos optaram por realizar tentativas não-solicitadas quando competiam contra um macho atractivo ou mais atractivo do que ele próprio, mas realizavam mais côrte quando competiam com um macho com maior área de pontos laranja. Uma vez que a área de laranja é um fenótipo plástico, realçado durante a côrte, este resultado sugere que machos guppies utilizam a côrte para aumentar a sua própria atratividade e, assim, a probabilidade de serem escolhidos pela fêmea. No capítulo 4 observei que os machos focais investem mais quando se aproximam da fêmea antes dos seus competidores. Uma vez que as fêmeas de guppies são mais receptivas aos primeiros machos que encontram, mas que machos que transferem esperma em último tendem a ganhar a competição espermática, este resultado sugere que os machos procuram primeiro beneficiar da escolha da fêmea e, depois, continuam a tentar acasalar com a mesma fêmea de forma a aumentarem a probabilidade de transferirem esperma em último e, assim, de ganharem a competição espermática.

Numa segunda fase investiguei qual o papel que machos espectadores podem ter na evolução dos comportamentos de côrte (capítulo 5). Assumindo que machos podem extrair informação sobre a qualidade competitiva de competidores com base na observação da sua côrte, será então plausível que estes espectadores explorem essa informação. Por exemplo, o macho espectador pode decidir lutar se detectar que o competidor está em baixa condição física. Por outro lado, o macho que corteja pode alterar este comportamento para enganar o espectador (por exemplo, exagerando a côrte). Como consequência, a côrte pode passar a ser dirigida também a competidores, mesmo na ausência de fêmeas. Assim, no capítulo 5, e com base em revisões sistemáticas, compilei informação sobre se a côrte macho-macho terá surgido em várias espécies de animais em contexto de competição e se estão descritas para as mesmas espécies o comportamento de *eavesdropping* (e outros relacionados). Os resultados recolhidos suportam esta hipótese, pois em vários artigos e para várias espécies os investigadores sugerem a competição como hipótese explicativa deste comportamento, e as evidências de *eavesdropping* são também maiores para as espécies para as quais esta hipótese foi sugerida. Em suma, estes resultados suportam que machos que inicialmente não estão envolvidos na interacção de côrte podem passar a ser alvo da mesma. Desta feita, investiguei uma útima questão: será que machos espectadores detetam côrtes desonestas dirigidas às fêmeas? Ou, de uma forma mais geral, será que um sinal desonesto quando é intercetado por um espectador pode representar uma pista desonesta para este indivíduo? Que consequências tem a interceção deste tipo de pista social? Este ponto é discutido num estudo concetual (capítulo 6), onde sugiro que a desonestidade pode ter evoluído para lá das interações emissor-recetor, sendo um produto evolutivo dos custos-benefícios para todas as partes envolvidas na rede de comunicação – emissores, recetores e espectadores.

Palavras-chave: comportamento de acasalamento, comunicação animal, informação social, plasticidade comportamental, selecção sexual.

Contents

CHAPTER 1.

General introduction

Chapter 1. General Introduction

In the past decades there has been a renewed interest in two old concepts in biology. First, on the idea brought by Lamarck (1809) that individuals across the animal kingdom adjust their behaviour to changeable abiotic and biotic conditions (**behavioural plasticity**; Snell-Rood, 2013). Second, the idea probably first given by Darwin (1872) that behavioural adjustment is highly dependent on available **social information** (Dall, Giraldeau, Olsson, McNamara, and Stephens, 2005; Danchin, Giraldeau, Valone, and Wagner, 2004; Grüter and Leadbeater, 2014). The exploration of such concepts has led to a change in our current view of animal behaviour. For instance, we now recognize that animals adjust their behaviour to social information acquired directly from interacting with other individuals but also as bystanders detecting others' actions, features or interactions. Hence, animal research has moved from a dyadic perspective of social information exchange to a more complex and inclusive **communication network** (McGregor and Peake, 2000).

Behavioural plasticity has become a hotspot theme on animal behaviour research, particularly in relation to social contexts[1]. This research has shown that behavioural plasticity helps animals cope with variable social information (Dingemanse and Wolf, 2013; Taborsky and Oliveira, 2012; Webber and Vander Wal, 2017), and has great implications on individual fitness (e.g. Cauchard et al., 2013; Lone et al., 2015; Karkarey et al., 2017), namely during reproduction (McGraw and Caswell, 1997). In fact, several studies have shown that plastic mating behaviours are determined by social information and have direct consequences for individual reproductive success (e.g. fantail darter fish

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[1] See, for example, special issues and most cited papers of main journals of animal behaviour (hyperlinks accessed on 2nd of February of 2018):
https://www.journals.elsevier.com/animal-behaviour/most-cited-articles
https://www.sciencedirect.com/journal/animal-behaviour/vol/103
https://academic.oup.com/beheco/pages/impact_factor

– O'Rourke and Mendelson 2013; yellow dung fly – Gress et al., 2017). The plasticity of mating behaviours, particularly the contribution of social information and the evolutionary consequences resulting from these contributions, are however incompletely understood. The goal of this thesis is to contribute to this framework.

1.1. Behavioural plasticity and flexibility

Behavioural plasticity is part of the spectrum of **phenotypic plasticity**. Phenotypic plasticity is the ability of a genotype to manifest itself in different phenotype forms according to the environmental conditions (Pigliucci, 2001; West-Eberhard, 1989). Such phenotype forms can be adaptive or not (Ghalambor, McKay, Carroll, and Reznick, 2007; West-Eberhard, 1989). Depending on this, these expressed phenotype forms can suffer different modifications along the evolutionary process, such as deletion, exaggeration, or attenuation (Schneider and Meyer, 2017; West-Eberhard, 1989). This way, research about the adaptive value of plastic phenotypes are of key relevance to fully comprehend the evolutionary history of a trait.

The phenotype forms can appear on different life stages of an individual and can either be fixed or reversible during the lifespan (West-Eberhard, 1989). The specific case of phenotypic plasticity in which the phenotype forms are reversible is general termed as **phenotypic flexibility** (Piersma and Drent, 2003; Piersma and Lindström, 1997; Taborsky and Oliveira, 2012). In this case, the phenotype can be expressed as discrete forms (e.g. changeable colour camouflage and mating tactics) or as a continuum (e.g. body mass and copulation duration).

Phenotypic plasticity manifests itself on different types of phenotypes, such as physiological, morphological, or behavioural (West-Eberhard, 1989; Whitman and

Agrawal, 2009). For example, Temnothorax ants adjust the composition of their cuticular hydrocarbons and body size to a temperature increase (Menzel, Zumbusch, and Feldmeyer, 2017; Molet, Péronnet, Couette, Canovas, and Doums, 2017). Attractive ornaments can be found in males or in females of the squinting bush brown butterfly depending on the developmental temperature (Prudic, Jeon, Cao, and Monteiro, 2011). Although, virtually all types of phenotypes can be adjusted (either in a fixed or reversible way) based on conditions encountered, behaviour seems to play a particularly important role on individuals adjustment to encountered conditions, as it is often the first phenotype to be modified when an environment changes (West-Eberhard, 1989; Wong and Candolin, 2015).

Based on this, increasing number of studies are now focused on how animals deal with quick changes induced by man, from an animal behaviour perspective. For example, since dragonflies quickly disperse when waters get polluted, reports about water quality commonly include measurements of this *taxa* biodiversity (Villalobos-Jiménez, Dunn, and Hassall, 2016). Other example is the assessment of pressure exerted by fisheries by evaluating penguins behaviour – these animals change their foraging behaviour when krill number decreases (Alonzo, Switzer, and Mangel, 2003). Also, a decrease of most territorial fish species in coral reefs except for the peacock grouper fish reveals coral mass mortality – this resistant species is maintained by changing their foraging behaviour (Karkarey et al., 2017).

Behavioural plasticity should be more beneficial under changeable environments (de Jong, 1995; Komers, 1997; Partridge and Harvey, 1988; Thompson, 1991), and increasing evidences show, indeed, that groups that face more variable environments exhibit, in general, higher plasticity. For example, Norwergian Northern gannets show plastic foraging tactics that follow the variable distribution (in space and time) of their vast range of preys (Pettex et al., 2012). Similarly, the way guppies establish social networks and the characteristics of their escape behaviours are determined by predation risk, which varies among populations (Heathcote, Darden, Franks, Ramnarine, and Croft, 2017; Templeton and Shriner, 2004). One important contributor for plastic responses is social information. As Taborsky and Oliveira (2012) briefly summarize: "the social domain is arguably the most complex and fluctuating component of an animal's environment as it involves interaction with other behavioural agents with inherently associated higher levels of unpredictability". Therefore, being able to make decisions in the social domain, and to adjust behaviour to the complex and variable social information available should be highly adaptive (**social competence**; Taborsky and Oliveira, 2012).

1.2. Social information

As already mentioned, animal behaviour is a response, in part, to external conditions. Information extracted from the biotic environment (**biological information** – Wagner and Danchin, 2010; see Figure 1) can be transmitted by genes (genetic information; e.g. inherited phenotypes), or present in the environment (**non-genetic information**). Non-genetic information includes all environmental facts, which in turn can be transmitted by physical elements (non-social information; e.g. temperature, light), or by individuals (**social information**). Social information includes all information transmitted by heterospecific and conspecific individuals, resulting from detecting, observing or interacting with others. Social information can have consequences to the individual fitness (Seppänen, Forsman, Mönkkönen, and Thomson, 2007; Valone and Templeton, 2002). For example, in several species alarm calls warn individuals about a predator, allowing them to escape on time and, therefore, increasing their chance of survival. Or male ornaments, relevant for female mate choice, that potentially increase the female reproductive success. Therefore, it is not surprising to find increasing research on the effect of social information on animal decision-making.

For a long time, most studies either focused on social information extracted from observing other individuals' actions (e.g., in 1989, Valone evaluated animals' decisions after observing others foraging), or on interacting individuals considering the dichotomy of sender-receiver (e.g. Darwin, in 1872, proposed that expressions inform others about an emotion). In 2000, McGregor and Peake identified a communication network where third parties are also involved in social information transmission. Since then, much research has moved the concept of **bystanders** (or **audience**) from an individual extracting information while observing others' actions (e.g. foraging), to include individuals that are not directly involved in an interaction but can extract information from observing others' interactions. Evidences that bystanders determine sender and receiver behaviours (**audience effect**), and vice-versa (**bystander effect**), provides support for the importance of communication networks in shaping behaviour during an individual's life (Danchin et al., 2004; Plath and Bierbach, 2011; Zuberbühler, 2008). However, communication networks also have long-term evolutionary implications.

Danchin and other researchers realized that to understand the evolution of animal communication it is important to separate social information into intentionally and inadvertently transmitted information (Danchin et al., 2004; Danchin et al., 2008; Wagner and Danchin, 2010; also recognized by Chris Barnard – Luís Vicente, personal communication). **Signals** represent **intentional social information**, while **social cues** represent **inadvertent social information**. A signal is, on average, beneficial both to the sender and receiver (Danchin et al., 2004; Maynard Smith and Harper, 2003; Searcy and Nowicki, 2005). This way, a signal evolved with the intent of allowing the sender to transmit information and the receiver to detect it. Contrastingly, a social cue is inadvertent, deriving from the non-signalling actions and interactions of animals in their daily activities (Danchin et al., 2004; Danchin et al., 2008; Wagner and Danchin, 2010). The social cue can be either beneficial, neutral or detrimental to the sender and to the bystander. Behaviour is one way in which animals transmit information, either intentionally or inadvertently (i.e. either as a signal or a social cue, respectively). A behaviour that originally was a social cue can become a signal during the evolutionary process if it benefits the sender by adaptively inducing a given response in a target receiver (Danchin et al., 2004). But, a social cue is most likely to evolve into a signal when it is beneficial to both sender and receiver. Still, the exact ways by which cues evolve into signals and <u>if and how bystanders (the communication network) keep affecting the subsequent evolution of signals remains largely unknown</u> (but see McGregor and Peake, 2000).

The theory predicts that a social cue evolves into a signal or a signal becomes exaggerated when it is mostly beneficial to both sender and receiver (Danchin et al., 2004, 2008; Maynard Smith and Harper, 2003). This seems to be the case of tidbiting in male fowls. Tidbiting probably had a primordial function of feeding but evolved as a ritualized display. This behaviour benefits the sender (male) by attracting potential sexual partners and benefits the receiver (female) since it gives information about food location and male quality (Smith and Evans, 2009). But if a signal is detrimental to the sender or to the receiver it should give rise to features and behavioural strategies that reduce information, like decreased conspicuousness or camouflage (Maynard Smith and Harper, 2003). For example, due to decades of agriculture intensification, horned larks' feathers became darker matching the soil colour (Mason and Unitt, 2018). Although the theory and the examples presented fall again into the dichotomy sender-receiver, bystanders can also exert an important evolutionary pressure. This was the case of singing males field crickets decrease at the Kauai Island, in the Hawaiian archipelago, caused by a parasitoid fly. Because males' song that attracted sexual partners also attracted the parasitoid, singing males became progressively less frequent, while silent morphs increased along in only a few generations (Zuk, Rotenberry, and Tinghitella, 2006). Similarly, in several fish species male colour conspicuousness is dependent of predators. Since these colours attract both sexual partners and predators, males tend to present less conspicuous colours when the risk of encountering a predator is higher (e.g. guppy – Ruell et al., 2013; mosquitofish – Giery and Layman, 2015). These examples support that to fully understand the evolution of a signal it is necessary to consider the balance between the costs and benefits for all parties involved in the communication network – sender, receiver, and bystander.

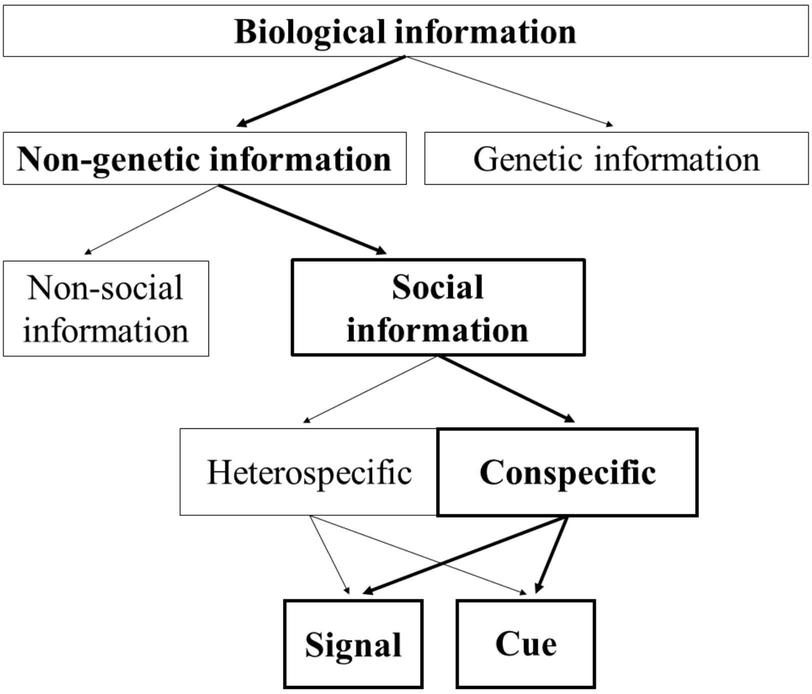


Figure 1. Adapted from *A taxonomy of biological information* (Wagner and Danchin, 2010). Components examined in the thesis are in bold.

The advances described above (Danchin et al., 2004; McGregor and Peake, 2000) meant that research on animal behaviour increasingly included the entire spectrum of social information. There is increasing evidences that animals collect different types of social information before deciding what to do, and the assumption is that more social information means less uncertainty (Dall et al., 2005); for example, about the current state and intentions of social partners, and the location and suitability of resources. Furthermore, social competence – the ability to collect and process complex social information – can be linked to an individual's fitness (Taborsky and Oliveira, 2012), particularly in contexts directly relevant to fitness, such as the mating context. Such ability can be expressed by adjusting mating decisions to encountered social contexts. This way, it is important to investigate decision-making on animals considering the social information available, and the correlation of this type of decision-making with individual fitness (for example, chance of mating or of producing more offspring).

1.3. Sexual selection theory and male mating decisions

In 1871, when Darwin proposed the sexual selection theory in *The descent of man, and selection in relation to sex*, he was focused on elaborate male traits. The challenge, as he saw it, was how such traits (**secondary sexual traits**) compromise males survival but, at the same time, increase their mating success. This classical view of sexual selection, thus, ascribes **mate choice** to females and **competition** for mating to males (**sex roles**), with males' secondary sexual traits shaping their level of attractiveness to females (**ornaments**) and their ability to fight against other males over access to females (**weapons**). Although some researchers disagree with Darwin's ideas (Milam, 2010;

Roughgarden, 2012; Roughgarden and Akçay, 2010; Wallace, 1871), a large body of evidence supports sexual selection theory (e.g. Fisher, 1930; Bateman, 1948; Cunningham and Birkhead, 1998; Andersson and Simmons, 2006; Clutton-Brock, 2009). Still, main findings support that sexual selection is more complex than previously thought (Andersson and Simmons, 2006; Bateman, 1948; Clutton-Brock, 2009; Cunningham and Birkhead, 1998; Fisher, 1930). These main findings are summarized in the next paragraphs.

We now know that mate choice is not restricted to females. In fact, **male mate choice** is present in most species (Clutton-Brock, 2009; Edward and Chapman, 2011; Tobias, Montgomerie, and Lyon, 2012). Furthermore, in some species there is **sex roles reversal**, in which the male is the choosing, and the female is the sex that spends more resources searching and competing for access to sexual partners (Bonduriansky, 2001; Bro-Jørgensen, 2007; Clutton-Brock and Mcauliffe, 2009; Eens and Pinxten, 2000). Nonetheless, in most animal species, the male is indeed the sex that spends more resources searching and competing for access to females (Andersson, 1994; Bateman, 1948; Clutton-Brock, 2009; Fromhage, Jennions, and Kokko, 2016). Thus, males generally face higher sexual selection pressures than females (Janicke, Haderer, Lajeunesse, and Anthes, 2016).

Males not only have to face competition with other males, but also with females. Competition between male and female results from their conflicting interests and is generally termed **sexual conflict** (Parker, 1979; Parker, 2006). There is a contrasting investment in gamete production between sexes, with males being able to produce, with low energy costs, several sperm cells in one ejaculate, while females have a higher expenditure than males to produce one bigger gamete – the egg (Bateman, 1948; Schärer et al., 2012; Kokko et al., 2013). Consequently, whereas males typically benefit from mating with several females, and from allocating more and better sperm in more fecund females, females gain advantage by being highly selective of male attractiveness to produce high quality offspring. This leads to an arms race, in which males can adopt strategies to overcome female mate choice (e.g. sneak – Gonçalves et al., 2008; Neff and Svensson, 2013), and where females may use strategies to control male fertilization success (e.g. **cryptic female choice** – Eberhard, 1996; discussed below).

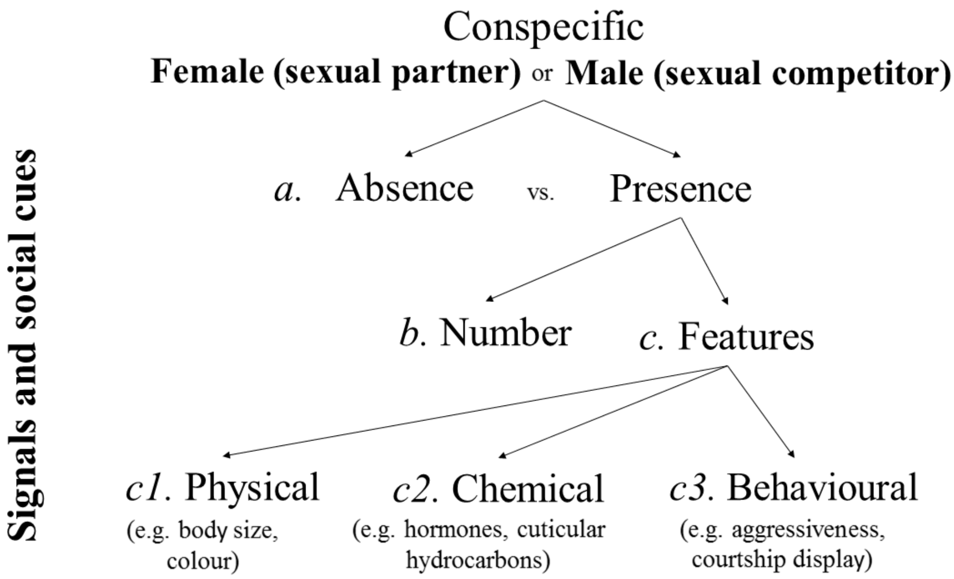
Even the classic competitive behaviours between males (**male-male competition**) can assume different forms, as they do not necessarily include fighting, nor even direct encounters. In Andersson's (1994) words, male-male "competition occurs whenever the use of one resource (in this case, mates) by one individual makes the resource harder to come by for others". One example of this would be when a male mate with a female whose sexual interest reduces afterwards, thus reducing competitors' likelihood of mating with the same female (female **sequential mate choice**; Halliday, 1983; Jennions and Petrie, 2000). Taking the different forms of competition (Andersson, 1994) together, a male can be in an advantageous position regarding a competitor if he: is able to find females earlier (**scramble competition**); remains sexually active for longer periods of time (**endurance rivalry**); is stronger or able to avoid encounters with other males (**contest competition**); is more attractive than competitors (benefiting from female **mate choice**); is able to mate regardless of the female interest (overcoming female mate choice; e.g. sneak); controls female receptivity or guards the female after mating (mate guarding); avoids other males mating and/or transferring sperm, or has mechanisms that give him advantages during fertilization (**sperm competition**).

Male fitness is thus determined not only by his **mating success**, but also by his **fertilization success**. Success in one domain is not necessarily highly correlated with success in the other. For example, when females mate with more than one male during a mating season (**polyandry**), male reproductive success is first dependent on being able to mate with the female, and, secondly of his sperm being more successful in fertilizing her eggs. In other words, there are selective processes acting before and after mating (**pre-** and **postcopulatory processes**). The postcopulatory processes include **cryptic female choice** (i.e. differential sperm retention; Eberhard, 1996), **sperm competition** (i.e. competition between the sperm of two or more males for the fertilization of a female's eggs; Parker, 1970; Parker, 1998), and **sperm cooperation** (i.e. collective movement of a male's sperm to increase his fertilization success; Foster and Pizzari 2010; Fisher et al. 2014).

In sum, male **reproductive success** is determined by both mating and fertilization success, which are constrained by processes acting before, during and after mating. However, both mating and fertilization success are partially determined during the mating context. First, it is obviously during mating attempts that male mating success vs non-success is defined – namely by interactions with competitor males. Second, it is during mating attempts that the female collects information about the male determinant to her mate choice decisions and subsequent cryptic choice. Third, it is during mating that male allocate his ejaculate (sperm and seminal fluid content and quantity).

Overall, this means that the mating context is a crucial moment during the reproductive cycle for male fitness. Signals and social cues transmitted by females or competitor males thus have considerable potential to affect the reproductive investment decisions made by males during such period. Males make a series of sequential decisions which include: approaching one or other female; the number of mating attempts to perform; which mating tactic to perform; how much investment to put on an ejaculate; and when to give up. It is predictable from evolutionary theory that individuals use, at least to some extent, information provided by signals and social cues before and during the mating context and adjust their behaviour accordingly. For example, female abundance, the presence of competitor males and their attractiveness can be crucial for male decisions, i.e. for a male to choose which female to approach or to approach females randomly; for a male to invest less or more trying to mate with a certain female; and for a male to give up sooner or later from the interaction with a female. In other words, male reproductive success will depend on the behavioural plasticity shown during the mating context in face of variable social factors encountered (Figure 2).

Examples of behavioural plasticity shown by males during the mating context support this hypothesis, being represented by fixed and reversible plastic mating behaviours. Mating strategies represent an example of fixed behavioural forms. In several species males are either displayers or sneakers – this last form is generally morphologically identical to females (Gross 1996; Gonçalves et al. 2008). Examples of reversible behavioural forms (**behavioural flexibility**) are the copulation duration, mate guarding, or mating tactics shown by the same individual – for example, each male guppy can perform courtship display or unsolicited attempts (Bretman, Gage, and Chapman, 2011). Additionally, researchers have found that bystanders influence males' decisions during mating. For instance, studies in a variety of species found that male mate choice shift in the presence of a conspecific male, leading males to change their initial sexual preference – eventually to deceive competitors (Plath and Bierbach, 2011; Castellano et al., 2016). But, studies have also shown the opposite – that bystanders' mate choice is determined by previously observed interactions between sexual partners and competitors. For example, a male can change his sexual preference, preferring females with a given phenotype, after observing the mate choice of competitor males – mate-choice copying (Bierbach, Kronmarck, Hennige-Schulz, Stadler, and Plath, 2011; Frommen, Rahn, Schroth, Waltschyk, and Bakker, 2008; Plath and Bierbach, 2011).



Social information

a.	Mating opportunity	Current competition risk (mating and sperm competition)
b.	Female abundance	Future competition risk (mating and sperm competition)
c1.	Female attractiveness (quality, condition and fecundity)	Male attractiveness and strength (quality and condition)
c2.	Species and sex identity, receptiveness	Species and sex identity, sexual interest
c3.	Receptiveness	Sexual interest, competitive ability and vigour

Figure 2. Representation of some conspecific factors that contribute to male mating decisions. In this thesis the focus is on the effect of conspecific adult females and males (sexual partners and competitors, respectively), and on the information they can transmit to males (as sexual partners and as competitors, respectively).

1.4. Thesis aims and outline

The overall goal of this thesis is to understand the effect of social information on individual decision-making. More specifically, I intend to evaluate the effect of conspecific social information on the plasticity of male mating behaviours, and the evolution of such behaviours. These aspects have been relatively neglected until now.

The **specific question** that I intend to contribute to solve is: how does the social context shapes male mating decisions? I focused on the social context both in terms of the availability of a sexual resource and the degree of sexual competition. By "mating decisions" I mean the time that males choose to spend following and attempting to mate with females, as well as the mating tactics they choose to employ. Different mating decisions may imply different costs and benefits. Following a female for longer periods and performing courtship displays may be more energy consuming and probably makes the male more vulnerable to predation than less time following or unsolicited mating attempts. At the same time, if the female is sexually interested, the male will probably produce more offspring by investing more time following and by courting her. The **goal of my thesis** was to understand how the social context shapes this type of plastic decisions in males, how social information may affect the evolution of male mating behaviours and, more broadly, animal communication.

To accomplish this, the following **topics** are **addressed**:

1) Contribution of conspecific social information to male mating behaviour adjustment:
    a. Sexual partner-related social information as a social cue of mating opportunities (chapter 2; Figure 2*a*, *b*, and *c1*):
        i. Time between females (time searching for females or females abundance)

        ii. Time between mating (mating opportunities or female accessibility)
    b. Sexual competitor-related social information as a social cue of pre- and post-mating competition risk:
        i. Competitor's presence (chapter 3 and 4; Figure 2*a*);
        ii. Competitor's sexual traits (chapter 3; Figure 2*c1*);
        iii. Order of arrival to the resource female (chapter 4; Figure 2*b*);

2) Contribution of conspecific social information to behaviour evolution:
    a. Impact of competitor (bystander) males on the evolution of male courtship display (chapter 5; Figure 2*c3*);
    b. General impact of bystanders to the evolution of animal communication (chapter 6).

For each topic, the **general hypotheses and predictions** are:

1) Contribution of conspecific social information to male mating behaviour adjustment:
    a. social information about the abundance and accessibility of females is relevant to male mating decisions and, so, males are predicted to invest more when the time between females or between mating opportunities is high;
    b. social information about competition risk is relevant to male mating decisions and, so, males are predicted to invest more when in the presence of competitor males, when competitor' relative attractiveness is high and when the male mating and/or fertilization success is compromised;

2) Contribution of conspecific social information to behaviour evolution:
    a. social information about a male's courtship display performance is relevant to competitor males as it can inform about displayer male' competitive ability and, so, the presence of a competitor male during a male-female courtship interaction is likely to exert a selective pressure on courtship display evolution, such as promoting male-male courtship display as a form of competition;
    b. gathering social information is in general adaptive and, so, bystanders should play a part on the evolution of communication, namely the evolution of signal dishonesty, if signal interception by the bystander is detrimental to the signaller.

Each chapter represents an independent manuscript, of which Chapter 4 has already been published and Chapter 6 submitted. Chapters included in the first topic (male behavioural adjustment; chapters 2 to 4) represent empirical studies, while the second topic (behaviour evolution) includes a systematic review study and a conceptual work (chapters 5 and 6, respectively). Although all experiments were performed with guppies at the University of St Andrews, the results were generalised, as much as possible, to other animal species.

Research under laboratory conditions allows investigators to control several variables, and potentially eliminate confounding effects (Campbell, Weiner, Starks, and Hauber, 2009). However, an extremely controlled environment is highly artificial, differing largely from what animal's experience in the wild, including the type and extent of social context (Campbell et al., 2009). This can limit applicability and interpretation of results (Campbell et al., 2009). For example, Dougherty and Shuker (2014) found that the dichotomous mate choice design overestimates the preference for a given sexual partner, particularly in species with sequential mate choice (such as guppies – Pitcher et al., 2003). To avoid such problems, the experiments described in this thesis were performed in mesocosm tanks (100 cm x 56 cm x 30 cm). Researchers from different areas have chosen to perform experiments in mesocosms (Nagrodski, Murchie, Stamplecoskie, Suski, and Cooke, 2013; Riebesell et al., 2013). A mesocosm system encompasses a bigger area, with a richer and more complex environment (e.g. with more individuals, and environmental enrichment, such as plants) than conventional laboratory experiments, while still allowing to control for some confounding variables. This type of system is not advisable if an extremely controlled environment is important to answer a scientific question. For example, by choosing a mesocosm system the researcher may have a quasi-experiment (where, for example, the social interactions between subjects are not chosen, nor controlled), instead of an experiment in which treatments are determined *a priori* (Cook, 2015). The experiments developed in this thesis are of this kind, i.e. with no control over social interactions during behavioural observations, though with controlled social conditions prior to the experiments. The greatest advantage of using a mesocosm system is that it reproduces a more natural environment. This is extremely relevant while studying the behaviour of social animals, since it allows individuals to behave and interact more freely than experiments restricted in space and group size (Devigili et al., 2015). This way, mesocosms are ideal when studies aim to consider several interactions (not exclusively dyadic interactions) and are designed to include a communication network.

Guppies used in this thesis were descendants from a wild population in the Lower Tacarigua River, Trinidad (Figure 3). This species is a model system in evolutionary ecology and animal behaviour research, particularly in relation to sexual selection. Guppy features are described in the next section, where I explain why this species is, in general, a good model in these research areas, and, specifically, a good model to study the questions presented in this thesis. Additionally, I present a brief description of the guppy ecology and behaviour.

Gone (or at least obsolete) is the image of fish as drudging and dim-witted pea brains, driven largely by instinct, with what little behavioural flexibility they possess being severely hampered by an infamous 'three-second memory'. Now, fish are regarded as steeped in social intelligence (...).

(Laland, Brown, and Krause, 2003)

1.5. Guppies

1.5.1. A model species

The guppy (*Poecilia reticulata* Peters, 1859) is a freshwater fish from the Poeciliidae family (Magurran, 2005a). Several Poeciliidae species have been used to study the evolution of reproduction, due to their diverse reproductive systems: from monogamy to promiscuity; with or without parental care; with internal or external fertilization; with total, partial, or no sperm competition; and with first, last, or no sperm precedence (Barbosa and Magurran, 2006). Particularly, the guppy has been extensively used in sexual selection research, among other research areas.

The guppy has features that makes it a good model for evolutionary ecology and animal behaviour: short generation time, individuals with a small size and that are easy to maintain in the laboratory, and retention of natural behaviour (Andersson and Simmons, 2006). Other advantages are that females are easily distinguished from males (both in morphology and behaviour) – revealing strong effects of sexual selection in this species (Andersson and Simmons, 2002) – and males are individually recognisable from their unique colour patterns (Magurran, 2005a). The species has a promiscuous mating system with both males and females mating multiply (Magurran, 2005a) – a feature that helps understand how pre- and postcopulatory sexual selection mechanisms are integrated (chapter 3 and 4). The species also has one of the highest frequencies of multiple mating in vertebrate species (Hain and Neff, 2007; Neff, Pitcher, and Ramnarine, 2008). This is relevant in understanding the impact that time searching for females has on males (chapter 2). The long history of study means that guppy ecology and behaviour have been extensively described, with reproductive behaviour especially well documented (Malte Andersson and Simmons, 2006; Houde, 1997; Magurran, 2005b). Additionally, because guppies show complex social interactions (with variable group sizes and unstable societies; Wilson et al., 2014), they are also used to study the implications of social information on the evolution of the brain and of behavioural flexibility[2].

1.5.2. Behavioural ecology

The guppy is native to Trinidad and Tobago (Magurran, 2005a), as well and as parts of NE South America, but currently is widespread in many different regions of the world (Figure 3). Indeed, the guppy is probably the tropical fish species with the widest distribution (Magurran, 2005a; Deacon et al., 2011). Its present distribution results from human action (escapes of ornamental strains and releases of fish for mosquito control; Houde, 1997; Deacon et al., 2011). Guppies have a great ability to survive and reproduce in new conditions as, for instance, a single pregnant female can start a new viable population (Deacon, Barbosa, and Magurran, 2014; Deacon et al., 2011). This invasion ability is partially explained by the species' behavioural plasticity. For example, guppies seem to gain easier access to food by shoaling with individuals of other species

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[2] See for example the work of two research groups (hyperlink accessed at 2nd of February of 2018): https://kaw.wallenberg.org/en/research/guppies-show-connection-between-behavior-and-brains-evolution; http://biology.mcgill.ca/faculty/reader/research.html

(Camacho-Cervantes et al., 2014). While behavioural plasticity helps guppies coping with new conditions, it is also possible that this species has evolved this ability due to facing constantly changing conditions (Bretman et al., 2011).

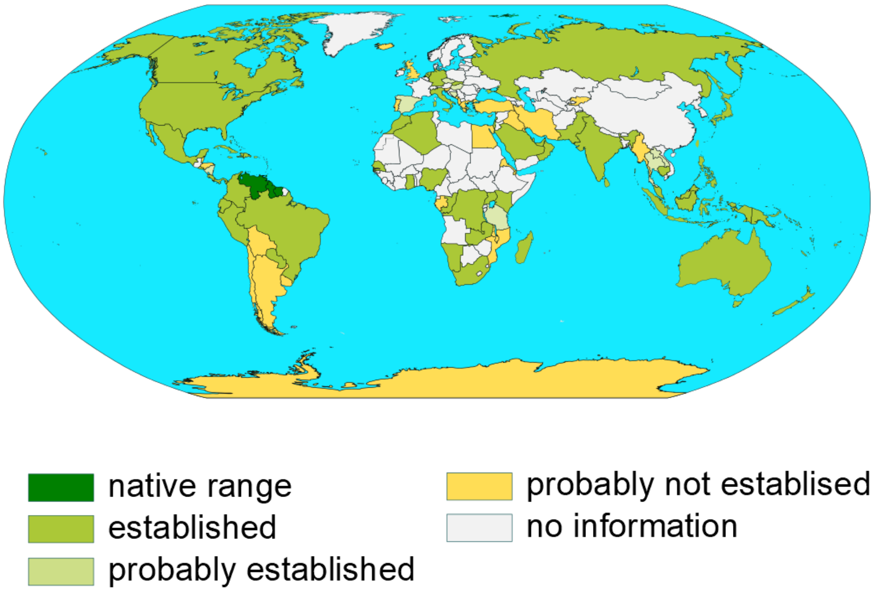

Figure 3. Global distribution and origins of *Poecilia reticulata*. Includes the guppy native range and current distribution because of introductions made by humans (adapted from Deacon et al., 2011).

Figure 4. Region of Trinidad and Tobago from which guppies tested in empirical studies presented in this thesis descend (Lower Tacarigua river). Image from google maps (https://maps.google.com/).

This species is present in rivers with contrasting and variable abiotic conditions of light, turbidity and stream width, among others (Houde, 1997; Magurran, 2005a). For example, guppies can be found in large rivers, as well as in intermittent streams and small pools (Reznick and Endler, 1982). Research shows that guppies adjust their behaviour to different abiotic conditions, namely by establishing more stable social bonds and forming larger shoals when water turbidity is higher (Borner et al., 2015).

Guppies also face contrasting biotic conditions, such as different levels of predation risk and variable group compositions. For example, several studies have addressed the effect of predation risk and of operational sex ratio on guppy behaviour. Schooling behaviour is more prominent and antipredator response is more intense in populations with high than with low predation risk (Brown, Macnaughton, Elvidge, Ramnarine, and Godin, 2009; Magurran and Seghers, 1991). Additionally, when guppies have access to information revealing presence of predators they tend to form more stable shoals (Hasenjager and Dugatkin, 2017; Heathcote et al., 2017). Another study found that male guppies invest more time following and trying to mate non-cooperatively with females in high-predation rivers (Magurran and Seghers, 1994). Similarly, evidences show that males try to mate non-cooperatively more often when females where previously near a predator (Evans, Kelley, Ramnarine, and Pilastro, 2002). As predation risk, operational sex ratio (OSR; i.e. the ratio of sexually receptive males to sexually receptive females; Kvarnemo and Ahnesjo, 1996) is highly variable for this species (Croft et al., 2003), and impacts male mating decisions such as mating tactic employed. Males perform less courtship displays and more non-cooperatively mating attempts when OSR is male-biased (Jirotkul, 1999). Males also behave differently when encountering females sequentially or simultaneously, performing more courtship displays when encounter females sequentially than at the same time (Jordan and Brooks, 2012). And male mate choice is dependent of having continuous contact with females or not, as males that are deprived from females during an interval of time show greater sexual interest towards a preferred female than when they are aware of females along the same interval of time (Barrett, Evans, and Gasparini, 2014).

1.5.3. Reproduction and male mating behaviour

Guppies are ovoviviparous livebearers fish with internal fertilization (Liley, 1966); females can carry offspring of different males and store sperm for more than six months (Constantz, 1984). The gestation time is three to four months per litter, and each litter is generally composed by less than 10 fry, although it can be of twenty or more (Houde, 1997). Differences between sexes become evident by the third and the fourth week of age, with females exhibiting a black mark in the anal region and males developing their typical colour patterns. At the fifth or the sixth week, the male gonopodium becomes visible, but sexual maturation is only completed when both females and males are about two months of age (Houde, 1997). By this age the sexual dimorphism is clear.

As mentioned previously, sexual dimorphism in this species is visible in morphology and behaviour (Andersson and Simmons, 2002). Females are, in general, bigger than males, and males more colourful than females (Houde, 1997; Magurran, 2005a; Figure 4). Social bonds also differ. While females associate with familiar conspecific females, males are constantly moving between female groups instead (Croft et al., 2003; Griffiths and Magurran, 1998). Therefore, due to male movements, guppies live in fission-fusion societies with fluid shoals (Wilson et al., 2014). Time-budgets are sex related: females spend most time foraging, while males dedicate most of their time and energy searching for females and attempting to mate (Griffiths, 1996).

Males can attempt to mate by performing one of two mating tactics: cooperative (courtship display) and non-cooperative (unsolicited) mating attempts (Houde, 1988; Magurran, 2005; Figure 4). In a courtship display, the male positions himself in front of the female and quivers his body in an s-shape movement. Ultimately, although infrequently and only if the female is receptive, the male may achieve mating as result of this behaviour. During an unsolicited attempt, the male may initially perform several gonopore nippings, i.e. he swims below the female and touches the female's gonopore with his mouth. Eventually, he inserts his gonopodium inside the female's gonopore (Baerends, Brouwer, and Waterbolk, 1955; Liley, 1966; Magurran, 2005a). Because gonopodial nippings and thrusts have a short duration and, therefore, are very hard to distinguish, I considered both as part of unsolicited attempts. Although, unsolicited mating tactic is commonly termed "sneaking", "coercive", or "forced attempt", I choose to use the term "unsolicited attempt" as it is more neutral in terms of an anthropogenic interpretation. As Magurran (2011) pointed out, "the fact that females are not exhibiting clear receptive responses does not necessarily mean that they are unwilling recipients of the sperm delivered via gonopodial thrusts". Besides, "sneaking" is sometimes confused with the mating strategy of sneak, performed by species with two types of males: the displayer (territorial) and the sneaker males (Gross, 1984). Guppies are different because the same male can perform a courtship display as well as a sneaking attempt.

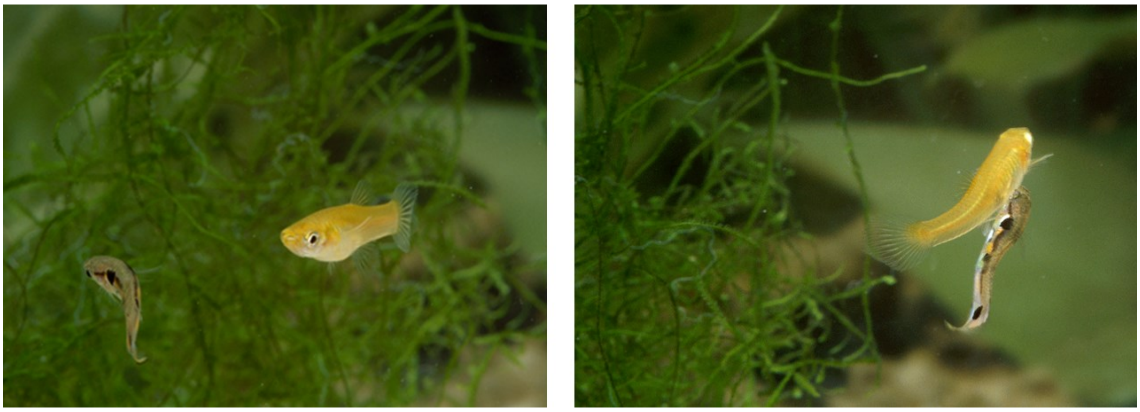

Figure 5. Mating context in guppies. Male (smaller and colourful) directing a mating tactic to a female (from the left to the right): a) courtship display, b) unsolicited attempt. Photographies of Sean Earnshaw, Biodiversity and Behaviour Group, University of St Andrews (https://synergy.st-andrews.ac.uk/diversity/).

Male mating success is in part determined by female mate choice. Female guppies prefer courting males (Magurran, 2005a). Additionally, females prefer brighter and colourful males (particularly for orange spots; Endler and Houde, 1995; Brooks and Endler, 2001; Pitcher et al., 2003; Evans et al., 2004; Barbosa et al., 2010; Auld et al., 2016). Females choose sequentially, being less choosy when they are virgin or receptive (Houde, 1997; Liley, 1966; Pitcher et al., 2003). Females are only receptive few days after parturition (Liley, 1966; Liley and Wishlow, 1974). Males prefer receptive and larger females (Guevara-Fiore, Stapley, and Watt, 2010; Ojanguren and Magurran, 2004).

After mating, and since females generally mate with several males (Magurran, 2005a), male fertilization success is dependent on postcopulatory processes. Guppies have partial last-male sperm precedence, i.e. the last male to mate before the female gives birth produces, in general, more offspring (Evans and Magurran, 2001; Pitcher et al., 2003). Last-male sperm precedence seems to be controlled by sperm competition, as a recent study found evidences that cryptic female choice favours first mated males (Magris, Cardozo, Santi, Devigili, and Pilastro, 2017).

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Modified image taken from Sean Earnshaw (Biodiversity and Behaviour Group, University of St Andrews)

CHAPTER 2.

Plasticity of male mating behaviours

Effect of sexual partners abundance and mating opportunities

Paper I. Use of Marginal Value Theorem to predict male mating decisions.

Inês Órfão, Alfredo F. Ojanguren, Miguel Barbosa, Luís Vicente, Anne E. Magurran and Susana A. M. Varela. Manuscript prepared to be submitted to journal Current Biology.

Summary

The Marginal Value Theorem predicts that optimal foraging time varies as a function of time spent searching for a patch with food (Charnov 1976). As with foraging time, time invested in a patch with females is also a vital factor for male fitness (Bateman 1948; Andersson 1994; Fromhage et al. 2016). This parallel gives a unique opportunity to use the known expectations of the Marginal Value Theorem on male mating effort, predicting that time following females and mating attempts should increase with time searching for females. Here, we investigated how male guppies (*Poecilia reticulata*) behave with a patch of females after spending different times between females. We found that males increased the time following females and performed more unsolicited mating attempts when time spent between females was longer. To disentangle the effects of time searching for females and of actual mating opportunities, we compared the behaviour of males that spent different time in between females and in between mating. Male mating effort was higher when males spent longer time between females than when they spent longer time between mating. Additionally, males that spend no time searching for females but spend lengthier times without mating became choosier, i.e. courted more attractive females. By using the Marginal Value Theorem, in the context of male mating decisions our study reveals that males invest more and risk less when their reproductive success is compromised by time invested searching for females.

Keywords: mate search, mating investment, mating tactic, mating success, decision-making, economic decision, optimality model

Highlights

- Males increase mating effort after spending more time between females.
- Time between females affects male mating effort more than time between mating.
- Males court attractive females when spend no time searching for females but were previously prevented from mating.
- Marginal Value Theorem helps predicting male mating decisions.

eTOC Blurb

Órfão et al. shows that the Marginal Value Theorem is useful to predict male mating decisions. Mating effort increases as a function of time searching for females. Mating effort is higher when time searching is lengthier. Males prefer more attractive females if have not mated recently and did not search for females.

2.1. Results and Discussion

The Marginal Value Theorem predicts that optimal time spent foraging in a patch is related to food depletion and with time spent travelling between patches (Charnov 1976; Figure 1). In the same way as food, sexual partners represent a valuable resource, which is limited in space and time (Darwin 1871; Andersson & Iwasa 1996; Andersson & Simmons 2006). Given the similarities, some researchers presented theoretical insights about the use of Marginal Value Theorem, as well as other optimal foraging theories, in the framework of sexual contexts (Parker & Stuart 1976; Louâpre et al. 2015). Furthermore, Parker and colleague found empirical evidences that copula duration of male dung flies is related with time searching for females (Parker & Simmons 1994; Parker 1970; Parker 1992). Still, the use of Marginal Value Theorem to predict male mating decisions in animal species remains poorly explored.

In the majority of species males invest more time and energy searching and attempting to mate than females (Bateman 1948; Andersson 1994; Fromhage et al. 2016; Clutton-Brock 2009). The probability of a male fertilizing one female decreases with increasing mating attempts, since along time there is a reduction of both female's sexual interest and male's sperm (Figure 1a). Consequently, like in Marginal Value Theorem, a male should leave a patch of females when the costs of exploiting the same sexual resource are higher than the benefits of searching and exploiting a new one (Figure 1b). However, this decision, as well as the decision of how much to invest in one resource, should also depend on the time invested searching for a new female (Figure 1c). The aim of this study is therefore to evaluate if male mating effort is in line with predictions of the Marginal Value Theorem.

For most species male sexual behaviour varies depending on mate encounter rate. (in guppies – Jordan & Brooks 2012; Barrett et al. 2014; Devigili et al. 2015; Cattelan et al. 2016; and in other species – Real 1990; Barry & Kokko 2010; Svensson et al. 2010; Head, Jacomb, et al. 2015). However, it is unknown whether this male variance in sexual behaviour occurs due to male perception of females' presence in the environment, or from difference in mating opportunities. Here, we evaluate whether male mating decisions are predictable from the time spent travelling between sexual partners and from time between encountering females time between successive mating.

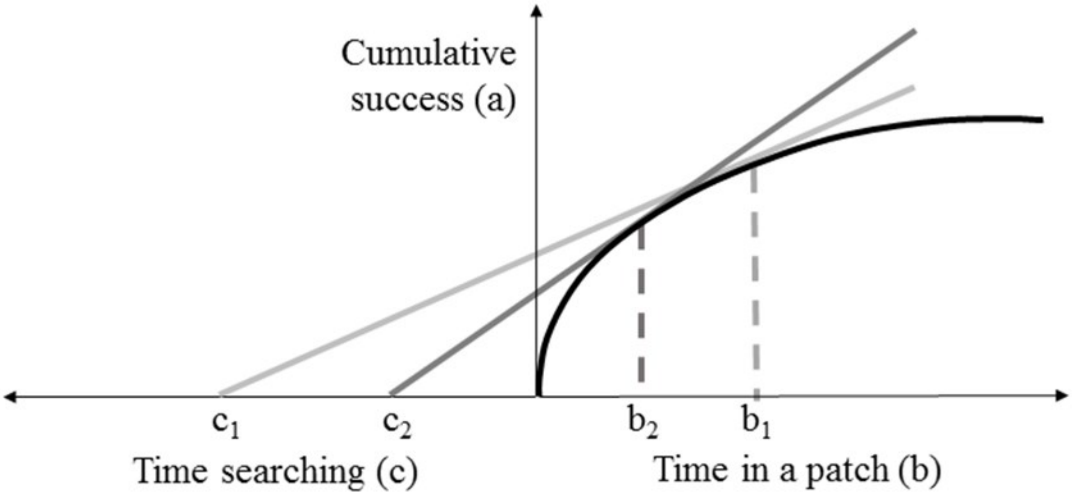

Figure 1. Representation of the Marginal Value Theorem. Its main variables, applied originally to the foraging behaviour and to the resource food, can also be applied to male mating behaviours and to the sexual resource (females). The three main variables considered are: a) cumulative success (resource intake for foraging, and fertilization success for mating), b) time in a patch (with food for foraging, and with females for mating), and c) time searching (for a patch with food for foraging, and for a patch of females for mating). The Marginal Value Theorem suggests that the optimal time spent in a patch (b_1 and b_2) depends on the time spent searching for that patch (c_1 and c_2, respectively). We add to consideration that time searching could be subdivided into time between resource (between patches with food for foraging, and between females for

mating) and time between resource intake (between meals for foraging, and between mating for mating). Consequently, the optimal time spent in a patch (b_1 and b_2) may also depend on the time between meals or mating (the cumulative curve).

We conducted experiments with guppies (*Poecilia reticulata*), a species in which females have a patchy distribution and the probability of males encountering females (time searching for females) differs across environments (Houde 1997; Griffiths & Magurran 1998; Magurran 2005). First (experiment 1), males were allocated to different treatments simulating time searching for females. We hypothesized that male mating decisions (time following, and frequency of mating attempts – a proxy of cumulative fertilization success), while following a patch of females, increase when the time between females is higher. Second (experiment 2), males were allocated to different treatments simulating time searching for females and time between mating. We hypothesized that mating opportunity has a great effect on male mating effort that time searching for females.

2.1.1. Experiment 1

Does time searching for females affect male mating decisions?

To test and quantify the effect of time searching for new females in shaping male mating decisions, males were divided between two isolation treatments for a 20 hour period (Figure 2 Experiment 1). This isolation period is based on the fact that male guppies in the wild can spend hours and even days until find females (Magurran 2005). 27 males were kept alone in a tank ('Total Isolation') and 29 were kept in a tank with a mixed-sex group ('No Isolation'/control). After this period, each male was allocated to a mesocosm tank with a patch of three females. We recorded the frequency of each male mating tactic (consensual courtship display and unsolicited mating attempt) and time spent following the female patch during 20 minutes. Since female sexual receptiveness affects male mating decisions (Evans et al. 2002; Guevara-Fiore et al. 2010), females were isolated and tested for receptivity before observation. The methodology is explained in detail in the supplemental information.

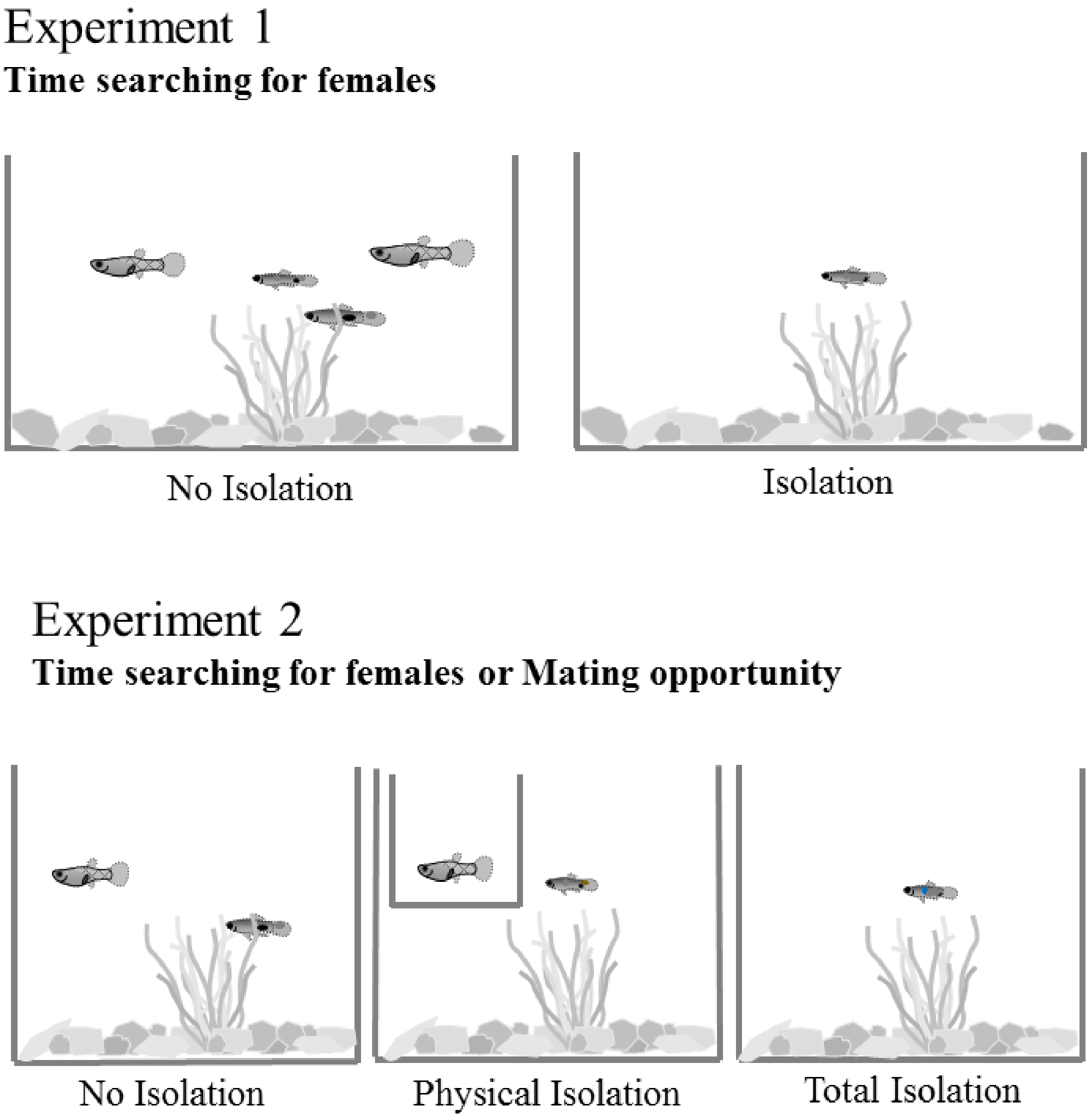

Figure 2. Isolation treatments for the two conducted experiments. 20 hours before behavioural observation focal males were assigned to different treatments. For the experiment 1 (above) males were assigned to one of two treatments (from the left to the

right): maintained in a mixed-sex group (No Isolation, n = 29), or maintained individually isolated in the tank (Total Isolation, n = 27). For the experiment 2 (below) focal males were assigned to one of three treatments (from the left to the right): maintained in a tank with one female (No Isolation, n = 14); maintained in a tank with a female but could not contact her, having only access to visual and to chemical cues (Physical Isolation, n = 13), or maintained isolated in the tank (Total Isolation, n = 15).

If we assume that a longer interval between encountering female patches equates to a reduced number of breeding opportunities by a male (the same way that longer time looking for a patch of food reduces the energy income), we predict that males allocated to the 'Total Isolation' treatment should be the ones investing more on mating behaviours. This way, we predict that males that have been isolated should spend more time following females and perform more mating attempts when given the opportunity to interact with a patch of females, than males that were not isolated from females.

Mating effort is higher when male spend more time in between females

Time between females had an effect on male mating decisions (the variable treatment was included in the best-fit model for time following females: $\chi^2_1 = 17.9$, n = 56, $p < 0.001$; for frequency of courtship display: $\chi^2_1 = 4.5$, n = 56, $p = 0.035$; and for frequency of unsolicited attempts: $\chi^2_1 = 13.0$, n = 56, $p < 0.001$). Males from 'Total isolation' spent more time following females than males from 'No Isolation' treatment (t = 4.2, $p < 0.001$; Figure 3a Experiment 1). Also, males from 'Total isolation' performed more unsolicited attempts (t = 3.4, $p = 0.001$; Figure 3c Experiment 1). However, totally isolated males performed less courtship displays that non-isolated males (t = -2.1, $p = 0.035$; Figure 3b Experiment 1).

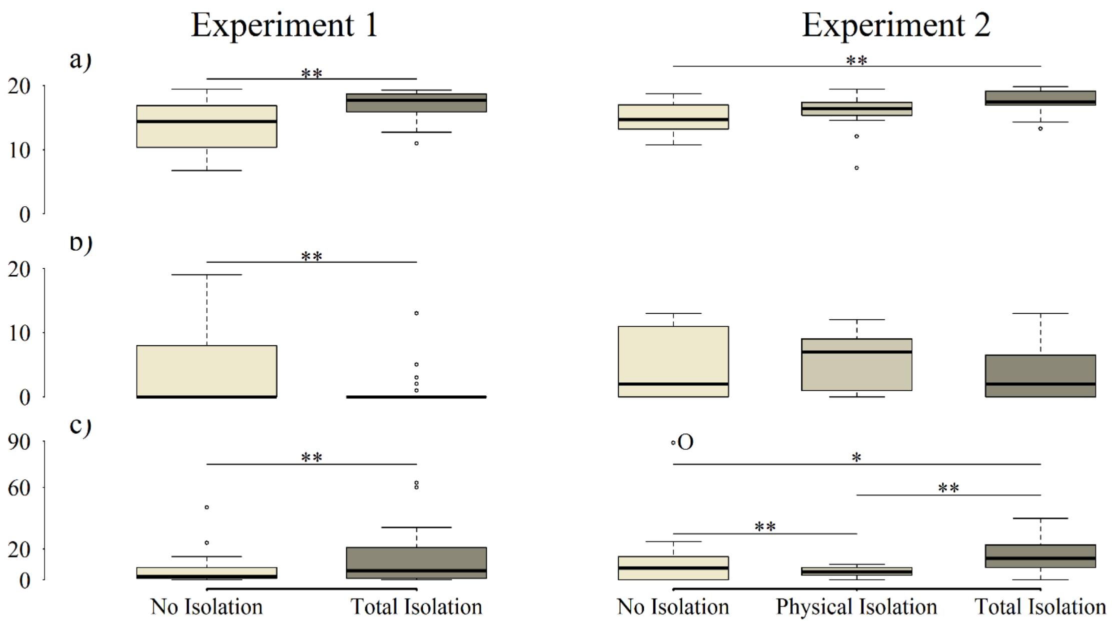

Figure 3. Mating effort by focal males (from top to bottom): a) time following females, b) frequency of courtship displays, and c) frequency of unsolicited attempts. 20 hours before behavioural observation focal males were assigned to different treatments. For the experiment 1 (left column) males were assigned to one of two treatments: maintained in a mixed-sex group (No Isolation, n = 29, left bar), or maintained individually isolated in the tank (Total Isolation, n = 27, right bar). For the experiment 2 (right column) focal males were assigned to one of three treatments: maintained in a tank with one female (No Isolation, n = 14, left bar), maintained in a tank with a female but could not contact her, having only access to visual and to chemical cues (Physical Isolation, n = 13, middle bar), or maintained isolated in the tank (Total Isolation, n = 15, right bar). Symbols above the boxplots represent significant differences between isolation treatments ("*" $p < 0.06$; "**" $p < 0.05$). In each boxplot the internal line represents the median. Lower and upper edges represent the 25% and the 75% quantile, respectively. Whiskers below and above the box edges represent, respectively, the minimum and the maximum points within the

1.5 interquartile range. Circles represent outliers. “O” indicates the outlier excluded from statistical analyses in experiment 2.

In general, males that spent a longer period of time without females invested more trying to mate than males that had been previously with females. Such result is in accordance with a predicted higher investment after spending more time searching for a resource by the Marginal Value Theorem. However, reduced frequency of courtship displays is surprising. This may result from a confounding variable. Males from the ‘No Isolation’ treatment that displayed significantly more courtship displays, were allocated to a mixed sex previous to observation. Since during observation there were no competitor males nearby, these males’ investment in courtship display is probably a side-effect of male-male competition. Accordingly, previous studies found that male guppies perform more courtship display when competition is weaker. For example, males perform more courtship displays in the absence than in the presence of another male (Farr 1976; Auld et al. 2015), and when operational sex ratio is female instead of male-biased (Jirotkul 1999; Chuard et al. 2016). To confirm this suspicion, male-male competition was controlled in experiment 2. In experiment 2 we also tested time between mating.

Male mating effort is higher with receptive females, regardless of time between females

Male mating effort was linked to females’ receptiveness (variable included in the best-fit model for time following: $\chi^2_1 = 4.8$, n = 56, $p = 0.029$; for courtship display: $\chi^2_1 = 5.2$, n = 56, $p = 0.022$; and for unsolicited attempts: $\chi^2_1 = 9.2$, n = 56, $p = 0.002$). When at least one female in the patch was receptive, males spent more time following the patch (t = 2.2, $p = 0.043$), performed more unsolicited attempts (t = 3.0, $p = 0.004$), and were more likely to perform any courtship display (t = 2.1, *p* = 0.038; females' receptiveness was included only in the zero model).

Higher effort on courtship display when females are receptive was expected (Farr 1980; Ojanguren & Magurran 2004; Guevara-Fiore et al. 2010). In contrast, previous studies found no or a negative correlation between receptiveness and unsolicited attempts (Guevara-Fiore et al. 2010; Farr 1980; respectively). Importantly, we did not evaluate male mating behaviours directed exclusively to the receptive females. This result may suggest that the presence of a receptive female in the patch increases male mating motivation, and males may try to mate with all females in the group. Accordingly, a previous study found that male guppies spend more time associated with shoals that contain receptive females (Guevara-Fiore et al. 2010).

We fail to detect an effect of interaction between females' receptiveness and isolation treatment on male mating behaviours (time following: $\chi^2_1 = 0.0$, n = 56, $p = 0.952$; courtship displays: $\chi^2_1 = 0.1$, n = 56, $p = 0.700$; unsolicited attempts: $\chi^2_1 = 0.0$, n = 56, $p = 0.868$). For this reason, and as females in the wild are commonly unreceptive (Liley 1966; Liley & Wishlow 1974), we only tested likely unreceptive females in experiment 2.

All best-fit models included mesocosm, isolation treatment and females' receptiveness (time following: $L_3 = 10.0$, n = 56, p = 0.067; courtship display: $\chi^2_1 = 7.7$, n = 56, $p = 0.005$ – isolation treatment was included in the count model, mesocosm and females' receptiveness in the zero model; unsolicited attempts: $F_{1,52} = 9.2$, n = 56, $p = 0.004$).

2.1.2. Experiment 2

Is the effect of time searching for females on male mating effort confounded with the effect of mating opportunities?

Here, we were interested in investigating male mating effort according to: 1) time searching for females and 2) mating opportunity. For that we exposed male guppies to different time between females (as a proxy of time searching for females) and time between mating (as a proxy of mating opportunity). Prior to observations, males were divided into three social treatments for a 20 hour period (Figure 2 Experiment 2). 15 males were kept alone in a tank ('Total Isolation'), 14 males were in a tank with one female, and could freely interact with her ('No Isolation'), and 13 males were also with one female, but had a physical barrier in between ('Physical Isolation'). Males from 'Physical Isolation' and from 'Total Isolation' treatments had in common the opportunity to mate – both were unable to mate during the time they were isolated. Males from 'Physical Isolation' and 'No Isolation' treatments had in common the perception of females – both were always with females (one female during the isolation period and three during the observation period). After the isolation treatment, each male was observed in a mesocosm with a patch of three females for a period of 20 minutes. Methodological details are given in the supplemental information.

Assuming that male behavioural decisions are primarily influenced by mating opportunity, males prevented from mating ('Total' and 'Physical Isolation') were expected to spend more time following a female patch and engage more vigorously in mating attempts than 'No Isolation' males. However, if the perception of females in the environment is more important than mating opportunity, then males are expected to invest more after spending some time without females ('Total Isolation'), than males that were previously with one female ('No' and 'Physical Isolation'). Also, males that were previously with one female ('No' and 'Physical Isolation') should have similar mating efforts.

Increased male mating effort is shaped by time in between females

Isolation was the only variable included in the best-fit models that explained the time males spent following females ($F_{1,39} = 4.4$, $n = 42$, $p = 0.019$), and the frequency of unsolicited mating attempts ($\chi^2_2 = 15.1$, $n = 41$, $p < 0.001$ – analysis excluding the outlier male that performed 89 attempts; mean ± SE of unsolicited attempts frequency: 11.7 ± 15.3; Figure 3c Experiment 2). In contrast, no variable explained the performance of courtship displays. Isolation had no effect on the frequency of courtship displays (count model: $\chi^2_2 = 1.7$, $n = 42$, $p = 0.419$), neither on the probability of males performing this mating tactic (zero model: $\chi^2_2 = 1.2$, $n = 42$, $p = 0.547$; Figure 3b Experiment 2).

Males spent more time following females after being deprived from females than after no isolation ('No Isolation' vs. 'Total Isolation': $t = 2.9$, $p = 0.006$; Figure 3a Experiment 2) and tend to perform more unsolicited attempts, albeit marginally significant ('No Isolation' vs. 'Total Isolation': $z = 1.9$, $p = 0.053$). Additionally, time following females did not differ between males that were with one female but could not mate and males that could mate ('Physical Isolation' vs. 'No Isolation': $t = -0.9$, $p = 0.362$), or with males that had no female near ('Physical Isolation' vs. 'Total Isolation': $t = 1.9$, $p = 0.062$). On the contrary, and against any of our predictions, males that were physically isolated performed significantly fewer unsolicited attempts than males from the other two treatments ('Physical Isolation' vs. 'No Isolation': $z = 2.1$, $p = 0.033$; 'Physical Isolation' vs. 'Total Isolation': $z = 4.3$, $p < 0.001$; Figure 3c Experiment 2).

Supporting the hypothesis that perception of female availability (time between females) influence male mating effort, we found that time following females by males with previous contact with one female was similar, regardless of mating or not ('No' and 'Physical Isolation'). Moreover, mating effort was higher when males did not find females, since males with no female in their vicinity ('Total Isolation') were the ones that invested more on mating attempts, more specifically on the unsolicited tactic. However, mating opportunity (time between mating) had an effect on male mating behaviour. Males that could not mate ('Total' and 'Physical Isolation') spent the same time following females. Additionally, males that encountered females but had contrasting mating opportunities ('No' and 'Physical Isolation') invested differently on unsolicited mating attempts. Nevertheless, because there was a clearer difference in time following between males that differed both on perception of females and on mating opportunity ('Total' and 'No Isolation'), time between mating seems only to be relevant combined with the time males spent to find females.

Interestingly, mating deprived males that had information about female presence during the entire time ('Physical Isolation') performed fewer unsolicited tactic. This contrasts with results found for time following: physically isolated males only differed from males that were totally isolated. Eventually, males from this treatment invested some time sampling females but allocated mating attempts to more attractive females. In fact, males from 'Physical Isolation', that did not have the opportunity to mate, have more to lose than males from 'No Isolation' treatment. But, they have less to lose than males from 'Total Isolation', who also did not have the opportunity to mate, but had a perception that females are difficult to find.

Are mating opportunities more important to male mate choice than time searching for females?

In guppies (Abrahams 1993; Herdman et al. 2004; Ojanguren & Magurran 2004; Guevara-Fiore et al. 2010) and in several species (Bonduriansky 2001; Edward & Chapman 2011) males may prefer receptive and larger females. Therefore, if males from the ‘Physical Isolation’ were choosier than males from the other two treatments, we predicted they would invest more in mating attempts towards receptive and larger females. This way, we compared the effect of female size on male mating behaviours between the different isolation treatments.

Firstly, we measured the standard length of females tested. Secondly, we calculated average patch length (i.e. the average standard length of the three females with which the focal male was observed). This was done because our goal was to examine male mating effort toward a patch of females and not to quantify male behaviour in relation to each single female. Finally, for the statistical analyses we included in the full model the isolation treatment, average patch length and interaction between them as explanatory variables. Females with different sizes were evenly distributed across the isolation treatments – there was no relationship between isolation treatment and average patch length ($F_{1,39} = 0.2$, $n = 42$, $p = 0.823$). For more details see methods in the supplemental information.

Males are choosier when they spend more time between mating, but the extent to which prefer different females depends on the time between females

Time spent following females varied with average female length ($F_{1,38} = 7.7$, $n = 42$, $p = 0.009$), but we found no significant effect of the interaction between this variable and the isolation treatment ($F_{1,38} = 3.1$, $n = 42$, $p = 0.059$). Thus, males from the three isolation treatments spent more time with a patch of females when they were, on average, smaller (Figure 4a and table 1a; $t = -2.8$, $p = 0.009$). The best-fit models for male mating attempts also included the average patch length, but, in contrast, included the interaction between this variable and the isolation treatment (courtship displays: $\chi^2_2 = 9.7$, $n = 42$, $p = 0.008$; unsolicited attempts: $\chi^2_2 = 9.0$, $n = 41$, $p = 0.011$ – analyses excluding the outlier).

Average patch length had no effect on the frequency of unsolicited attempts (count model: $\chi^2_1 = 0.2$, $n = 41$, $p = 0.619$; Figure 4c and table 1c), but was included in the best-fit model that explained the probability of males performing this mating tactic (zero model: $\chi^2_3 = 9.1$, $n = 41$, $p = 0.028$). The percentage of males that performed at least one unsolicited attempt was of 93.3% for the ‘Total Isolation’ treatment, 84.6% for ‘Physical Isolation’, and 69.2% for the ‘No Isolation’ treatment. Still, no significant differences between treatments were found (‘No’ vs. ‘Physical Isolation’: $t = 0.6$, $p = 0.516$; ‘No’ vs. ‘Total Isolation’: $t = 0.0$, $p = 0.997$; ‘Physical’ vs. ‘Total Isolation’: $t = 0.0$, $p = 0.997$).

In relation to the cooperative mating tactic, we found that males from ‘Physical Isolation’ treatment performed more courtship displays with larger females than males from ‘Total Isolation’ treatment ($z = -3.1$, $p = 0.002$; Figure 4b and table 1b). In the case of the ‘Total Isolation’ treatment, males directed more courtship displays towards females that had, on average, standard lengths close to the average of all females tested (average females’ length: 1.954; see Figure 4b). Also, males from the ‘Total Isolation’ treatment were less likely to direct courtship displays to smaller females than males from ‘No Isolation’ ($z = -2.1$, $p = 0.037$).

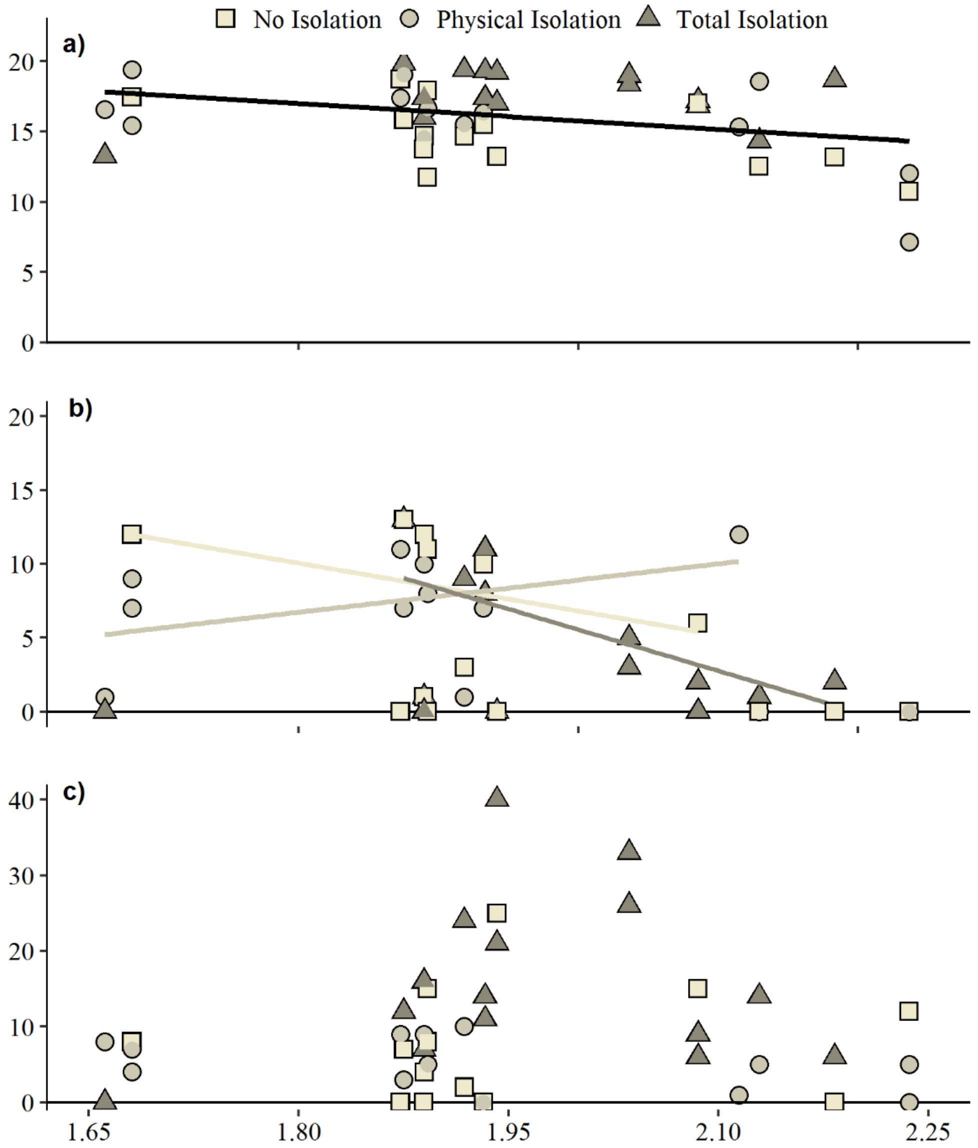

Figure 4. Mating effort by focal males in experiment 2 and in relation to females' average standard length (from top to bottom): a) time following females, b) frequency of courtship displays, and c) frequency of unsolicited attempts. 20 hours before behavioural observation focal males were assigned to one of three treatments: maintained in a tank with one female (No Isolation, n = 14, represented as squares), maintained in a tank with a female but could not contact her, having only access to visual and to chemical cues (Physical Isolation, n = 13, represented as circles), or maintained isolated in the tank (Total Isolation, n = 15, represented as triangles). Regression lines in figures a) and b) represent trends estimated with linear model ("lm") and generalized linear model ("glm"), respectively.

Table 1. Summary table of the best-fitted models to explain for male mating behaviours observed during experiment 2 in relation to isolation treatment and females' attractiveness.

Response variable	Model type	Distribution	Explanatory variables	Contrasts	Z-value/ T-value	P-value	
a) Time following	LMER	Normal	Treatment	Physical vs. Total Isolation	2.3	0.025	*
				Physical vs. No Isolation	-0.8	0.425	
				Total vs. No Isolation	-3.2	0.003	**
			Average length		-2.8	0.009	**
b) Courtship display	Hurdle Count model	Negative Binomial	Treatment x Average length	Physical vs. Total Isolation	-3.1	0.002	**
				Physical vs. No Isolation	-1.5	0.145	
				Total vs. No Isolation	1.8	0.079	
	Zero model		Treatment x Average length	Physical vs. Total Isolation	0.0	0.997	
				Physical vs. No Isolation	0.0	0.997	
				Total vs. No Isolation	-2.1	0.037	*
c) Unsolicited attempt	Hurdle Count model	Negative Binomial	Treatment	Physical vs. Total Isolation	4.3	<0.001	***
				Physical vs. No Isolation	2.1	0.033	*
				Total vs. No Isolation	-1.9	0.053	.
	Zero model		Treatment x Average length	Physical vs. Total Isolation	0.0	0.997	

Response variable	Model type	Distribution	Explanatory variables	Contrasts	Z-value/ T-value	P-value
				Physical vs. No Isolation	-0.6	0.561
				Total vs. No Isolation	0.0	0.997

Legend: "·" $p < 0.1$; "*" $p < 0.05$; "**" $p < 0.01$ and "***" $p < 0.001$

Analyses were separated based on the mating behaviour directed to a patch of females: a) time following females, b) frequency of courtship displays, and c) frequency of unsolicited attempts. The initial full model included as explanatory variables: isolation treatment (No Isolation, n = 13; Physical Isolation, n = 13; and Total Isolation, n = 15), females' average standard length and their interaction (represented by "x").

These results indicate that physically isolated males were choosier. Previous studies found evidence that males are choosier when mate encounter rate, and chance of future mating are higher (mate encounter rate – Jordan & Brooks 2012; Head et al. 2015; Barry & Kokko 2010; future mating change – Edward et al. 2010). Interestingly, we found that totally isolated males were also choosier. These two isolation treatments had in common the mating opportunity, which indicates that time between mating is the main responsible for male mate choice. Furthermore, our findings show that males with lower opportunity to mate invested in more or less attractive females depending on the time spent searching for females. Thus, males that were aware of females for a longer period ('Physical Isolation') court larger and more fecund females. But when females were hard to find ('Total Isolation') males invested on averagely attractive females – not courting smaller females, but directing more courtship displays to females with average size.

2.2. General Conclusions

Our study lends support for the use of optimal behaviour theories in the sexual context. Specifically, we show that more time searching for the resource female lead to a higher male mating effort, according to the Marginal Value Theorem. Perception of females in the environment (time between females) is the main contributor to male mating effort. On the other hand, mating opportunities (time between mating) played a second role on male mating effort but determined male choosiness.

Time searching for females lead males to invest in the mating tactic with lower mean reproductive success – unsolicited attempts (Evans & Magurran 2001). However, this is also the tactic with lower variability outcome, since allow males to mate even without female consent (Houde 1988; Magurran 2001). This result suggests that males that spend more time without seeing females are being risk averse. Corroborating this, under lengthier time searching for females, males directed courtship to females of average size. Since bigger females are more fecund, but also attract more males (Herdman et al. 2004; Bonduriansky 2001; Edward & Chapman 2011), with this strategy males may avoid competition. Simultaneously, by not courting smaller (less fecund) females, male reproductive success is less compromised. By other hand, when males could not mate but spend no time searching for females, they prefer to court bigger, more attractive females. In this case, males are investing in a tactic with more variable success – either males are able to mate and produce more offspring, or females reject them – and in more choosy females. Therefore, males seem to respond with a risk prone strategy (see risk-sensitive model – Caraco et al. 1980, 1990).

The allocation of courtship displays to more attractive females when females are easier to find is in accordance with previous research (short vs. longer interval between females – Svensson et al. 2010; simultaneous vs. sequential encounter with females –

Head, Jacomb, et al. 2015; Barry & Kokko 2010; Jordan & Brooks 2012). What is new in our study is the finding that time searching for females lead males to employ different mate choice strategies depending on the time between mating. This way, we found that male mating decisions based on time searching for females are not exclusively linked to mate encounter rate. While we found that male guppies employ more unsolicited attempts when spend more time between females, other research show that male guppies perform more unsolicited attempts and less courtship displays when the probability of finding females is higher (simultaneous vs. sequential encounter with females – Jordan & Brooks 2012; short vs. longer time between unfamiliar females and to find one female – Devigili et al. 2015; Cattelan et al. 2016). Importantly, in these later studies, social conditions were kept constant for days, while in our study conditions were maintained just for some hours. This may suggest that mating tactics depend on environment predictability. In the wild, probability of mate encounters social will depend on social conditions (e.g. population density, operational sex ratio).

Taken together our analysis showcases the utility of optimality models in elucidating reproductive decision making (Louâpre et al. 2015). Future studies can build on this to investigate the reason behind the evolution of other sexual decisions (e.g. which sexual partner to exploit), using background theory extensively tested and validated in the foraging context.

Author Contributions

The main contributors to the conception and design of the work were (descendent order) SAMV, IO, AM and AO. IO was responsible for the acquisition and analysis of data. Everyone contributed to the interpretation of data. IO and MB drafted the first versions of the article and all revised the article critically.

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Supplemental Information

Experimental design

The study was conducted at the Scottish Oceans Institute (University of St Andrews) with guppy descendants from a wild population of the Lower Tacarigua River, Trinidad. Before observations, males were kept individually in adjacent isolation tanks (34 cm x 23 cm x 25 cm) with similar conditions of temperature and light for a period of 20 hours. To exclude possible effects related with competitor males, a visual barrier was placed between the isolation tanks. After the isolation period, males were transferred to a small container in the observation tank during a 15-minute acclimatization period. After, males were released and observed for a period of 20 minutes.

To ensure temperatures were kept in the range found in Trinidad streams (Reeve et al. 2014) and were equivalent to what individuals had experienced during their lifetime in the laboratory, isolation and observation tanks contained heaters. Temperatures were monitored each 30-minutes using a data logger (MicroLite Lite 5016, Fourier Systems) in each isolation and observation tank. For males in the "No Isolation" treatment in experiment 1, we recorded the temperature of stock tanks from which we removed them.

Each observation was performed in one of twomesocosm tanks (100 cm x 56 cm x 30 cm). Mesocosms were covered with gravel in the bottom and contained plants. An aerating system was turned off during observations. Observations were conducted between 9:00 and 13:15 (Houde 1997; Magurran 2005) and were performed from above. Males were tested with females removed from a different stock tank, as familiarity decrease male sexual interest (Kelley et al. 1999; Mariette et al. 2010). All individuals were fed with flake food during the morning before daily observations.

To account for female receptiveness all tested females were kept isolated in a tank (34 cm x 23 cm x 25 cm) for a period of three days before observation. This period is based on the fact that female guppies are only receptive when virgins or few days immediately following parturition (Liley 1966; Liley & Wishlow 1974). Females tested were likely non-virgin (previously in mixed-sex stock tank) and were considered to be receptive if they had gave birth during this period (Liley 1966). Since female guppies, in opposition to males, form strong social bonds (Griffiths & Magurran 1998; Croft et al. 2003), females were kept in adjacent tanks without any visual barrier to minimize stress and preserve familiarity. For the same reason three familiar females (original from the same stock tank and from adjacent isolation tanks) were introduced in each mesocosm the afternoon before the experimental day.

All tested individuals were transferred to another tank, ensuring individuals were tested only once. However, the same group of females was kept in each mesocosm the entire observation day to avoid stress. Therefore, 3 to 6 males were tested with same female group.

Behavioural recordings

Each male was observed with a group of three unfamiliar females for a period of 20 minutes by focal sampling. Focal sampling started when we removed the male from the small container used during the acclimatization period. We recorded time following the females and the frequency of courtship displays and of unsolicited mating attempts. We considered that males were following a female (or a patch of females) when they approached any female and swam or stayed near her (maximum at 3 body-length). During a courtship display a male would place himself in front of the female and quiver his body in an s-shape movement, and, rarely this behaviour would lead to a full consensual mating. When performing an unsolicited attempt, male tries to insert his gonopodium inside the female' gonopore without the females' cooperation, and, previously, he may touch the female' gonopore with his mouth – gonopore nipping (Baerends et al., 1955; Liley, 1966; Magurran, 2005).

A total of 56 and of 42 focal samples were performed during the first and the second experiment, respectively. All observations were recorded with JWatcher v1.0. software (Blumstein & Daniel 2007).

Experiment 1: Does time searching for females affect male mating decisions?

The population studied consisted of 56 males and 60 females. Two treatments were considered: males were transferred from a stock tank to an isolation treatment tank and kept alone ('Total Isolation', n = 27), or were kept in a stock tank with a mixed-sex group ('No Isolation', n = 29). Females used during observations were randomly selected from the stock tanks, and, so, could be receptive or unreceptive.

Experiment 2: Is the effect of time searching for females on male mating effort confounded with the effect of mating opportunities?

The population studied consisted of 42 males and 48 females. All tested males were transferred to an isolation treatment tank, with a maternity box inside. Each male was allocated to one of three treatments: kept alone in the tank ('Total Isolation', n = 15); kept with one female inside a maternity box, to prevent any physical contact and, therefore, mating ('Physical Isolation', n = 13); or kept with one female outside the maternity box, so they could swim freely with the female ('No Isolation', n = 14). To avoid using receptive females (due to results from experiment 1; but see also Farr 1980; Guevara-Fiore et al. 2010; Ojanguren & Magurran 2004), only females that did not gave birth in a period of three days were used – so, were likely unreceptive (Liley & Wishlow 1974).

Females placed in the isolation treatment tanks were previously used in observations, and, therefore, were very likely unreceptive.

Are mating opportunities more determinant to male mate choice than time searching for females? – Calculation of the average female length

After observations, females were placed in a Petri dish with water and photographed from above. We measured the standard length of each female recurring to ImageJ software (Schneider et al. 2012). Then, we calculated the average standard length of females of the same patches (average female length).

Statistical analyses

All analyses were performed using the software R 3.2.3 (R Core Team 2015). Except in specified cases, analyses were similar for data of both experiments. The significance level was set at $p = 0.05$. To test if the total time following a female was dependent on time searching for females (experiment 1), and on time between mating (experiment 2), we used linear mixed-effects models (LME from the package nlme in R; Pinheiro et al. 2016). The isolation treatment was included in the models as a fixed term, and the mesocosm and the female group as random terms (female group nested inside mesocosm). In experiment 1, females' receptiveness and its interaction with isolation treatment were included as fixed terms. In both experiments, diagnostic plots were used to evaluate models' homoscedasticity and normality. Models with random terms were compared using a likelihood ratio test (LRT), and models with no random terms were compared using linear models (LM), both from the package stats (R Core Team 2015).

To evaluate if males invested more in mating attempts after spending time searching for females, we compared the frequency of sexual behaviours (courtship displays and unsolicited mating attempts) between males from different isolation treatments. Due to excess of zeros in most of the cases, the frequency of sexual behaviours was modelled with a hurdle model (Zuur et al. 2009; Zeileis et al. 2008). For tht we used the package pscl in R (Jackman 2015). Hurdle models consider separately a binomial distribution with a logit link function to model the zeros and a poisson or a negative binomial distribution to model the count (non-zeros). Isolation treatment and mesocosm were included as explanatory variables in the models of both experiments. Females' receptiveness and its interaction with isolation treatment was considered for the model of experiment 1. In both experiments, the best fitted model was selected based on likelihood ratio test of nested models (Zuur et al. 2009), specifically on LRTEST from the package lmtest in R (Zeileis & Hothorn 2002). Since the overdispersion of the frequency of unsolicited attempts recorded in experiment 1 was not zero-inflated (diagnosed by frequency plot of the data), the dispersion parameter was inferior to 15 (theta = 13.4) and did not result from outliers (theta = 7.3), we applied a generalised linear model (GLM from the package stats in R; R Core Team 2015) with a logit link function for a quasipoisson error structure (Zuur et al. 2009; Thomas et al. 2013). Model selection was based on a backward step-wise procedure with an analysis of variance (anova).

To evaluate if males from the 'Physical Isolation' treatment were choosier than males from the other two treatments in experiment 2, we analysed the following male mating behaviours: time following females, frequency of courtship displays, and of unsolicited attempts. For that, we used the previously described statistical methods, but including the average female length and its interaction with the isolation treatment as explanatory variables.

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Modified image taken from Sean Earnshaw (Biodiversity and Behaviour Group, University of St Andrews)

CHAPTER 3.

Plasticity of male mating behaviours

Effect of competitor's presence and sexual traits

Paper II. Me against who? Male guppies adjust mating behaviour according to competitor's attractiveness.

Inês Órfão, Miguel Barbosa, Alfredo F. Ojanguren, Luís Vicente, Susana A. M. Varela and Anne E. Magurran. Manuscript prepared to be submitted to journal Ethology.

Abstract

Competition during mating and the relative difference in attractiveness between two competing males can affect the reproductive success of each male. Therefore, the presence of a competitor and his attractiveness are predicted to determine male investment in a mating context. Here, we address this hypothesis, and ask how male guppies (*Poecilia reticulata*) adjust mating tactics (courtship display and unsolicited attempts) in response to competitor presence and secondary sexual traits. First, we compared focal male mating behaviours under two distinct competitive scenarios: 1) with two females (no-competition) and 2) with two females, but in the presence of another male (competition). Since males facing competition have less chance of mating, we predicted that males would perform fewer courtship displays in the competition scenario. Instead, because males can achieve mating without female consent by performing unsolicited attempts, we also predicted that males would perform more this mating tactic in the competition scenario. Second, we measured male phenotype characteristics that can influence female mate choice (standard body length, orange and black body area), and asked if competitor absolute and relative (compared to the focal male) attractiveness, as defined by these characteristics, determined focal male mating decisions. Since males have less chance of being chosen by females when competing against attractive or more attractive competitors, we also predicted that in the presence of such type of competitor males would invest more on unsolicited attempts and less on courtship displays. We found that focal males adjust their mating behaviours in response to the competing scenario, as well as to the attractiveness of the competitor. Males performed more unsolicited mating attempts when in a presence of a competitor, regardless of his sexual traits. Importantly, when in a presence of a large or a larger competitor, male guppies performed less courtship displays, and performed less of both tactics when competitor had a large or larger area of black spots. Contrastingly, they engaged in significantly more courtship displays when competing against males with greater area of orange pigmentation – a plastic phenotype enhanced during courtship. Overall, we show that male guppies invest less when competing against attractive competitors. But, risk higher investment through courtship displays while competing against males with attractive orange pigmentation, probably because, by doing so, focal males also enhance their own attractiveness for the orange-colouration plastic phenotype.

Keywords: behavioural plasticity; female mate choice; intrasexual competition; ornament; phenotypic plasticity; secondary sexual character; sexual behaviour; social information.

3.1. Introduction

Research on sexual selection has long focused on the impact of male-male competition for access to females on male fitness (Darwin 1871; Andersson 1994). Due to relevance of this sexual mechanism, studies have considered how males adjust their mating behaviour to the presence and attractiveness of competitor males (e.g. Plath & Bierbach 2011; Chaudhary, Mishra & Omkar 2017; Tuni et al. 2017). More recently, the difference in attractiveness between two competing males has been also considered (e.g. Leonard & Hedrick 2009; Mautz & Jennions 2011). However, as most research has separately considered competitor presence, competitor absolute and relative attractiveness, the integrated impact of these cues of competition risk on male mating behaviours is still incompletely understood.

Male reproductive success is determined by the presence of competitor males during mating. Firstly, a competitor can decrease male mating success through sexual interference or sneaking (Darwin 1871; Andersson 1994). For example, leafhopper males from the species *Aphrodes makarovi* win mating success over a competitor by approaching the female in silence or by making a call that overlaps the female response to the competitor call (Kuhelj & Virant-Doberlet 2017). Secondly, if females mate multiply, the presence of a competitor male can decrease male fertilization success. For example, a competitor male can expose the male to sperm competition (when mate with the female), deprive or constrain the other male of transferring sperm (Parker et al. 2013). For example, wax moth males increase copulation under the playback of a courtship song, apparently to prevent competitor male from mating (Jarrige et al. 2016). To adjust to competitor presence males can change their mating interactions with females. This is corroborated by numerous studies that compared male mating behaviours in the absence and presence of a competitor male. In general, males change their initial preference (e.g.

Ziege et al. 2009; Wronski et al. 2012; Auld & Godin 2015) and reduce courtship activity when there is a male audience (e.g. Westerman, Drucker & Monteiro 2014; Setoguchi et al. 2015, but see Fisher & Rosenthal 2007; Dzieweczynski, Lyman & Poor 2009). Additionally, a male stands different chances of success depending on how attractive the competitor is.

Male sexual traits are key to female pre- and post-mate choice (Andersson 1994; Eberhard 1996). During mating, a competitor male can decrease the probability of another male's mating success if he is preferred by the female – female mate choice (Darwin 1871; Andersson 1994). And, in species with multiple mating, females are also able to differentially retain sperm of several males, according to their preference – cryptic female choice (Eberhard 1996). Therefore, the reproductive success of a male is compromised by competitor attractiveness. Consequently, males are expected to be able to assess competitor attractiveness, and to modulate their mating investment accordingly. While most studies have focused on how female mate choice is affected by male sexual traits (Hunt et al. 2009; Hill 2015), few have tried to uncouple the role of phenotypic differences between competing males in shaping the male mating decisions.

Here, using the Trinidadian guppy (*Poecilia reticulata*), we take an integrated approach and investigate simultaneously the effect of three constrains imposed by a competitor during mating on male mating decisions. Specifically, we ask if and how males adjust their mating tactics to the: 1) presence of a competitor, and competitor attractiveness, in 2) absolute and 3) relative terms.

In guppies, male body size and proportion of pigment area can determine female mate choice and, so, male mating success (Houde 1997; Magurran 2005). Females prefer males with larger and brighter carotenoid-based spots (Endler & Houde 1995; Brooks &

Endler 2001; Pitcher et al. 2003; Evans et al. 2004; Barbosa et al. 2010; Auld et al. 2016), and, in some populations, with larger melanin-based spots (Endler and Houde 1995) and body size (Reynolds & Gross 1992; Karino & Matsunaga 2002; Magellan et al. 2005). But female mate choice is also determined by male mating behaviour. Male guppies perform two mating tactics: the consensual courtship display and the unsolicited attempts (Magurran 2005). There is evidence that males that perform courtship displays are preferred by females (Farr 1980b; Bischoff et al. 1985; Houde 1997; Nicoletto & Kodric-Brown 1999), and obtain greater paternity success (Evans and Magurran 2001). Unsolicited attempts, however, allow males to circumvent female sexual interest, giving them some paternity success (Houde 1988; Magurran 2005). The mating tactic employed is dependent on the social context, namely the degree of competition. For instance, males often perform more unsolicited attempts when in the presence of another male than when alone with females (Farr & Herrnkind 1974; Magellan et al. 2005; Auld et al. 2015a). Studies also showed that male guppies adjust the frequency and type of mating tactic in response to the perceived attractiveness of a male audience. For example, individuals engage in less mating behaviour activity when in the presence of a colourful (Dugatkin & Sargent 1994; Gasparini, Serena & Pilastro 2013; but see Auge et al. 2016), more colourful (Yoshikawa et al. 2016), or larger competitor (Auld et al. 2017). But, researchers have not addressed simultaneously the impact of competitor presence and attractiveness, nor the effect of different attractive phenotypes on male guppies' decisions.

We predict that a male guppy should, in general, perform more unsolicited attempts when in a presence of a competitor, because males face the risk of being interrupted by the competitor during courtship and because females may prefer the competitor male over him. Additionally, when the competitor male is attractive (i.e. large and/or colourful) or proportionally more attractive (i.e. larger and/or more colourful), the male should engage in more unsolicited attempts than in consensual courtship displays. In contrast, when the competitor is less attractive male should perform more courtship displays, since the risk of losing female preference is lower and males produce more offspring when perform this tactic. We tested these predictions by recording mating tactics of individual male guppies in a no-competition scenario and in a competition scenario, and by quantifying focal and competitor body size, and number and area of colour pigmentation.

3.2. Materials and Methods

Experimental setup and design

Experiments were conducted at the Scottish Oceans Institute (University of St Andrews) with descendants from wild guppies from the Lower Tacarigua River, in Trinidad. We used a focal sampling procedure (Altmann 1974) to record male mating behaviour. Each focal male was observed in a tank containing two females, and in two scenarios: 1) alone with these females (no-competition scenario), and 2) with another male (competition scenario). The order with which scenarios was presented to each male was randomised. Each trial lasted 15 minutes and the frequency of courtship display and unsolicited attempts was recorded using JWatcher v1.0 (Blumstein & Daniel 2007).

Prior to observations all individuals were kept in mixed-sex ratio stock tanks, with similar densities. In the afternoon before observations, males were haphazardly chosen and transferred to a holding tank (59 cm x 29 cm x 35 cm). Pairs of females were transferred to three experimental tanks each (59 cm x 29 cm x 35 cm). Because, generally, familiarity between males and females reduces male sexual interest (Mariette et al. 2010;

but see Zajitschek, Evans & Brooks 2006), females and males were chosen from different stock tanks. To reduce stress, females were kept in one experimental tank until the end of the observation day. This way, the females were observed with different males (two to three focal males). All individuals were fed with flake food one hour before the experiments started.

Observations were performed between 9 am and 3 pm. Focal and competitor males were randomly selected from the holding tank and transferred to the experimental tank where they were kept in a transparent container at least for 15 minutes to acclimatize to the new conditions. Experiments started when the container was removed, and males could swim freely. At the end of each day all individuals were removed from the experimental and holding tanks and replaced by new ones. The individuals used in the experiments were transferred to an independent stock tank, to avoid being used again. We tested a total of 72 males (divided in focal and competitor males, being distinguished by their colour patterns – Magurran 2005) and 17 female pairs.

The experimental tanks had an aerating system and the bottom was covered with gravel. The water temperature was maintained with a thermostat heater with a range similar to what is found in the wild rivers of Trinidad (24.1º C and 27.0 ºC; average (±SD) of 25.7 ± 0.8ºC; Reeve *et al.* 2014). To prevent individuals of different experimental and holding tanks from seeing each other, opaque divisions were added to all sides of the tanks, except the observation side of the experimental tanks.

Measurement of males' phenotypes

To measure focal' and competitor' attractiveness three male phenotypes were considered: body size and orange and black colouration.

From different possible measures of male guppies' colouration, colour area represents best the colour pattern variance (Endler and Houde 1995). To measure the body area covered with colour male guppies are generally anaesthetized (e.g. Brooks & Endler 2001; Barrett et al. 2014; Auld et al. 2016). Here, however, we recorded male individual colouration using a standardized scheme, as in a previous study (Figure 1; Deacon, Barbosa & Magurran 2014). This allowed us to obtain a proxy of male colour area without using an invasive method. Each record was created during the acclimatization period when males swam in a limited area but could detect females, and, therefore, their colours were more easily identified – in guppies the colour of males change according to sexual motivation (Baerends et al. 1955). Also, although most researchers consider the absolute or the proportion of body area covered with a pigmentation of one of the male's side chosen *a priori* (e.g. Brooks & Endler 2001; Barrett et al. 2014; Auld et al. 2016), we opted to represent the most colourful side of each male since males tend to expose this side when courting (Gross et al. 2007). Two of the main colour pigmentations on male guppies were considered: 1) carotenoid-based (orange, red, and yellow colours), and 2) melanin-based (black colouration; Brooks & Endler 2001). These colour pigmentations were named as 'orange' and 'black', respectively.

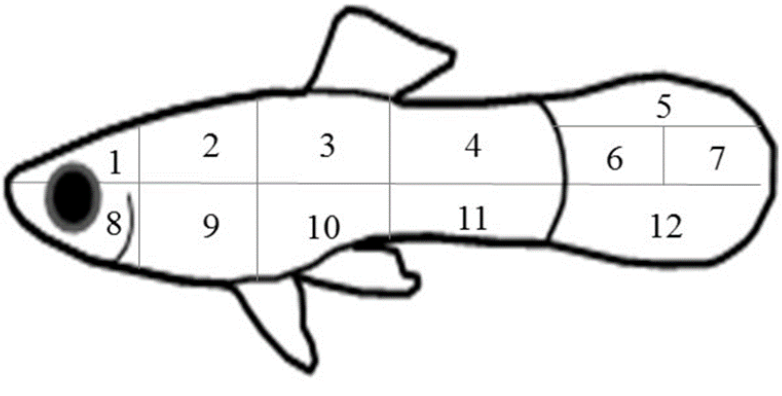


Figure 1. Scheme used to measure proxies of orange and black spots areas (adapted from Deacon et al. 2014).

In guppies, measures of male body size typical include standard length, tail length, and total length – both standard and tail length (e.g. Bischoff et al. 1985; Reynolds & Gross 1992; Endler & Houde 1995; Karino & Matsunaga 2002). But, since previous studies found contradictory effects of male' tail length on female preference (e.g. Endler & Houde 1995; Karino & Matsunaga 2002), we chose to measure male standard length. To measure this variable (from the mouth tip to the base of the tail), each male was transferred to a Petri dish with water after observations. While in the Petri dish, males were photographed from above with a scale below, and the image produced was analysed using ImageJ software (Schneider et al. 2012).

These measures were used to evaluate the competitor absolute and relative attractiveness (Table 1). Absolute attractiveness includes the measurements of the following competitor phenotypes: standard length, area of orange, and area of black spots. Relative attractiveness includes the comparison of competitor and focal male phenotypes. For that, we calculated the ratio of each phenotype as the difference between competitor and focal phenotype, divided by the sum of competitor and focal phenotype. When the ratio was positive the competitor was more attractive for that trait. Additionally, ratio amplitude represents the degree of difference between competitor and focal males attractiveness.

Table 1. Mean and standard deviation of males' phenotypes.

Phenotypic trait	**Male**	**Mean**	**± SE**
Orange area	Focal	4.94	1.19
	Competitor	4.86	1.25
Black area	Focal	2.86	1.13
	Competitor	2.42	0.97
Standard length (cm)	Focal	1.50	0.13
	Competitor	1.43	0.10

Statistical analyses

All statistical analyses were performed using R 3.4.1 (R Core Team 2017). To evaluate if the frequency of each mating tactic (courtship display and unsolicited attempts) employed by focal males during a mating context was dependent on competitor presence and/or attractiveness, we used Generalized Linear Mixed Effects models (GLMM) with the GLMER package from library lme4 (Bates 2011). This way, the frequency of courtship displays and of unsolicited attempts were analysed separately, being included as the response variables in models with a Poisson residual distribution. Additionally, all full models included experimental tank and female pair as random factors. Models' selection was based on a backward step-wise procedure with analysis of variance (anova). The significance was set at $P = 0.05$.

First, to evaluate the effect of another competitor male presence on the frequency of mating tactics performed by the focal males, we included the competitive scenario (with two categories: no-competition and competition) as a fixed explanatory variable. Since focal males were tested in both competitive scenarios, other two variables were included as explanatory variables: the scenario order experienced by each male was included as a fixed term (with two categories: no-competition followed by competition, and competition followed by no-competition); and, to account for the replication effect, the focal identification was included as a random factor.

Second, to evaluate the effect of competitor attractiveness on male mating decisions we considered the frequency of courtship displays or unsolicited attempts performed only during the competition scenario as the response variable, and competitor phenotypes as explanatory variables. We tested, separately, the effect of competitor absolute and relative attractiveness. This way, to evaluate absolute attractiveness we considered the following competitor sexual traits: areas of black and of orange spots, and standard length. For the competitor relative attractiveness, we considered the ratio of colour area (black and orange spots), and the ratio of standard length. Since in these analyses we were no longer considering replicates (each focal sample was considered only once), the focal male identification was not included as a random factor in the model.

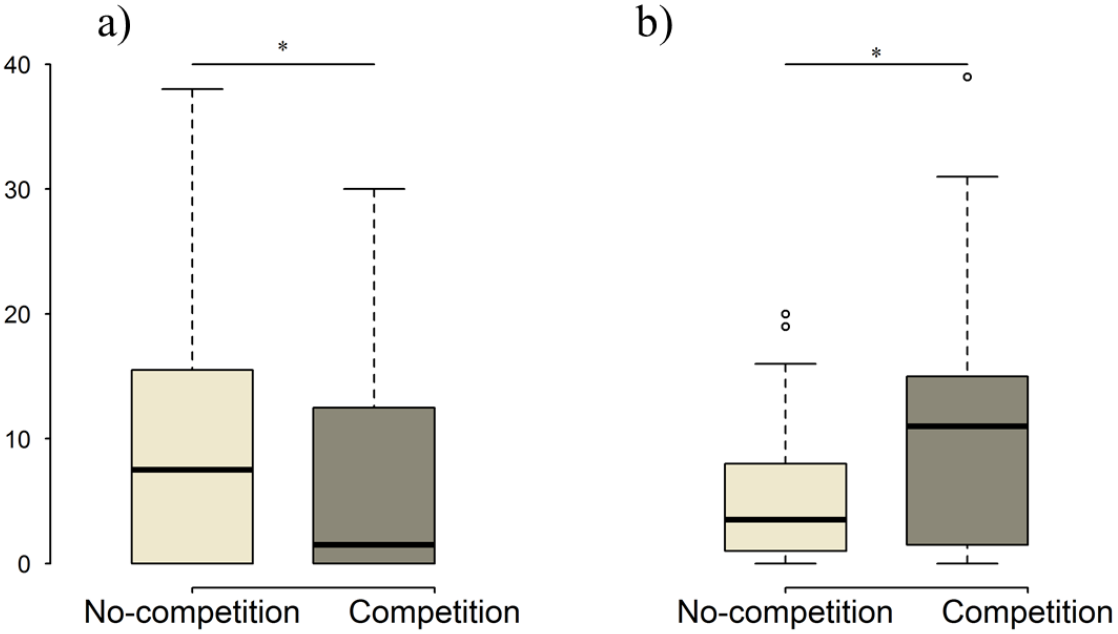
3.3. Results

Competitor presence

For the frequency of both mating tactics the best explanatory models included the competitive scenario (courtship display: $\chi^2_1 = 25.5$, $N = 72$, $P < 0.001$; unsolicited attempts: $\chi^2_1 = 42.9$, $N = 72$, $P < 0.001$). Focal males performed fewer courtship displays ($z = -5.0$, $P < 0.001$; Figure 2a), but more unsolicited attempts ($z = 6.5$, $P < 0.001$; Figure

2b) when a competitor male was present. The order of the competition scenario had no effect on the performance of any of the mating tactics (courtship display: $\chi^2_1 = 0.1$, $N = 72$, $P = 0.760$; unsolicited attempts: $\chi^2_1 = 0.4$, $N = 72$, $P = 0.504$).

Figure 2. Frequency of mating tactics performed in two competitive scenarios. Mating tactics were: a) courtship displays (on the left side of the figure), and b) unsolicited mating attempts (on the right side). Focal males ($N = 36$) were observed in two scenarios: with two females (no-competition), and with two females and a competitor male (competition).



Pairwise comparisons between competitive scenarios of the frequency of each mating tactic were obtained from the best-fit GLMER models. Asterisks and lines above the plots show significant differences (*$P < 0.05$). In each boxplot, the internal line represents the median. Lower and upper edges represent the 25% and the 75% quantile, respectively. Whiskers below and above the box edges represent, respectively, the minimum and the maximum points within the 1.5 interquartile range. Circles represent outliers.

Competitor attractiveness

The best models to explain the frequency of courtship displays in the competition scenario included female pair as random factor, and all measured phenotypes as fixed variables – while considering as response variables both competitor absolute ($\chi^2_1 = 21.9$, $N = 36$, $P < 0.001$), and relative attractiveness ($\chi^2_1 = 8.5$, $N = 36$, $P = 0.004$). This way, the best models included the competitor standard length, and the body areas covered with black and with orange spots (Table 2 and Figure 3). Focal males performed more courtship displays in the presence of a small competitor or smaller than him. Similarly, males performed more courtship in samples where the competitor had few or fewer black spots than him. In contrast, focal males performed more courtship displays if the other male had a large or larger area covered by orange.

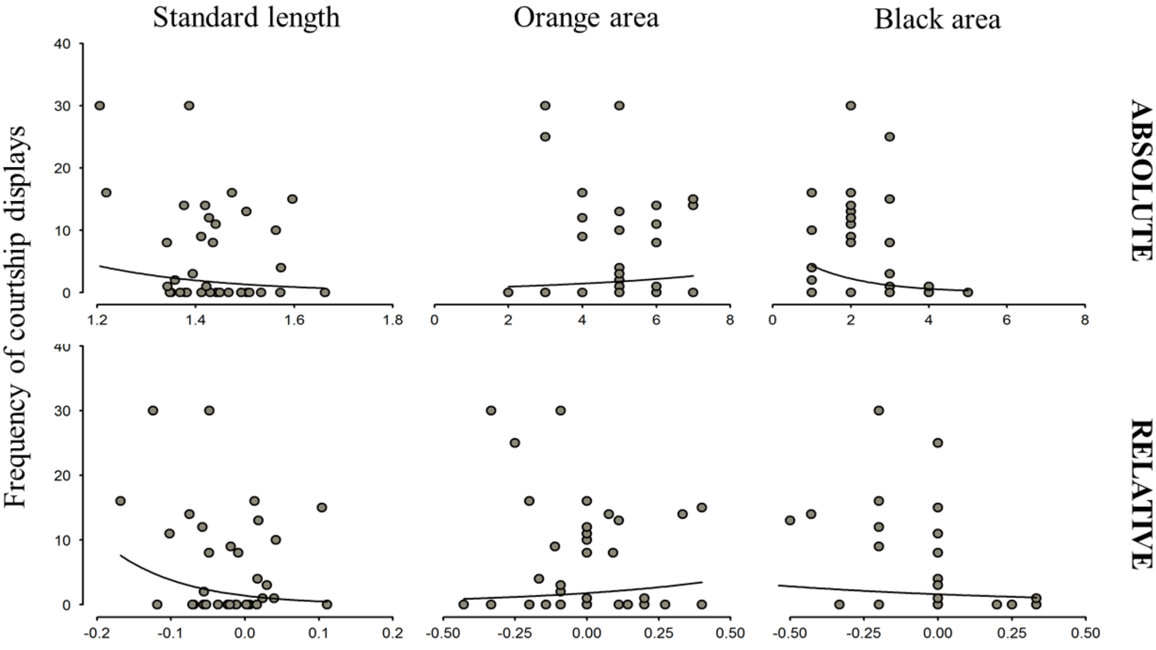


Figure 3. Frequency of courtship displays in relation to competitor phenotypes. Competitor phenotypes considered were (from left to right): standard length, orange area, and black area; and either in absolute terms and relative to focal male phenotypes (from top to bottom). The lines in each graph represent the predicted effect of each explanatory

variable included in the best GLMER model on the frequency of courtship displays, assuming the mean value for all other explanatory variables in the model.

In terms of frequency of unsolicited attempts, the best models also included female pair as random factors, and orange and black colour areas as fixed terms – for both absolute ($\chi^2_1 = 12.0$, $N = 36$, $P < 0.001$), and relative attractiveness ($\chi^2_1 = 12.9$, $N = 36$, $P = 0.004$). The competitor standard length had no effect on the performance of this mating tactic (Table 2 and Figure 4). Focal males performed more unsolicited attempts when in the presence of a competitor with a small body area covered with orange or black spots. Similarly, males invested less in this mating tactic if the competitor was less colourful, both for orange and black pigmentation.

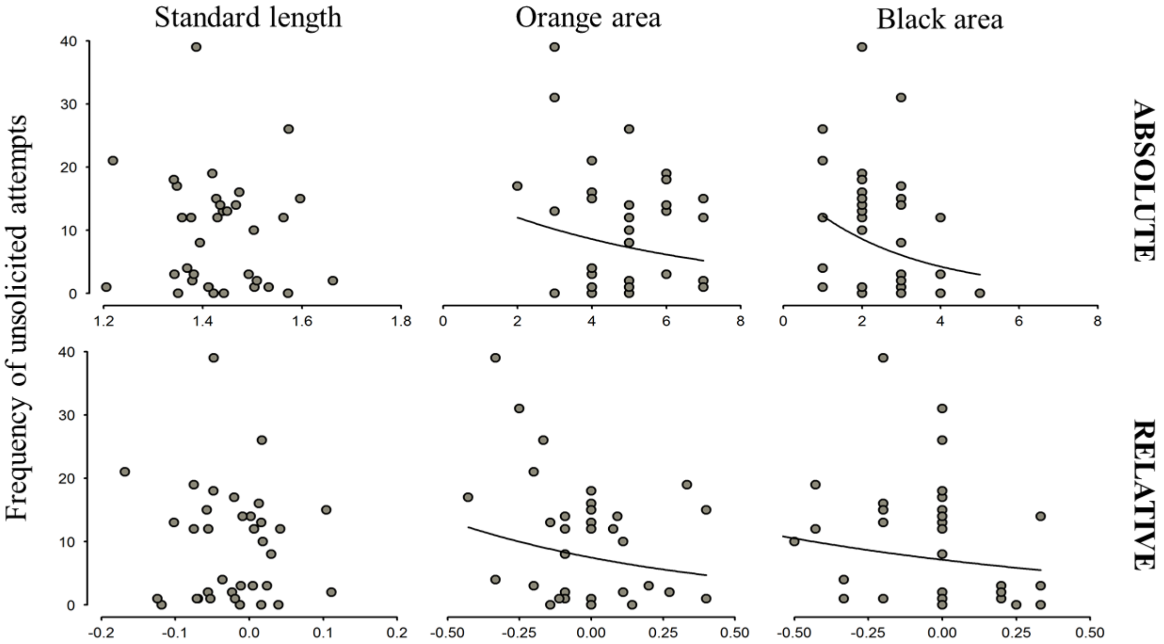


Figure 4. Frequency of unsolicited attempts in relation to competitor phenotypes. Competitor phenotypes considered were (from left to right): standard length, orange area, and black area; and either in absolute terms and relative to focal male phenotypes (from top to bottom): absolute and relative (to focal phenotypes). The lines in each graph

represent the predicted effect of each explanatory variables included in the best GLMER model on the frequency of unsolicited attempts, i.e. the predicted values for the competitor phenotype considered in each plot, and assuming the mean value for all other explanatory variables in the model. Plots with no line represent no significant effect of competitor phenotype on this mating tactic, and so cases where the best model did not include that competitor male phenotype.

Table 2. Summary table of the GLMER models for the effect of competitor sexual traits on the frequency of mating tactics performed by males.

Mating tactic	**Competitor attractiveness**	**Phenotypic trait**	χ^2_1	**P-value**	**Z-value**	**P-value**
Courtship display	Absolute	Orange area	8.9	0.003	2.9	0.003
		Black area	21.9	<0.001	-4.4	<0.001
		Standard length	24.6	<0.001	-4.7	<0.001
	Relative	Orange area	8.5	0.004	2.9	0.004
		Black area	6.0	0.015	-2.4	0.015
		Standard length	157.3	<0.001	-4.9	<0.001
Unsolicited attempt	Absolute	Orange area	12.0	0.001	-3.4	<0.001
		Black area	18.0	<0.001	-4.1	<0.001
		Standard length	0.7	0.417	-	-
	Relative	Orange area	12.9	<0.001	-3.5	<0.001
		Black area	10.9	<0.001	-3.2	<0.001
		Standard length	1.1	0.295	-	-

Analyses were conducted separately for each mating tactic (courtship display and unsolicited attempt), and for competitor absolute and relative attractiveness ($N = 36$; except for standard length, where $N = 35$, since one of the competitor male was not measured).

3.4. Discussion

Our results show that males adjust the frequency of mating tactics to competitor presence, and to competitor absolute and relative attractiveness. In general, male guppies performed more unsolicited attempts when a competitor male was nearby than when there was no competition. But, investment on each mating tactic depended on how attractive the competitor was. Overall, males invested more in courtship displays and in unsolicited attempts if the competitor male was unattractive, or if the competitor was less attractive than himself. However, the decision of performing courtship was reversed when the was more attractive for orange colouration. Competitor standard length had no impact on unsolicited attempts. So, here we show that males base their mating decisions on several indicators of likelihood of losing reproductive success due to male-male competition during mating Additionally, we show that male behavioural adjustment is dependent on different competitor sexual traits.

Competitor presence

Males performed more unsolicited attempts and fewer courtship displays when in the presence of another male, regardless of the scenario encountered first (no-competition or competition). This result is in line with other published work discussed below.

Previous studies with guppies have found an increase in the frequency of unsolicited attempts when another male was present (Farr & Herrnkind 1974; Farr 1980; Magellan et al. 2005; Auld et al. 2015). Because there is evidence that this tactic allows males to successfully transfer sperm (Matthews and Magurran 2000), and to mate more rapidly without female consent (Houde 1988; Magurran 2005), it seems to be an adaptive tactic under competition. In line with this reasoning, our results suggest that this tactic gives a reproductive advantage in scenarios where males risk losing female mate preference, or where competitor males can interrupt the male mating attempts. Similarly, in species other than guppies, males perform more alternative mating tactics and strategies when the competition risk is higher than when it is lower. This occurs, for example, when another male is present (e.g. in the threespine stickleback *Gasterosteus aculeatus* – Candolin & Vlieger 2013), under high male density or male-biased sex ratio (e.g. two-spotted spider mite *Tetranychus urticae* – Sato, Sabelis & Egas 2014; Japanese medaka *Oryzias latipes* – Weir 2013), and when males compete against dominant males (e.g. Rhesus macaques *Macaca mulatta* – Overduin-De Vries et al. 2012; reviews – Taborsky 2001; Gonçalves, Oliveira & McGregor 2008).

Previous studies that considered wild guppies also reported a decrease in courtship behaviour in response to the presence of a competitor (Magurran 2005; Auld et al. 2015). In contrast, studies that tested domestic strain of guppies found an increase in this tactic (Farr & Herrnkind 1974; Farr 1976, 1980). Therefore, it is likely that these different behavioural patterns are related with domestication (e.g. domestic strain do not face predation). Studies on many other species have also reported a reduction in mating behaviour in the presence of a competitor in the mating arena (e.g. courtship frequency – Aragón 2009; Westerman et al. 2014; Lane et al. 2015; Setoguchi et al. 2015; time spent constructing nuptial gifts – Tuni et al. 2017). Similarly, when the risk of competition is higher – for example, when population density is high or operational sex ratio is male-sex biased – males tend to reduce courtship performance (Bretman et al. 2011).

Competitor attractiveness

When in the presence of an attractive or a relatively more attractive competitor a male stands less chances of being chosen by the female. Under this scenario, we predicted that males would decrease the frequency of consensual courtship displays and, instead, increase the frequency of unsolicited mating attempts. Our results are in partial agreement with these predictions. Focal males performed less courtship displays when competing with a competitor male attractive or more attractive for black colour and body size. However, we failed to detect an increase in the frequency of unsolicited attempts in response to a highly attractive competitor. Instead, focal males invested more on this tactic when competing with a male that had few or fewer body area covered with black (melanin pigmentation), and when he was also less attractive for orange colour (smaller body area covered by carotenoids pigmentation). This way, we found that males invest in both mating tactics in the presence of a male with less attractive phenotypes, and, so, probably a low-quality competitor. In accordance, previous studies found that male guppies prefer to associate with competitor males with which females associate less (Dugatkin & Sargent 1994), choose to approach females that had been previously in the company of less attractive males (Gasparini et al. 2013), and redirect their mate choice to a different female or give up quicker moving away from the female if a larger or colourful competitor is nearby (Auld et al. 2017; Yoshikawa et al. 2017; respectively). However, a study performed in the wild found, instead, that male guppies do not prefer shoals composed by less attractive competitors (Auge et al. 2016). This apparently contrasting result may be because under natural conditions males are not in a position to choose their competitors. But even if males cannot choose their competitors during a mating context, it is still advantageous to adopt different mating strategies in response to competitor quality or condition. Indeed, this is what our results suggest.

Interestingly, the effect of competitor orange colour contradicts for the effect found for other competitor phenotypes, as focal males increased the frequency of consensual courtship displays when the competitor male had a large or larger orange area. When a male guppy performs a courtship display his orange colour becomes more conspicuous (Houde 1997). Eventually, when performing more courtship displays a male is enhancing this trait to equal or overcome competitor attractiveness. In accordance with this result, Jirotkul (2000) also found that when male guppies compete against competitors with large orange area they invest more on courtship, i.e. they court for longer time. The same way, male three-spined sticklebacks enhance their carotenoid-colour when competing with more colourful males, by performing more courtship (Kim and Velando 2014), and male common eggfly butterflies adjust their courtship behaviour to enhance their attractive colours (White et al. 2015). Future studies should further investigate the correlation between courtship behaviours and male attractive colours, namely in competitive contexts.

Another interesting finding of our study is that focal males responded differently to competitors with greater areas of melanin or carotenoid-based coloration. While males invest less in both mating tactics when competing against males with greater area of melanin pigment, they also invest less in unsolicited mating attempts but more in courtship displays if competitor males have larger orange area. This may be due to males increasing the brightness of their orange spots during courtship and, hence, increase their chances of mating success. But may also increase their chances of fertilization success, as orange spots are positively correlated with sperm viability (Locatello et al. 2006) and fertilization success (Evans et al. 2003; Pilastro et al. 2004), while black spots are not (Evans et al. 2003). This way, when males compete with a competitor with great carotenoid pigmentation they probably stand a chance of successfully mating with the female by performing courtship display, and by enhancing their colour. Then, future studies should address the outcome of such strategy on male fertilization success. Currently, studies have shown that female guppies prefer sperm of more orange males (Pilastro et al. 2004; Barbosa et al. 2010), and cryptic female choice has a great impact on male fertilization success (Magris et al. 2017). Moreover, Órfão et al. (2018) found that male guppies tend to invest more in mating behaviours when their risks of losing both mate and fertilization success are high – though competitor attractiveness was not assessed in this study. Future studies should address how competitor absolute and relative attractiveness affects both male mating and fertilization success. Furthermore, it would be interesting to investigate the contribution of competitor males behaviour to male mating decisions, particularly, considering that some secondary sexual traits are correlated with propensity to perform one of the two tactics – colourful males perform more courtship displays and less unsolicited attempts (Jirotkul 2000; Evans 2010; Kiritome et al. 2012), while larger males perform more unsolicited attempts (Magellan et al. 2005).

Competitor male standard length elicited more courtship display from focal males, when competitor males were small, but, contrary to our expectations, it had no effect on the performance of unsolicited attempts. Although female guppies tend to prefer larger males (Reynolds and Gross 1992; Karino and Matsunaga 2002; Magellan et al. 2005), studies have shown that smaller males sire more offspring (Evans et al. 2003; Becher & Magurran 2004). Therefore, when males face a small or a smaller competitor, performing courtship display increase their probability of winning mating success. On the contrary, when males face a large or a larger competitor they stand few chance of being chosen by female, so they reduce courtship display. However, males can be in advantageous during fertilization by performing unsolicited attempts, since can transfer sperm even without female sexual interest (Houde 1988; Magurran 2005).

3.5. Concluding remarks

Although we did not account for all male guppy attractive traits, and the method used to measure colour area has a degree of subjective, our results consistently show that male guppies adjust the mating tactics differently according to several competitor sexual traits. This suggest that multiple cues collected from the social environment are relevant to male mating decisions, other than male-female interactions and female mate choice (Candolin 2003; Lozano 2009). Future studies should consider the effect of other male attractive traits, such as orange brightness and chroma (Endler and Houde 1995), as well as other more neglected traits – such as olfactory attractive cues (e.g. in guppies – Magurran 2005; but see Partridge et al. 2008). Our study, in line with previous ones (Yoshikawa et al. 2016; Auld et al. 2017), shows that male guppies can evaluate other males' phenotypes in relation to themselves. Future studies should investigate this in other species. Furthermore, this raises a question that deserves some research: how are males aware of their phenotypes, particularly of their colour? One possibility is that they get this information from conspecifics behaviours, either other males (as we show here, males change their behaviour according to other male' phenotypes), or female responses – namely behaviours that demonstrate sexual interest.

Additionally, we prove that, although males show a general strategy in the presence of another male, males make complex mating decisions, adopting a specific strategy according to the degree of competitor attractiveness. In other words, we found that males decisions are based on different ways of evaluating competition risk. Males invest more by performing more mating attempts when have higher chance of being chosen by females than a competitor male, based on different information: 1) if there is no competitor; 2) if the competitor is unattractive, 3) if the competitor is less attractive than himself, and 4) if male can enhance his own attractiveness. Therefore, our results support a coevolution between male mating behaviours and male secondary sexual traits shaped by male-male competition.

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Modified image taken from Sean Earnshaw (Biodiversity and Behaviour Group, University of St Andrews)

CHAPTER 4.

Plasticity of male mating behaviours

Effect of competitor's presence and order of arrival to the resource female

Paper III. How pre- and postcopulatory sexual selection influence male mating decisions in a promiscuous species.

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Abstract

When females mate multiply, male reproductive success depends on both pre- and postcopulatory processes, including female choice and sperm competition. However, these processes can favour different mating tactics in males. Here we use the Trinidadian guppy (*Poecilia reticulata*) system to understand how this conflict is resolved. We ask whether knowledge of recent female mating history leads males to adjust their mating effort in respect of the time devoted to mating activity, and the frequency and the sequence of mating tactics employed. To do this we quantified male mating behaviour in three competitive scenarios: 1) Single, when a focal male arrives near a single female and remains alone with her; 2) First, when a focal male is joined by a rival male; and 3) Second, when a focal male arrives after a rival male. We hypothesize that males adjust their behaviour based on arrival order. If female sequential mate choice is the main process shaping male mating behaviours (favouring First males in guppies), males should avoid competition and invest most when Single. Alternatively, if last-male sperm precedence is the major driver of decision making, males should invest more in mating attempts in the Second scenario. Greatest investment when First implies an intermediate strategy. We find that order of arrival influences mating decisions with most mating activity during the First scenario instead of the Single and Second scenarios. This result suggests that both pre- and postcopulatory processes influence mating investment, and that individual males make contingent decisions to maximize both mating and fertilization success.

Keywords**:** courtship display; female sequential mate choice; male-male competition; polyandry; sneak; sperm competition; unsolicited attempts.

4.1. Introduction

When females mate with multiple males within the same breeding season, often referred as polyandry, males gain more mating opportunities but face, at the same time, the challenge of cryptic female choice (Eberhard 1996) and sperm competition (Parker, 1970; 1998). This means that both precopulatory and postcopulatory processes influence the evolution of male sexual traits.

There is considerable interest in the contribution of secondary sexual traits to male reproductive success both during and after mating, and how they are influenced by pre- versus postcopulatory processes (reviewed by Evans & Garcia-Gonzalez, 2016). However, the relative influence of these two selective forces on male mating sexual traits continues to be debated (Collet et al., 2012; Pischedda & Rice, 2012; Pélissié et al., 2014; Buzatto et al., 2015; Devigili et al., 2015; Turnell & Shaw, 2015). Recent studies have focused on physical and sperm traits (e.g. body and sperm length, respectively; Evans & Garcia-Gonzalez, 2016), but few have considered mating behaviours (Buzatto et al., 2015; Devigili et al., 2015; Turnell & Shaw, 2015).

As with other male sexual traits, mating behaviours are subject to both pre- and postcopulatory sexual selection pressures (Andersson & Simmons, 2006), and are correlated with male reproductive success (Pélissié et al., 2014; Buzatto et al., 2015; Devigili et al., 2015; Turnell & Shaw, 2015; Fisher et al., 2016). Unlike most physical traits however, behaviours can be adjusted in light of the social context. For instance, mating behaviours can both promote mating and fertilization success by stimulating or circumventing female mate choice (e.g. courtship displays and unsolicited mating attempts, respectively; Gross, 1984; Andersson, 1994), and by avoiding or overcoming mating competition (e.g. mate guarding and sneak, respectively; Andersson, 1994; Neff & Svensson, 2013).

Male order of arrival at or near a female can greatly affect male reproductive success, both at the pre- and postcopulatory levels (Evans & Garcia-Gonzalez, 2016; Pélissié et al., 2014; Pischedda & Rice, 2012). For instance, when females choose sequentially (Real, 1990; Jennions & Petrie, 1997) a male's mating success will vary if he is the first or last to arrive near a female, depending on whether females are less or more discriminating (choosy) towards a first than a second male. Similarly, when sperm competition occurs, male fertilization success can only be maximized if he mates first or last, depending on whether there is first- or last-male sperm precedence (Birkhead & Hunter, 1990; Wedell, Gage, & Parker, 2002; Dosen & Montgomerie 2004; Plath & Bierbach, 2011). Female mating history can thus play a crucial role in determining which mating behaviours a male should adopt.

In natural conditions males may have little opportunity to evaluate female mating history (Parker, Ball, Stockley, & Gage, 1997), raising the question of how males cope with this uncertainty. Considering this, we hypothesize that, if mating order has no effect on male mating decisions, a male should approach and invest in mating attempts whenever near a female. In contrast, a male could adjust his behaviour based on whether he arrives before or after a rival male.

Males face a particularly challenging decision when pre- and postcopulatory processes favour different mating orders. This arises, for example, when females are less choosy towards the first male they encounter, but where sperm precedence favours a male that mates afterwards. According to the trade-up hypothesis, females benefit from being less choosy with a first male, particularly when males are scarce, because they can ensure the fertilization of all their eggs (Halliday, 1983; Jennions & Petrie, 2000). Females may then become progressively choosier, and mate with any higher-quality males they subsequently encounter to enhance the genetic quality of their brood. This hypothesis has been supported in species of birds (e.g. Gabor & Halliday, 1997), mammals (e.g. Klemme, Eccard, & Ylönen, 2006), insects (e.g. Bateman, Gilson, & Ferguson, 2001), fishes (e.g. guppies; Pitcher, Neff, Rodd, & Rowe, 2003), and reptiles (e.g. Laloi, Eizaguirre, Fédérici, & Massot, 2011).

Here, we ask whether males adjust their mating decisions over a short time scale based on the order at which they encounter a female. We further examine whether female mate choice or sperm precedence have the strongest influence on this decision making. To answer these questions, we studied the mating behaviours of male Trinidadian guppies (*Poecilia reticulata*) in mixed-sex groups. In these tests we mimicked the situation in the wild by allowing free interactions between individuals. First, we determined if a focal male approach a female before or after another male (approach decision), and, second, whether his investment in mating behaviours depends on order of arrival (behavioural adjustment; Figure 1). The Trinidadian guppy is a freshwater livebearing fish. In this species, the precopulatory process of female mate choice should favour the first male to approach since females are less discriminatory towards him than towards subsequent males (Houde, 1997; Liley, 1966; Pitcher et al., 2003). However, sperm competition (postcopulatory process) favours the last male to mate since mixed paternity broods (Becher & Magurran, 2004) are predominantly sired by these males (Evans & Magurran, 2001; Pitcher et al., 2003).

Male guppies perform two mating tactics: consensual courtship displays and unsolicited mating attempts (Magurran, 2005). Courtship displays result in the greatest paternity success (Evans & Magurran, 2001). Unsolicited mating attempts, on the other hand, do not require female cooperation (Houde, 1988; Magurran, 2005) and typically result in the transfer of only modest amounts of sperm (Pilastro & Bisazza, 1999). This mating tactic is more frequent when other males are present (Magellan et al., 2005; Magurran, 2005).

We hypothesize that, if males are able to evaluate female mating history based on their own assessment of male-male competition, they will adjust their mating behaviour based on whether they are with a female alone (Single), approached before (First) or after (Second) a rival male (Figure 1). In more detail, if female mate choice gives the most advantage to males, they should avoid competition during mating, and invest more in following and trying to mate when they are alone with a female (Single), particularly using the mating tactic that allows them to transfer more sperm (courtship display). In this case, we expect male guppies to approach females with no rival male following them, to court more and repeatedly, and to spend more time with a female when Single. On the other hand, if sperm precedence gives the most advantage to males, they should invest more when there is competition, particularly in the mating tactic that transfers fewer amounts of sperm, but may help secure last sperm precedence (unsolicited attempt). In this case, we predict that male guppies should approach females with at least one rival male following them, perform more unsolicited mating attempts, and spend more time with a female when Second. However, if both pre- and postcopulatory processes are important, males should invest more when First, again due to potential advantages in terms of female choice, and, simultaneously, to secure sperm precedence. Figure 1 summarises these scenarios. Alternatively, if information available to males during the current encounter conveys little fitness benefits to them, then order of arrival should not influence their behaviour towards the female. Here we expect no difference in mating behaviour if a male is the only, the first or the second to approach a female (Figure 1).

<table>
<tr><td></td><td colspan="4">Hypotheses</td></tr>
<tr><td>Questions
Males adjust to short-term order of arrival?</td><td colspan="3">Yes</td><td>No</td></tr>
<tr><td>Which selective process has higher contribution to male reproductive success?</td><td>Female mate choice (precopulatory)</td><td>Sperm precedence (postcopulatory)</td><td>Both</td><td>-</td></tr>
<tr><td></td><td colspan="4">Predictions</td></tr>
<tr><td>Male mating decisions
(1) Approach decision
(at this point only scenarios Single and Second occur)</td><td>Single</td><td>Second</td><td>Single</td><td>Single,
Second</td></tr>
<tr><td>(2) Behavioural adjustment
(a) Time spent following
(b) Mating tactics
(c) Mating behaviours sequence</td><td>
Courtship display
(transfer more sperm)</td><td>
Unsolicited attempt
(transfer sperm in last)</td><td>First
</td><td>Single,
First,
Second</td></tr>
</table>

Figure 1. Questions, hypotheses and predictions on male mating decisions. Observed competitive scenarios: Single (*N* = 45), the focal male (black) was the single male following a female (grey) for the entire time; First (*N* = 45), the focal male was the first to approach the female and a rival male (white) arrived later; Second (*N* = 62), the focal male approached the female after the rival male. Males make mating decisions at different points: (1) whether to approach a female or not (approach decision), (2) after approaching, they decide how and how much to invest (time spent following, mating tactics and mating behaviours sequence). The scenario below each of the hypothesis represents the predicted outcome. In addition, the framework identifies the mating tactic we expect to dominate under each scenario. Drawings are adapted from Liley (1966).

4.2. Materials and Methods

Experimental setup

We used descendants from wild guppies from the Lower Tacarigua River, in Trinidad. Following other studies with guppies (e.g. Deacon, Ramnarine, & Magurran, 2011), observations were carried out in two mesocosm tanks (100 cm x 56 cm x 30 cm). Behavioural observations in mesocosms have the advantage of allowing individuals to behave and interact more freely (Devigili et al., 2015). Each mesocosm contained gravel, an aerating system and two thermostat heaters. The range of temperatures (24.1ºC to 25.7ºC) was similar to that found in the wild (Reeve et al., 2014).

Inside each mesocosm we placed one mixed sex group of fish: four males and three females. Wild females are only receptive either as virgins or in few days immediately following parturition (Liley, 1966; Liley & Wishlow, 1974), thus male guppies are expected to typically encounter non-receptive females in the wild. This way, to better simulate natural situations, all females used in our experiments came from a stock tank, thus likely to be non-virgin and non-receptive.

Two groups of three females were haphazardly chosen (standard length mean ± SE: 2.09mm ± 0.45) from the same stock tank and allocated to each of the two mesocosms in the afternoon on the day before the observations. This allowed females to acclimate to the new conditions. Simultaneously, 16 males were transferred from stock tanks to four maintenance tanks (30 cm x 15 cm x 20 cm). Males were kept in all-male groups to ensure they were not sperm limited and, therefore, were sexually active during the observation period. On the day of the observations, males were haphazardly chosen (standard length mean ± SE: 1.48mm ± 0.15) and assigned to one of the mesocosms 15 minutes before observations started. To avoid familiarity during observations, males came from different stock and maintenance tanks from that of other males and females. During each observation day, we tested two male groups in each mesocosm with the same female group, meaning each female group was used twice.

All observations were conducted between 9 am and 1 pm. All females were fed with flake food one hour before the beginning of the observation day, while males were fed on the previous evening to increase mating behaviour frequency (Sartori & Ojanguren, n.d.). In total, we tested 152 males and 60 females. At the end of an observation day, all tested individuals were transferred to a tank identified as observed group to ensure that males were tested only once, and females were only tested in one day (with two male groups).

Experimental design

The behaviours of the four males in the mesocosm were observed one at a time and registered using JWatcher v1.0. (Blumstein & Daniel, 2007). To ensure each male was sampled only once, prior to observation each male was identified based on their unique colour patterns (Magurran, 2005). Each male behaviour was registered while following a female, starting with the approach to her and ending when he left that female. As a result, the duration of each observation was neither controlled by the observer nor had the same length for each focal male. Instead, the duration of each observation was determined by the focal male's decision to swim away from the female. Each focal male experienced one of three possible competitive scenarios: Single, First and Second (Figure 1). Single was defined as when a male approached a solitary female and remained as the only male following her the entire time. First was defined as a situation when the male approached a solitary female, but was subsequently joined by at least one rival male. Second was defined as a situation where the male approached a female that was already being followed by at least one rival male. Therefore, we conducted a quasi-experimental design (Cook, 2015) in the sense that males were not allocated to a given scenario, but scenarios emerged from males (focal and rivals) decisions.

Behavioural recording

Males made a sequence of mating decisions: (1) whether to approach a female alone or already being followed by one or more males (approach decision), and (2) whether and how to adjust their mating behaviour while following the female (behavioural adjustment) As behavioural adjustment we considered: (a) time spent following the females, (b) mating tactics performed (courtship displays or unsolicited mating attempts), and (c) sequence of mating behaviours.

We employed two sampling methods: focal and scan sampling. Focal sampling was used to record male mating decisions and classify the pertaining competitive scenario. During focal sampling we recorded the total time each focal male followed a female, the frequency of mating tactics performed (courtship displays or unsolicited attempts), and the sequence of mating behaviours (from the time the male approached a female until he swam away from her). Scan sampling was used to calculate the probability that males had of finding females swimming alone (non-competitive encounter) or with a rival male (competitive encounter). This method allowed us to have a proxy of competition. Each focal sampling started when the focal male approached a female and ended when he left the female. Scan sampling was performed between focal samplings.

We characterized the prevailing competitive scenario during each focal sample by recording the presence of any other male near the female, as well as the order of arrival of the focal male. Changes in the competitive scenario during the time a focal male followed the female were not considered. For example, we considered a scenario as First even if the rival male swam away while the focal male was still following the female.

We started each trial with a scan sample and alternated between scans and focal observations. Each trial consisted of five scan samples of the females and four focal observations, one assigned to each male at a time. A total of 184 scans and 152 focal samples were performed.

Statistical analyses

All analyses were performed using the software R 3.2.3 (R Core Team, 2015); the significance alpha was set at $P = 0.05$.

Approach decision

To evaluate if males preferred non-competitive versus competitive encounters, relative to a random expectation, we first estimated the frequency of each encounter during the scan samples. In other words, we estimated the probability of females being alone or with at least one male following them. These probabilities were calculated by dividing the frequency of scan samples with females swimming alone, and with females with at least one male following them, by the total number of scan samples, respectively. A chi-square goodness-of-fit test was then used to evaluate males' approach decision against the null expectation.

Behavioural adjustment while following a female

Time following

To test if total time following a female was dependent on the competitive scenarios, we ran a linear mixed-effects model (LME) from the package nlme (Pinheiro et al., 2014). The model included time following as the response variable, the competitive scenario (Single, First, or Second) as a fixed term, and the mesocosm and the female group as random terms, with the female group nested inside the mesocosm. Diagnostic plots revealed departure in residuals homogeneity in the response variable. Therefore, time following the female was log-transformed. Models were compared with a LM model with no random terms using a likelihood ratio test (LRT).

Additionally, we ran an unpaired t-test statistic to compare the time that First males spent with a female before the approach of another male with the total time following a female by Single males. This allowed us to infer if time following a female when in the First scenario was dependent on the male's order of arrival alone, and not on the time that he previously invested with that female.

Mating tactics frequency

We considered the three competitive scenarios to analyse the effect of other males' presence, and order of arrival, on the focal male's frequency of courtship displays and unsolicited mating attempts. For the First scenario, only the behaviours performed after the approach of a rival male were considered. To account for the excess of zeros, the frequency of mating behaviours was analysed considering separately zeros and non-zeros using a hurdle model (package pscl; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). This procedure considers a binomial distribution with a logit link function to model the zeros and a Poisson or a negative binomial distribution to model the non-zeros. Response variables were the frequency of mating tactics (courtship displays and unsolicited attempts, analysed separately), while competitive scenario and mesocosm were added as explanatory variables, both for the count (non-zeros) model and for the zero model. A likelihood ratio test of nested models with a backward step-wise procedure was used to select the best-fit model (LRTEST from the package lmtest; Zuur et al., 2009).

Mating behaviours sequence

Our aim was to examine if the probability of each behaviour was dependent on the behaviour that preceded it, and on the competitive scenario the focal male found himself in. As before, when the focal male was the first to approach a female, we only considered the behaviours performed after the approach of a rival male. We evaluated sequences of two behaviours (one followed immediately by another). There were three possible precedent behaviours: approach, courtship display and unsolicited attempt. Each of these behaviours was analysed separately. In all cases, a sequence terminated with either a courtship display, or an unsolicited mating attempt, or a swim away. Hence, for each analysis three sequence types were considered: precedent behaviour-courtship display, precedent behaviour-unsolicited attempt, and precedent behaviour-swim away.

Our data do not fulfil the assumptions of Markov Chain Analysis, since the transition times between behaviours were not homogeneous, and the probability of behaviours was not uniform (Bakeman & Gottman, 1997). We, therefore, applied generalized linear mixed-effects models (GLMER from the package lme4; Bates, Mächler, Bolker, & Walker, 2014), with a logit link function for a binomial distribution to each precedent behaviour. The response variable was the occurrence/non-occurrence of each sequence type. This means that in the data set, each focal male was represented three times, one time for each of the three sequences, using “1” to indicate the occurrence of one sequence and “0” the non-occurrence. Fixed terms considered were the competitive scenario and sequence type, as well as their interaction. Random terms were female group nested in the mesocosm, as well as the repeated observations of each focal male (the pseudo-replicates). Model selection was based on a backward step-wise procedure with an analysis of variance (anova).

Ethical notes

Before observations all individuals were kept in mixed-sex stock tanks at similar densities. The stock tanks had gravel in the bottom, and an aerating system. The room was kept at a controlled stable temperature. The period of light was controlled (12:12h light:dark cycle). Individuals were fed daily. Before the observations, all individuals were transferred to observation tanks (mesocosms) to acclimate to the new conditions. Female guppies are often more sensitive to changes than males, because of their stronger and more stable social bonds (Griffiths & Magurran, 1998; Croft et al., 2006). Therefore, females were transferred to the mesocosms several hours before the observations and were kept with other familiar females, while the males were transferred 15 minutes before observations started. After observations all individuals were transferred to stock tanks with no fish (labelled as "observed individuals" during the experiment). All animals were individually and carefully caught and moved between tanks with a net.

All behavioural observations were carried out at the School of Biology at the University of St Andrews. The premises where the observations were carried out comply with the UK guidelines for the treatment of animals in behavioural research and teaching, set by the UK Home Office (PCD 60/2609). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

4.3. Results

Approach decision

In 45.7 % of the scan samples (out of a total of 184) females were not followed by any male. However, 59.2 % of focal males approached females when they were alone. There was no significant difference between the frequency with which males chose or avoided competition relative to the null expectation ($\chi^2_1 = 1.5$, $N = 152$, $P = 0.220$). After approaching the female, the focal male remained the Single one following the female in 29.6 % of the cases; was the First to approach but other male approached afterwards 29.6 % of the time; and was the Second to approach in 40.8 % of the cases.

Behavioural adjustment while following a female

Time following

For the time males spent following females, only the competitive scenario remained in the best explanatory model ($F_{2,149} = 16.2$, $N = 152$, $P < 0.001$). On average, males spent 25 seconds following a female. No significant difference was found between the time spent following a female when males were Single versus when they were Second ($|t| = 0.6$, $P = 0.568$). However, males that First approached a female spent more time following her compared to males that were Single or the Second to arrive at the female (Single vs. First: $|t| = 5.1$, $P < 0.001$; First vs. Second: $|t| = 4.9$, $P < 0.001$; Figure 2). Additionally, the total time males spent following the female when they were First was independent of the time invested prior to the approach of another male. The time that First males spent with a female before the approach of another male was not different from the total time following when males were in the Single scenario (unpaired t-test: $|t|_{195} = 0.6$, $N_1 = 90$, $N_2 = 107$, $P = 0.555$).

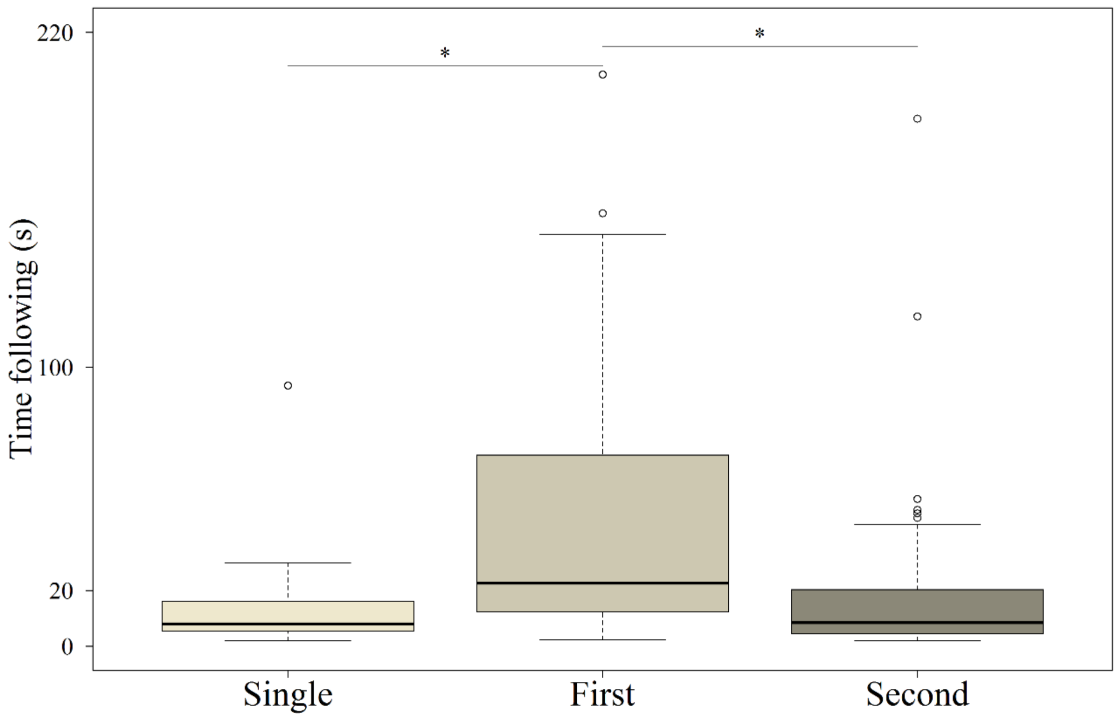

Figure 2. Time spent by focal males following a female under the three competitive scenarios: when the focal male was the single male following a female the entire time (Single, *N* = 45); when he was the first to approach a female (First, *N* = 45); and when he was the second (Second, *N* = 62). Pairwise comparisons of time spent following between competitive scenarios were obtained from the best-fit LME model. Asterisks and associated lines above the plots show which sets of competitive scenarios differed significantly from one another (**P* < 0.05). In each boxplot the internal line represents the median. Lower and upper edges represent the 25% and the 75% quantile, respectively. Whiskers below and above the box edges represent, respectively, the minimum and the maximum points within the 1.5 interquartile range. Circles represent outliers.

Mating tactics frequency

Most focal males did not perform any courtship display (86.2 %) or unsolicited attempt (78.9 %) when following females. From those that performed mating attempts, they did it only once in most of the cases: 81.0 % and 75.0 % of the samples for courtship displays and unsolicited attempts, respectively. Specifically, for the frequency of courtship displays, and after model selection, none of the explanatory variables (competitive scenario and mesocosm) was included in the best-fit model ($\chi^2_2 = 8.9$, $N = 152$, $P = 0.064$; Figure 3*a*). By contrast, the best-fit model that explained the frequency of unsolicited attempts included the competitive scenario ($\chi^2_2 = 8.2$, $N = 152$, $P = 0.017$; Figure 3*b*). Focal males that approached a female First performed more unsolicited attempts than males that were the Single following the female (z = 2.3, $P = 0.031$), or males that were the Second (z = 2.3, $P = 0.031$).

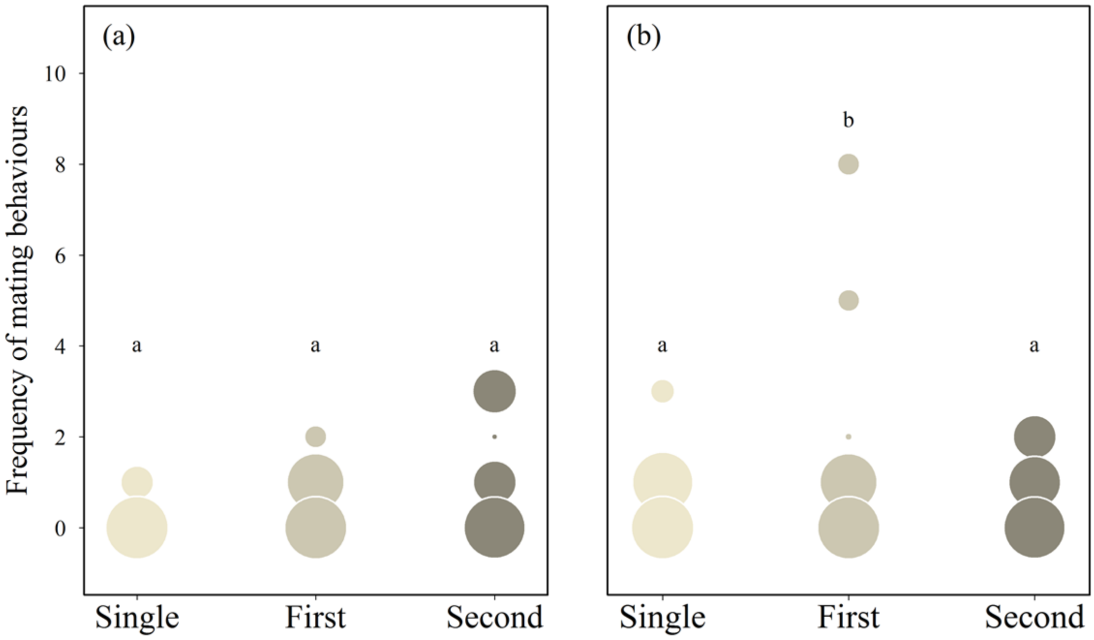

Figure 3. Frequency of mating tactics in relation to competitive scenario. Mating tactics were either (a) courtship displays (on the left side of the figure) or (b) unsolicited mating attempts (on the right side). There were three scenarios: when the focal male was the single male following a female the entire time (Single, $N = 45$); when he was the first to approach a female (First, $N = 45$); and when he was the second (Second, $N = 62$). The

diameter of the circles is proportional to the sample size. Pairwise comparisons between competitive scenarios of the frequency of each mating tactic were obtained from the best-fit hurdle models. Asterisks and associated lines above the plots show which sets of competitive scenarios differed significantly from one another (*$P < 0.05$).

Mating behaviours sequence

For the analyses of the behavioural sequences that started with approach, the best-fit model ($\chi^2_4 = 10.2$, $N = 456$, $P = 0.037$) excluded the random terms and included the interaction between sequence type and competitive scenario. Based on the best model, males had higher probability of swimming away after an approach than of performing courtship displays or unsolicited attempts across all scenarios (Figure 4*a*, Table 1). However, this probability was higher when they were the Second to approach a female (see in Table 1, for comparison between Second and the other two scenarios).

Only 21 focal males performed at least one courtship display, meaning that only those males were included in the analysis of the behavioural sequences that started with a courtship display. Males never performed some sequences in some competitive scenarios (courtship display-courtship display when focal males were Single with the females, and courtship display-unsolicited attempt when males were the First to approach the females; Figure 4*b*). Differences between competitive scenarios were not significant, as the best-fit model included sequence type as the only explanatory variable ($\chi^2_2 = 21.9$, $N = 63$, $P < 0.001$). Males had significantly higher probability of swimming away after a courtship display, than of performing a second courtship ($z = 3.985$, $P < 0.001$) or an unsolicited attempt ($z = 4.283$, $P < 0.001$). But, when males did not swim away, they were equally likely of performing a courtship and an unsolicited attempt ($z = -0.867$, $P = 0.386$).

For the analyses of the behavioural sequences starting with unsolicited attempts, only the 32 focal males that performed at least one unsolicited attempt were considered. The best-fit model excluded the random terms (mesocosm and female group) and included the interaction between the competitive scenario and the sequence type ($\chi^2_4 = 11.0, N = 96, P = 0.026$). The best model revealed that the probability of swimming away after an unsolicited attempt was higher when males were Single and Second than when they were First (Figure 4*c*; Table 1). Additionally, there were no significant differences between the probabilities of First males swimming away, performing a courtship display or repeating an unsolicited attempt.

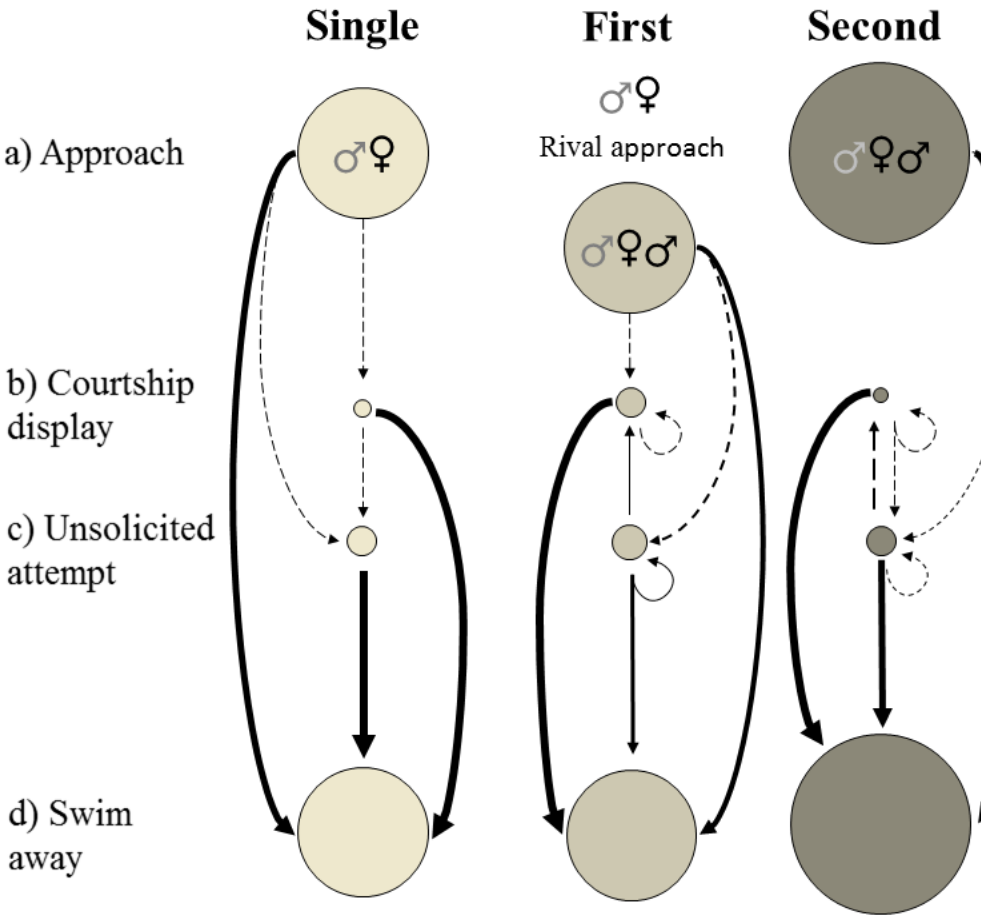

Figure 4. Mating behaviours sequences in three different competitive scenarios. The three scenarios were (from left to right; with focal male represented in grey): when the focal male was the single male following a female the entire time (Single, *N* = 45); when he was the first to approach a female (First, *N* = 45); and when he was the second (Second, *N* = 62). Each arrow represents the sequence of two behaviours. Each sequence could start with (a) approach to a female, (b) courtship display, or (c) unsolicited attempt; and end with (b) courtship display, (c) unsolicited attempt, or (d) swim away. Arrow width represents the estimated probability of that sequence. Sequences that were not observed or were rare (with an estimated probability lower than 0.1) were not included. For each scenario, continuous arrows represent behavioural sequences that were significantly more likely to occur than sequences represented by dashed arrows. We obtained the probability estimates of each behaviour sequence from the best-fit statistical GLMER models. The

diameter of the circles is proportional to the frequency of each behaviour for each scenario.

Table 1. Summary table of the GLMER models for the behavioural sequences on different competitive scenarios.

Precedent Behaviour	Sequence type			Competitive scenario	Z-value	P-value
Approach						
	Approach-Swim away	vs	Approach-Courtship display	Single	-5.013	**<0.001**
				First	-4.795	**<0.001**
				Second	-6.232	**<0.001**
				Single vs First	-0.057	0.955
				First vs Second	-2.015	**0.044**
				Single vs Second	-2.108	**0.035**
			Approach-Unsolicited attempt	Single	-4.429	**<0.001**
				First	-3.500	**<0.001**
				Second	-6.743	**<0.001**
				Single vs First	0.854	0.393
				First vs Second	-2.532	**0.011**
				Single vs Second	-1.620	0.105
Unsolicited attempt						
	Unsolicited attempt-Swim away	vs	Unsolicited attempt-Courtship display	Single	-2.948	**0.003**
				First	0.000	1.000
				Second	-1.736	0.083
				Single vs First	2.586	**0.010**
				First vs Second	-1.332	0.183
				Single vs Second	1.515	0.130
		vs	Unsolicited attempt-Unsolicited attempt	Single	-2.948	**0.003**
				First	-0.409	0.682
				Second	-2.128	**0.033**
				Single vs First	2.384	**0.017**
				First vs Second	-1.423	0.155
				Single vs Second	1.185	0.236
	Unsolicited attempt-Unsolicited attempt	vs	Unsolicited attempt-Courtship display	Single	0.000	1.000
				First	0.409	0.682
				Second	0.514	0.608
				Single vs First	0.198	0.843
				First vs Second	0.152	0.879
				Single vs Second	0.296	0.768

Analyses were conducted separately based on the behaviour that initiated the sequence type (precedent behaviour): approach to a female (*N* = 152; all males approached a female) or unsolicited attempt (*N* = 32; number of focal males that performed the unsolicited tactic). Each sequence type includes the precedent behaviour and the following behaviour (courtship display, unsolicited attempt, or swim away). Sequences that started with courtship display are not shown because the best-fit model did not include the interaction between sequence type and competitive scenario. P-values in bold represent significant differences.

4.4. Discussion

Our findings indicate that males adjust their mating behaviour in response to the order in which they arrive at a female, investing more on mating behaviours when they precede a rival. Specifically, males spent more time following females and performed more and repeated mating attempts when they were the First to arrive at the mating context, than males that remained Single with the female or arrived Second. The scenario First was the situation where males' chance of being preferred by females (female sequential mate choice) and of fertilizing more eggs (sperm precedence) could be compromised by a rival's approach. By investing more in this scenario, males probably try to reduce such risks. Taken together our results demonstrate that males make contingent mating decisions depending on the competitive environment. However, they do this by investing more in unsolicited mating attempts than in courtship displays.

Approach decision

Males approached a female regardless of the presence or absence of other males, in line with the frequency of occurrence of competitive encounters in the population. This result indicates that order of arrival near a female does not influence a male's decision to approach a female.

Since male guppies express weak social bonds with females and are constantly moving between groups of females (Griffiths & Magurran, 1998; Croft et al., 2006), avoiding or seeking encounters with other males may be difficult in the wild. In fact, we found that, even in cases where a male approached a solitary female (choosing a non-competitive encounter), he was often joined by a rival male. It is likely that guppies do not avoid other males, because doing so does not prevent sperm competition, as it has been shown for the field cricket (*Gryllus campestris*; Fisher et al., 2016). At the same time, it seems unlikely that male guppies actively seek out females accompanied by rival males.

Behavioural adjustment while following a female

We predicted that if a male's order of arrival near a female was important in shaping male mating behaviour, we would find different patterns of mating behaviour across the three scenarios. More specifically, we expected more investment in mating behaviours when Single if males were prioritizing female mate choice, or more investment when Second if males were prioritizing sperm precedence. The First scenario was an intermediate one, where males would try to secure both the advantage of female choice and sperm precedence. We found evidence for the First scenario, with First males investing more in females than Single and Second males. However, they did so through unsolicited mating attempts instead of courtship display. Additionally, males postponed the decision to swim away, and invested more on mating attempts (courtship and unsolicited) after an unsolicited attempt when were First. This indicates that, despite seemingly being non-selective about their order of arrival, males do not invest equally in all three competitive scenarios, but invest more in the scenario where neither pre- nor postcopulatory processes dominate.

There is evidence that female guppies choose higher displaying males (Magurran 2005) and that male reproductive success is linked to the frequency of courtship displays (Evans & Magurran, 2001). Since we found that focal males did not change the frequency of courtship displays between competitive scenarios, this could indicate that investment on courtship displays is more dependent on female traits, like receptivity (Farr, 1980; Guevara-Fiore, Stapley, & Watt, 2010), than on the male's order of arrival. Males typically achieve mating after courtship display only if females are receptive (Magurran, 2005). Given that receptive females are more responsive to males that approach them first (Pitcher et al., 2003) and males are able to distinguish among receptive and non-receptive females (Guevara-Fiore, Stapley, Krause, Ramnarine, & Watt, 2010), a higher investment in courtship displays could still be expected with receptive females in scenarios where males were alone with the females or the first to approach. Although we did not control for female receptivity, our experimental approach mimics closely female-male dynamics in nature, where receptive females are rare (Liley, 1966; Liley & Wishlow, 1974). In such natural conditions, our results indicate that courtship displays are not used differently across the three competitive scenarios, suggesting that reproductive advantages are obtained from such displays irrespective of whether males are Single, First or Second with a female. Future studies could examine the relevance of female receptivity in male mating behaviours relative to order of arrival.

In contrast to courtship displays, both the frequency of unsolicited mating attempts and the sequence of behaviours performed after these attempts were dependent on the order of arrival. Males that were the First to approach the female performed more unsolicited attempts, and were more likely to repeat that behaviour or to court than to swim away than Single and Second males. It has been previously shown that male guppies increase unsolicited mating attempts in response to increased mate competition (Magellan et al., 2005; Magurran, 2005). Indeed, males can mate more rapidly and repeatedly without female cooperation by performing unsolicited attempts (Houde, 1988; Magurran, 2005). Furthermore, by re-mating, males ensure a higher chance of being the last one to mate or of transferring more sperm. The greater proportion of unsolicited mating attempts found in our study when males were the First to approach suggests that males are less willing to abandon the female in that scenario. This could represent a strategy either to transfer more sperm (correlated with unsolicited attempts frequency; Matthews, Evans, & Magurran, 1997), to be the last to transfer sperm and ensure last sperm precedence, or to inform rival males that the female has mated. Supporting this last hypothesis, a theoretical study suggests that males should only transfer such information to rival males when they are in disadvantage on sperm competition (Engqvist & Taborsky, 2017). In fact, the First scenario is the one where the risks are higher, as males have more to lose than those who approached Second or those that were alone with a female the entire time.

To assess if First males tried to be the last to mate, we performed additional statistical analyses on the order by which males left the females and found that males left the females later than rivals more frequently when they were the First to arrive than when they were Second (see Appendix Table A1). We also found that males invest more time and make more mating attempts when they left the female after the rival male in both First and Second scenarios (see Appendix Table A1, Figures A1 and A2), suggesting that

First males tried to secure sperm precedence. These findings are compatible with higher investment in mating attempts (more sperm transference) in high sperm competition scenarios predicted by theoretical models (Parker et al., 1997), and supported by empirical studies in several other species (e.g. dominant versus sneaker males, Collet et al., 2012; and absence versus presence of competitor males, Kelly & Jennions, 2011). Sperm competition is potentially high in guppies, as females can store sperm of multiple males for months (Herdman et al., 2004; Magurran, 2005) and a single brood is generally fathered by two or more males (Becher & Magurran, 2004; Elgee et al., 2012; Devigili et al., 2015). Males' investment in time and energy with a female, when they are the First to approach the female, could give them a significant fitness advantage relative to sperm competition. In fact, previous studies found that male guppies invest less in mating behaviours when they lose the opportunity of mating first with a female (Dosen & Montgomerie, 2004; Jeswiet et al., 2011).

Our study provides additional support for the finding by Magris and colleagues that, when female cryptic choice is controlled (by artificial insemination), there is first- instead of last-male sperm precedence (Magris et al., 2017). The greater mating investment by First males reported in our study may increase a male's chances during cryptic female choice. Moreover, sperm competition could explain why First males performed more unsolicited attempts and repeated more mating attempts after this tactic. This was why sperm precedence was reversed in Magris et al. (2017) study where males' equal ejaculate size competed for fertilization.

For female sequential mate choice, we predicted higher investment when males were Single, than when they were First and Second. We failed to detect an increase time following and in the frequency of courtship displays when males were Single (as discussed above). What we did find was that males spent more time with females when they were First and invested more in mating attempts (both unsolicited and courtship) after an unsolicited attempt than in the other scenarios. This suggests that female sequential mate choice is not the main mechanism shaping male mating behaviours, but that in concert with sperm precedence it selects for male mating tactics. We cannot exclude the possibility that males also improved their chances of being preferred by the females when arriving First. The fact that males invested in courtship displays after an unsolicited mating attempt supports this possibility, as do studies showing that female guppies cryptic choice favours the sperm of preferred males (Gasparini & Pilastro, 2011; Pilastro, Simonato, Bisazza, & Evans, 2004).

4.5. Conclusions

Our work advances understanding of how pre- and postcopulatory sexual processes shape male mating behaviours, when the order in which males approach a female (before or after a rival male) provides contrasting competition advantages. We provide, for the first time, empirical evidence about the interplay between male uncertainty about long-term female mating history, short term male behavioural flexibility, and antagonistic pre- and postcopulatory processes. We show that male guppies use information based on the order in which they approach a female and invest more in mating attempts when the risk of losing both female preference and sperm precedence is higher. In light of the growing evidence of female multiple mating across taxa (Arnqvist & Nilsson, 2000; Barbosa & Magurran, 2006; Parker & Birkhead, 2013; Taylor, Price, & Wedell, 2014), we suggest that future studies (including comparative approaches) ask how males (and females; Shuster, Briggs, & Dennis, 2013) shape their mating decisions based on their knowledge of their mates' mating history. Such new studies should consider the degree of polyandry in a population (based on the number of females that mate multiply, as well as the maximum average number of matings per female; Taylor et al. 2014); the degree of uncertainty about female mating history; whether sequential mate choice is in place or not; and the degree of sperm precedence (if partial or total; see Turnell & Shaw, 2015). For example, in species like guppies that have high frequency of female multiple mating, but unlike guppies have total sperm precedence, postcopulatory pressures may have a higher contribution to the evolution of male mating behaviours. In such cases, uncertainty about female mating history and order of arrival should be irrelevant, if males assume that they are always the first or the last to mate, and have strategies to control sperm competition (e.g. mate guarding; Ridley, 1980, 1989), or to avoid sperm competition (e.g. mating plugs; Dougherty, Simmons, & Shuker, 2016). On the other hand, precopulatory selection may act more strongly on male mating behaviours in species with partial sperm precedence and less polyandry than in guppies (Turnell & Shaw, 2015). Indeed, as we have shown here, the outcome of selection on male behaviour is subject to subtle drivers that vary in intriguing and complex ways amongst species.

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Appendices

Table A1. Summary table of the best models encountered to explain male mating investment relative to their chance of winning last sperm precedence.

Response variable	Model type		Distribution	Explanatory variables	Contrasts	Z-value/ T-value	P-value
Leaving order	GLMER		Binomial	Competitive scenario	First vs Second	-2.2	0.025
Time following	LMER		Normal (log-transformed)	Competitive scenario	First vs Second	-3.8	<0.001
				Leaving order	Earlier vs Later	4.8	<0.001
Courtship display	Hurdle	Zero model	Poisson	Leaving order	Earlier vs Later	5.0	<0.001
Unsolicited attempt	Hurdle	Count model	Negative Binomial	Competitive scenario	First vs Second	-2.2	0.0310
		Zero model		Leaving order	Earlier vs Later	5.0	<0.001

Two competitive scenarios during which focal males faced competition were considered (First and Second). Response variables were: leaving order (i.e., leave the female earlier/later than rival male), frequency of courtship displays, frequency of unsolicited attempts, and time following females. Explanatory variables considered for all the models were: competitive scenario (First and Second), mesocosm, female group (nested inside the mesocosm) and leaving order (except when it was used as the response variable). For leaving order (as the response variable) the best-fit model included the competitive scenario ($\chi^2_1 = 5.0$, $N = 107$, $P = 0.025$). Both leaving order and the competitive scenario were included in the best-fit model for time following ($F_{104,149} = 23.5$, $N = 107$, $P < 0.0001$; Figure A1). For frequency of courtship displays, only leaving order (as explanatory variable) influenced this behaviour (included in the zero model; $\chi^2_1 = 30.0$, $N = 107$, $P < 0.001$; Figure A2a). In relation to frequency of unsolicited attempts, the competitive scenario was included in the count model and leaving order was included in the zero model ($\chi^2_1 = 29.2$, $N = 107$, $P < 0.001$; Figure A2b). None of the best models included the interaction between leaving order and competitive scenario.

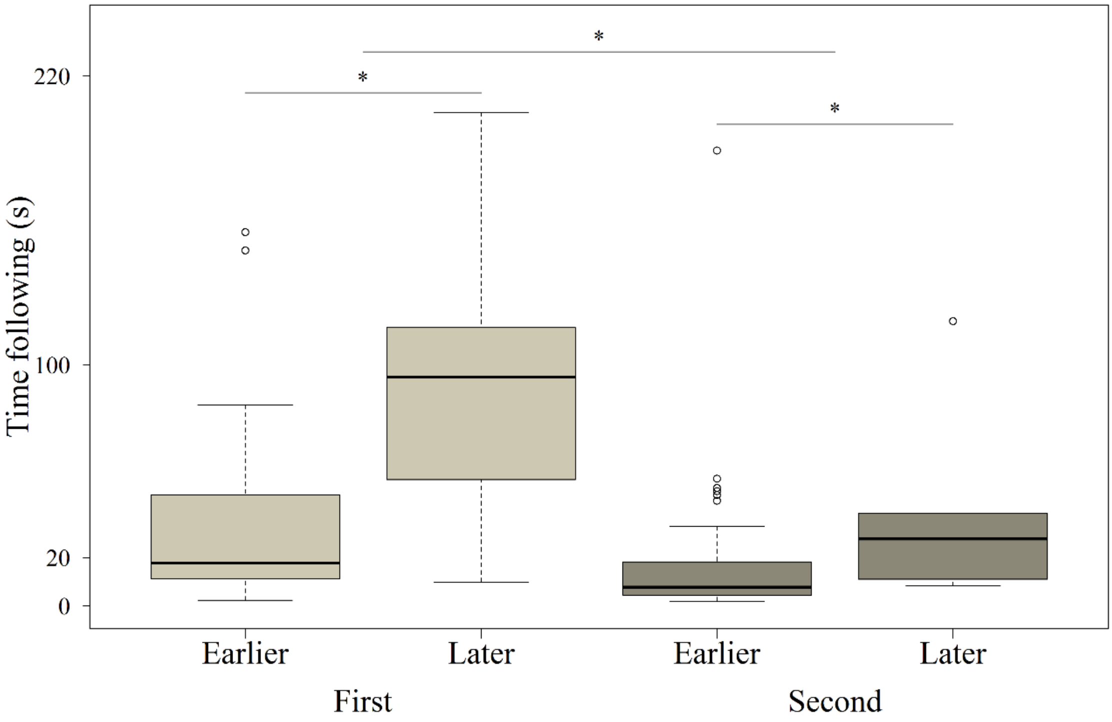

Figure A1. Time spent by focal males following a female between scenarios with different advantage in relation to sperm precedence. Two competitive scenarios related with order of arrival near a female were considered: when the focal was the first to approach a female (First); and when he was the second (Second). And, within these competitive scenarios, males were divided in two additional scenarios depending on the order they left the female (i.e. probability of mating at last): when the focal male leave the female earlier than the rival (lower probability of mating after the rival - Earlier); and when the focal leave the female later than the rival (higher probability of mating after the rival - Later). Pairwise comparisons of time spent following between scenarios were obtained from the best-fit LME model. Asterisks and associated lines above the plots show which sets of competitive scenarios differed significantly from one another (*$P < 0.05$). In each boxplot the internal line represents the median. Lower and upper edges represent the 25% and the 75% quantile, respectively. Whiskers below and above the box edges represent, respectively, the minimum and the maximum points within the 1.5 interquartile range. Circles represent outliers.

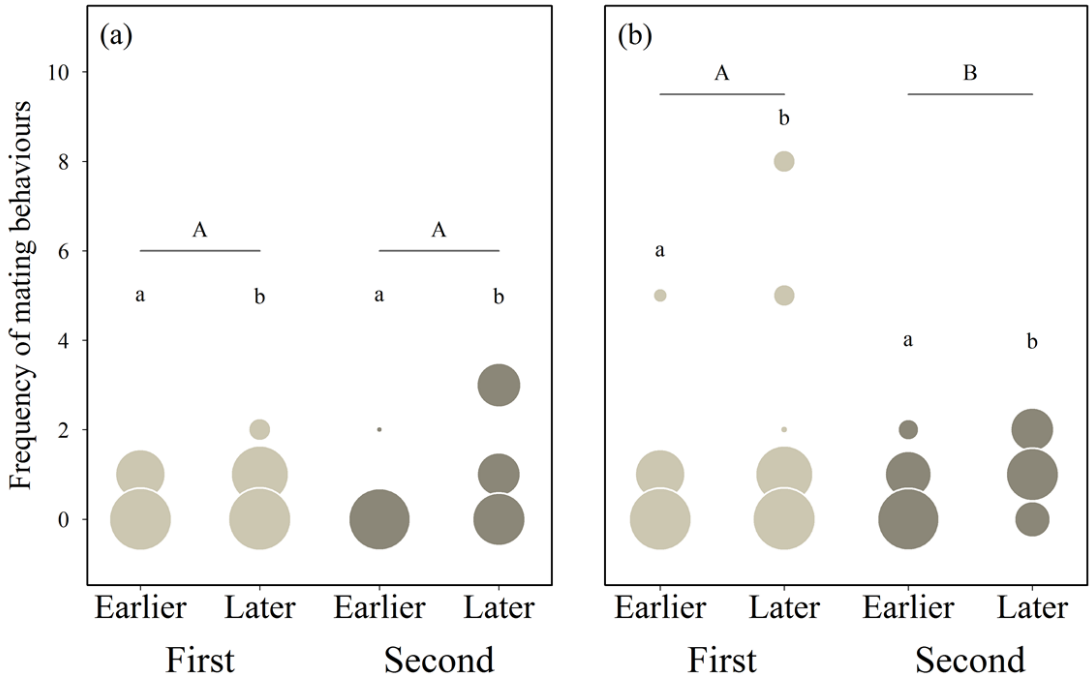

Figure A2. Frequency of mating tactics between scenarios with different advantage in relation to sperm precedence. Mating tactics were either (a) courtship displays (on the left side of the figure) or (b) unsolicited attempts (on the right side). Two competitive scenarios related with order of arrival near a female were considered: when the focal was the first to approach a female (First); and when he was the second (Second). And, within these competitive scenarios, males were divided depending on the order they left the female (i.e. probability of mating at last): when the focal male leave the female earlier than the rival (lower probability of mating after the rival - Earlier); and when the focal leave the female later than the rival (higher probability of mating after the rival - Later). The diameter of the circles is proportional to the sample sizes. Pairwise comparisons of the frequency of each mating tactic between scenarios were obtained from the best-fit hurdle models. Asterisks and associated lines above the plots show which sets of competitive scenarios differed significantly from one another (*$P < 0.05$).

Modified image taken from Sean Earnshaw (Biodiversity and Behaviour Group, University of St Andrews)

CHAPTER 5.

Courtship display evolution

Effect of bystander males

Paper IV. The role of male-male competition on the evolution of male-male courtship display: a systematic review.

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Abstract

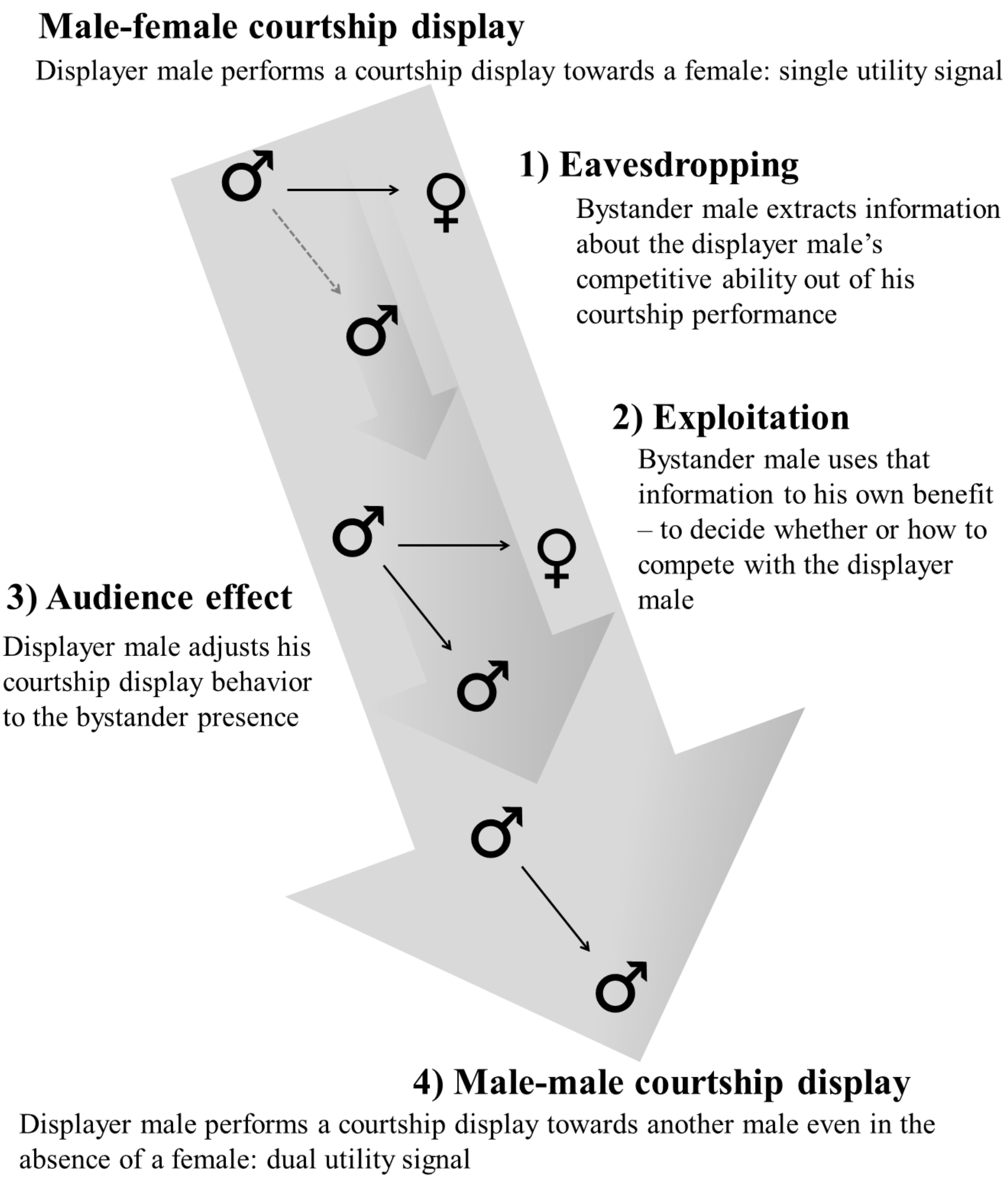
There is increasing evidence of male courtship display directed to other males across the animal kingdom. However, it is not clear how this behavior evolved. Here, we hypothesize that it emerged as an evolutionary byproduct of male-male competition in some species, acquiring a second function of deterring competitors. More specifically, we hypothesize the following evolutionary steps: bystander males 1) eavesdrop male courting females; 2) exploit information transmitted; then, displayer males 3) change their display in the presence of bystanders; 4) direct this behavior to the bystander males. For that, we performed systematic reviews. First, we located research articles reporting male-male courtship display. For these articles, we listed species mentioned and explanations suggested. Finally, we searched articles reporting eavesdropping, exploitation and audience effect during male-female courtship display for the species listed. We predicted that researchers investigating this behavior had considered the competition hypothesis. Moreover, we predicted that evidence of evolutionary steps, separately as well as combined, should be more frequent in species where male-male courtship display has been linked to competition. Our results show that the hypothesis of male-male competition driving male-male courtship display is largely considered, although it is not the most common. In species where this hypothesis has been suggested there are significantly more evidences of eavesdropping, and more evidences of combined with the other steps. In sum, we show that it is very likely that male courtship display has a dual function in several animal species, and that intrasexual competition has been the driving force.

Key words: communication network; courtship display; double function; intrasexual competition; male-male courtship; male-male sexual behavior; same-sex sexual behavior; sexual display; sexual selection; social information.

Running header**:** Evolution of male-male courtship display.

Lay summary:

In several animal species males can be found displaying courtship behavior to other males. This behavior seems to have an adaptive function evolving similarly in different species. Based on literature reviews we found that competition has been largely suggested to explain this behavior, and that in some species it first appeared because bystander male eavesdrop and exploit other male courtship display, ultimately leading the displaying male to redirect this behavior as a form of deterring competitor males.



Male-female courtship display

Displayer male performs a courtship display towards a female: single utility signal

1) Eavesdropping

Bystander male extracts information about the displayer male's competitive ability out of his courtship performance

2) Exploitation

Bystander male uses that information to his own benefit – to decide whether or how to compete with the displayer male

3) Audience effect

Displayer male adjusts his courtship display behavior to the bystander presence

4) Male-male courtship display

Displayer male performs a courtship display towards another male even in the absence of a female: dual utility signal

Figure 1. Suggested evolutionary path leading to male-male courtship display. Male courtship display, initially directed to sexual partners (females), can be shaped by male-male competition, following these evolutionary steps: 1) eavesdropping, and 2) exploitation of male-female courtship display by bystander males, 3) audience effect by the displayer male, and, ultimately, 4) male-male courtship display.

5.1. Introduction

For the last decade, the increasing evidences of same-sex sexual behavior in animal *taxa*, including male-male courtship display, has been intriguing researchers. Consequently, some hypotheses have been proposed to explain why these behaviors occur in the first place (for a review see Bailey and Zuk 2009). Some researchers have suggested that this is a form of male-male competition (e.g. Bailey and Zuk 2009; Caballero-Mendieta and Cordero 2012). However, the competition hypothesis remains to be tested.

Male courtship display informs females about the quality, motivation and condition of prospective mates (Andersson 1994; Andersson and Simmons 2006; Chandler et al. 2012; Kuijper et al. 2012). Therefore, it has been shaped by intersexual selection, but not exclusively. Male courtship display is also determined by intrasexual competition. For example, in several species males change the courtship display (e.g. exaggerate or attenuate) in the presence of another male (Earley 2010; Plath and Bierbach 2011). This suggests that the displayer male is deceiving the bystander – eventually about his quality as a potential competitor, motivation to mate with that female, or about his condition. Therefore, males can also transfer information during displays to other males. Male courtship display has, therefore, been suggested to have a dual function, i.e., that it attracts sexual partners and, at the same time, deters sexual competitors (Fisher 1930; Berglund et al. 1996).

The hypothesis of dual function has only been tested for male physical traits (ornaments and weapons; e.g. Morris et al. 2007), and the classic view is that they have first evolved through intrasexual selection, being later co-opted in the context of female choice (Berglund et al. 1996). This is the case, for example, of antlers' evolution in male deer, which they use to fight with competitors, but that females also find attractive since males that win fights are also better-quality mates (Berglund et al. 1996). Contrastingly, Morris et al. (2007) found evidence that in the swordtail fish, *Xiphophorus* spp., the vertical bars was primarily selected to attract females before it was co-opted to deter aggression in competitor males, suggesting an ancestral role of female choice in the evolution of male ornaments. Since courtship display has a fundamental role in female choice, we assume that its primary function is to attract females. With this rational, we ask if male-male courtship display evolved secondarily due to intrasexual competition and propose a possible evolutionary pathway.

We hypothesize that increasing bystander pressure by competitor males should have shaped the evolution of this behavior. This way, we expect four evolutionary steps (Figure 1). At first, when a male display to a female in the presence of other males, bystander males can extract information about the competitive ability of the displayer (eavesdropping; Wiley 1983; Danchin et al. 2008; see Glossary). Eventually, the bystander can benefit with that information, exploiting the displayer's male mating investment (McGregor 2005) – for example, by initiating a fight (if the displayer male is judged as of inferior quality) or by sneaking (if the displayer male is of better quality). Then, if the costs of inadvertently transferring information to a bystander overcome the benefits of ignoring his presence, the displayer male is expected to change the purpose of the courtship display signal by incorporating the bystander in his targeted audience (audience effect; Matos and Schlupp 2005). Examples of the latter can be the redirection of the display to a less initially preferred female as a way to deceive the competitor (Plath and Bierbach 2011), reduction, exaggeration or matching of courtship display intensity and frequency with that of the competitor male (e.g. Vignal et al. 2004; Fisher and Rosenthal 2007; Auld and Godin 2015). Lastly, males may start displaying to competitor males directly, even in the absence of females, either to discourage them to court females, or to inhibit their aggression.

Glossary

Courtship display: Conspicuous behavior generally performed by males, that attracts sexual partners, stimulate mate choice, and, thereby, promote the mating success of the displayer. It is used to put on evidence secondary sexual traits, the ornaments, such as bright colors, or conspicuous appendages (e.g. crowns and tails). Sometimes these ornaments can be features that extend the males' physical phenotype (Dawkins 1989), but evolved with the same purpose (e.g. bower constructions by satin bowerbird males that determine female choice).

Bystander: An individual that performs eavesdropping.

Eavesdropping: When a bystander extracts information from actions or interactions of other individuals in which he is not directly involved (Wiley 1983; McGregor 1993, McGregor and Dabelsteen 1996; McGregor and Peake 2000; McGregor 2005; Danchin, Giraldeau and Cèzilly 2008). When bystanders extract information from the signaling actions of other individuals, i.e., when they intercept their signals (e.g. mating songs produced by males to attract females), this is called **interceptive eavesdropping** (Wiley 1983; Bradbury and Vehrencamp 1998; Peake 2005). When bystanders extract information from the interactions between senders and receivers (e.g. when a male and a female are engaged in a sexual interaction), this is called **social eavesdropping** (McGregor 2005; Peake 2005; Bonnie and Earley 2007; Earley 2010).

Exploitation: When a bystander uses the extracted information (i.e. changes his behavior; Otter et al. 1993; McGregor and Dabelsteen 1996) to his own benefit, with costs to the individuals that produce the information (Danchin, Giraldeau and Cèzilly 2008). For example, when a male attempt to sneak, to sexually interfere, or to assess female receptiveness from the courtship display investment of another male. Again, we considered features that extend the males' physical phenotype (e.g. satin bowerbird males steal decorations from other males; Borgia and Mueller 1992).

Audience effect: When an individual is aware of the presence of a bystander (the audience) and changes his behavior because of that (Marler and Evans 1996; Danchin et al. 2004; Danchin, Giraldeau and Cèzilly 2008). This behavioral change includes withholding information, exaggeration or, in contrast, attenuation (see deception).

Deception: When individuals transmit a non-reliable information to manipulate the behavior of another individual. This benefits the signaler but does not benefit or can even harm the receiver. Includes lying, withholding information, bluffing (or exaggeration), and attenuating (Bradbury and Verhencamp 1998; Searcy and Nowicki 2005).

To test this hypothesis, first, we performed a systematic review to find out in which species male-male courtship display has been described. Second, we searched within the text of these articles for the hypotheses given to explain this behavior. Third, we searched for evidences of eavesdropping, exploitation and audience effect during male courtship displays within the species listed in the systematic review. Fourth, we compared the frequency of those behaviors in the listed species where male-male courtship display has been hypothesized to be competition driven and where it is thought to be driven by other evolutionary pressures. Our expectations were that evidences of eavesdropping, exploitation, audience effect, as well as evidences of the three behaviors combined (i.e., evidences of more than one evolutionary step per species) are higher in species where the competition hypothesis has been given, than in species where researchers suggested other hypotheses.

5.2. Materials and Methods

Systematic review

The systematic review took place between April 6, 2016 and June 23, 2017. We used the Web of Science bibliographic database from our institution. Web of Science is a major biology database that encompasses over 50 000 scholarly books, 12 000 journals and 160 000 conference proceedings. Other search engines were not considered, since they were unlikely to index the sort of articles we were considering (e.g. PubMed). Therefore, articles retrieved by Web of Science are expected to be robust and sufficient.

In the first systematic review, we located articles addressing male-male courtship display. This general term includes courtship display and other sexual behaviors, such as mounting. The use of this general term increased the probability of finding all or at least most of the relevant articles related with male-male courtship display published so far. Since we were only interested in non-human animal behavior, we conducted our search in the following Web of Science research areas: “BEHAVIORAL SCIENCES OR PSYCHOLOGY OR ZOOLOGY OR EVOLUTIONARY BIOLOGY OR REPRODUCTIVE BIOLOGY OR SOCIOLOGY OR MARINE FRESHWATER BIOLOGY OR ENVIRONMENTAL SCIENCES ECOLOGY OR SOCIAL SCIENCES OTHER TOPICS OR SCIENCE TECHNOLOGY OTHER TOPICS OR ENTOMOLOGY OR SOCIAL ISSUES OR FISHERIES OR COMMUNICATION”. Within these research areas, we searched for articles with: "same-sex sexual behavio*" OR "same-sex sexual display" OR "same-sex courtship" OR "sexual display toward? *other male" OR "sexual behavio* toward? *other male" OR "sexual display toward? *other male" OR "male-male sexual display" OR "male-male courtship" OR "male-male sexual behavio*" OR "sexual display between males" OR "sexual behavio* between males" OR "sexual display between males". These expressions were searched in the Web of Science advanced search engine, in the field “Topic (TS)”. This field allows to search for words or short sentences in the articles’ title, abstract and keywords, as well as in indexing fields such as systematics, taxonomic terms and descriptors, and in “keywords plus” (keywords added by “Thomson Reuters editorial expertise in science”).

126 articles were retrieved (Figure 2). Two independent readers analyzed all the articles and selected the ones that described male-male sexual behaviors in the title or

abstract (70 articles; Appendix “References A1”), and from these, those that described male-male courtship display in the full-text for further analysis (56 articles; Appendix “References A2”). This step took place between April 6, 2016 and May 4, 2016.

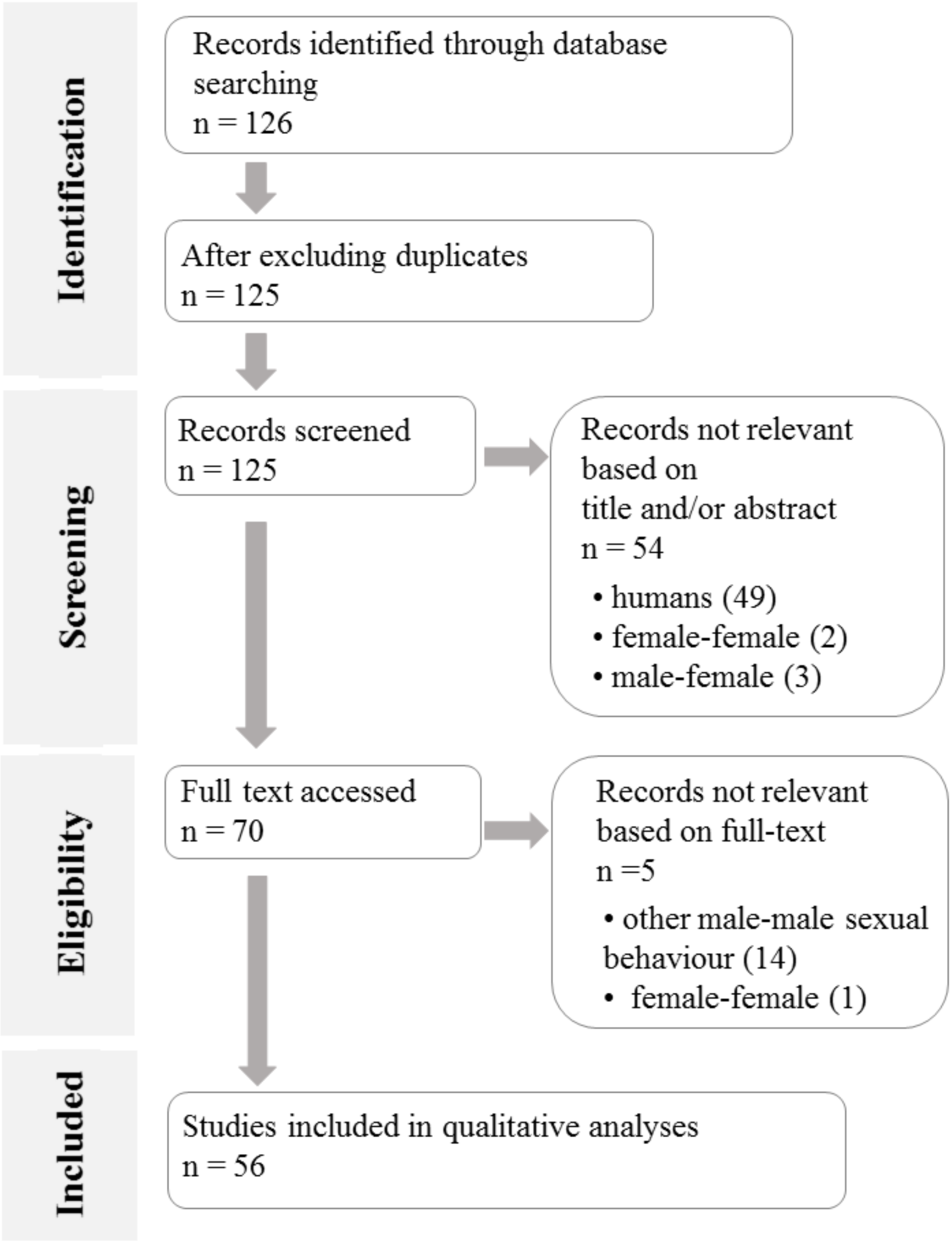


Figure 2. PRISMA flow diagram with search results for male-male courtship display in non-human animals. The systematic review followed the four steps proposed by Moher et al. (2009): identification, screening, eligibility and included data. In the identification

step, to reduce chances of missing any relevant article identifying and/or describing male-male courtship display that used another term, we searched for broader terms related with same-sex sexual behavior. In the screening and eligibility steps we excluded records that did not reported male-male courtship display. These non-relevant records reported male-male sexual behavior in humans, male-female or female-female sexual interactions, or other male-male sexual behaviors (e.g. mounting, pairing). In the included step we finished with articles with qualitative data (species name and behavior).

From the 56 articles, we listed all species where male-male courtship display was described (146) and searched for the hypotheses given for this behavior and for each species. Two independent readers classified the hypotheses into different categories (Appendix "Table A1", describing all hypotheses encountered, common terms used and definition). We organized hypotheses in three categories: "competition", "other" (all other hypotheses given that do not include competition; e.g. "social glue"), and "none" (when no explanation was given). In cases where researchers gave more than one explanation, we considered that competition was suggested if it was one of the explanations given or considered the category "other" if competition was not suggested. In cases where researchers were against one hypothesis but did not suggest any other, we considered that no hypothesis was given. In cases that we were not sure if researchers were suggesting any hypothesis, we considered that no hypothesis was given. The classifications attributed by each of the readers were compared to dismiss any disagreement. (See Appendix "Table A2", describing all hypotheses encountered listed by species).

In the second systematic review we searched for evidences of eavesdropping, exploitation and audience effect within the courtship display context in the species listed before. Again, we searched in Web of Science, in the field "Topic (TS)". We searched the scientific and common name of each species, combined with the terms: "(sexual behavio*" OR "sexual display" OR "court*") AND ("conspecific" OR "observer" OR "public" OR "viewer" OR "spectator" OR "eavesdrop*" OR "bystander" OR "receiver" OR "presence" OR "audience" OR "exploit*")". Once more, we used the general term "sexual display" to increase the probability of finding all or at least most of the relevant articles. Like for the first systematic review, we first read the title and abstract of retrieved articles and then the full-text. However, contrary to the first systematic review, where we screened the text of all possible articles, here when one article provided the evidence for eavesdropping, exploitation or audience effect we ended the process. This way, data used for analyses were retrieved from 63 articles only: 35 for eavesdropping, 36 for exploitation and 31 for audience effect (see Appendix "References A3" and "Table A3", listing evidences of these behaviors per species with male-male courtship display). Since we found an extensive number or articles on *Drosophila* species, instead of conducting an independent search for each of these species, we searched the term "Drosophila". This search was conducted from June 9 to June 26, 2017.

Statistical analyses

To test if the occurrence of eavesdropping, exploitation and audience effect during male-female courtship display are more evident when the competition hypothesis has been suggested to explain male-male courtship display, only the species for which any hypothesis was given (competition or other) were considered (n = 64). First, we tested evidences of each behavior (evolutionary steps), and, second, evidences of the three combined behaviors following a given sequence (no evidence, only eavesdropping, eavesdropping and exploitation, or all the behaviors). All analyses were performed using the software R 3.2.3 (R Core Team 2015). The significance level was set at $p = 0.050$.

First, we tested if each behavior could be an evolutionary step leading to the male-male courtship display. We employed generalized linear models (GLM from the package lme4; Bates et al. 2014), with a logit link function for a binomial distribution to each behaviour. The response variable was the evidence or non-evidence and had a binomial distribution, with "0" representing no articles (no evidence), and "1" representing at least one article reporting the behavior. The explanatory variable was the "competition hypothesis", with two categories: "competition" and "other".

Second, we tested if the three behaviors could be part of an evolutionary path leading to male-male courtship display. For the analysis of the three behaviors combined, the explanatory variable was the same as above and the response variable had a multinomial distribution, with four categories: "1", no articles found for any behavior; "2", articles found only for eavesdropping; "3", articles found for eavesdropping and exploitation or only for exploitation (in the latter case we considered that species have both exploitation and eavesdropping, because the bystander has necessarily to perform eavesdropping before performing exploitation); and "4", articles found for eavesdropping (whether implicit or explicit), exploitation and audience effect. In this analysis we employed an ordered logistic regression models (polr from the package MASS; Venables and Ripley 2002), since we considered no eavesdropping by bystander males as the original evolutionary step, followed by derived behaviors (eavesdropping as second evolutionary step, then exploitation, and in last audience effect; Figure 1). At last, for the calculation of predicted probabilities of finding evidences of a higher evolutionary step for species that competition hypothesis was suggested compared to species that other hypothesis was given we used the stargazer function from the package sartgazer (incorporates the values given by the ordered logistic regression model; Hlavac 2015).

5.3. Results

Species with male-male courtship display

The 146 species where male-male courtship display has been described belong to six taxonomic classes (see Appendix “Table A2”). Most were insects (62 species, 42.5%), followed by birds (53 species, 36.3%), mammals (25 species, 17.1%), ray-finned fish (i.e. Actinopterygii; 3 species, 2.1%), spiders (i.e. Arachnida; 2 species, 1.4%), and reptiles (1 species, 0.7%).

Hypotheses suggested

Most articles that describe male-male courtship display for a given species do not suggest any hypothesis for this behavior (45.7%; Table 1 and Appendix “Table A2”). In articles where hypotheses were suggested, the most common was mistaken identification (50.0%), followed by competition (20.0%). More than one hypothesis was given to explain the courtship display between males in some species (e.g. *Drosophila melanogaster*). This was more common in species that belong to the most studied classes (Aves, Mammalia and Insecta; see previous subsection “Species with male-male courtship display”).

Table 1. Number of species per hypothesis and per class.

Hypothesis	Class Actinopterygii	Arachnida	Aves	Insecta	Mammalia	Reptilia	Total
None	2		50	22	17		91
Mistaken identification	1	2	1	38	2	1	45
Competition	1		6	7	3	1	18
Displaced or abnormal	1		1	3	2		7
Practice			1	4			5
Social glue			1		1		2
Sexual preference				2			2
Thermoregulation				1		1	2
Early experience				1	1		2
Byproduct				5	2		7

The hypotheses names are the ones commonly used (for a review see Bailey and Zuk 2009). The definition of each hypothesis is presented in Appendix "Table A1". Because the same or different authors can suggest different hypothesis for male-male courtship display per species, the total number inside the table is superior to the number of species considered.

The inter-readers agreement regarding the classification of the hypotheses into each category was of 88% (29 disagreements out of 241 – total number of times that hypotheses have been suggested for the 146 species in the 56 articles included in the qualitative analysis). To solve the disagreements, consensus was reached after detailed discussions.

Evolutionary path

There was no evidence of eavesdropping (115 species), exploitation (120 species), or audience effect (125 species) within the context of courtship display for most species. Where there were evidences of these behaviors, the most predominant one was eavesdropping (29 species), followed by exploitation (21 species), and audience effect (16 species). In two cases, authors suggested eavesdropping, but it was not clear whether they were actual evidences or were authors' suggestion, so we did not count these species. The same happened for exploitation and audience effect in 5 cases. In the 19 species where competition have been suggested, evidence of eavesdropping has been described for 9 of them, exploitation for 5, and audience effect for 4 species (Appendix "Table A3").

The best-fit model to explain the occurrence of eavesdropping during male-female courtship display included the hypothesis given for male-male courtship display ($\chi^2_1 = 8.2$, n = 64, $p = 0.004$). Evidences of exploitation tend to be related with the hypothesis suggested ($\chi^2_1 = 3.5$, n = 64, $p = 0.060$). But, the best-fit model for audience effect did not include this variable ($\chi^2_1 = 2.0$, n = 64, $p = 0.160$). There were statistically more evidences of eavesdropping in species where competition was pointed out as a hypothesis for the evolution of male-male courtship display in comparison to species where other explanations were given ($z = 2.8$, $p = 0.005$). This way, evidences of eavesdropping were found in a greater proportion of species for which competition has been suggested than when another hypothesis was given (Figure 3). Although there was a similar tendency for exploitation, the difference was marginally significant (z = 1.8, $p = 0.060$). For the audience effect the difference was non-significant (z = 1.4, $p = 0.154$).

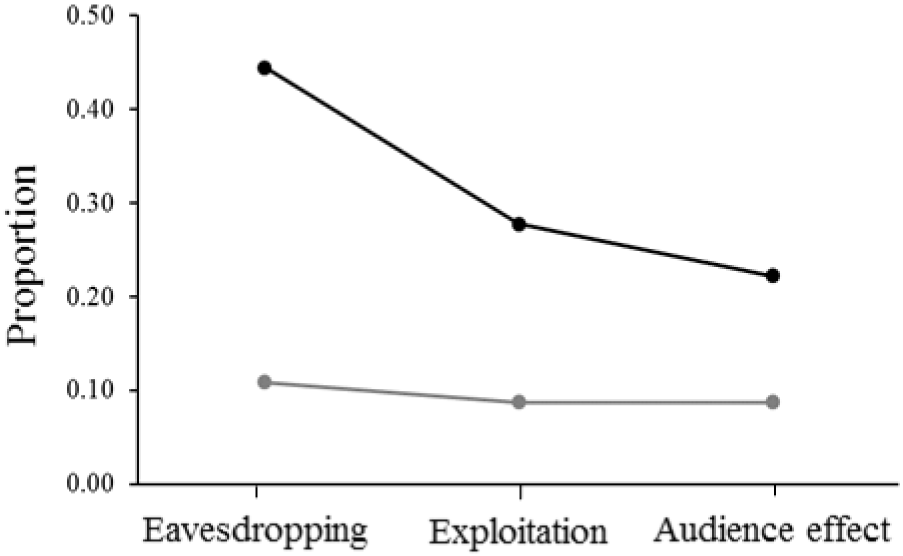


Figure 3. Proportion of species with evidences of the three evolutionary steps according to the hypothesis suggested to explain male-male courtship display. The evolutionary steps are (from the left to the right in the x axis): eavesdropping and exploitation by bystander males, and audience effect by the displayer male. The species considered are the ones found in the first systematic review and represent species in which male-male courtship display has been reported (n = 146). The black line represents the proportion of species for which the competition hypothesis has been suggested, and the gray line for which other hypotheses has been given.

Concerning the multinomial ordered analysis, we were 5.9 more times likely to find evidences of more and higher evolutionary steps combined for species where competition has been suggested than for species for which researchers suggested other hypotheses (t = 2.6, n = 59, $p = 0.01$). This analysis excluded 5 additional species that could not be easily inserted in one stage of the evolutionary path – in three species we found evidences of eavesdropping and of audience effect, but not of exploitation, and in two there was only evidence of audience effect.

5.4. Discussion

Here we found that intrasexual competition is one of the main hypothesis suggested to explain the behavior of male-male courtship display. Additionally, and as we predicted, we found more evidences of behaviors that are probably determinant to the appearance and fixation of male-male coursthip display as a result of the pressure exerted by competitor males (eavesdropping, exploitation and audience effect). This supports our hypothesis that courtship display gained, along the evolutionary time, a secondary function due to constrains imposed by male audiences during mating interactions with females; i.e. from attracting females it evolved to be simultaneously effective at intimidating or deterring competitor males.

Although there are increasing evidences of male-male courtship display in several species, we suspect that this is still underestimated. First, because in species with no sexual dimorphism researchers may hardly distinguish the two sexes, and, thereby, are more likely to assume that two individuals in a sexual interaction are a male and a female than otherwise. And second, because this behavior is generally considered abnormal and, hence, neglected. Even though, increasing research are now focused on male-male courtship display, and on this behavior adaptive value.

The most frequently proposed hypothesis is the mistaken identification, where males are judged to have accidentally mistaken a competitor male with a female (non-adaptive). The competition hypothesis, that proposes an adaptive value for this behavior, comes only in second place. Moreover, one should not forget that the majority of articles did not provide any explanation, and the ones which did rarely tested their hypotheses. However, hypotheses can only become relevant when there are evidences that support them.

In an attempt to find evidence for the competition hypothesis, we predicted that, for male-male courtship display to evolve in a population, three behaviors should take place first: 1) eavesdropping and 2) exploitation of information by bystander males, and, as a response, 3) audience effect by the displayer male that modifies his courtship display in the presence of the bystander. Such evolutionary path should be expected only if male-male competition is the mechanism responsible for courtship display between males. Therefore, evidences for such evolutionary steps should be higher in species for which the competition hypothesis has been suggested. Our expectations were partially confirmed, since we found that competition driven species exhibit the above behaviors more frequently than others. Particularly, we found significant evidence for eavesdropping, but not for audience effect, and exploitation was only marginally related with the male-male competition hypothesis. Finally, we found support for an evolutionary association of eavesdropping with exploitation and audience effect, suggesting that these behaviors could be steps of an evolutionary path towards male-male courtship display. These findings deserve some thought.

Eavesdropping is not necessarily a costly behavior – animals can remain hidden, sometimes without leaving their territories while collecting information about conspecifics – and is useful in different contexts. So, it is likely that it is widespread across the animal kingdom. Thereby, it is more likely to find articles that mention eavesdropping directly or indirectly, than articles that mention exploitation or audience effect. For example, in lekking species – where males aggregate in displaying arenas that are visited by females – we can assume that there is male-male eavesdropping without researchers mentioning it. In other words, to acknowledge the existence of exploitation and audience effect authors need to be looking for these behaviors, while for eavesdropping this is not necessary, as we can infer its existence through indirect evidence. Hence, it is reasonable to assume that exploitation and audience effect should be more frequent than what we found in the literature. On the other hand, these two behaviors are less likely to be evolutionary stable, considering that they benefit one individual but harm the other. Hence, they could have appeared and disappeared throughout the course of evolution. Therefore, it is possible that male-male courtship display has evolved in some species through this evolutionary path, but that the evolutionary steps of exploitation and audience effect no longer take place.

5.6. Concluding remarks

To conclude, our results provide some support to the hypothesis that the information contained in a male courtship display, intentionally transmitted to females, can also be inadvertently transmitted to competitor males, and eventually used by these competitor males. Hence, during evolution displaying males may gained more benefits by directing courtship display behaviors to competitor males, than by using other forms of competition, eventually involving higher risks and/or costs.

Notwithstanding, we acknowledge that our study has some limitations: we did not include in the systematic review search terms all possible keywords of male-male courtship display (e.g. double-function or dual-function), neither all possible keywords for the evolutionary path (e.g. deception can be related with audience effect). Future studies should fill these gaps. For the evolutionary path, it would be interesting to compare evidences of eavesdropping, exploitation and audience effect between phylogenetic branches, and verify if, in general, there are more evolutionary steps in species that branched more recently, and none of the behaviors or only eavesdropping in more basal ones. Future studies could also search for other variables that could have affected the evolution of male-male courtship behavior in some species and not in others (e.g. mating systems).

Previous studies suggest that the selective pressure exerted by same-sex bystanders is higher for males than for females (e.g. Kniel et al. 2016). Based on this, researchers have been focused on male behavioral plasticity, in the sense that they study the immediate adjustment of sexual behaviors during male-female sexual interactions if bystander males are present (Zuberbühler 2008). However, as our findings suggest, intrasexual competition is likely to have changed male sexual behaviors in a more extreme way, by leading such behaviors to gain a new function, and hence giving a purpose to male-male courtship display in the absence of females.

It was in this context that Berglund et al. (1996) suggested the dual function of male sexual traits, though only in relation to physical traits. These authors considered the evolutionary path of sexual traits having primarily evolved for male-male competition (serving as weapons) and that were later used to attract females (serving as ornaments). Their review give some support for this suggestion. Contrastingly, other researchers found evidences that support an evolution from ornaments to weapons (Morris et al. 2007). Although, these can vary between species, we suspect that male courtship displays originated, in general, to attract females. Otherwise it would be hard to explain why researchers have, for so long, focused and found evidences for the correlation between this behavior and female mate choice (including Darwin, 1871). Anyone that had the opportunity of observing a male courtship display, naturally infers that the behavior is primarily directed to females. It is also possible that courtship display behaviors evolved originally to deter rivals and that such function has been subsequently lost in most species. Again, phylogenetic analyses would be helpful to ascertain the ancestral state evolutionary path of this behavior.

Therefore, by taking our results into account, we realize that intrasexual selection can play an important role not only in the evolution of behavioral flexibility, but also in the evolution of male courtship display dual function (Sullivan-Beckers and Cocroft 2010). More broadly, research on male courtship display must seek for behavioral patterns that may have evolved conditioned not only by male-female sexual interactions, but also by the entire communication network, as Doutrelant and others (2001) have suggested.

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Appendices

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References A2. Systematic review of same-sex sexual behavior: records of male-male courtship display.

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Table A1. Hypotheses suggested to explain male-male courtship display.

Table A2. Hypotheses suggested for male-male courtship display by species.

Table A3. Species with male-male courtship display, respective references supporting this and the evidences of eavesdropping, exploitation and audience effect during male-female courtship display.

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Table A1. Hypotheses suggested to explain male-male courtship display.

Hypotheses	**Sub-hypothesis**	**Description**
Adaptive value		
Competition	Defense	Can be used to defend a territory or resource (food or female)
	Intimidation	Can be used to obtain a resource
	Aggressiveness inhibitor	Inhibits aggression in competitor males
	High competition	Inhibits aggression in environments where male-male encounters are frequent
	Sexual interference	Takes place during a mating attempt of another male, and eventually stops it
	Dominance hierarchy	Helps to define or maintain a hierarchical position
	Condition assessment	Allows to assess the phenotypic condition of a competitor male
Social glue		Promotes affiliative relationships between males
Practice		Unexperienced juveniles learn from adult males
Non-adaptive value		
Displaced or abnormal behavior	Isolation or no opportunity to mate	When males have no access to females for a certain period of time
	High competition environment	When males face a high rate of encounters with another males
	Mutations	When induced (laboratory) or natural (rare) mutations change males' sexual behaviors
Byproduct		When, for example, males perform this behaviour immediately after stopping hibernation
Mistaken identification	Sex recognition	When males do not distinguish another males from females
	Species recognition	When male do not distinguish individuals of their own species with those of another species
Not specified (possibly adaptive)		
Early experience	Isolation or no opportunity to mate	When males have no access to females for a certain period of time
	High competition environment	When males face a high rate of encounters with another males
Sexual preference		When males can choose between females and males, but prefer males
Thermoregulation		

Table A2. Hypotheses suggested for male-male courtship display by species.

Class	Species	Hypotheses		Reference
Actinopterygii				
	Oreochromis mossambicus	displaced behaviour	(high sexual motivation)	Amorim et al. (2003)
		mistaken identification	(sex recognition)	
		competition	(high competition environment / male-male encounters)	Oliveira and Almada (1998)
	Poecillia reticulata	none		Bailey and Zuk (2009)
	Pungitius pungitius	none		Oliveira and Almada (1998)
Arachnida				
	Oedothorax fuscus	mistaken identification	(sex recognition) (species recognition)	Shcarf and Martin (2013)
	Oedothorax gibbosus	mistaken identification	(sex recognition) (species recognition)	
Aves				
	in general	competition	(high competition environment / male-male encounters)	MacFarlane et al. (2010)
		early experience	(high competition environment / male-male encounters) (isolation/no opportunity to mate)	
		displaced behaviour	(high competition environment / male-male encounters)	
	altricial birds	early experience		MacFarlane et al. (2007)
	precocial birds	abnormal behaviour	(high competition environment / male-male encounters)	
	Penguins (in general)	against mistaken identification	(sex recognition)	Pincemy et al. (2010)
	Anas discors	none		MacFarlane et al. (2007, 2010)

Class	Species	Hypotheses		Reference
	Anas platyrhynchos	none		
	Anser anser	none		
	Apenodytes patagonicus	against mistaken identification	(sex recognition)	Pincemy et al. (2010)
	Aratinga (Eupsittula) canicularis	none		MacFarlane et al. (2007, 2010)
	Biziura lobata	none		
	Branta canadensis	none		MacFarlane et al. (2007)
	Callaeas cinerea	none		MacFarlane et al. (2010)
	Calypte anna	none		MacFarlane et al. (2007, 2010)
	Chiroxiphia caudata	none		
	Chiroxiphia pareola	none		
	Chlamydera maculata	none		
	Cygnus atratus	competition	(territory/resource defence)	MacFarlane et al. (2010)
			(social conflict / aggression)	
		social glue		
		none		MacFarlane et al. (2007)
	Dinopium benghalense	none		MacFarlane et al. (2010)
	Diomedea (Phoebastria) immutabilis	none		MacFarlane et al. (2007, 2010)
	Euplectes orix	none		
	Falco tinnunculus	none		
	Fringilla coelebs	none		

Class	Species	Hypotheses		Reference
	Gymnopithys bicolor (leucaspis)	none		
	Haematopus ostralegus	none		
	Larus (Chroicocephalus) ridibundus	none		MacFarlane et al. (2007, 2010)
	Larus (Leucophaeus) atricilla	none		
	Melanerpes formicivorus	competition	(inhibit aggression)	MacFarlane et al. (2010)
		none		MacFarlane et al. (2007)
	Melopsittacus undulatus	competition	(assess other condition)	Abbassi and Burley (2012)
	Menura novaehollandiae	abnormal behaviour	(high competition environment / male-male encounters)	MacFarlane et al. (2010)
		none		MacFarlane et al. (2007)
	Mionectes oleagineus	none		MacFarlane et al. (2007, 2010)
	Parotia lawesii	none		MacFarlane et al. (2010)
	Perissocephalus tricolor	none		MacFarlane et al. (2007, 2010)
	Phaenostictus mcleannani	none		
	Phaethornis superciliosus	none		
	Phalacrocorax aristotelis	none		
	Phalacrocorax carbo	none		

Class	**Species**	**Hypotheses**		**Reference**
	Philomachus pugnax	none		
	Pica pica	none		
	Pluvialis apricaria	none		
	Poliocephalus poliocephalus	none		
	Porphyrio porphyrio	none		
	Ptilonorhynchus violaceus	practice		MacFarlane et al. (2010)
		competition	(high competition environment / male-male encounters)	
		none		MacFarlane et al. (2007)
	Ptiloris magnificus	none		MacFarlane et al. (2007, 2010)
	Ptiloris victoriae	none		
	Pygoscelis adeliae	none		
	Pygoscelis papua	none		
	Riparia riparia	none		MacFarlane et al. (2010)
	Rupicola rupicola	competition	(high competition environment / male-male encounters)	MacFarlane et al. (2010)
		none		MacFarlane et al. (2007)
	Scenopoeetes dentirostris	none		MacFarlane et al. (2007)
	Scopus umbretta	none		MacFarlane et al. (2007, 2010)
	Sericulus chrysocephalus	none		MacFarlane et al. (2007, 2010)
	Sialia sialis	none		MacFarlane et al. (2007, 2010)
	Struthio camelus	none		MacFarlane et al. (2007, 2010)
	Taeniopygia guttata	competition	(territory/resource defence)	Elie et al. (2011)

Class	Species	Hypotheses		Reference
	Tringa nebularia	none		MacFarlane et al. (2007, 2010)
	Tringa totanus	none		MacFarlane et al. (2007, 2010)
	Tryngites subruficollis	none		MacFarlane et al. (2007)
Insecta				
	in general	mistaken identification	(sex recognition)	Scharf and Martin (2013)
		competition	(dominance)	Wang et al. (2011)
	flies and wasps	competition	(female defence)	Scharf and Martin (2013)
			(sexual interference)	
	Beetles, flies (table 1)	abnormal behaviour	(artificial environment)	Scharf and Martin (2013)
	Drosophila	none		Chen et al. (2011); Chertemps et al. (2012); Certel et al. (2010); Liu et al. (2008a); Kitamoto (2002); Hing and Carlson (1996)
		thermoregulation		Liu et al. (2009)
		byproduct	(genes)	Zhang and Odenwald (1995)
		against mistaken identification	(sex recognition)	Wang et al. (2011)
	Acraea andromacha	none		Caballero-Mendietaa and Cordero (2012)
	Allomyrina dichotoma septentrionalis	abnormal behaviour	(artificial environment)	Scharf and Martin (2013)
		mistaken identification	(sex recognition)	
	Anomalochrysa maclachlani	none		
	Aphidius ervi	competition	(sexual interference)	Scharf and Martin (2013)

Class	Species	Hypotheses		Reference
		mistaken identification	(sex recognition)	
	Bactrocera oleae	mistaken identification	(sex recognition)	Benelli et al. (2013)
	Bemisia tabaci	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Bicyclus anynana	none		Caballero-Mendietaa and Cordero (2012)
	Byrsotria fumigata	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
		competition	(sexual interference) (female defence)	
	Cephalonomia tarsalis	competition	(sexual interference)	Scharf and Martin (2013)
	Ceratitis capitata	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Cerotainia albipilosa	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Choristoneura fumifera	mistaken identification	(sex recognition)	Scharf and Martin (2013)
		none		Caballero-Mendietaa and Cordero (2012)
	Chrysoperla lucasina	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Ciulfina biseriata	against mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Cotesia rubecula	mistaken identification		Scharf and Martin (2013)
		competition	(territory/resource defence)	

Class	Species	Hypotheses		Reference
	Dacus cucurbitae	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Danaus gilippus (berenice)	none		Caballero-Mendietaa and Cordero (2012)
	Drosophila persimilis	byproduct	(genes)	Dai et al. (2008)
	Drosophila affinis	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
		practice		
	Drosophila ananassae	byproduct	(genes)	Dai et al. (2008)
	Drosophila birchii	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
		practice		
	Drosophila erecta	byproduct	(genes)	Dai et al. (2008)
	Drosophila heteroneura	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Drosophila melanogaster	mistaken identification	(sex recognition)	Vijayan et al. (2014); Scharf and Martin (2013); Toda et al. (2012); Meissner et al. (2011); Agrawal and Riffell (2011); Dukas (2010)
		byproduct	(genes)	Vijayan et al. (2014); Ganter et al. (2011); Anaka et al. (2008)
			(physiology)	Yamamoto and Seto (2014)
		practice		Bailey and Zuk (2009)
		abnormal behaviour		Bailey and Zuk (2009)
			(high competition environment / male-male encounters)	Bailey et al. (2013)
			(high sexual motivation)	Krstic et al. (2013)

Class	Species	Hypotheses		Reference
			(induced/rare mutations)	Weng et al. (2013); Wang et al. (2011)
		none		Ejima (2015); Hoskins et al. (2015); Dalton et al. (2009); Dai et al. (2008); Liu et al. (2008a, b); Chan and Kravitz (2007); Lacaille et al. (2007); Gaines et al. (2000); Crossley et al. (1995)
		early experience		Meissner et al. (2011)
		against sexual preference		
		thermoregulation		Ganter et al. (2007)
		against sexual preference		Meissner et al. (2011)
	Drosophila montana	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Drosophila paulistorum	none		Scharf and Martin (2013)
	Drosophila sechellia	none		Dai et al. (2008)
	Drosophila serrata	none		Scharf and Martin (2013)
	Drosophila silvestris	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Drosophila simulans	none		Dai et al. (2008)
	Drosophila virilis	none		Dai et al. (2008)
	Drosophila willistoni	none		Dai et al. (2008)
	Drosophila yakuba	none		Dai et al. (2008)
	Euleia fratria	none		Scharf and Martin (2013)

Class	**Species**	**Hypotheses**		**Reference**
	Euphydryas editha	none		Caballero-Mendietaa and Cordero (2012)
		mistaken identification	(sex recognition)	Scharf and Martin (2013)
		competition	(sexual interference)	
	Eupoecilia ambiguella	none		Caballero-Mendietaa and Cordero (2012)
		mistaken identification	(sex recognition)	Scharf and Martin (2013)
		competition	(territory/resource defence)	
	Eurycotis floridana	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Glossina morsitans morsitans	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Gnatocerus cornutus	competition		Lane et al. 82016)
	Grapholitha molesta	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Gromphadorhina portentosa	none		Logue et al. (2009)
	Gryllus bimaculatus	abnormal behaviour	(handicap)	Murakami and Itoh (2003)
	Gryllus veletis	byproduct	(traits)	Boutin et al. (2016)
	Hylobittacus apicalis	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Junonia coenia	none		Caballero-Mendietaa and Cordero (2012)
	Lariophagus distinguendus	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Magicicada cassini		(sex recognition)	Scharf and Martin (2013)

Class	Species	Hypotheses		Reference
		mistaken identification	(species recognition)	
	Magicicada septendecim	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Megacopta punctatissima	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Musca domestica	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Periplaneta americana	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Periplaneta brunnea	mistaken identification	(sex recognition) (species recognition)	Scharf and Martin (2013)
	Phytoecia rufiventris	mistaken identification	(sex recognition)	Scharf and Martin (2013); Wang et al. (1996)
	Pieris rapae crucivora	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Piezodorus hybneri	none		Scharf and Martin (2013)
	Prochyliza xanthostoma	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Protophormia terrae-novae	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Psyttalia concolor	none		Benelli et al. (2012)
		practice		Benelli and Canale (2013)
		mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Rhagoletis mendax	none		Scharf and Martin (2013)
	Teleogryllus oceanicus	mistaken identification	(sex recognition)	Scharf and Martin (2013)

Class	**Species**	**Hypotheses**		**Reference**
	Thyanta pallidovirens	mistaken identification	(sex recognition)	Scharf and Martin (2013)
	Thymelicus lineola	none		Pivnick et al. (1992)
Mammalia				
	Barbastella barbastellus	none		Riccucci (2011)
	Capra hircus	competition	(dominance)	Ungerfeld et al. (2014)
		early experience	(high competition environment / male-male encounters)	Ungerfeld and Gonzalez-Pensado (2008)
	Corynorhinus rafinesquii	mistaken identification	(sex recognition)	Riccucci (2011)
		abnormal behaviour	(artificial environment)	Riccucci (2011)
	Desmodus rotundus	abnormal behaviour	(artificial environment)	Riccucci (2011)
	Eptesicus serotinus	byproduct	(other behaviours)	Riccucci (2011)
	Miniopterus schreibersii	none		Riccucci (2011)
	Myotis bechsteinii	none		Riccucci (2011)
	Myotis capaccinii	none		Riccucci (2011)
	Myotis daubentonii	none		Riccucci (2011)
	Myotis lucifugus	none		Riccucci (2011)
	Myotis myotis	byproduct	(other behaviours)	Riccucci (2011)
	Myotis mystacinus	none		Riccucci (2011)
	Myotis nattereri	none		Riccucci (2011)
	Nyctalus leisleri	none		Riccucci (2011)
	Nyctalus noctula	mistaken identification	(sex recognition)	Riccucci (2011)

Class	Species	Hypotheses		Reference
	Ovies aries	competition	(dominance)	Ungerfeld and Gonzalez-Pensado (2008)
		none		Bailey and Zuk (2009)
	Ovis canadensis	competition	(dominance, high competition environment / male-male encounters)	Ungerfeld and Gonzalez-Pensado (2008)
	Pipistrellus pipistrellus	none		Riccucci (2011)
	Plecotus auritus	none		Riccucci (2011)
	Pteropus giganteus	social glue		Riccucci (2011)
	Pteropus livingstonii	none		Riccucci (2011)
	Pteropus poliocephalus	none		Riccucci (2011)
	Pteropus rodricensis	none		Riccucci (2011)
	Rhinolophus ferrumequinum	none		Riccucci (2011)
	Rhinolophus hipposideros	none		Riccucci (2011)
Reptilia				
	Thamnophis sirtalis parietalis	against mistaken identification thermoregulation		Bailey and Zuk (2009)
		competition	(territory/resource defence?)	
		mistaken identification	(sex recognition)	Shine et al. (2000)

Table A3. Species with male-male courtship display, respective references supporting this and the articles providing evidences of eavesdropping, exploitation and audience effect during male-female courtship display.

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
Acraea andromacha	Caballero-Mendieta and Cordero (2012)			
Allomyrina dichotoma septentrionalis	Scharf and Martin (2013)			
Anas discors	MacFarlane et al. (2007, 2010)			
Anas platyrhynchos	MacFarlane et al. (2007, 2010)	Johnsgard (1960)	Davis (2002)	Davis (1997)
Anomalochrysa maclachlani	Scharf and Martin (2013)	Tauber et al. (1990)		
Anser anser	MacFarlane et al. (2007, 2010)			
Apenodytes patagonicus	Pincemy et al. (2010)			
Aphidius ervi	Scharf and Martin (2013)			
Aratinga (Eupsittula) canicularis	MacFarlane et al. (2007, 2010)			
Bactrocera oleae	Benelli et al. (2013)	Benelli et al. (2014, 2016)		
Barbastella barbastellus	Riccucci (2011)			
Bemisia tabaci	Scharf and Martin (2013)			
Bicyclus anynana	Caballero-Mendieta and Cordero (2012)	Holveck et al. (2015)	Holveck et al. (2015)	Westerman et al. (2014)
Biziura lobata	MacFarlane et al. (2007, 2010)			
Branta canadensis	MacFarlane et al. (2007)			
Byrsotria fumigata	Scharf and Martin (2013)			
Callaeas cinerea	MacFarlane et al. (2010)			
Calypte anna	MacFarlane et al. (2007, 2010)	Clark (2012)	Clark (2012)	

Species	**Reference** **Male-male courtship display**	**Eavesdropping**	**Exploitation**	**Audience effect**
Capra hircus	Ungerfeld et al. (2014) Ungerfeld and Gonzalez-Pensado (2007)	Price et al. (1991)		Lacuesta and Ungerfeld (2012)
Cephalonomia tarsalis	Scharf and Martin (2013)			
Ceratitis capitata	Scharf and Martin (2013)	Papanastasiou et al. (2011) Hendrichs et al. (1994) Shelly (2000) Benelli et al. (2014)	Papanastasiou et al. (2011) Shelly (2000)	
Cerotainia albipilosa	Scharf and Martin (2013)	Scarbrough (1978)		
Chiroxiphia caudata	MacFarlane et al. (2007, 2010)			
Chiroxiphia pareola	MacFarlane et al. (2007, 2010)			
Chlamydera maculata	MacFarlane et al. (2007, 2010)		Wojcieszek et al. (2007)	
Choristoneura fumifera	Caballero-Mendieta and Cordero (2012); Scharf and Martin (2013)			
Chrysoperla lucasina	Scharf and Martin (2013)			Noh and Henry (2015)
Ciulfina biseriata	Scharf and Martin (2013)			
Corynorhinus rafinesquii	Riccucci (2011)			
Cotesia rubecula	Scharf and Martin (2013)			
Cygnus atratus	MacFarlane et al. (2007, 2010)			
Dacus cucurbitae	Scharf and Martin (2013)			
Danaus gilippus (berenice)	Caballero-Mendieta and Cordero (2012)			
Desmodus rotundus	Riccucci (2011)			
Dinopium benghalense	MacFarlane et al. (2010)			
Diomedea (Phoebastria) immutabilis	MacFarlane et al. (2010; 2007)			

	Reference			
Species	**Male-male courtship display**	**Eavesdropping**	**Exploitation**	**Audience effect**
Drosophila affinis	Scharf and Martin (2013)			
Drosophila ananassae	Dai et al. (2008)			
Drosophila birchii	Scharf and Martin (2013)			
Drosophila erecta	Dai et al. (2008)			
Desmodus rotundus	Riccucci (2011)			
Dinopium benghalense	MacFarlane et al. (2010)			
Diomedea (Phoebastria) immutabilis	MacFarlane et al. (2010; 2007)			
Drosophila affinis	Scharf and Martin (2013)			
Drosophila ananassae	Dai et al. (2008)			
Drosophila birchii	Scharf and Martin (2013)			
Drosophila erecta	Dai et al. (2008)			
Drosophila heteroneura	Scharf and Martin (2013) Agrawal and Riffell (2011) Anaka et al. (2008) Bailey and Zuk (2009) Bailey et al. (2013) Chan and Kravitz (2007) Crossley et al. (1995) Dai et al. (2008) Dalton et al. (2009) Ejima (2015) Gaines et al. (2000) Ganter et al. (2011) Hoskins et al. (2015) Krstic et al. (2013) Lacaille et al. (2007)			

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
	Liu et al. (2008b)			
	Scharf and Martin (2013)			
	Vijayan et al. (2014)			
	Weng et al. (2013)			
	Yamamoto and Seto (2014)			
	Ganter et al. (2007)			
	Meissner et al. (2011)			
	Sellami et al. (2012)			
	Toda et al. (2012)			
	Scharf and Martin (2013)			
	Dukas (2010)			
	Wang et al. (2011)			
Drosophila melanogaster	Agrawal and Riffell (2011)	Maguire and Price (2015)	Ng et al. (2014)	Rouse and Bretman (2016)
	Anaka et al. (2008)			
	Bailey and Zuk (2009)			
	Bailey et al. (2013)			
	Chan and Kravitz (2007)			
	Crossley et al. (1995)			
	Dai et al. (2008)			
	Dalton et al. (2009)			
	Dukas (2010)			
	Ejima (2015)			
	Gaines et al. (2000)			
	Ganter et al. (2007)			
	Ganter et al. (2011)			
	Hoskins et al. (2015)			
	Krstic et al. (2013)			

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
	Lacaille et al. (2007) Liu et al. (2008b) Meissner et al. (2011) Scharf and Martin (2013) Sellami et al. (2012) Toda et al. (2012) Vijayan et al. (2014) Wang et al. (2011) Weng et al. (2013) Yamamoto and Seto (2014)			
Drosophila montana	Scharf and Martin (2013)			
Drosophila paulistorum	Scharf and Martin (2013)			
Drosophila persimilis	Dai et al. (2008)			
Drosophila sechellia	Dai et al. (2008)			
Drosophila serrata	Scharf and Martin (2013)			
Drosophila silvestris	Scharf and Martin (2013)			
Drosophila simulans	Dai et al. (2008)			
Drosophila virilis	Dai et al. (2008)			
Drosophila willistoni	Dai et al. (2008)			
Drosophila yakuba	Dai et al. (2008)			
Eptesicus serotinus	Riccucci (2011)			
Euleia fratria	Scharf and Martin (2013)			
Euphydryas editha	Caballero-Mendieta and Cordero (2012); Scharf and Martin (2013)			
Euplectes orix	MacFarlane et al. (2007, 2010)	Metz et al. (2007)	Metz et al. (2007)	
Eupoecilia ambiguella	Caballero-Mendieta and Cordero (2012) Scharf and Martin (2013)			

Species	**Reference** **Male-male courtship display**	**Eavesdropping**	**Exploitation**	**Audience effect**
Eurycotis floridana	Scharf and Martin (2013)			
Falco tinnunculus	MacFarlane et al. (2007, 2010)			
Fringilla coelebs	MacFarlane et al. (2007, 2010)			
Glossina morsitans morsitans	Scharf and Martin (2013)			
Gnatocerus cornutus	Lane et al. (2016)	Lane et al. (2015)		Lane et al. (2015)
Grapholitha molesta	Scharf and Martin (2013)			
Gromphadorhina portentosa	Logue et al. (2009)	Clark (1998)	Clark (1998)	Clark (1998)
Gryllus bimaculatus	Murakami and Itoh (2003)			
Gryllus veletis	Boutin et al. (2016)		Boutin et al. (2016)	Boutin et al. (2016)
Gymnopithys bicolor (leucaspis)	MacFarlane et al. (2007, 2010)			
Haematopus ostralegus	MacFarlane et al. (2007, 2010)	Spoon et al. (2007)		Spoon et al.(2007)
Hylobittacus apicalis	Scharf and Martin (2013)		Thornhill (1980)	
Junonia coenia	Caballero-Mendieta and Cordero (2012)			
Lariophagus distinguendus	Scharf and Martin (2013)			
Larus (Chroicocephalus) ridibundus	MacFarlane et al. (2010; 2007)			
Larus (Leucophaeus) atricilla	MacFarlane et al. (2010; 2007)			
Magicicada cassini	Scharf and Martin (2013)			
Magicicada septendecim	Scharf and Martin (2013)			
Megacopta punctatissima	Scharf and Martin (2013)	Himuro et al. (2006)		Himuro et al. (2006)
Melanerpes formicivorus	MacFarlane et al. (2007, 2010)			
Melopsittacus undulatus	Abbassy and Burley (2012)			
Menura novaehollandiae	MacFarlane et al. (2007, 2010)			

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
Miniopterus schreibersii	Riccucci (2011)			
Mionectes oleagineus	MacFarlane et al. (2007, 2010)			
Musca domestica	Scharf and Martin (2013)			
Myotis bechsteinii	Riccucci (2011)			
Myotis capaccinii	Riccucci (2011)			
Myotis daubentonii	Riccucci (2011)			
Myotis lucifugus	Riccucci (2011)			
Myotis myotis	Riccucci (2011)			
Myotis mystacinus	Riccucci (2011)			
Myotis nattereri	Riccucci (2011)			
Nyctalus leisleri	Riccucci (2011)			
Nyctalus noctula	Riccucci (2011)			
Oedothorax fuscus	Scharf and Martin (2013)			
Oedothorax gibbosus	Scharf and Martin (2013)			
Oreochromis mossambicus	Amorim et al. (2003) Oliveira and Almada(1998)	Wackermannova et al. (2017)	Nelson (1995)	
Ovies aries	Ungerfeld and Gonzalez-Pensado (2008) Bailey and Zuk (2009)	Ungerfeld et al. (2007) Price et al. (1998)	Price et al. (1991)	
Ovis canadensis	Ungerfeld and Gonzalez-Pensado (2008)		Ungerfeld and Lacuesta. (2015)	Ungerfeld and Lacuesta (2015) Lacuesta and Ungerfeld (2012) Ungerfeld (2012) Ungerfeld and González-Pensado (2009)
Parotia lawesii	MacFarlane et al. (2010)			Patel et al. (2007)

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
Periplaneta americana	Scharf and Martin (2013)		Murfin (1992)	
Periplaneta brunnea	Scharf and Martin (2013)			
Perissocephalus tricolor	MacFarlane et al. (2007, 2010)	Trail (1990)	Trail (1990)	Trail (1990)
Phaenostictus mcleannani	MacFarlane et al. (2007, 2010)			
Phaethornis superciliosus	MacFarlane et al. (2007, 2010)			
Phalacrocorax aristotelis	MacFarlane et al. (2007, 2010)			
Phalacrocorax carbo	MacFarlane et al. (2007, 2010)			
Philomachus pugnax	MacFarlane et al. (2007, 2010)	Cummings and Gelineau-Kattner (2009) Jiguet and Bretagnolle (2001) Hugie and Lank (1997)	Candolin and Reynolds (2002) Hugie and Lank (1997)	Hugie and Lank (1997)
Phytoecia rufiventris	Scharf and Martin (2013) Wang et al. (1996)			
Pica pica	MacFarlane et al. (2007, 2010)			
Pieris rapae crucivora	Scharf and Martin (2013)			
Piezodorus hybneri	Scharf and Martin (2013)			
Pipistrellus pipistrellus	Riccucci (2011)	Voigt-Heucke et al. (2016) Sachteleben and von Helversen (2006)	Voigt-Heucke et al. (2016)	Voigt-Heucke et al. (2016)
Plecotus auritus	Riccucci (2011)			
Pluvialis apricaria	MacFarlane et al. (2007, 2010)			
Poecillia reticulata	Bailey and Zuk (2009)	Castellano et al. (2016)	Auld and Godin (2015)	Auld and Godin (2015) Auld et al. (2015)

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
Poliocephalus poliocephalus	MacFarlane et al. (2007, 2010)			
Porphyrio porphyrio	MacFarlane et al. (2007)			
Prochyliza xanthostoma	Scharf and Martin (2013)			
Protophormia terrae-novae	Scharf and Martin (2013)			
Psyttalia concolor	Benelli et al. (2012) Benelli and Canale (2013) Scharf and Martin (2013)			
Pteropus giganteus	Riccucci (2011)			
Pteropus livingstonii	Riccucci (2011)			
Pteropus poliocephalus	Riccucci (2011)			
Pteropus rodricensis	Riccucci (2011)			
Ptilonorhynchus violaceus	MacFarlane et al. (2007, 2010)	Reynolds et al. (2008) Borgia and Mueller (1992)	Borgia and Mueller (1992)	
Ptiloris magnificus	MacFarlane et al. (2007, 2010)			
Ptiloris victoriae	MacFarlane et al. (2007, 2010)			
Pygoscelis adeliae	MacFarlane et al. (2007, 2010)			
Pygoscelis papua	MacFarlane et al. (2007, 2010)			
Pungitius pungitius	Oliveira and Almada(1998)			
Rhagoletis mendax	Scharf and Martin (2013)			
Rhinolophus ferrumequinum	Riccucci (2011)			
Rhinolophus hipposideros	Riccucci (2011)			
Riparia riparia	MacFarlane et al. (2010)			

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
Rupicola rupicola	MacFarlane et al. (2007, 2010)	Omena Junior (2009) Trail (1990)		
Scenopoeetes dentirostris	MacFarlane et al. (2007)	Frith and Frith (1995)	Frith and Frith (1995)	
Scopus umbretta	MacFarlane et al. (2007, 2010)			
Sericulus chrysocephalus	MacFarlane et al. (2007, 2010)	Borgia and Mueller (1992) Lenz (1994)	Borgia and Mueller (1992) Lenz (1994)	Lenz (1994)
Sialia sialis	MacFarlane et al. (2007, 2010)			
Struthio camelus	MacFarlane et al. (2007, 2010)			
Taeniopygia guttata	Elie et al. (2011)	Lin et al. (2014) Jesse and Riebel (2012) Jarvis et al. (1998)		Jesse and Riebel (2012) Gleeson (2007) Vignal et al. (2004) Jarvis et al. (1998) Dunn and Zann (1997)
Teleogryllus oceanicus	Scharf and Martin (2013) Bailey and French (2012)	Lane et al. (2015) Reichard and Anderson (2015)	Tinghitella and Zuk (2009)	Thomas et al. (2011)
Thamnophis sirtalis parietalis	Bailey and Zuk (2009) Shine et al. (2000)	Shine et al. (2005a)	Shine et al. (2005a)	Shine et al. (2005a, b)
Thyanta pallidovirens	Scharf and Martin (2013)			
Thymelicus lineola	Pivnick et al. (1992)			
Tringa nebularia	MacFarlane et al. (2007, 2010)			
Tringa totanus	MacFarlane et al. (2007, 2010)			
Tryngites subruficollis	MacFarlane et al. (2007, 2010)	Lanctot et al. (1998)	Lanctot et al. (1998)	

Species	Reference Male-male courtship display	Eavesdropping	Exploitation	Audience effect
		Trail (1990)	Trail (1990)	

Modified image taken from Sean Earnshaw (Biodiversity and Behaviour Group, University of St Andrews)

CHAPTER 6.

Behaviour evolution

Effect of bystanders on dishonest signals

Paper V. Interceptive cues and the evolution of signal dishonesty.

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Abstract

Signals are specialized traits of senders that transmit intentional information to targeted receivers and whose adaptive function is the equally specialized responses by the receivers. Social cues, on the other hand, derive from the non-signalling actions and interactions of senders that inadvertently transmit information to non-targeted bystanders. Social cues did not evolve to convey information to others, but the responses of bystanders may have evolved to exploit this valuable source of information. Hence, because they are inadvertently produced, and by consequence cannot be manipulated by the sender, social cues are considered honest information and accordingly highly reliable. However, what remains to be recognized is that inadvertent information can also derive from signals when these are intercepted by non-targeted bystanders: a signal that is intentionally transmitted to the receiver, is also a social cue if inadvertently transmitted to non-targeted bystanders. Here we draw attention to this shift of a signal into a social cue, to which we call "interceptive cue". By recognizing this new type of social cue, a new corollary becomes evident: social cues are not always honest, because interceptive cues derived from dishonest signals will also transmit dishonest information. Consequently, this will engage bystanders, as much as intended receivers, in erroneous decision-making. This raises the question of whether dishonesty evolved exclusively in the context of sender-receiver interactions, or also as a counter-adaptation to the exploitation of interceptive cues by bystanders. We propose the hypothesis that signal dishonesty is an evolutionary product of the interactions between all parties involved in a communication network – senders, receivers and bystanders.

Keywords: social information; communication network; signals; social cues; interceptive eavesdropping; intentional social information; inadvertent social information; information reliability; signal honesty.

6.1. Introduction

All living beings communicate with each other through the transfer of different types of information. Collared flycatchers (*Ficedula albicollis*), for example, prospect the environment in search for suitable places to breed and extract information from the breeding success of conspecifics by observing parental feeding activity (Doligez et al., 2002; Doligez et al., 2004). Hawaiian flatwing male crickets (*Teleogryllus oceanicus*) exploit the sexual calling song of singing males to encounter sexually receptive females (Zuk et al., 2006; Pascoal et al., 2014). Female Trinidadian guppies (*Poecilia reticulata*) observe the mating interactions of other females and copy their decisions (Dugatkin, 1996; Godin & Hair, 2009). Bats use echolocation (Simmons et al., 1979; Seibert et al., 2015) to detect and localize their prey. Communication, however, is not exclusive of animals, as plants, for example, produce volatile organic compounds to self-induce resistance to attacks by pathogens or herbivores (Heil & Karban, 2009), or to recruit pollinators and herbivore enemies (Pichersky & Gershenzon, 2002). Even bacteria use quorum sensing signalling to coordinate the expression of extracellular enzymes that degrade macromolecules (Keller & Surette, 2006). The information that is used in all these examples is inherently different, deriving either from signals or social cues, but being, in both cases, of key importance to the decisions or opportunities that each of these organisms will face in the future.

Signals and social cues are characterized and distinguishable by two major characteristics: intentionality – which is exclusive of signals – and reliability – which has been described in the literature as the main trait of cues (Maynard Smith & Harper, 2003; Danchin et al., 2004; Théry & Heeb, 2008). However, the assumption that cues are always reliable needs to be revised. Here, we defend that when signals are intercepted by bystanders – individuals not targeted by the sender – the transmission of information is no longer intentional but inadvertent. Under these circumstances, signals should be considered cues – to which we call “interceptive cues”. By doing so, it becomes recognizable that when intercepted signals convey dishonest information – which reduces signal reliability – the cues that derive from them can also convey dishonest information, potentially engaging bystanders, as much as intended receivers, in erroneous decision-making. With this new definition of a type of a cue, the concepts of signal and social cue become more coherent. Moreover, it opens the possibility for new hypotheses and predictions about the evolution of signals that have not yet been addressed.

In this paper, we review the concepts of communication, signal and social cue, presenting how they fundamentally differ in terms of the intentionality and reliability of the information they provide (see Table 1). We then introduce the concept of the interceptive cue (see also Table 1) with a few examples and discuss its importance to the understanding of the evolution of communication systems, namely the evolution of signal dishonesty, but also signal conspicuousness and function.

Table 1. Differences between signals, interceptive cues and non-interceptive cues

		Social cues	
	Signals	**Interceptive cues**	**Non-interceptive cues**
Definition:			
	Specialized behaviours, physical features or markings (e.g. chemical, visual, acoustic or electrical) that evolved to convey information to a given receiver, in a given context.	Social cues that derive from signals. Specialized behaviours, physical features or markings (e.g. chemical, visual, acoustic or electrical) that convey information to a given bystander, in a given context, but that did not evolve to do so.	Social cues that do not derive from signals. Non-specialized (non-signalling) behaviours (actions or interactions) or physical traits that convey information to a given bystander, in a given context, but that did not evolve to do so.
Type of information:			
	Intentional social information.	Inadvertent social information.	Inadvertent social information.
	Produced by selection.	Not produced by signal selection.	Not produced by signal selection.
	Communication in the strict sense.	Communication in the broad sense.	Communication in the broad sense.
		Interceptive eavesdropping.	Interceptive and social eavesdropping.

		Social cues	
	Signals	**Interceptive cues**	**Non-interceptive cues**
Communication system:			
Sender	Intentional Produces information to targeted receivers.	Inadvertent Releases information to opportunistic receivers, the bystanders.	Inadvertent Releases information to opportunistic receivers, the bystanders.
Receiver	Intentional Receives information from the signalling actions of designated senders.	Intentional or inadvertent. Intercepts or perceives information from the signalling actions or interactions of senders.	Intentional or inadvertent. Intercepts or perceives information from the non-signalling actions or interactions of senders.
Message	Adaptive representation of a state or condition about the sender that the receiver can perceive and respond to, benefiting the sender.	Representation of a state or condition about the sender to which the bystander can perceive and respond to, being neutral, positive or negative to the sender.	Facts about the sender (actions and interactions) that the bystander can perceive and use for future action, being neutral, positive or negative to the sender.

		Social cues	
	Signals	**Interceptive cues**	**Non-interceptive cues**
Information reliability:			
Accuracy	The precision with which the signal is associated with a specific context.	The precision with which a signal (and consequently the interceptive cue) is associated with a specific context.	The precision with which the non-interceptive cue (from the performance or the decisions made by the sender) is associated with a specific context.
	Signal errors reduce information accuracy (they are inadvertently produced).	Signal errors produce erroneous interceptive cues and reduce information accuracy.	Performance always provides accurate information. Wrong decisions may produce erroneous non-interceptive cues, reducing information accuracy.
Honesty	The rectitude with which the signal represents the normal message to which receivers adaptively evolved to respond to.	The rectitude with which a signal (and consequently the interceptive cue) represents the normal message to which receivers (and possibly bystanders) adaptively evolved to respond to.	The rectitude with which the non-interceptive cue represents facts about the sender (actions and interactions) to which bystanders may have adaptively evolved to respond to.
	Dishonest signals deviate from the normal message (they are intentionally produced).	Dishonest signals produce dishonest interceptive cues that deviate from the normal message.	Non-interceptive cues always provide honest information.

6.2. Communication in broad and strict sense

Taken together, the examples described above fit the definition of **communication in the broad sense**. The definition considers as communication «any interaction taking place between an individual acting as sender who delivers some information to another individual that acts as a receiver who uses this information to make a decision» (Théry & Heeb, 2008). According to information theory (Shannon, 1948), an event is said to carry information about another event or circumstance, if the first is predictive of the second. In a biological setting, a sender is said to provide information to a receiver if the sender's behaviour and/or physical traits (e.g. a sexual ornament or an alarm call) are predictive of the sender's current state or intentions (e.g. a male's sexual attractiveness), or with events or circumstances in the environment (e.g. the presence of a predator) (Owren et al., 2010). The information acquired by the receiver is therefore reducing the receiver's uncertainty about the sender and/or about the environment (Shannon, 1948; Danchin et al., 2008; Scott-Phillips, 2008; Schmidt et al., 2010).

Importantly, the interaction between sender and receiver does not need to be direct, as in many cases organisms play the role of non-targeted receivers (the bystanders). In these cases, bystanders gather information on the actions or interactions of other individuals, a behaviour known as **eavesdropping** (McGregor, 1993; Mcgregor & Dabelsteen, 1996). If the information gathered is based on the actions of senders, it is a case of **interceptive eavesdropping** (Wiley, 1983; Bradbury & Vehrencamp, 1998; Peake, 2005). If the information gathered is based on the interactions between senders and receivers, it is a case of **social eavesdropping** (McGregor, 2005; Peake, 2005; Bonnie & Earley, 2007; Earley, 2010). There is interceptive eavesdropping in flatwing male crickets that detect and use the sexual signals of other males to seize females, and there is social eavesdropping in female guppies and collared flycatchers that use the outcome of the breeding interactions of other breeding pairs to decide with whom to mate and where to breed, respectively. Therefore, an individual acting as a sender of information could be inadvertently transferring information to a certain audience, that is, without the intention of doing so. In the taxonomy of biological information, proposed by Wagner and Danchin (2010), organisms in these circumstances are producing **inadvertent social information**, also known as **social cues**.

On the other hand, when the interaction taking place between a sender and a receiver is a direct one – that is, when the sender is intentionally performing specialized behaviours, or carrying specialized colour markings, or producing specialized chemicals and other attributes – this is called **intentional information**, or **signals** (Barnard, 2004; Théry & Heeb, 2008; Wagner & Danchin, 2010). Together with the response by the receiver, this is what constitutes a system of **true communication** or **communication in the strict sense** (Barnard, 2004). In the examples above, this is the case of the calling crickets, bats, plants and bacteria. In particular, when the individual acting as the sender is also the information receiver, it constitutes a system of self-communication (Théry & Heeb, 2008). The use of echolocation by bats, and of organic volatile compounds to self-induce resistance by plants are examples of self-communication.

The term "intentionality", in the context of a signal, should not be confused with intelligence, consciousness, or higher-order cognitive abilities. These abilities could be involved in communication, but this assumption is not necessary and it would exclude non-animals from any definition of true communication. The intentionality of a signal is related to the evolutionary process, which means that the signal has an adaptive function (Maynard Smith, 2000; Maynard Smith & Harper, 2003; Scott-Phillips, 2008; see also Townsend et al., 2017): the individuals producing the signal (i.e. the correlation between events), as well as those receiving it, (i.e. perceiving that correlation), have an advantage and are selected positively because of it, while the individuals that do not produce, nor receive, the signal are not. In other words, the signal can be seen as an adaptive *representation* of a given message (the correlation between events), produced by the sender in a given context, to which the receiver will respond to (Artiga, 2014; but see Rendall et al., 2009 and Owren et al., 2010). In the game-theoretic sense, the adaptive *representation* is dependent on the minimal accuracy of the signal – the conditional probability that the receiver will correctly associate a signal with a context – that must be achieved for the sender and the receiver to gain enough benefits from the information exchange (Bradbury & Vehrencamp, 2000). For example, in the case of the Hawaiian crickets, the *representation* of the males' calling song – that they produce from their territories – would be something like *females, you can meet me here*. The minimal accuracy would be the correct association by the females of the males' calling song with their location in the context of mating. In other organisms, such as plants, the *representation* could simply be the outcome of the genetic and biochemical mechanisms that allow the perception of the signal (Heil & Karban, 2009), and the minimal accuracy the correct association between the signal – a given volatile organic compound – and a certain type of tissue damage in the context of an herbivore attack, for example.

There is intentional communication even towards bystanders, if the sender becomes aware of their presence and, by that fact, changes the behaviour accordingly. This is called the **audience effect** (McGregor & Peake, 2000; McGregor, 2005; Matos & Schlupp, 2005; Earley, 2010), which makes bystanders become intended receivers of the information, though they might not be directly interacting with the sender. In the fighting fish (*Betta splendens*), for example, when two males are in a fighting interaction – signalling towards each other with aggressive behaviours –, and if a female audience approaches and becomes noticed by the males, the nature of the male-male interaction changes. In this scenario, male fighting fish reduce the intensity of aggressiveness and increase the intensity of signals used by females in male-female interactions. Instead, if a male audience approaches and matches in size with the displaying males, there is no decrease in the displayed aggressiveness (Doutrelant et al., 2001). Many other examples of audience effect have been described and reviewed in the literature (McGregor & Peake, 2000; Matos & Schlupp, 2005; Earley, 2010), acknowledging the importance of considering the entire communication network when it comes to understanding the contexts in which signals are produced and have evolved.

6.3. Intentionality and reliability of signals

Signals are, therefore, intentional information that is produced by senders to targeted receivers, having evolved when they convey, with a minimal accuracy, a valuable message to the receiver in a specific context (Bradbury & Vehrencamp, 2000; Maynard Smith, 2000; Maynard Smith & Harper, 2003; Scott-Phillips, 2008). The minimal accuracy of a signal often needs to be quite high for the message to be reliable and consequently elicit a response from the receiver (Bradbury & Vehrencamp, 2000). For example, male crickets need a good accuracy in producing their sexual call within the same acoustic range, and female crickets need a good cognitive and phonotaxis accuracy in detecting, interpreting and following that one signal. However, the signal does not need to be always correct for the communication system to evolve. Sender errors are part of the communication system, as highly accurate signals may be costly to produce to the sender and unnecessary, as well as costly to perceive by the receiver (Bradbury & Vehrencamp, 2000 and references therein).

However, signal reliability does not only depend on minimal accuracy levels but on honesty as well. Accuracy is an intrinsic property of the evolving communication system and cannot be manipulated by the sender, but honesty can. When signals are dishonest, they do not entirely represent the *normal* message to which receivers adaptively evolved to respond to (Maynard Smith & Harper, 2003; Artiga, 2014), representing a cost, instead of a benefit, to the receiver. Sexual signals, for example, may be dishonest when low condition males invest as much as high condition males in sexual signalling, dissociating courtship vigour from the male condition, and trapping females into low-quality mating interactions. Such dishonest signalling allows low condition males to secure current reproduction, although their exaggerated energetic investment may prevent future breeding attempts (the terminal reproductive investment hypothesis; Clutton-Brock, 1984; Kokko, 1997; Wong & Candolin, 2005; Harrison et al., 2013).

Despite the possibility of dishonest signalling, communication by signals should, on average, benefit the receiver, as the receiver response is also an adaptive response. In other words, the part of dishonest information carried by signals should not be too frequent, or too costly, for the receiver to keep associating a certain signal with the right context. Otherwise, receivers would be selected to ignore those signals (Bradbury & Vehrencamp, 2000; Maynard Smith & Harper, 2003; Théry & Heeb, 2008; Davies et al., 2012; Artiga, 2014). In the terminal reproductive investment example, males producing honest sexual signals should be common enough in the population, so that the costs of mating with dishonest males do not affect females too often. Alternatively, females may use other, sometimes more reliable signals of male condition, like male-male competition, to facilitate mate choice (Wong & Candolin, 2005).

This equilibrium also applies to communication systems between heterospecifics, such as the cases of mimicry and aggressive mimicry (Artiga, 2014), where one species (the mimicking one) exploits a sensory bias of the other (the model species). These mimicries are maintained because responding to the model species (the honest signaller) still provides more benefits to receivers than the costs of responding to the mimicking species (the dishonest signaller), or because the frequency of encounters with mimics is lower than that of encounters with models (Maynard Smith & Harper, 2003). Classic examples of these include: the edible polymorphic butterfly (*Papilio memnon*) that mimics various distasteful model species (Batesian mimicry; Mallet & Joron, 1999); the predator firefly (*Photuris versicolor*) that preys on other firefly species using deceptive mating signals (Lloyd, 1975; Gronquist et al., 2006); and vocal mimicry by song birds (Kelley et al., 2008; Flower, 2010), where one species mimics calls or songs of one or several other species to gain some sort of advantage, like giving the false impression that a certain habitat patch is already occupied by potential competitors or predators, as a way to avoid competition or to steal food.

6.4. Intentionality and reliability of social cues

Cues, contrary to signals, are inadvertently produced and consequently have not evolved within a system of true communication (Danchin et al., 2008). Because they are not produced by selection for communication (Danchin et al., 2008; Wagner & Danchin, 2010), organisms do not intentionally manipulate the way the information is transmitted. They are simply engaged in performing well their activities, and «are selected to perform as well as possible, rather than to inform others» (Danchin et al., 2004). Therefore, because their actions or interactions did not evolve as signals, cues are considered highly reliable sources of information (Valone & Templeton, 2002; Danchin et al., 2004; Théry & Heeb, 2008; Danchin et al., 2008). However, what has been poorly recognized is that cue reliability, similarly to signals, also depends on accuracy and honesty.

Cues are only highly accurate if based on the performance of organisms. Performance provides truthful information about the current condition of the individual that produces the information, or about the current quality of the resources being used (Danchin et al., 2004; Wagner & Danchin, 2010). This is not necessarily the case for cues extracted from behavioural decisions made by others. This is because individuals sometimes make wrong decisions, which may engage bystanders in sub-optimal decision-making behaviours (Giraldeau et al., 2002). For example, a cue based on performance is the breeding success of collared flycatchers extracted from the parental feeding activity of breeding pairs, which inform about the suitability of a breeding patch (Doligez et al., 2002; Doligez et al., 2004). Because breeding success is not a signal, pairs neither reduce nor increase the number of chicks they can successfully feed to convey less accurate information to others. Instead, they are engaged in raising as much as offspring as they presently can. Contrastingly, a cue based on a behavioural decision is that of guppy females that copy the mate choices of other females (e.g. Dugatkin, 1996; Godin & Hair, 2009). This behaviour is called mate-choice copying and is thought to be advantageous because it generally allows females to assess more effectively and faster the quality of potential mates (reviewed in Vakirtzis, 2011 and Varela et al., 2017, in revision). However, this type of cue may sometimes convey less accurate information if the demonstrator female makes a wrong decision, leading the bystander to copy the acceptance of a low-quality male or the rejection of a high-quality male. This can potentially engage several observer females into erroneous informational cascades (Gibson & Höglund, 1992; Giraldeau et al., 2002). Therefore, copying from reliable model individuals (e.g. older and more experienced) is preferable and has been revealed in several species (e.g. Hill & Ryan, 2006).

This demonstrates that the information that can be extracted from social cues may not always be totally accurate. Similarly, it may not always be totally honest. Indeed, cues that derive from signals – the interceptive cues – cannot be considered sources of honest information if derived from dishonest signals (more details in the next sections and in Table 1).

6.5. When signals become interceptive cues

It is not rare that examples such as that of the *Trachops cirrhosus* bat, who localizes its prey – the túngara frog (*Engystomops pustulosus*) – by its courtship song (Tuttle & Ryan, 1981; Ryan et al., 1982; Trillo et al., 2013), raises doubts amongst university students of animal behaviour about the definition of a signal. This is a classic case of signal exploitation by a predator, and the students raise the question of whether the frog sexual signal should be considered a cue to the predator instead of a signal since the frog does not produce a courtship song with the intention of attracting the predator. This can also happen intraspecifically, as is the case of the Hawaiian flatwing male crickets, who localize their singing conspecifics by their courtship songs (Zuk et al., 2006; Pascoal et al., 2014).

This doubt is legitimate because the definition of a signal is intentional social information and when signals are intercepted by non-targeted receivers the condition of intentionality does no longer apply. Danchin et al. (2008) recognise this, by classifying intercepted signals as social cues. We suggest developing the concept further, giving the name of "**interceptive cue**" to this type of information and clearly distinguishing interceptive cues from non-interceptive cues (see Table 1).

The concept of interceptive cue is associated with the concept of interceptive eavesdropping (Wiley, 1983; Bradbury & Vehrencamp, 1998; Peake, 2005). The difference between the two is that interceptive eavesdropping is the communication system – a bystander intercepting information from the signalling actions of senders – and the interceptive cue is the actual information – the *representation* – within the communication system that is detected by the bystander. Then, depending on the value of the information, the bystander will respond in some specific way to the interceptive cue. The response – known as the eavesdropping or bystander effect (Oliveira et al., 1998; Earley & Dugatkin, 2002; Dugatkin, 2014) – will then affect both the fitness of the bystander and that of the sender. For *T. cirrhosus* bats, their response to the males túngara frogs' courtship song is their predatory attack on its prey (Trillo et al., 2013).

6.6. When interceptive cues are dishonest

The two major characteristics that distinguish interceptive cues from non-interceptive cues are that the former are derived from signals and that, when signals are dishonest, interceptive cues will also convey dishonest information to the bystander, as much as the signal itself will convey dishonest information to the targeted receiver. Non-interceptive cues, on the other hand, because they are not derived from signals, they are not manipulated by the sender and, hence, are always sources of honest information (Table 1).

For example, many species use false alarm calls of predator warning so that other individuals, sharing the same feeding patch, will hide and the caller can steal their food (e.g. Whiten & Byrne, 1988; Kaplan, 2004; Flower, 2010). Bystander individuals – that were extracting information from the profitability of that feeding patch or that were in adjacent feeding patches – might hear the same false alarm calls and use similar avoidance behaviours towards a non-existent common predator. Sexual signals, which are typically quite conspicuous, when they are dishonest they will often be received not only by intended female receivers but intercepted by bystander females as well, who could equally decide to mate with those males. Dishonest sexual signals could also be intercepted by bystander rival males who may decide to begin a territorial fight or retreat from a potential one.

As far as we know, the fact that intercepting dishonest signals generates dishonest interceptive cues has never been acknowledged. However, the concept of interceptive cue and its quality of being honest or dishonest are important increments to the taxonomy of biological information (Wagner & Danchin, 2010) and to communication theory, in general. This is so, because intercepting honest or dishonest cues may have different consequences to the bystander and, eventually, affect the evolution of signals and communication systems.

6.7. Interceptive cues and the evolution of signals

Knowing that signals did not evolve to be detected by bystanders, but that a bystander– the communication network – may be at a signal's reach and affect its outcome (McGregor & Peake, 2000; McGregor, 2005), the outstanding question is what are the evolutionary consequences of bystander responses to interceptive cues to the way a signal is produced? It is likely that the evolutionary trajectory of the signal, namely the proportion of dishonest information, will depend on the costs and benefits to the sender from having its signal intercepted and to the bystander from intercepting the signal (see Rowell et al., 2006). Three evolutionary outcomes are possible: (1) if signal interception is neutral to the sender, the signal is not expected to change; (2) if the sender derives benefits from the bystander response to the interceptive cues, the bystander should become an intended receiver and the interceptive cue an actual signal to the bystander; contrarily, (3) if the bystander response to the interceptive cues is costly to the sender, the signal should evolve to try to exclude the bystander from the communication network. In both positive and negative scenarios, the evolutionary outcome could be a change in the signal conspicuousness, the signal function and/or the proportion with which signal dishonesty is employed, changing, at the same time, the bystander's adaptive response. Particularly, what we wish to highlight is that in a sender-receiver communication system the proportion of dishonesty in a signal can be modelled by the presence of the bystander.

1) Neutral interceptive cues to the sender

One example of neutral interceptive cues to the sender is when signallers and bystanders do not share the same food preferences while sharing the same ecological space. For example, when food sources are divisible they can be shared with no competition. And those that found the food may vocalize to attract their mates and inadvertently call the attention of interceptive bystanders as well. Food calls are known from bird species that generally feed in flocks, like the house sparrow (*Passer domesticus*; Elgar, 1986), the siskin (*Carduelis spinus*; Senar & Metcalfe, 1988) and the domestic chicken (*Gallus gallus domesticus*; Marler et al., 1986). Known advantages of calling flock mates to these food sources is protection against predators, via dilution effects and more efficient vigilance (Mangel, 1990), and mate attraction, via males signalling to females that they found food and subsequently becoming more attractive to them (Marler et al., 1986; Hauser, 1997). Dishonest food calls could be produced if senders seek for protection or to attract mates, while not having found any food (Hauser, 1997).

Interceptive bystanders, despite hearing the food calls, would not join the flock and, therefore, would not represent neither a benefit – which could arise if bystanders' presence would increase the dilution effect – nor a cost – if bystanders were attracted to the same type of food – to the sender. This neutral influence from the presence of bystanders would, thereby, not affect the evolution of the signal, nor its proportion of honest and dishonest information. Only if paying attention to the signal would carry a cost to the bystander – by becoming distracted it might lose a feeding or mating opportunity, or become more vulnerable to predation –, the bystander may be selected to ignore the signal or to avoid habitat overlap with the sender.

2) Beneficial interceptive cues to the sender

Beneficial interceptive cues to the sender can be found, for example, in invasive plants (Vilà et al., 2009), which originally evolved signals to attract pollinator species present in their native distribution area. When plants invade a new area, their signals may be intercepted by a new set of pollinator species. In this case, plants benefit with the inadvertent information they provide and, with time, the signal, and its proportion of honest and dishonest information will likely evolve to more accurately target the new set of pollinator species. In other words, the signal becomes more conspicuous, or salient, to the sensory spectrum of the new pollinators. Adaptive radiation in plants is, indeed, a well-documented phenomenon when a species spread into new environments and face new sets of pollinators (Kay & Sargent, 2009). Well documented is also the evolution of dishonest pollinator rewards by plants, as pollen, nectar, fragrances and resins are costly to the sender in terms of seed production and plant growth (Pélabon et al., 2012). It is, therefore, likely that at some point in the coevolutionary process between the signalling efforts by plants and the pollinator responses by bystanders that the evolution of dishonesty will also occur. Dishonesty could even be a plant primary strategy – as is the case of at least one-third of orchid species (Cozzolino & Widmer, 2005) – through mimicry of honest model species or exploitation of perceptual pollinator biases (Schaefer & Ruxton, 2009). Since exploitation of perceptual biases has been suggested as the evolutionary precursor of mimicry (Schaefer & Ruxton, 2009), and a perceptual bias increases a pollinator probability of intercepting signals not addressed to them, then interceptive cues could also be the evolutionary precursors of intended exploitative signals.

3a) Costly interceptive cues to the sender and signal conspicuousness

Costly interceptive cues, on the other hand, are expected to affect the sender's signalling behaviour, namely signal conspicuousness. In the example of male túngara frogs, the evolutionary trajectory of male sexual signals has been deviating from the predators' acoustic abilities to detect the signal, generating an arms race between senders and bystanders (Tuttle & Ryan, 1981; Ryan et al., 1982; Trillo et al., 2013). However, *T. cirrhosus* bats not only use interceptive cues to locate their prey but also social cues generated from the frogs' calling action – the water ripples, which are a by-product of the males inflating their vocal sac to produce sound (Halfwerk et al., 2014). By using both cues, bats are more accurate in finding their prey than if using only the vocalizations or the water ripples, imposing significant costs to the frogs calling behaviour. As a response, frogs try to reduce the amount of inadvertent information they provide to their predators, by moving into dense vegetation cover, where the water ripples propagate for shorter distances (Halfwerk et al., 2014). The interceptive cues, on the other hand, can only be reduced by changing, throughout evolutionary time, the acoustic structure of the males' song (Tuttle & Ryan, 1981).

Similarl to túngara frogs, Hawaiian crickets also face predation pressure from an acoustically orienting predator, the parasitoid fly *Ormia ochracea* (Zuk et al., 2006; Pascoal et al., 2014). But here, the deviation from the acoustic perception of the predator was radical, as a mutation that first appeared on the island of Kauai changed the male wing structure, making singing impossible (Zuk et al., 2006; Bretman & Tregenza, 2007). This flatwing male morph is not detected by the parasitoid fly and, hence, the mortality of flatwing males is much reduced. The advantage was so significant that, in only 12 to 20 generations since the mutation appearance, most males (circa 90%) are now flatwing (Zuk et al., 2006; Pascoal et al., 2014). This poses important challenges to the species reproduction, as females still use the acoustic signal of the remaining singing males to localize mates, and the remaining singing males are not only more exposed to the parasitoid fly, but also to competition from flatwings that act as satellites to intercept females. At present, the frequency of the parasitoid fly is very low in Kauai, releasing the selective pressure on singing (Bretman & Tregenza, 2007). For their part, flatwing males are also adapting to their silent condition, showing an evolutionary divergence of their chemical signals from those of singing males (Simmons et al., 2014).

3b) Costly interceptive cues to the sender and signal function

Instead of reducing signal conspicuousness, a different outcome of costly interceptive cues is the evolution of signal dual utility, with the original function of the signal being co-opted to a second context. This can happen when females eavesdrop on male-male competitive interactions, extracting information from the quality of the males' armament or from the outcome of their contests to generalize about their potential quality as mates. Or the other way around, when males eavesdrop on other males' sexual signals, extracting information from the quality of their ornaments and condition or from the outcome of their mating attempts to generalize about their winning probability in a prospective dominance contest. This is the dual utility hypothesis that suggests that male armaments and ornaments are as good indicators of an individual's fighting ability as of his quality as a mate (Berglund et al., 1996; Wong & Candolin, 2005). By this reasoning, if the signal has first evolved by intersexual selection, male bystanders that intercept the signal exert an additional selective pressure over the information that the signal provides. The outcome is the male bystander becoming an intended receiver, much like the original female receiver. This evolutionary pathway has been suggested to explain the dual utility of vertical bars in the northern swordtail fish (*Xiphophorus* spp.) (Morris et al., 2007), and many other examples have been given for the co-option of male armaments into ornaments, like the red deer's (*Cervus elaphus*) roar (Berglund et al., 1996).

All these examples are focused on male physical or acoustic traits. But the same reasoning can also be applied to sexual behaviour traits: if a male bystander eavesdrops on the sexual behaviour of a displaying male towards a female, reducing the conspicuousness of the sexual signal would not be advantageous in face of the female audience. Hence, the most likely evolutionary trajectory is the co-option of the sexual signal to a male-male competitive context. As a result, males may display sexually directly towards each other, but with a different function of the male-female interaction – that of intimidation. There are several examples in the literature of male same-sex sexual behaviour (reviewed in Bailey & Zuk, 2009). One of the proposed functions for this behaviour is indeed male-male sexual competition. Here, we suggest that the evolutionary pathway for the arisen of this behaviour may be the use, by rival males, of interceptive cues.

3c) Costly interceptive cues to the sender and signal dishonesty

In the examples presented above, we assumed signal honesty. But costly interceptive cues to the sender are also likely to affect the proportion with which signal dishonesty is employed. Explicitly, our hypothesis is that the evolution of signal dishonesty is not only dependent on sender-receiver interactions, but also on how costly the exploitation by bystanders of the interceptive cues is to which they adaptively evolved to respond to. The costs and benefits resulting from bystander and receiver responses to a signal will affect the equilibrium between the part of the honest and dishonest information that will evolve in the signal (see also Rowell et al., 2006).

For instance, male fiddler crabs (*Uca* spp.) have one enlarged claw with dual utility, used both to attract females and to intimidate rival males (Lailvaux et al., 2009; Bywater & Wilson, 2012). Males wave their claw to signal performance, but there is signal dishonesty when claw size does not reliably relate with claw strength (Bywater & Wilson, 2012). Weaker claws result from low investment in the maintenance of the original claw muscle (Bywater & Wilson, 2012), or from low muscle growth and poor mechanical functioning of replacement claws (Bywater et al., 2015). In fact, after fights with rivals or attacks from predators, males sometimes lose their original claw. They can regenerate a replacement one that is similar in size to the original, but significantly weaker and different in shape (Bywater et al., 2015). The frequency of dishonest signalling, both for original and replacement claws, is dependent upon the risk of punishment by honest receiver males that may decide to engage in a fight with dishonest males (Bywater & Wilson, 2012). Males with replacement claws do, indeed, avoid fights with other males (Reaney et al., 2008), and males with original claws investment more in muscle maintenance when in high than when in low-density populations (Bywater & Wilson, 2012). Accordingly, Bywater & Wilson (2012) suggested that claw strength reliability is dependent upon variation in the population density of potential receivers. From our perspective, a population of potential receivers can also be seen as a population of potential bystanders. And like intended receivers, a high population density of bystanders can affect the risk of punishment to the sender, as a male that loses a fight is more likely to lose future fights with bystanders. This is so because a bystander is generally more aggressive towards a male that loses a fight than towards a winning rival – the bystander effect (Earley & Dugatkin, 2002). Consequently, the density of bystanders in fiddler crabs, but in species in general, may have an important role in modulating the proportion of dishonest signalling in a population (see also Rowell et al., 2006).

Alarm calls are also good examples of a signal type that can be frequently dishonest. A well-known case of deceptive alarm calls is that by fork-tailed drongos (*Dicrurus adsimillis*) that make meerkats (*Suricata suricatta*) and pied babblers (*Turdoides bicolor*) to flee away abandoning their food items, which are then stolen by the deceptive drongos (Flower, 2010). Drongos fake their own alarm chink calls and those from glossy starlings (*Lamprotornis nitens*), among other species, using vocal mimicry to deceive heterospecifics about the presence of a predator. Hence, all species with which drongos share their habitat – the South African Kalahari desert – are potential intended receivers and bystanders of their true and false alarm calls. What is remarkable in this system is that the frequency of false alarms is quite high, as almost half of the alarms observed by Flower (2010) were false. Probably as a response to this high frequency of deceptive information, pied babblers were no more likely to abandon their food after drongo-specific true or false alarm calls than after non-alarm calls. Meerkats, on the other hand, did abandon their food more often after true or false drongo-specific alarm calls. In contrast, when drongos mimicked glossy starlings alarm calls, both pied babblers and meerkats abandoned their food more often than after a non-alarm call, though pied babblers tended to respond more towards glossy starlings' true alarm calls than drongo-mimicked false alarm calls. Hence, when drongos specifically target meerkats to steal food (Flower, 2010), we could ask how bystander pied babblers influence meerkats response towards drongos' dishonest signalling strategy. If pied babblers are more selective of drongos' alarm calls than meerkats, as they seem to be, meerkats could learn to use pied babblers' behaviour as an additional source of reliable information about a predator's approach. Consequently, drongos would have to adjust the proportion of dishonest information that would still successfully deceive both meerkats and pied babblers. One way to test this hypothesis would be to verify whether meerkats are more likely to abandon their food when pied babblers do the same and whether drongos more often use mimicked glossy starling alarm calls to deceive meerkats when pied babblers are present. On the other hand, drongo-specific false alarm calls could remain quite effective in deceiving meerkats even in the presence of pied babblers, if the cost of not fleeing away when the alarm happens to be true is higher to meerkats than to pied babblers.

The evolution of signal dual utility due to interceptive cues also opens the way to the evolution of asymmetric signal dishonesty, that is, an asymmetric proportion of dishonesty transmitted to a signal's dual audience (Rowell et al., 2006). As seen above, claw waving in fiddler crabs is used to signal performance to both females and rival males. However, females are sensitive to wave rate – they prefer males that wave faster – but not to claw shape and strength (Callender et al., 2013). By consequence, they do not distinguish between original and replacement claws. This means that the reliability of claw waving as a signal of performance to females and rival males is asymmetric: the proportion of dishonest signalling towards females is higher than that towards rival males, as the costs of punishment by honest rival males could be severe. Females, on the other hand, can have access to signal honesty and facilitate mate choice, by promoting competition between males (Montgomerie & Thornhill, 1989; Berglund et al., 1996; Wong & Candolin, 2005). This puts females in the role of bystanders and make them users of honest interceptive cues instead of dishonest signals. In the pipefish (*Syngnathus typhle*), for example, a species with reversed sex-roles, females display a temporary body marking to both males and rival females to signal attractiveness and dominance, respectively. Males, however, prefer to choose a mate after having observed a female-female fighting interaction than after having been courted directly by a female (Berglund & Rosenqvist, 2001).

6.8. Concluding remarks

It is of general knowledge that interceptive eavesdropping, mainly when costly to the sender, could originate evolutionary arms races between the properties of the signal produced by the sender and the abilities of the bystander to detect the signal (Peake, 2005). What was lacking in the literature was an unequivocal usage of the concepts of *signal* and *social cue*. If we consider the unique and distinguishable characteristics of signals as intentional social information, and of social cues as inadvertent social information (Wagner & Danchin, 2010), then, it becomes clear that when a signal is intercepted by a non-targeted and non-detected bystander it should no longer be considered a signal but a social cue instead. At the level of the sender-receiver interaction, the signal remains a signal, but at the level of the inadvertent sender-bystander interaction, the signal becomes a cue – an *interceptive cue* as we named it – because it was not produced to intentionally communicate with the bystander. Understanding this shift from a signal into a social cue helps to acknowledge that the concepts of intentional and inadvertent information are essential to a better comprehension of a communication system and of how it evolves. Here, we have highlighted this shift and by that means demonstrated that when interceptive cues derive from dishonest signals they constitute dishonest information as well. This corollary change the previous one that social cues are always honest information because they derive from inadvertent information that cannot be manipulated. By recognizing that a social cue, when derived from a signal, can be dishonest, new predictions can be made about the evolution of signals, namely about the evolution of dishonesty. When a signal is dishonest, and by consequence the interceptive cue as well, it could represent a cost to the bystander as much as to the targeted receiver. Hence, the frequency of dishonest information could determine whether the bystander will keep intercepting the signal or not. This is important, because the strength of the selective pressure exerted by the bystander over the sender may change the equilibrium proportion of honest and dishonest information produced by the sender, as a counter-adaptation. Complementarily, this will affect the very own sender-receiver interaction, that is, the benefits that the sender will obtain from its regular audience of targeted receivers. Therefore, signal dishonesty may not only be viewed as an evolutionary product of sender-receiver interactions but of the interactions between all parties involved – senders, receivers and bystanders – affecting the overall dynamics of the entire communication network.

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CHAPTER 7.

General discussion

Chapter 7. General Discussion

This thesis brings novel knowledge about the relevance of social conspecific information to male mating decisions and to the evolution of male mating behaviours. Overall, my work shows that in guppies, and potentially in other species, male mating decisions are highly plastic and are based on multiple and complex social information (chapters 2 to 4). Additionally, the empirical findings described in this thesis suggest that male behavioural plasticity is adaptive, since males adjust their behaviour based on social information in a way that affect their reproductive success. Finally, results presented in the last chapters (chapters 5 and 6) highlight the important role of bystanders in the evolution of male courtship display and of dishonesty in animal communication. In sum, this thesis contributes to understanding the role of social information in individual decision-making and to behavioural evolution.

The key findings of this thesis will be briefly discussed in this chapter (section 7.1.). In what follows I discuss the limitations of the studies described (section 7.2.), identify future directions (section 7.2.), and finish with some concluding remarks (section 7.3.).

7.1. Key findings

Male mating decisions depend on collected social information

The observation that males alter their mating decisions in response to social cues and signals is not novel (e.g. Johansson and Jones, 2007; Paxton et al., 2007; Bretman et al., 2011; Chaudhary et al., 2017; Houslay et al., 2017). However, work described here sheds new light on the impact of conspecific information on male mating behaviours (chapters 2 to 4). The reasons for this are twofold. First, most studies are still focused on female mate choice and male contests separately (e.g. Procter et al., 2012; Barquero et al., 2015; Lehtonen et al., 2016; Carballo et al., 2017; Kuriwada, 2017; Montiglio et al., 2017; Muschett et al., 2017), while here I show the importance of the communication network to male mating decisions, including both females (with their mating history) and competitor males. Second, the impact of social information to male mating decisions is still incompletely understood, for different reasons: previous studies have not compared confounding variables, such as female abundance and male mating opportunities (e.g. Barry and Kokko, 2010; Jordan and Brooks, 2010; Head et al., 2015); or have examined variables separately, such as competitor presence, and absolute and relative attractiveness (e.g. Leonard and Hedrick, 2009; Mautz and Jennions, 2011; Chaudhary et al., 2017); or evaluated key variables from a different perspective, like studying the correlation between male mating behaviour and sperm competition risk but without including the effect of female mate choice (e.g. Fisher et al., 2016; Tuni et al., 2017).

Male guppies adjust their mating behaviour to multiple conspecific social information

The experimental studies presented in this thesis show that male guppies base their mating decisions on multiple aspects of social information (chapters 2, 3 and 4). This information is collected at different stages of the reproductive period (before as well as during a mating encounter) and could impact current and future mating outcomes and fertilization success.

In chapter 2, I found that males base their decisions on the time interval between encounters with females mating (time between females), investing more on mating behaviours when such time is greater. Previous studies are consistent with this, having demonstrated a correlation between male mating investment and mate encounter rate (e.g.

Segoli et al., 2006; Ferkin and Ferkin, 2016; Houslay et al., 2017; but see Barrett et al., 2014). The novelty of my work is that I found that both time between females and time between actual mating opportunities determined the mating behaviours of males, such as mating effort, mating tactics employed, and mate choice.

Correspondingly, my work allowed to understand that male mating behaviour depends also on different social information associated with competition risk. In chapter 3, I found that the mating tactics employed are related to both the presence of other males and to their secondary sexual traits. Moreover, results presented in chapter 4 show that adjustments of male mating behaviour to competition risk – both at the mating and sperm competition levels – depend not only on the presence of another male, but also on males' order of arrival to a female.

Male guppies adjust their mating behaviour to complex conspecific social information

Besides showing that male guppies use different types of social information, I found that they adjust their behaviour to complex social information, i.e. with direct and indirect implications and with opposing outcomes for their reproductive success (chapter 2 and chapter 4, respectively). In addition, I found that male guppies adjust their mating behaviour in complex ways, as they adopted different strategies to different signals and social cues (chapters 2 to 4).

Differential behaviour between sexes has been related with fitness asymmetries (Bateman, 1948; Burns and Broders, 2015; Canal, Jovani, and Potti, 2012; Shuster, 2009). While for males the limiting factor for their fitness is the sexual resource (females), for females instead it is the habitat/food resource which is determinant to raise their offspring (Emlen and Oring, 1977). For this reason, the male sex is general the one that invests more time and energy searching for sexual partners. So, male reproductive success is highly dependent on the number of mating opportunities (Andersson, 1994; Bateman, 1948; Clutton-Brock, 2009; Fromhage, Jennions, and Kokko, 2016). Interestingly, results in chapter 2 show that, on a first stage, the interval between encounters with females highly impacts male guppies mating effort. Such result suggests that males perceive time taken to find females as a key indicator of future mating opportunities. Consistent with this, previous studies have also shown that male choosiness seems to be related with mate encounter rate and chance of future mating opportunities in other species (e.g. Barry and Kokko, 2010; Head, Jacomb, et al., 2015). My finding with guppies go beyond this correlation by showing that males combine information about time searching for females and time between mating. In fact, it is only when in the presence of females that the time between mating opportunities determines male mate choice. For example, males were choosier when they spent more time without mating and, simultaneously, spent no time searching for patches with females. These findings reveal considerable complexity in male mating decisions that may enhance their fitness.

Further support for this complex decision-making system was provided by my other study on male attractiveness (chapter 3), where I found that males adopted different strategies in response to social cues and signals that provided complementary information on competitor attractiveness – body size (a social cue), and black and orange colouration (signals). This may be explained by the fact that these traits are correlated with female mate choice to different degrees (e.g. large areas of orange pigmentation are preferred by female guppies over other male traits – Endler and Houde, 1995 – included in my study), or because they are correlated in different ways with male fertilization success (e.g. while attractive colourful males produce more offspring due to sperm competition, in contrast, unattractive smaller males produce better competitive sperm – Evans et al., 2003; Pilastro et al., 2004). In this sense, such different types of information are linked with competitor attractiveness but may not transmit precisely the same information.

Furthermore, we found that males model their mating decisions according to their order of arrival to a female, which is related with the likelihood of losing one or both of mating opportunity and sperm competition (chapter 4). Therefore, social information collected during a mating encounter may help males to deal with complex contexts which can have opposing contributions to their mating and fertilization success.

Male mating decisions in response to social information are probably adaptive

The empirical studies presented here strongly support the idea that males adopt strategies to increase their chance of mating and of fertilization success, as well as strategies to overcome lost opportunities and risky contexts. For example, I found that males invested more in mating behaviours after spending more time without females (chapter 2). This investment was in the less risky mating tactic – unsolicited attempt (with which males can mate quickly, regardless of female consent; Houde, 1988; Magurran, 2005). Also, male guppies were choosier when spent more time without mating if they were always in the presence of a female. This suggests that males prefer better quality females to compensate for missed mating opportunities when they can afford to be choosy (when it was easy to find females in a recent experienced environment). This way, males can risk spending energy and time trying to mate with a highly fecund female (generally choosier in several species; e.g. Judge et al., 2014; Rueger et al., 2016; Kuczynski et al., 2017), because, if they fail (for instance, because the female is not sexually interested), they can quickly approach another female and try to mate with her.

Males also invested more in the less costly mating tactic (unsolicited attempt) when there was another male following the same female (chapter 3). In this context males risk losing mating and fertilization success: they face a higher chance of being interrupted when trying to mate (sexual interference), may lose female mate choice if they are less attractive than the competitor male, and will probably face sperm competition. The higher investment in the less costly mating tactic thus seems the best strategy to employ in such a risky situation. This result is in accordance with previous studies that found that males invest less trying to mate when competition risk is high (e.g. under high population density, or male-biased sex ratio – Shine et al., 2003; Casalini et al., 2010; and when competitor males are present – Rouse and Bretman, 2016; Tuni et al., 2017).

However, although the findings described in chapters 2 and 3 support the conclusion that males avoid taking risks, I also found that males sometimes opt for risky strategies. For example, also in chapter 3, I found that male guppies invest in the riskier mating tactic (courtship display) when the competitor male was very attractive for the main sexual trait in guppies (area of orange pigment), that is, when focal males were less likely of being chosen by the female. My interpretation is that males may have adopted a strategy to overcome competition risk, by enhancing their own attractiveness, since courtship display puts on evidence the orange pigment. As discussed in chapter 3, this hypothesis is supported by studies in other species (three-spined sticklebacks and common eggfly butterflies – Kim and Velando, 2014; White et al., 2015; respectively).

Further support for the view that males sometimes invest in a risky strategy is provided in chapter 4, where I show that males invest more when both mating and fertilization success may be compromised. By investing more when they approached a female before a competitor male, males may “give it all”. Under this scenario, males are in advantage due to female sequential mate choice (females are less choosy with the first males that they encounter) but have the disadvantage of sperm precedence (in guppies there is last-male sperm precedence). So, males do not abdicate from following those females because, by leaving the female only after the competitor male, they can try to be the last to mate, and hence the last to transfer sperm. Guppies, along with some species (e.g. Arnqvist and Nilsson, 2000; Barbosa and Magurran, 2006; Parker and Birkhead, 2013; Taylor, Price, and Wedell, 2014), have a promiscuous mating system where paternity is generally shared. Therefore, males can hardly ever find a perfect mating context (such as encountering a virgin female, with no competitor males nearby), and hence hardly ever avoid sperm competition. Thus, males seem to have evolved an ability to distinguish risks taken in each context – whether they are alone with a female or they are the first or second to arrive. Then, males invest more when these risks are reduced or when mating effort may compensate the faced risks.

Overall, the experiments in this thesis suggest that conspecific social information is extremely important to male mating decisions in guppies. This supports the claim that social information reduces environment uncertainty (Dall, Giraldeau, Olsson, McNamara, and Stephens, 2005). Accordingly, an interesting finding in chapter 4 is that, even though there is a great uncertainty about female mating history, a male uses the information that is immediately available to him regardless of what the female had experienced before his approach and will experience after he leaves. Since environmental conditions are constantly changing (particularly the social environment – Taborsky and Oliveira, 2012), it is probably advantageous to collect multiple relevant information to shape decision-making and try to gain advantage from it whenever possible – even if the "lifetime" of this information is very short.

Furthermore, since these results support that behavioural adjustment to social contexts is adaptive, then social information is likely to play a central role in the evolution of male mating behaviours. This is supported by results of the systematic review presented in chapter 5, as well as by the theoretical framework presented in chapter 6, discussed in the next section.

Social information is relevant to behaviour evolution

Male-male courtship display may have evolved due to bystander pressure in some species

The concept of dual function in secondary sexual traits, proposed by Berglund et al. (1996), concerns the evolution of sexual traits that started out with a single function and later acquired a second function. This hypothesis, as these authors first proposed, suggests that weapons used by males to intimidate competitors, have later been used as ornaments to attract females. Although it was not explicit in this study, the authors were considering a communication network where third parties (females, in this case) have considerable influence on the evolution of sexual traits. For example, if females prefer mating with males that have better or more conspicuous weapons (informing about a male's condition and/or ability to win a fight), then it is likely that males increasingly use these weapons to attract females (e.g. in ritualized courtships), and later that these weapons become more exaggerated (e.g. increased in size). This and other studies on signal dual utility or dual function made an important contribution to the field by moving from the traditional compartmentalized view of ornaments versus weapons, to a more nuanced understanding (Fisher, 1930; Kuriwada, 2017; Morris, Tudor, and Dubois, 2007; Snijders, van Oers, and Naguib, 2017). Nonetheless, this hypothesis has been tested mainly in relation to male physical traits (e.g. Kekäläinen et al., 2010; Leitão et al., 2015).

By considering a behavioural trait (courtship display) and its evolution from an ornament into a weapon (i.e. from its assumed primary function of attracting females to the additional function of transmitting information to competitor males), this thesis extends understanding on male-male competition in novel ways. With a systematic review (chapter 5), I found evidences supporting that in some species the pressure exerted by bystander males shaped the evolution of male courtship display. In addition to causing a behavioural adjustment of the displayer male in the presence of the bystander (audience effect), it leads this behaviour to acquire a new type of receiver, the competitor male. In other words, I found evidences that more than causing a change on male-female courtship interaction, the intrasexual competition can shape this mating behaviour more extremely, to a scenario where it manifests even in the absence of females. In fact, previous studies provide evidence that males can deter competitors with courtship displays (e.g. Kuriwada, 2017). This opens the possibility for males to exaggerate this behaviour towards other males the same way as they exaggerate courtship towards female targets.

Frequently, males that display dishonestly to females are lower quality individuals (Brautigam and Persons, 2003; Nielsen and Holman, 2012; Rypstra, Walker, and Persons, 2016; Svensson, Nymam, and Kvarnemo, 2004). This raises the question of whether bystander males also intercept such dishonest displays. If that is the case, the outcome when a bystander male intercepts a dishonest display should differ from the outcome when a display is honest (e.g. the bystander male may withdraw from a fight when the displayer is of inferior competitive ability because its courtship performance was dishonest). To my knowledge, the consequences for the bystander male that detects a dishonest courtship display has not been considered in the literature. Such consequences should have implications for the evolution of male courtship displays, as well as for the evolution of signal honesty in general.

Social cues can be dishonest, and bystanders may play a part on the evolution of dishonest communication

When males are signalling their courtship displays to receptor females, this signal may be intercepted by other females and competitor males (interceptive eavesdropping; Wiley, 1983; Bradbury and Vehrencamp, 1998; Peake, 2005). As I propose in chapter 6, signals that are intercepted become social cues to the bystanders since they were not intentionally produced to communicate with them (taxonomy of biological information; Danchin et al., 2008; Wagner and Danchin, 2010). This way, when signals are dishonest, the interceptive cues are dishonest as well. This means that the bystander can obtain dishonest information from the interceptive cue and make erroneous decisions in much the same way as the targeted receptor.

As with dishonest courtship displays, other dishonest signals can also be intercepted and used by bystanders in a variety of contexts (e.g. food and habitat quality – Danchin et al., 2004; Valone, 2007). Therefore, the theory of how dishonest information is evolutionarily maintained in a population (Maynard Smith and Harper, 2003) should be extended to include the response by the bystander. Consequently, the assumption that at equilibrium honest signals in a population have to be more frequent than dishonest ones so that receivers can continue to respond, should now be re-evaluated, since the balance between honesty and dishonesty is likely dependent not only on receptor but also on bystander responses. If bystander responses to a dishonest social cue are beneficial to senders, the proportion of dishonest information may increase. How receptors cope with an increased proportion of dishonest information should probably be related with the costs of that information, and it is something that deserves further investigation. Male courtship display is an excellent example of this, as dishonesty could play a role in intimidating competitor males. How would females cope with increased levels of courtship dishonesty when male-male competition is high? This could be studied in species where the courtship display has a dual function, such as those listed in chapter 5.

7.2. Limitations and future research

Are male mating behaviour decisions advantageous to them?

The studies presented here suggest an adaptive value of male mating behaviour adjustment linked to the use of information transmitted by conspecifics. It is now necessary to compare mating and fertilization success of males that adopt different strategies, although having access to the same social information. Furthermore, it is necessary to compare the outcome of males that perform the same strategy but in different social contexts. So, for example, I hypothesized that a male’s decision of performing more unsolicited attempts than courtship displays when he approaches a female before another male (chapter 4) improves his fitness. Now, to test this effectively, it is necessary to evaluate if males in the same social context (first to approach the female) mate more times and produce more offspring when adopting this strategy (perform more unsolicited attempts) than when invest in the other mating tactic (courtship display). Such studies should also compare fitness variables of males that invest more in the same mating tactic (unsolicited attempts) but in different social contexts (e.g. when the male approaches the female first *versus* when he approaches the female in second).

Special attention should be given to fertilization success. Although there is increasing evidence showing that post-copulatory processes affect male reproductive success (in guppies and in several other species – McDonald et al., 2017; Gasparini et al., 2018; Ruhmann et al., 2018), much remains unknown. Accordingly, I made predictions about the correlation between male mating behaviours and his investment in sperm, in chapters 2, 3 and 4. Therefore, it would now be interesting to quantify male investment in sperm number and seminal fluid composition in the social contexts that I have tested. This would confirm if the decisions made by males were indeed strategies to improve their success in terms of sperm competition (post-copulatory process) or in terms of mating success (pre-copulatory process). Alternatively, it could also be sperm cooperation, which remains less studied (Fisher, Giomi, Hoekstra, and Mahadevan, 2014; Foster and Pizzari, 2010). For example, males may allocate components of the seminal fluid that increase their sperm competitive ability (e.g. increasing sperm velocity due to cooperation among sperm cells within the same ejaculate) just when sperm competition risk is higher (e.g. when a competitor male approaches, but not when a male is the only one following a female).

The unexpected result found in chapter 3 (that male guppies tend to perform courtship display while competing with an attractive male for orange area) also deserves further investigation. Since I have measured only orange area, future studies could also measure orange brightness of competing males and compare these traits with the frequency of their courtship displays. Ideally, as during courtship orange brightness increases, brightness should be measured while males are courting. However, since guppies are small, and courtship is quick, this is a challenging task. Another possibility is to observe male responses to competitor males when the courtship (behavioural) trait is decoupled from the (physical) orange trait. A study of this kind was performed by Kodric-Brown and Nicoletto (2001) for female choice. Using computer-modified videos, these authors found that females were more attracted to male animations with orange colouration (rather than without) if both animations showed low display rates, but this was no longer the case when both animations displayed at high rate. This suggests that males with small areas of orange colouration could compensate by displaying more if more colourful competitors are present. This was not tested by Kodric-Brown and Nicoletto (2001), nor variations of orange area and brightness. With computer-modified videos this additional studies could be easily made.

What information each social cue and signal transmits? Is it relevant to identify the information transmitted?

The signals and social cues of male attractiveness considered in the different chapters of this thesis are related, that is, they provide information about male relative quality. However, we do not know what information content each of them exactly conveys. For example, chapter 3 considered male traits that are known to determine female mate choice in guppies (body size, and melanin and carotenoid-based colouration; Houde, 1997; Magurran, 2005), but we do not know what information each male trait is transmitting to females, though all traits should be related somehow with male quality, condition and/or motivation. For instance, orange brightness seems to be related with male condition because it changes over a male's lifetime and is related with male carotenoid intake (Grether, 2000; Reznick, Butler, and Rodd, 2001). However, males with a richer diet in carotenoids are not more resistant to parasitism than males with low carotenoid intake (Kolluru et al., 2006). So, what kind of information about male quality the orange colouration is providing? Could it be that it informs females about a male ability to find food (and hence a good general physical condition, including eventually good quality sperm), but not about male resistance to parasitism? Eventually this is the case, but more studies are needed. Still, the current knowledge (about guppies and other species) is sufficient enough to recognize that male ornaments are important in female mate choice, whatever the exact information they convey.

Researchers on animal communication have different views about the relevance of identifying the type of information transmitted. Some authors consider this is an extremely hard task or even an irrelevant one. Some claim that only the effect or influence of a signal/cue is relevant instead of the information that it actually conveys (Owren, Rendall, and Ryan, 2010; Scott-Phillips, 2008). Others consider that by using the word "information" we are anthropomorphising communication between animals and taking a language approach (Rendall, Owren, and Ryan, 2009). On the other hand, there are researchers who argue that decoding information is key to understanding a receiver/bystander response to a signal/cue, and the evolution of this signal/cue (Laidre and Johnstone, 2013; Seyfarth and Cheney, 2016). I agree that by taking this last approach we can move our understanding of animal communication to the next level. As Font and Carazo (2010) put it so well "On a proximate level, information allows receivers to make informed decisions; on an ultimate level, it is the reason why receivers respond to signals". Furthermore, if we do not know which information is transmitted, then we do not know if the use of multiple signals and social cues is a mere way of reinforcing the transmission of a given information, or, contrastingly, if it transmits different information relevant to individual fitness. Thus, I consider important to investigate which information signals and social cues are conveying.

Can the results be extrapolated to other populations of guppies?

I observed individuals from a population kept for several generations under stable laboratorial conditions. Based on the assumption that behavioural plasticity is more relevant for individuals that face changeable environments (de Jong, 1995; Partridge and Harvey, 1988; Thompson, 1991), it is plausible that individuals of wild populations show more plasticity than individuals kept in captivity, and that this could be part of a life-history strategy to cope with environmental uncertainty (Dingemanse and Wolf, 2013; Sol, Sayol, Ducatez, and Lefebvre, 2016; Trillmich, Günther, Müller, Reinhold, and Sachser, 2015). However, the results that I obtained showed that the males tested presented plastic behaviour, having adjusted their mating decisions according to different social conditions. Therefore, we could ask if the reason for this is because the time kept under stable conditions was not enough to counter select such behavioural plasticity. On the other hand, given that social information is one of the most complex and unstable components of an environment (Taborsky and Oliveira, 2012) and that the population I used faced social complexity (animals were kept in large, mixed-sex groups, and individuals were regularly transferred between tanks), it is likely that individuals of this population conserved the behavioural plasticity of the founding generation, at least in the social domain.

Future studies could also compare different populations of guppies. For example, by evaluating the effect of competitor males' sexual traits on focal males' mating decisions (as in chapter 3), when males are from populations for which sexual traits contribute differently to female mate choice. For instance, in some guppy populations females prefer large males but not in others (Endler and Houde, 1995). Although I observed that competitors' body size had no effect on male unsolicited mating attempts, it is possible that this result would differ if I tested other populations where male body size plays a greater role in female preference. In this case, I predict that males would perform more unsolicited attempts while competing with a large or larger male. Another possible study would be to consider populations with different predation risk. There is substantial evidence that wild guppy populations differ in behavioural patterns depending on predation risk, biased towards one or other mating tactic (Magurran and Seghers, 1990;

Luyten and Liley, 1991). For instance, males from the population studied here (Lower Tacarigua river) tend to perform more unsolicited attempts than populations descended from other Trinidadian rivers where the predation risk is lower (Magurran and Seghers, 1990). Eventually, such reduction in courtship display performance reduce males' conspicuousness and, hence, make the males less vulnerable to predators. Even so, I suspect that when comparing mating tactics of males descended from rivers where the predation risk differs (higher versus lower), the main results would still be similar to the ones described in this thesis, because the behaviours observed are likely adaptive under social conditions in general. For example, males of both populations should always gain advantage if they invest more in mating behaviours after spending more time without females (chapter 2), compete against less attractive males (chapter 3), and arrive first near a female (chapter 4).

Can the experimental results presented in this thesis be extrapolated to species other than guppies?

As explained in the Introduction section, the guppy is a good model to study the impact of social information on male mating decisions (Andersson and Simmons, 2006). Furthermore, variables considered in these empirical studies (such as time searching for females, competitor presence and attractiveness) are generally predictive of male reproductive success across animal taxa with typical sex roles (the male as the mate searcher sex, and the female as the choosier sex). Therefore, my results can provide valuable knowledge for future studies with other species. For instance, in several species male reproductive success is highly variable, being dependent on mate searching time and/or mating opportunities. Also, competitor males of several species can compromise other males' mating success, for example by interrupting mating, or by being preferred by females. Moreover, my results could be particularly relevant to species with opportunities for postcopulatory processes (i.e. with female multiple mating), since, for example, in a monogamous species a male does not have to face contrasting outcomes related with the order of arrival to a female (considered in chapter 4), since the sexual selection process relevant to his reproductive success is female mate choice, and postcopulatory processes (sperm competition and cryptic female choice) play no part.

What to expect more on the individual level?

This thesis gives important insights into behavioural plasticity from a population perspective, since I found that while different mating strategies are chosen by males in different contexts, the same strategies are consistently chosen by different individuals in the same contexts. Therefore, the results presented in this thesis show individual plasticity but, at the same time, population consistency. Some researchers, on the other hand, are considering individual plasticity making comparisons between individuals of the same species and populations (Dingemanse and Wolf, 2013; Stamps, 2016). Furthermore, some researchers are evaluating the behavioural plasticity of keystone individuals (i.e. of individuals that have a major relevance on a group), to determine how the behaviour of these animals affects that of the group and vice-versa (Modlmeier et al., 2014). In the context of sexual interactions, we could ask, for example, if the mating decisions of dominant males could lead subordinates to engage or avoid mating interactions with the same female or with all females in the group and with what level of investment.

How much do bystander males contribute to the evolution of male courtship display?

In chapter 5 I suggest an evolutionary pathway for courtship display driven by the eavesdropping of other males and, ultimately, leading to a redirection of this behaviour from the female target to a new target – the competitor males. I performed a systematic review to find whether evidences of male-male courtship display across the animal kingdom were correlated with evidences of eavesdropping and other related behaviours (exploitation and audience effect). Although I recognize that our results are embryonic as they do not show causality, they suggest a range of new and informative studies. First, it would be important to directly test whether male-male courtship display is a form of competition. Species in which this has been suggested (listed in chapter 5) will be a good starting point. Second, comparative studies on the evolution of male-male courtship displays with and without eavesdropping by competitor males, will allow to test for causality between these behaviours. In other words, it will allow to test for the hypothesis that male-male courtship display is more likely to be selected when there are bystander males than when not, evolving as a form of intrasexual competition. Third, if male-male courtship display is a form of competition, than it could be interesting to study what role it has in male contests and/or sperm competition: it could improve male success in fights, or deter competitors from fighting (i.e. by being a real threat signal); it could be an alternative competitive strategy used by inferior males to avoid costly contests (Andersson, 1994); or it could be used by males to avoid competitors from using other competition forms (e.g. sexual interference), eventually reducing sperm competition. Studies that evaluate the correlation between this behaviour and the type of males that perform and receive it (i.e. identify if males are subordinate or dominant, less or more attractive, etc.) will be extremely helpful. From another perspective, we can also ask if ritualized intimidation behaviours were, at some ancestral point, sexual behaviours. In fact, sometimes it is hard to differentiate attractive from intimidation ritualized displays. For example, in several butterfly species males perform displays towards other males in the same way as they display towards females, and researchers are intrigued by whether males are making erroneous displays or if such displays are a form of competition (Takeuchi, 2017). This brings again the discussion of whether weapons evolved into ornaments or vice-versa (Berglund et al., 1996; Morris et al., 2007). Phylogenetic studies comparing species where one or both ritualized displays (sexual and intimidating) occur can help understanding the evolutionary link between these two behaviours.

Which implications have dishonest social cues for the evolution of male mating behaviours?

The conceptual work described in this thesis (chapter 6) raises a series of questions. Although, in theory, it is expected that dishonest signals produce dishonest cues when intercepted by bystanders, it is necessary to test this hypothesis. Since a dishonest signal represents a cost to receivers, an interceptive dishonest cue should also represent a cost to bystanders. In the context of sexual interactions, this could be tested by observing responses of bystander males to exaggerated sexual signals of low condition males (the terminal reproductive investment hypothesis; Clutton-Brock 1984; Wong and Candolin 2005; Harrison et al. 2013). If, for example, bystander males avoid contests with such dishonest displayers (the same way they avoid with honestly signalling males), then the cue is indeed dishonest. But, if instead bystander males still decide to compete with the deceiving male, then we could assume that the dishonest cue was not effective in deterring the bystander. On the other hand, the female could receive the dishonest signal as honest, and mate with the male. In such a scenario, females could benefit from the bystander response, as it would help them to better discriminate between honest and dishonest males. This interplay between the displayer male, the female and the bystander could even facilitate the evolution of a dual function of courtship display. In line with this, and as suggested before, another relevant study would be to compare the prevalence of dishonest signals (courtship display or other) across generations in the presence and absence of a bystander. Such a study should include the evaluation of costs and benefits for senders, receivers and bystanders.

7.3. Concluding remarks

Overall, the results presented in this thesis show that social information has a substantial impact on male mating decisions. Specifically, I have shown that male guppies adjust their behaviour to information transmitted by conspecific females and males (sexual partners and sexual competitors), and that the responses of competitors to courtship displays may have given raise to male same-sex sexual behaviour. These results are interpreted in terms of the specificities of each social context in which males are placed and of its impact on male mating and fertilization success. Finally, I emphasize the importance of bystanders to the evolution of dishonest communication in animals. Taken together, research presented here shows that individual decision-making and, more broadly, the behavioural ecology of a species, can only be understood by considering the entire communication network in which animals take part (McGregor and Peake, 2000; Sih, Hanser, and McHugh, 2009).

Results presented in this thesis support the view that social information is pivotal in behavioural adaptation (Dall et al., 2005). This adaptation is commonly expressed in the form of behavioural plasticity, and behavioural plasticity is part of what Taborsky and

Oliveira (2012) called “social competence”. Social competence is the ability of animals to detect and respond to social information, which is characterized by being highly variable in space and time (each social partner produces social information differently). The social environment is, therefore, complex and by consequence requires high levels of behavioural flexibility. My findings agree with this view but also highlight that, while individuals in a population contribute to a variable and ever changing social environment, they also display features that transmit consistent information all the time. For example, the social context that a male face during mating is highly variable – he can encounter a female alone or with a competitor male nearby, and the competitor can be less or more attractive than himself. This requires that males adjust their behaviour to each circumstance. However, the ways to do it may not vary much – e.g. the presence of a competitor always indicates a higher risk of sexual interference, and of the male losing mate choice or sperm competition. Males can, therefore, respond by investing more when they are the first to arrive near a female, or by performing more courtship if the competitor is more attractive. These are simple rules that probably increase male fitness in most situations.

In summary, male guppies, as well as other species that are likely to face changeable environments, benefit from manifesting plastic behaviours, as this ability enhances their fitness. This is particularly important when considering complex environments (namely under the social domain) and the rapid environmental changes currently induced by the human (Sih, 2013; Sol, Lapiedra, and González-Lagos, 2013; Wong and Candolin, 2015).

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